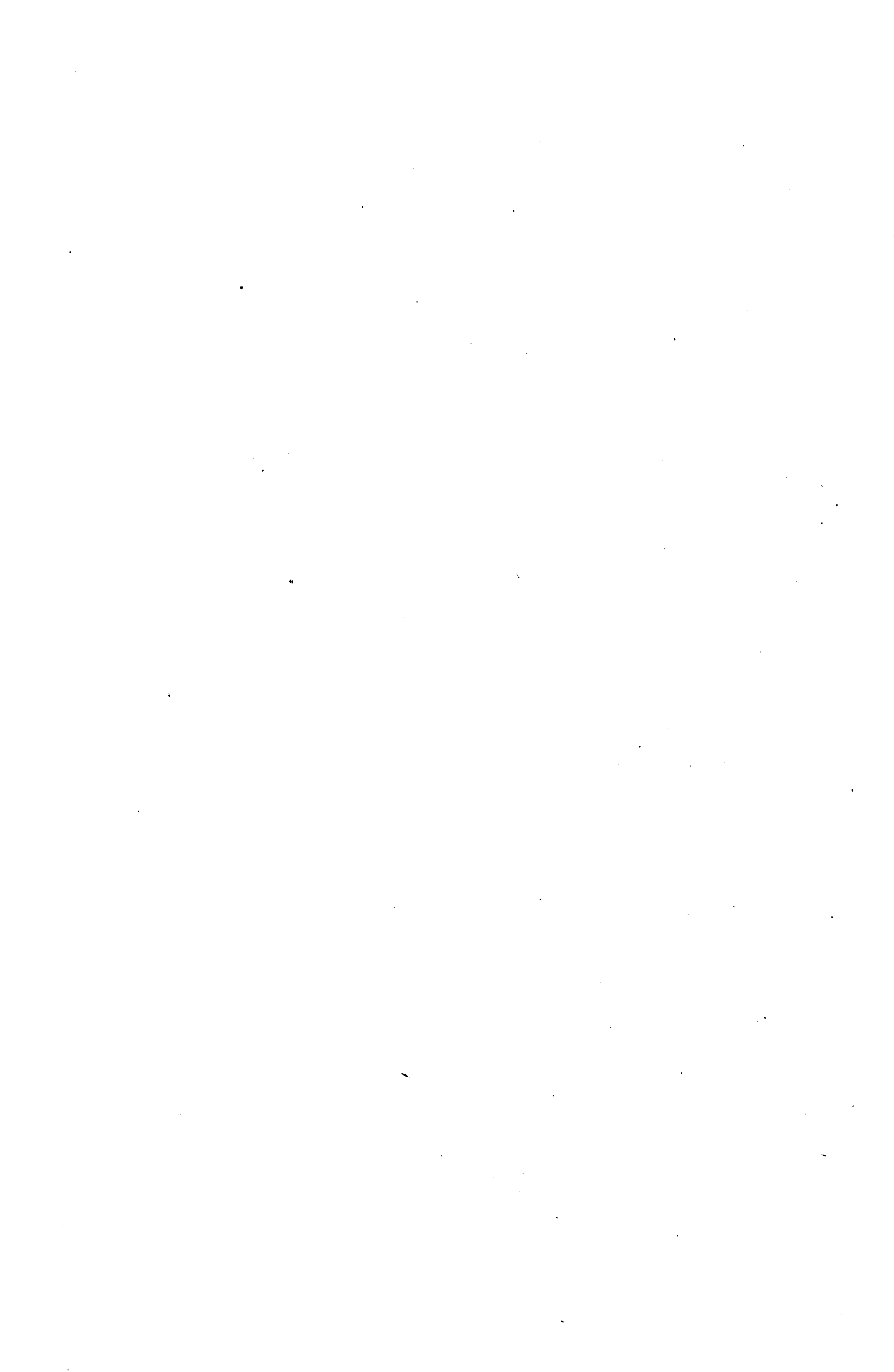

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# *Thaxteriellopsis lingicola* and its *Moorella* anamorph*

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MS received 14 October 1981 ; revised 18 December 1981

**Abstract.** A *Moorella* anamorph referable to *M. speciosa* Rao and Rao, is described for *Thaxteriellopsis lignicola* Sivanesan, Panwar and Kaur. The connection is established from a study of single ascospore cultures of the fungus established for several (6) South Indian collections. Both the teleomorph and anamorph are described and brief notes are given of characteristics of this fungus in culture.

**Keywords.** *Thaxteriellopsis lignicola* ; *Moorella speciosa* ; teleomorph ; anamorph.

## 1. Introduction

During the survey of microfungi under the project 'Fungus Flora of South India', several collections of a Loculoascomycete with setose ascomata were made. Single ascospore cultures of the fungus produced an anamorph referable to the hyphomycete genus *Moorella*. The *Moorella* anamorph was also found in association with the Loculoascomycete on the natural substrate. The teleomorph was identified as *Thaxteriellopsis lignicola* Sivanesan, Panwar and Kaur, and the anamorph as *Moorella speciosa* Rao and Rao.

The monotypic genus *Thaxteriellopsis* was erected by Sivanesan *et al* (1976) with *T. lignicola* collected from Mount Abu, India as the type. It is characterized by non-ostiolate, setose, cupulate ascomata, superficial on a subiculum, with bitunicate asci containing hyaline or subhyaline ascospores, which are transversely multiseptate. Some of the ascospores, in addition, develop one longitudinal or oblique septum, rarely two in one or two of its cells.

In all the collections studied, the fungus agrees in all details with the type description of *T. lignicola* except for the difference in the number of the septa in ascospores. The transversely multiseptate ascospores, with one or two longitudinal and oblique septa in some, typical of *T. lignicola* were seen only in one collection from the Silent Valley, Kerala (Herb. FSI 3363). In the remaining collections the ascospores were predominantly transversely 5-septate. The present study shows that in *T. lignicola* the ascospores are initially 5-septate ; the centrum is hyaline to white, as seen in most of the collections. As the ascospores mature, additional transverse, longitudinal and oblique septa develop ; the centrum becomes light brown, as seen in the single collection from the Silent Valley. The additional

* Memoir No. 352 from Centre of Advanced Study in Botany.

transverse, longitudinal and oblique septa develop in any sequence and are forme
within any cell, without any regular pattern, in the originally 5-septate ascospore

## 2. Description of the fungus

*Thaxteriellopsis lignicola* Sivanesan, Panwar and Kaur, Kavaka 4 : 39, 197
Anamorph : *Moorella speciosa* Rao and Rao, Mycopath. Mycol. appl. 22 : 5
1964. (figures 1–19.)

Colonies on the substrate conspicuous, superficial, widely effused, black, consi
ting of velvety growth of anamorph, interspersed with scattered to gregarious asc
mata of teleomorph. Surface mycelium composed of septate, branched, da
brown, creeping hyphae 4–7$\mu$ wide, constricted at septa when cells are short an
swollen, smooth when the cells are long cylindrical. Immersed mycelium consi
ting of septate, branched, light brown or dark brown hyphae ca. 5$\mu$ wide.

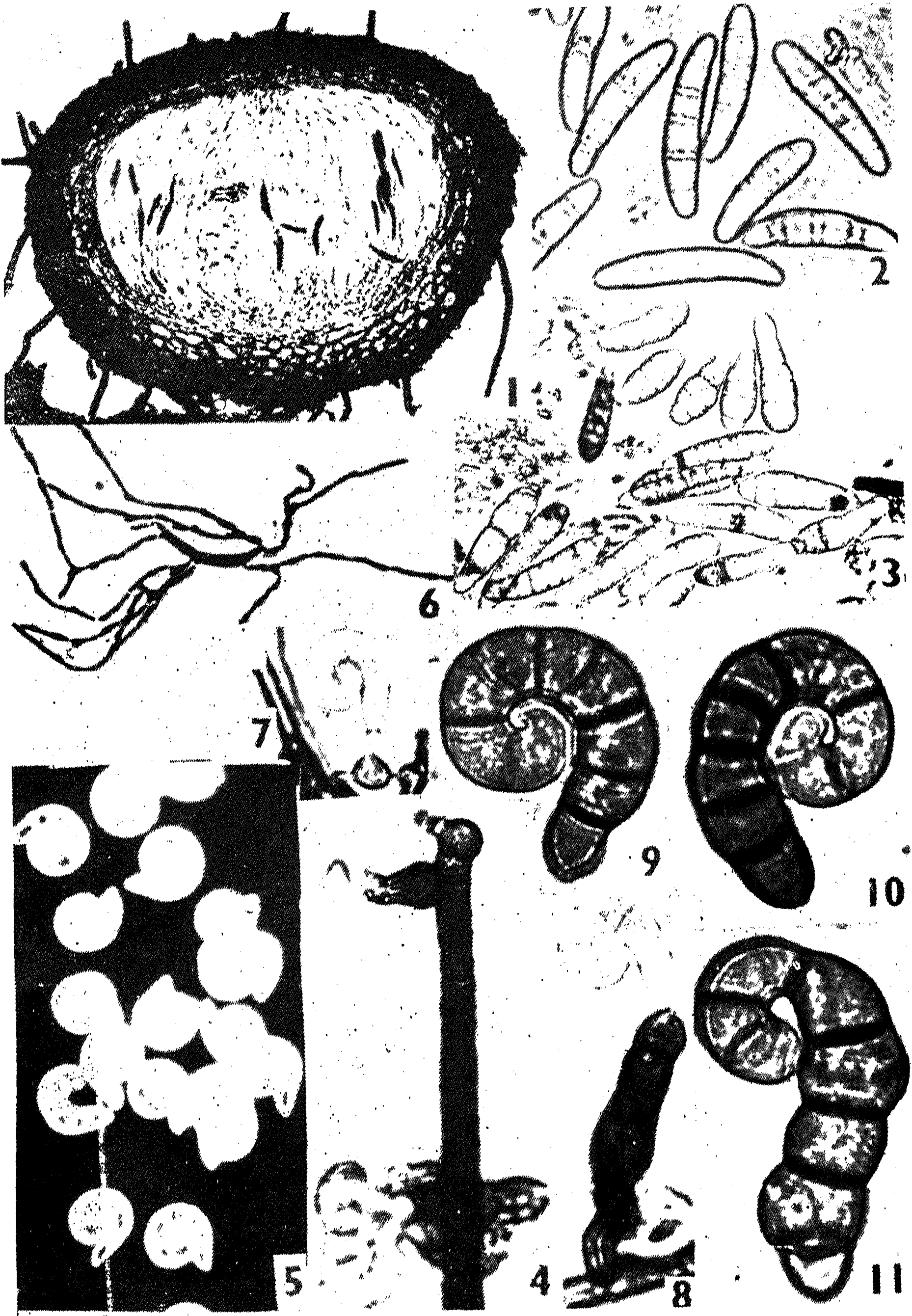
Ascomata superficial, connected to the creeping mycelium by septate, brow
pendant hyphae ca. 5$\mu$ wide, originating from the lower half of the ascoma, son
seated on a stroma directly on the substrate, black, cupulate, in water becomi
spherical to broadly spherical or obconic-spherical, 200–300$\mu$ high, 250–37
diam., non-ostiolate, with numerous dark brown, septate, unbranched setae, wi
rounded ends, mostly on the upper half of the ascoma. Setae up to 250$\mu$ lon
4·5–6·0$\mu$ wide. Wall of the ascomata somewhat fleshy, 35–50$\mu$ thick, pseud
parenchymatous, composed of 6–9 layers, made up of polygonal cells, 5–20
5–13$\mu$ ; cells of 'ostiolar' region comparatively smaller, 5–10 × 3–5$\mu$ ; eac
cell with a large oil droplet, oozing out in teased mounts ; outer layers dark brow
gradually becoming light brown to hyaline towards the inner layers ; cells of t
inner layers flattened.

Asci in a basal hymenium, bitunicate, long-cylindrical to cylindric-clavate, shor
pedicellate, 90–150 × 15–30$\mu$, generally 8-spored ; sometimes fewer-spored.

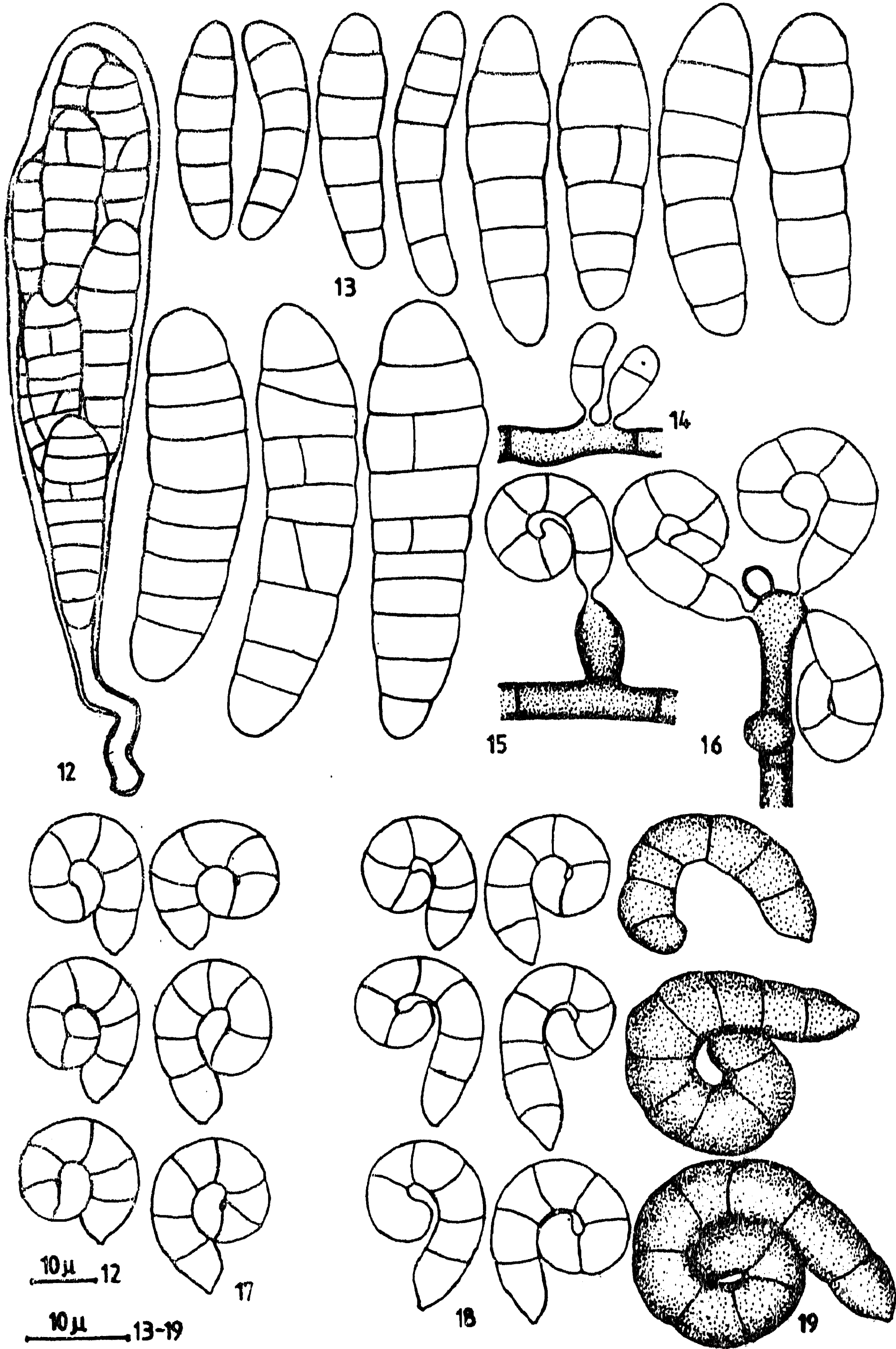
Pseudoparaphyses present, attached both to the roof and the basal hymeniu
septate, hyaline, branched, ca. 1·5$\mu$ wide.

Ascospores irregularly tristichous, 23–50 × 5·5–13 (–16)$\mu$, initially with
transverse septa. Mature ascospores with additional transverse, longitudin
and oblique septa, up to 6–12-transversely septate and often with up to 3 long
tudinal and/or oblique septa, clavate to clavate-fusiform or fusiform ; general
the second and thir dcells from apex broader, tapering towards the rounded end
curved to straight, thin-walled, slightly constricted at septa in large spores, faint
striate, hyaline to smoky brown in mass.

Conidiophores erect, straight or slightly bent, arising directly from creepi
mycelium, septate, smooth, up to 420$\mu$ long, blackish brown and 7–11$\mu$ wide a
base, gradually becoming pale brown and 4–7$\mu$ wide at apex, bearing up to si
whorls of branches at intervals, with one to six branches per whorl ; branch
1–4-celled, 4·5–7·5$\mu$ wide. Conidiophore often terminating in an apical who
of branches or a conidiogenous cell. Terminal cell of each branch conidiogenou
Conidiogenous cells hyaline, light brown to brown, flask-shaped to globose o
cylindrical, often proliferating, polyblastic, denticulate. Conidia helicoid, tightl
coiled 1–1½ times, borne on short cylindrical denticles ca. 1$\mu$ long, 3–10 septat
generally 6-septate, 11–15$\mu$ diam. ; filaments 4–6$\mu$ wide, hyaline, finally becomin
smoky brown with faint striations.




**Figures 1–11.** *Thaxteriellopsis lignicola* and its *Moorella* anamorph. **1.** Vertical section of ascoma (Herb. FSI 3359) × 200. **2.** Predominantly 5—septate ascospores (Herb. FSI 3151) × 700. **3.** Mature ascospores showing longitudinal and oblique septa (Herb FSI 3363) × 480. **4.** Part of a conidiophore with conidiogenous cells and a conidium initial (Herb. FSI 3151) × 1200. **5.** Conidia from natural substrate (Herb. FSI 3363) × 750. **6.** Germinated ascospore (Herb. FSI 2128) × 375. **7.** A conidiogenous cell with a developing conidium (from culture, Herb. FSI 3151) × 1200, **8.** A short conidiophore with a solitary terminal conidium (from culture, Herb. FSI 3151) × 1065. **9, 10, 11.** Mature brown conidia from a 6 month's old culture (Herb. FSI 3151) × 1600.




**Figures 12–19.** *Thaxteriellopsis lignicola* and its *Moorella* anamorph. **12.** Ascus (Herb. FSI 3363). **13.** Ascospores (Herb. FSI 3363). **14.** Young conidia developing directly on a hypha (from culture, Herb. FSI 1423). **15.** A conidium borne on a single conidiogenous cell produced laterally on a hypha (from culture, Herb. FSI 1423). **16.** A conidiophore with several conidia produced from its apical part (from culture, Herb. FSI 1423). **17.** Conidia from natural substrate (Herb. FSI 3151). **18.** Conidia (from culture, Herb. FSI 3151). **19.** Brown conidia from a 6 month's old culture (Herb. FSI 3151).

Collections examined :

All collections by G. Sekar.

Karnataka State

On indet. wood, Kemphole Forest, near Sakleshpur, Hassan District, 31 October 1977, Herb. FST 1423 ; on indet. wood ; Mundwar Pimpley Forest, near Londa Belgaum District, 22 November 1980, Herb. FST 3204 ; on indet. bark, Karwar, North Kanara District, 18 November 1980, Herb. FSI 3151.

Kerala State

On indet. wood, Silent Valley, Palghat District : Kumattanthod, 5 December 1980, Herb. FSI 3225, 3230 ; Dam site, 6 December 1980, Herb. FSI 3257 ; camp site, 7 December 1980, Herb. FSI 3261 ; near Valaiyampara Estate, 8 December 1980, Herb. FSI 3271 ; Mathrithod, 11 December 1980, Herb. FSI 3321 ; Valaiyaparathod, 13 December 1980, Herb. FSI 3356, 3359. On wood and bark of *Syzygium cumini* (Linn.) Skeels, Valaiyaparathod, Silent Valley, Palghat District, 13 December 1980, Herb. FSI 3363. On indet. wood, Karapara Forest, Palghat District, 21 December 1980, Herb. FSI 3413, 3417 ; 24 December 1980, Herb. FSI 3461.

Tamil Nadu State

On wood of *Syzygium cumini* Seegaltheri, Kalakad, Tirunelveli District, 13 February 1979, Herb. FSI 2048. On indet. wood, Mundanthurai Forest, Tirunelveli District, 16 February 1979, Herb. FSI 2128. On indet. bark, Mundanthurai Forest, Tirunelveli District, 18 February 1979, Herb. FSI 2166. On wood of Mango, Kakkanalla bridge, Mudumalai Forest, Nilgiri District, 12 January 1981, Herb. FSI 3480.

## 3. Cultural studies

Ascospores germinate on potato dextrose agar within 8 h at 25–30° C. Germ tubes are produced from any one or all the cells of ascospores (figure 6), more frequently from the end cells. Single spore isolations on potato dextrose agar are slow growing, attaining 1 cm. diameter in 10 days. The colonies are olive green to olivaceous brown, velvety, with compact margin, restricted in growth, with aerial mycelium and dark brown submersed mycelium. The conidiogenous cells are either intercalary or terminal on hyphae, or on a simple conidiophore (figures 8 and 16). Typical dark brown erect conidiophores with whorls of branches bearing conidiogenous cells are produced after three months in culture. Conidia are produced on narrow, cylindrical denticles, ca. 1$\mu$ long and are loosely or tightly coiled or just bent and curved. Conidium size is comparable to that on the natural substrate, but some may be a little larger. In a long standing culture, conidia (figures 9–11 and 19) become brown, up to 12-septate and measure up to 23$\mu$ diameter with filaments 9$\mu$ wide, constricted at the septa.

## 4. Discussion

*Thaxteriellopsis* is a member of the Loculoascomycetes and belongs to the Pleosporales; in having *Pleospora* type of centrum. It is closely related to *Thaxteriella*, as pointed out by Sivanesan *et al* (1976), and possibly to *Tubeufia*. The anamorph

of *Thaxteriellopsis,* as shown here, is *Moorella* and that of *Thaxteriella* and *Tubeufia* are *Helicoma* and *Helicosporium* respectively. It is interesting to note that all the three teleomorphs mentioned above have somewhat fleshy ascomata, with usually hyaline ascospores and their anamorphs are dematiaceous hyphomycetes producing helicoid conidia on denticles. This is the first time a *Moorella* anamorph is connected with a teleomorph.

Barr (1980) notes that the illustration and description of *Thaxteriellopsis lignicola* are suggestive of *Boerlagiomyces* Butzin. However, even if this were to be confirmed by further study of the types, the generic name *Thaxteriellopsis* will have to be retained according to the rules of priority. The name *Thaxteriellopsis lignicola* is therefore used here for the teleomorph of our fungus.

## Acknowledgements

One of the authors (GS) is grateful to University Grants Commission for the award of Junior Research Fellowship, under the project 'Fungus Flora of South India'. They also thank Mr V Kaviyarasan for photographic assistance.

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# A contribution to the embryology of *Alysicarpus monilifer* D.C.

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MS received 25 April 1981 ; revised 4 February 1982

**Abstract.** Embryology of *Alysicarpus monilifer* D.C. is described. The anther wall comprises the epidermis, the fibrous endothecium, two middle layers and uniseriate glandular tapetum. Pollen is shed at the 2-celled stage. The ana-campylotropus ovule is crassinucellate and bitegmic. The archesporium in the ovule is hypodermal and cuts off a parietal cell. Embryo sac development conforms to the Polygonum type. Endosperm development is nuclear. A chalazal part of the endosperm remains free nuclear and is haustorial in function. Embryogeny conforms to Period I Megarchetype IV and Series $B_2$. The embryogenic classification of the tribe is discussed in the light of the present observation and earlier reports.

**Keywords.** Embryology ; *Alysicarpus*.

## 1. Introduction

The tribe Hedysareae of the Papilionaceae includes 47 genera and 7000 species distributed in the tropical and subtropical regions of the world (Rendle 1925). Of these only 14 genera are known embryologically. The researches of Souèges (1947, 1953a, 1953b, 1955, 1956), Johansen (1950), Rau (1951, 1953, 1954), Smith (1956), Goursat (1961), Kapuskar (1964) and Deshpande *et al* (1976) reveal the embryogeny of 15 species belonging to 11 genera of this tribe. The embryology of *Alysicarpus monilifer* D.C. is described here.

## 2. Material and methods

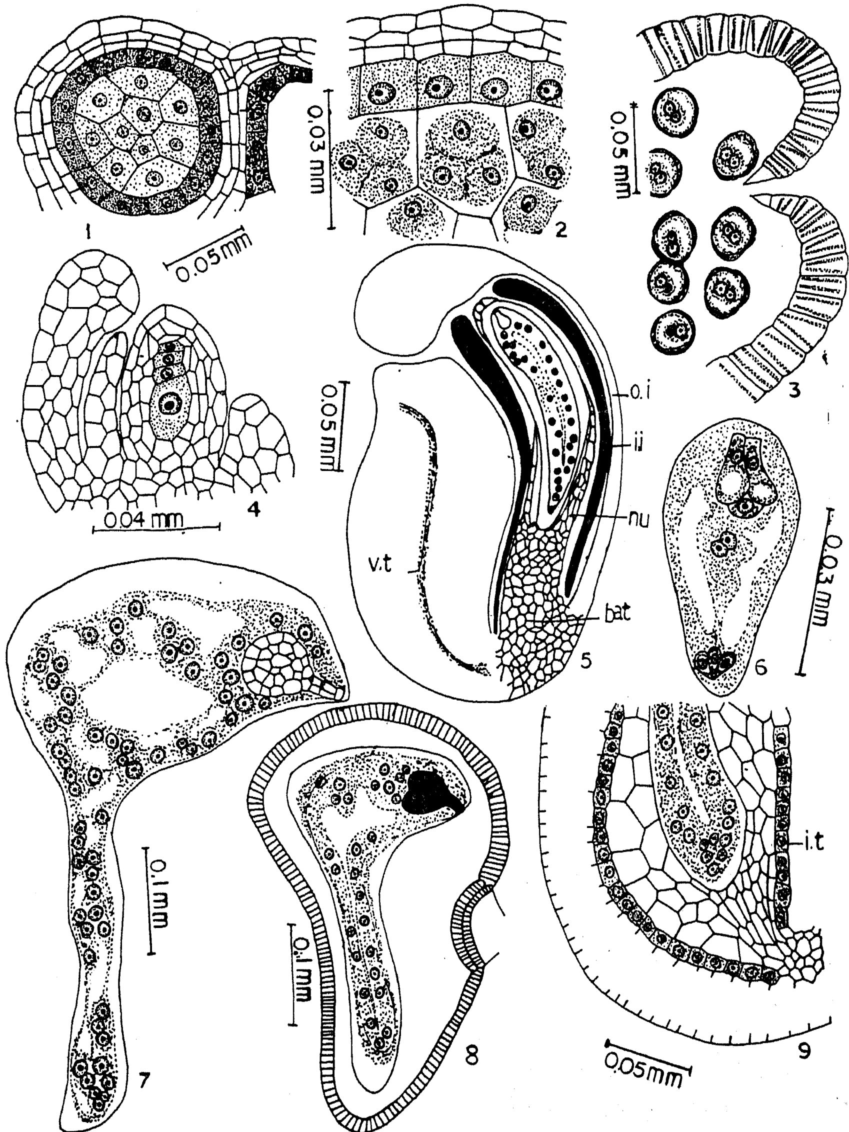
Buds, flowers and fruits of varying ages were collected from the plants growing in the university campus, during the rainy season and were fixed in FAA. Customary methods of dehydration and embedding, etc. were followed. Sections cut between 8 and 10 microns thick were stained with safranin and fastgreen. The endosperm was observed in whole mounts after making suitable dissections.

## 3. Observations

### 3·1. *Microsporogenesis and the development of pollen*

The archesporium in the anther is hypodermal represented by a single row of 4–5 cells. The primary parietal layer by further divisions forms 2–3 parietal layers of which the innermost forms the secretory tapetum (figure 1). The tapetal cells remain uninucleate throughout. Some of the cells on the connective side adjoining

the tapetum also behave like the tapetal cells in assuming a dense cytoplasm and deeply stained nuclei. The hypodermal parietal layer forms the fibrous endothecium (figure 3). The two middle layers are crushed in the mature anther. Division of the microspore mother cell is simultaneous and cytokinesis takes place by furrowing (figure 2). The pollen is shed at the 2-celled stage.




**Figures 1–9. 1.** T.S. anther lobe showing wall layers and sporogenous tissue ; **2.** Same showing cytokinesis in pollen mother cells ; **3.** Same showing fibrous endothecium and two celled pollen grains ; **4.** L.S. portion of ovule showing a linear tetrad of megaspores ; **5.** LS ovule showing the integuments endosperm and barrier tissue at the globular stage of embryo ; **6.** Mature embryo sac ; **7.** Nuclear endosperm showing chalazal elongation ; **8.** LS young seed showing the embryo and endosperm ; **9.** LS portion of the ovule at the chalazal regions showing the incipient integumentary tapetum and thick walled barrier tissue.

### 3·2. *Megasporogenesis and female gametophyte*

The ovule at maturity is campylotropous bitegmic and crassinucellate (figure 5). The single celled archesporium in the ovule is hypodermal and cuts off a parietal cell. The megaspore mother cell undergoes the usual meiotic divisions resulting in a linear tetrad of magaspores of which the chalazal is functional (figure 4). The development of the mature embryo sac conforms to the Polygonum type. The mature megagametophyte is 8-nucleate with an egg apparatus, two polar nuclei and three antipodal cells (figure 6). The antipodal cells are ephemeral and degenerate prior to fertilization.

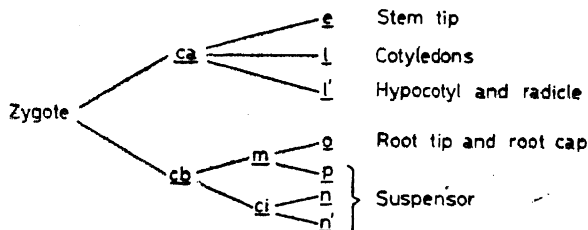
### 3·3. *Endosperm*

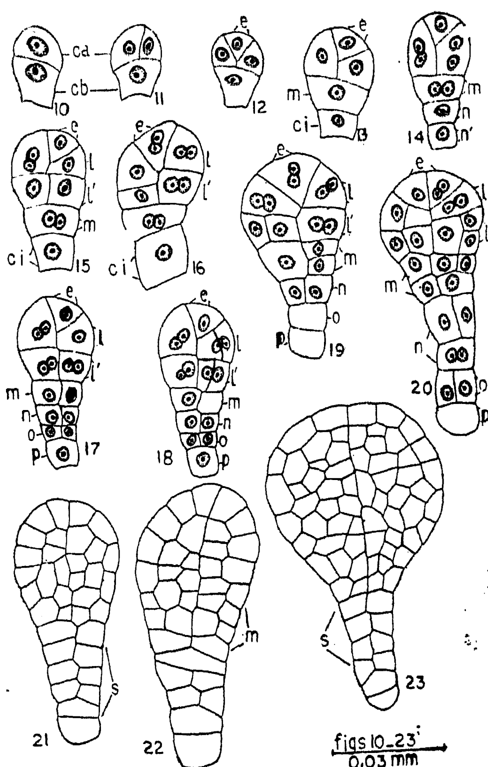
The primary endosperm nucleus divides much earlier than the zygote and the development of the endosperm is of the nuclear type. The endosperm remains free nuclear until the embryo reaches the early dicotyledonous stage (figure 8). During the course of its development the embryo sac enlarges enormously and encroaches on the surrounding nucellar tissue. This is more prominent at the micropylar region where it comes in direct contact with the innermost layer of the inner integument. At the chalazal region the tubular free nuclear part assumes a haustorial role (figure 7). The active growth of the endosperm haustorium at the chalazal end is arrested by the development of thick walled barrier tissue (figure 5). The cells of the innermost layer of the inner integument have dense contents and are prominent (figure 9).

### 3·4. *Embryo*

The developmental sequence in the embryogeny is illustrated in figures 10 to 23. The zygote divides transversely resulting in a two celled proembryo. The apical cell (*ca*) divides by an obliquely vertical wall resulting in two unequal cells (figure 11). Another oblique division in the larger derivative cell of the tier (*cal*) cuts off an epiphyseal initial (*e*) (figure 12). The basal cell (*cb*) undergoes a transverse division resulting in *m* and *ci*. Further divisions in the apical quadrant demarcate the tiers *l* and *l′*. Periclinal divisions in both the tiers differentiate the dermatogen. The epiphyseal initial undergoes a vertical division and by further divisions contributes to the stem tip in the mature embryo.

The cell *m* divides by a vertical wall and both the cells divide transversely. Their upper derivatives contribute to the root tip and the root cap, while the derivatives of the lower cells together with those of tiers *n* and *n′* contribute to the long and massive suspensor. A schematic representation of the zygote derivatives and their destinations in the mature embryo is given below :






**Figures 10–23.** Stages in the development of embryo.

Thus the embryogeny according to Souèges scheme (Crété 1963) falls under Period I, Megarchetype IV and Series $B_2$.

## 4. Discussion

The archesporium in the ovule is hypodermal in all the species investigated so far in the tribe the sole exception being *Desmodium paniculatum* where a subhypodermal archesporium was reported by Rembert (1969). However, this needs verification in view of its rare occurrence in the family Papilionaceae.

In *Alysicarpus monilifer* wall formation in the endosperm is initiated after the differentiation of the cotyledonary lobes in the embryo and is further restricted to the upper half of the embryo sac, while the chalazal part functions as the haustorium. A similar feature has earlier been reported by Rau (1953) in *Desmodium triflorum*, *D. tortulosum*, *D. pulchellum*, *Eleiotis soraria* and *Aeschynomene indica*; by Kapuskar (1964) in *Aeschynomene aspera* and by Deshpande *et al* (1976) in *Zornia diphylla*. *Stylosanthes mucronata* (Rau 1953) is so far the only member of the tribe where a cellular endosperm is not organised at all in the developing seed.

The tribe Hedysareae shows considerable variation in the mode of embryo development and in the nature and organisation of the suspensor. The variation met with in the embryogeny of the tribe is summarized in table 1.

**Table 1.** Variation in the embryo development among the members of the tribe Hedysareae.

| Species investigated | Author | Embryo type, after Johansen 1950 | Embryo type, after Souéges 1948 |
|---|---|---|---|
| *Coronilla minima* | Souéges 1947 | Coronilla variation of the Onagrad type | Period I, Megarche-type VI, series A |
| *Desmodium canescens* | Rau 1954 | do. | do. |
| *D. canadense* | Rau 1954 | do. | do. |
| *Hedysarum coronarium* | Souéges 1956 | do. | do. |
| *Hippocrepis comosa* | Souéges 1955 | do. | do. |
| *Lespedeza violacea* | Rau 1954 | do. | do. |
| *Ornithopus perpusillus* | Souéges 1953a | do. | do. |
| *)nobrychis sativa* | Souéges 1953b | do. | do. |
| *Scorpiurus verniculata* | Goursat 1961 | do. | do. |
| *Zornia diphylla* | Deshpande and Bhasin 1976 | do. | do. |
| *leschynomene indica* | Rau 1951 | do. | do. |
| *1. aspera* | Kapuskar 1964 | do. | do. |
| *Desmodium laevigatum* | Rau 1954 | do. | Period I, Megarche-type VI, Series B |
| *llysicarpus monilifer* | Present study | Alysicarpus variation of the Onagrad type | Period I, Megarche-type IV, Series B |
| *lrachis hypogea* | Smith 1956 | Solanad type | Period I, Megarche-type V, Series C |
| *Iedysarum nutans* | Johansen 1950 | Caryophyllad type | Period II, Megarche-type IV, Series A |

The embryogeny in a majority of the species follows a more or less uniform pattern agreeing with that originally described by Souèges (1947) in *Coronilla minima* and belongs to the first embryogenic group under Period I, Megarchetype VI and Series A. According to the system of Johansen (1950) the embryogeny in all these species conforms to the Onagrad type, but does not fit into any of the variations proposed by him. Hence it is now proposed to erect a new variation designated as 'Coronilla variation' under the Onagrad type to accommodate all these genera of the tribe Hedysareae, belonging to Period I, Megarchetype VI and Series A, where $cb = s$.

Rau (1954) observed that *Desmodium laevigatum* differs in its embryogeny from the rest of the members of the tribe which necessitates further investigations to evaluate the significance of this variation.

*Alysicarpus monilifer* shows further deviation from the fundamental type, where the embryogeny falls under the second group along with *Desmodium laevigatum* but belongs to the fourth megarchetype in period I where $cb = iec + co + s$. In this respect *Alysicarpus* differs from the rest of the Hedysareae and resembles members of Phaseoleae and Galegeae.

According to Johansen's system, the embryogeny in *Alysicarpus monilifer* conforms to the Onagrad type but does not fit into any of the variations proposed under that type. The embryogeny shows resemblence to the Trifolium variation of the Onagrad type in the possession of the epiphyseal initial, but differs in the destination of the derivatives of the basal cell *cb*. In Trifolium variation, based on the embryogeny in *Trifolium minus* (Souèges 1927) the entire basal cell *cb* contributes to the suspensor ($cb = s$) whereas in *Alysicarpus* the derivatives of the basal cell contribute to the root tip and root cap in addition to suspensor ($cb = iec + co + s$).

Hence, a new variation designated as *Alysicarpus* variation is proposed to accommodate the type, having an epiphyseal initial and where the derivatives of the tier *cb* contribute to the root tip and root cap in addition to the suspensor. *Alysicarpus* is so far the only genus in this tribe where an epiphyseal initial is demarcated in the tier *ca* and $cb = iec + co + s$. In this respect it resembles *Rhynchosia suaveolens* of Phaseoleae, and *Tephrosia strigosa* of Galegeae (Seshavatharam 1969) suggesting a relationship between these tribes and Hedysareae embryogenically.

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# Non-inheritance of isomerism in cocoyams

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**Abstract.** The present communication describes the non-inheritance of isomerism in prefoliation and contortion of spathes in cocoyams. Importance of isomerism from the point of taxonomy and agronomy has been discussed. It is expected that a study on the orientation of leaf promordia at shoot apex and their spiral descendance would help in unravelling the hidden laws in shoot morphogenesis of cocoyams.

**Keywords.** Isomerism; cocoyams; dextro; levo; contortion; prefoliation; taro; tannia.

## 1. Introduction

Isomerism in biological materials was described by many workers under different names such as asymmetry (Davis 1962) bioisomerism (Meyen 1973; Venkateswarlu and Hrishi 1977) enantiomorphism (Davis 1974), isomerism (Bahadur and Venkateswarlu 1976 a,b), chirality (Venkateswarlu 1978) and radial symmetry (Davis 1978).

Isomerism in prefoliation and contortion of spathes in cocoyams was described in detail (Venkateswarlu and Hrishi 1977, Venkateswarlu 1978). In some crops this phenomenon is correlated with yield (Davis 1972). The present study is taken up to see if the corm yield is associated with prefoliation and if so whether this trait is transmitted through cormels and true seeds. Kasinov (1969) described that this character is transmitted through fronds in *Lemna gibba* L. The present paper describes the investigations carried out on the pattern of prefoliation in tuber and seedling progenies in taro and only tuber progenies in tannia.

## 2. Materials and methods

All the specimens used were taken from the collection of germplasm of edible aroids maintained at the Central Tuber Crops Research Institute, Trivandrum. The method of scoring a plant into levo and dextro was the same as that reported earlier (Venkateswarlu and Hrishi 1977; Venkateswarlu 1978). Observations on transmission of prefoliation through cormels in taro and tannia were recorded in 1978. The seed tubers were selected from labelled plants and sown. Observations on prefoliation were recorded at weekly intervals. Based on the prefoliation

pattern, plants were classified as levo, dextro and bichiral. Observations were also made on prefoliation pattern on the main pseudostem and on the suckers developing from the main clump.

For inheritance studies in taro seedlings, the nature of contortion in spathe was recorded at the time of effecting crosses and labelled as C95 $L \times$ C 271 $D$ etc. as the case may be. Seeds obtained from the cross were sown and observations were recorded from the spathes produced in the seedlings. Observations were also made on prefoliation in taro seedlings.

## 3. Results and discussion

### 3·1. *Observations on Prefoliation*

The phyllotaxy in cocoyams is alternate. Unfurling of leaves is either clockwise (levo) or anticlockwise (dextro) (figures 1–6). Plants were labelled as levo, dextro and bichiral based on their morphology during the growth period. The corm and cormels of labelled plants were sown during 1978 to see whether the nature of prefoliation is transmitted vegetatively. The prefoliation pattern of seed setts and the resulting new pseudostems in both taro and tannia are presented in table 1.

Out of 60 levo tubers planted in taro, 28 grew into levo, 28 as dextro and 4 bichiral types *i.e.*, levo and dextro in 1 : 1 ratio. Total dextro tubers planted in taro were 21, out of which 9 developed as levo, 12 as dextro. Interestingly, no bichiral plants were produced when dextro tubers were planted.

In tannia, bichiral plants were produced from both levo and dextro tubers. In all, 38 levo tubers were planted, from which 11 levo, 21 dextro and 6 bichiral plants were produced. From 42 dextro tubers sown, 17 behaved as levo, 21 as dextro and 4 as bichiral. There seems to be no association between the seed sett and the preudostems in cocoyams similar to that of *Costus speciosus* (Davis 1978).

Observations were also made to know whether the new suckers developing from the seed sett differ in the nature of isomerism in prefoliation in taro. The data are presented in table 2. From the data presented in tables 1 and 2, it is obvious that the isomerism in 'prefoliation of cocoyams is not transmissible vegetatively which is contradictory to the results of Kasinov (1969) in *Lemna gibba*. Further the whole phenomenon is complicated by an irregular pattern the suckers show.

Similar observations were noticed in the seedlings of taro also. Levo and dextro seedlings were produced almost in a 1 : 1 ratio indicating the non-genetic nature of the character. This is similar to the findings of Davis (1962) in coconut.

### 3·2. *Contortion of spathes in taro seedlings*

An attempt was also made to study the inheritance pattern of spathe contortion in taro seedlings. Crosses were made in all possible combinations ($L \times L$, $L \times D$, $D \times D$ and $D \times L$). At the time of flowering, the nature of contortion in spathes was recorded. Seedlings of all the crosses flowered except of $D \times D$. The data on the asymmetry of spathe are presented in table 3. It is clear from the data that both levo and dextro spathes were produced in almost equal numbers within the same plant. Davis(1962) also reported similar 1 ; 1 ratio of left and

**Figures 1–6.** **1.** Levo prefoliation in taro, **2.** Dextro prefoliation in taro, **3.** Levo contortion of spathe in taro, **4.** Dextro contortion of spathe in taro, **5.** Levo prefoliation in tannia, **6.** Dextro prefoliation in tannia,

**Figures 7-8. 7.** Levo dextro prefoliation in the same clump of taro, **8.** Levo and dextro prefoliation in the same clump of tannia.

Table 1. Isomerism in prefoliation of seed setts and new pseudostems.

| Name of the crop | Nature of seed sett | No. of seed setts planted | Isomerism in new pseudostems | | | |
|---|---|---|---|---|---|---|
| | | | Levo | Dextro | Bichiral | Total |
| Taro | Levo | 60 | 28 | 28 | 4 | 60 |
| | Dextro | 21 | 9 | 12 | ... | 21 |
| | Total | 81 | 37 | 40 | 4 | 81 |
| Tannia | Levo | 38 | 11 | 21 | 6 | 38 |
| | Dextro | 42 | 17 | 21 | 4 | 42 |
| | Total | 80 | 28 | 42 | 10 | 80 |

right spiralled plants in coconut in all the four combinations indicating that this character is not genetically controlled.

Furthermore, the fact that both levo and dextro rotatory leaves and spathes are produced on the same plant suggests that this phenomenon is non-genetic (figures 7,8). At the same time, it is interesting to see some plants behaving either as levo or dextro throughout their growth period with respect to both prefoliation and contortion (Venkateswarlu 1978). Such plants were termed unichiral.

Isomerism, a fairly common mechanism in biological materials has attracted the attention of several workers. This is exhibited in different characters like prefoliation in leaves, contortion in floral parts, twining in stems and coiling of shells in molluscs.

This phenomenon was widely used over the years by taxonomists, phylogeneticists and agronomists . Hutchinson(1964) used twisting of keel petals and styles for taxonomic purposes. Smartt (1976) pointed out that the Asiatic species of *Phaseolus* show clock-wise contortion of style and the American species show counter clock-wise contortion. More recently, Jos and Venkateswarlu (1978) used twining nature in Asian yams to describe their distribution. It was suggested that the phylogeny of dextral types might be basically different from that of sinistral types even among the Asian yams.

In palms (coconut, arecanut and toddy palm) the nature of leaf spiral determines the yield. Right spiralled plants were found to be higher than their counterparts (Davis 1972). Bahadur *et al* (1978) probed into the causes resulting in higher yield in right spiralled coconut. Their study revealed that the right spiralled coconuts were physiologically superior to the left spiralled ones and therefore the higher fruit production in right handers was correlated to their efficient metabolism.

In *Dioscorea esculenta*, the levo plants were found to give more yield than the dextro plants under artificially controlled conditions. But the vericals were better than lefts by 42·63% and the rights by 55·57% (Davis 1972).

Table 2. Isomerism in prefoliation of main stem and the suckers in taro.

| Nature of main stem | No. of tubers planted | Prefoliation in suckers | | |
|---|---|---|---|---|
| | | Levo | Dextro | Total |
| Levo rotation | 8 | 4 | 15 | 19 |
| Dextro rotation | 3 | 3 | 3 | 6 |
| Total | 11 | 7 | 18 | 25 |

Table 3. Isomerism in spathes of seedling families in taro.

| Sl. No. | Cross particulars | Levo | Dextro | Total |
|---|---|---|---|---|
| 1. | 95 *L* × 271 *L* | 1 | 2 | 3 |
| 2. | 271 *L* × 251 *L* | 1 | 1 | 2 |
| 3. | 271 *L* × 135 *L* | 4 | 3 | 7 |
| 4. | 271 *L* × 268 *D* | 3 | 3 | 6 |
| 5. | 271 *L* × 271 *D* | 1 | 2 | 3 |
| 6. | 271 *D* × Kovvur *L* | 2 | 1 | 3 |
| | Total | 12 | 12 | 24 |

Similar studies conducted in cocoyams did not show any convincing results. Moreover, since this character is not even transmitted vegetatively, the results will not have any bearing on its utility as selection criterion.

At the moment, we are not in a position to understand the factors responsible for the expression of levo, dextro, and bichiral nature in the prefoliation and contortion of spathes in cocoyams. Recent study by Davis (1978) on *Costus speciosus*, suggests the involvement of one or more important laws governing plant growth and he hopes that a study on the orientation of leaf primordia at shoot apex and their spiral descendance may help in unravelling the hidden law in shoot morphogenesis.

Similar study in cocoyams may also reveal some interesting facts responsible for the mechanism as the new leaves and spadices arise from shoot apex.

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# Apomixis in *Cenchrus glaucus* Mudaliar et Sundaraj

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**Abstract.** A detailed cytoembryological investigation in *Cenchrus glaucus* revealed that it is an obligate apomict producing only aposporous embryosacs. Microsporogenesis disclose chromosomal irregularities and megasporogenesis occasionally occur and the development of sexual embryosac is completely absent. On the other hand, aposporous initials develop into 4-nucleate embryosacs. Autonomous development of the embryo is of common occurrence.

**Keywords.** *Cenchrus glaucus* ; obligate ; apospory.

## 1. Introduction

*Cenchrus glaucus* Mudaliar et Sundaraj, a pentaploid perennial tropical species belongs to the tribe Paniceae of Panicoideae. The occurrence of apomixis in this genus was first investigated by Fisher *et al* (1954) in *Pennisetum ciliaris* and *Cenchrus setigerus*. Later Snyder *et al* (1955) outlined a detailed study on the formation of aposporous embryosacs in *C. ciliaris*, a polymorphic facultative apomict. Preliminary investigation has disclosed that another species of *Cenchrus, C. glaucus* an aneuploid with $2n = 45$, where in microsporogenesis is characterized by abnormalities such as univalents, multivalents and lagging chromosomes, reproduces by gametophytic apomixis. The present study reports the results of cytoembryological studies revealing the formation of only aposporous embryosac (obligate apomict) and the complete absence of sexual embryosac.

## 2. Material and methods

Clones were collected from Agricultural College, Coimbatore and were grown in the Departmental Botanical Garden, Manasagangotri, University of Mysore, Mysore. Identification of the material was confirmed by the Botanical Survey of India, Coimbatore. A voucher specimen of the material is deposited in the Herbarium, Department of Botany, University of Mysore, Mysore, India.

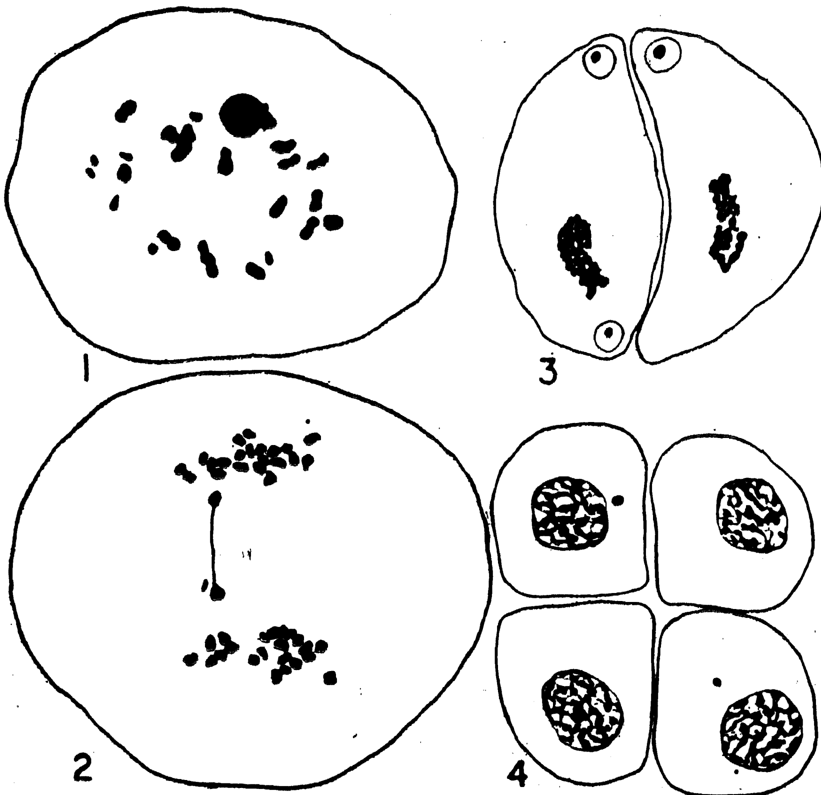
Florets at appropriate stages of development were collected betweeen 10 a.m. and 1 p.m. and fixed in a mixture of 3 : 1 absolute alcohol and acetic acid, later on stored in 70% alcohol. Pollen mother cells were smeared in 2% acetocarmine for the study of meiosis. For mitotic studies, root tips squashes were made follow-

ing Tijo and Levan's (1950) technique. Embryological studies were carried out following conventional methods of dehydration, infiltration and embedding. Sections were cut at 10–14 microns in thickness and stained in Heidenhain's iron alum haematoxylin.

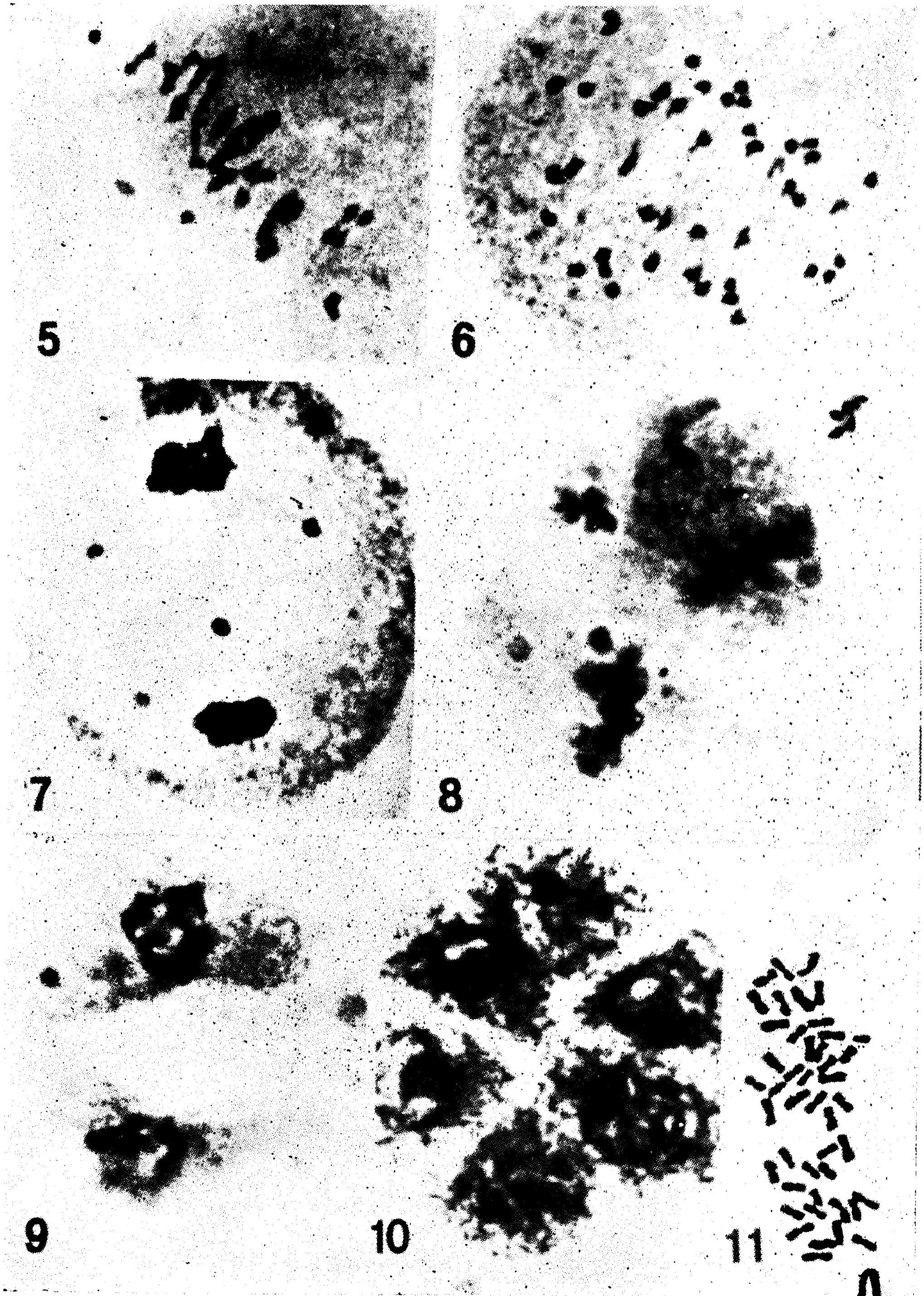
## 3. Results

### 3·1. *Microsporogenesis*

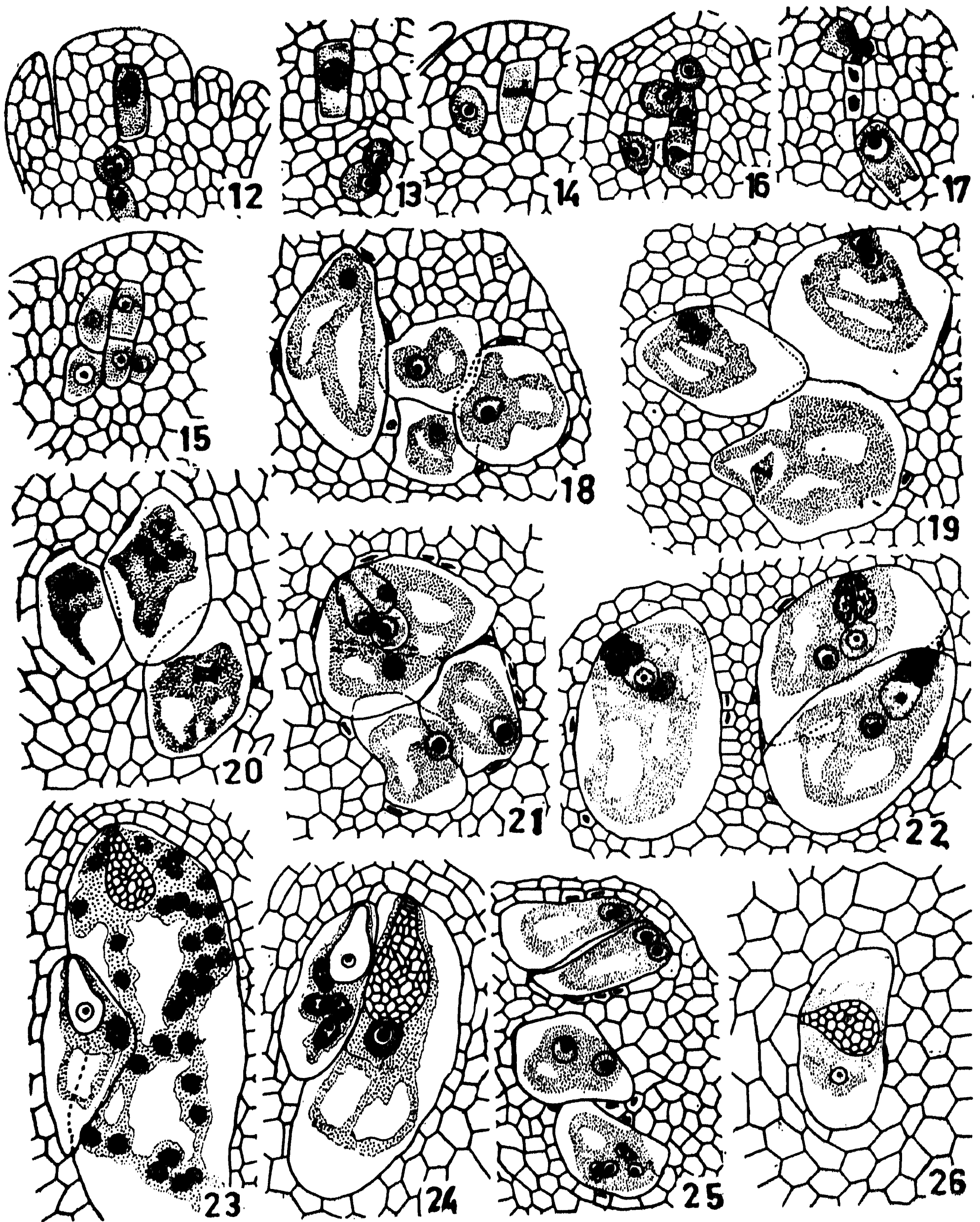
During diakinesis and metaphase I, tetravalents, univalents in addition to bivalents are formed (figures 1, 5). Chromosome number was confirmed in root-tip (figure 11). Occasionally, a bridge and a fragment configuration indicating structural changes were seen at anaphase I (figure 2). Anaphase distribution is marked by irregularities such as irregular distribution (figure 6), laggards (figure 7) and micronuclei formation at dyad stage (figure 8). Second division irregularities are also pronounced with precocious movement of chromosomes at anaphase (figure 9). Figure 3 reveals a dyad in division stages with 2 micronuclei in one




**Figures 1–4.** Microsporogenesis. **1.** diakinesis stage showing tetravalents, trivalents, univalents and bivalents, **2.** anaphase I showing bridge fragment configuration, **3.** dyad in division showing single micronucleus at each pole in one of the dyad cells, while in the other, one micronucleus, **4.** tetrad with chromosomal material being left behind.




**Figures 5–11.** Microsporogenesis. **5.** metaphase I with multivalents, bivalents and univalents, **6.** anaphase with irregular distribution of chromosomes, **7.** late anaphase with lagging chromosomes, **8.** dyad showing micronuclei, **9.** dyad in division with micronucleus in one of the cells and also showing precocious movement of chromosomes, **10.** polyad formation, **11.** somatic cell with 45 chromosomes (magnification × 750).




**Figures 12–26.**

(For figure captions see page 35)

of the dyad cells, while in the other a single micronucleus at one pole. On the other hand, figure 4 reveals chromosomal material being left behind in a tetrad. All these above irregularities lead to polyad formation (figure 10). Pollen sterility is about 51%.

### 3·2. *Megasporogenesis and megagametogenesis*

Megasporogenesis begins with the differentiation of an hypodermal archesporial cell which functions as the megaspore mother cell (figure 27) and proceeds through the usual meiotic divisions. Figures 16 and 17 reveal degenerating dyad and tetrads. In addition, several ovular sections showed the inception of the meiotic divisions (figures 13, 14, 15). The presence of either sexual embryosacs or the stages leading to their formation were not observed in any ovule.

### 3·3. *Aposporous embryosac*

Aposporous initials numbering from 1 to 8 in most of the ovules differentiated in the vicinity of megaspore mother cell (figures 12, 13, 14) or its meiotic products (figures 15, 16, 17) within the nucellus. In the early stages of development aposporous initial which consists of a single nucleus located at one end of the cell with a prominent vacuole developing below it (figures 18, 29) undergoes the first mitotic division resulting in 2 nuclei and both the nuclei are located at the same pole (figures 19, 28). The next division would result in a 4-nucleate embryosac and all the 4-nuclei remaining at the same pole (figures 20, 30). Later on, organization of the four nuclei takes place, 3 nuclei contribute to form an egg apparatus of 2 synergids and an egg and a solitary polar (figures 21, 31). However, synergid nuclei degenerate very early, leaving an embryosac with an egg and a polar nucleus (figures 22, 32). Occasionally, the 4-nuclei without organization undergo further divisions resulting in the formation of 6 to 8 nuclei all grouped together (figure 25). The development of aposporous embryosac may be simultaneous (figures 19, 22, 29) or may be non-simultaneous (figures 20, 21, 31).

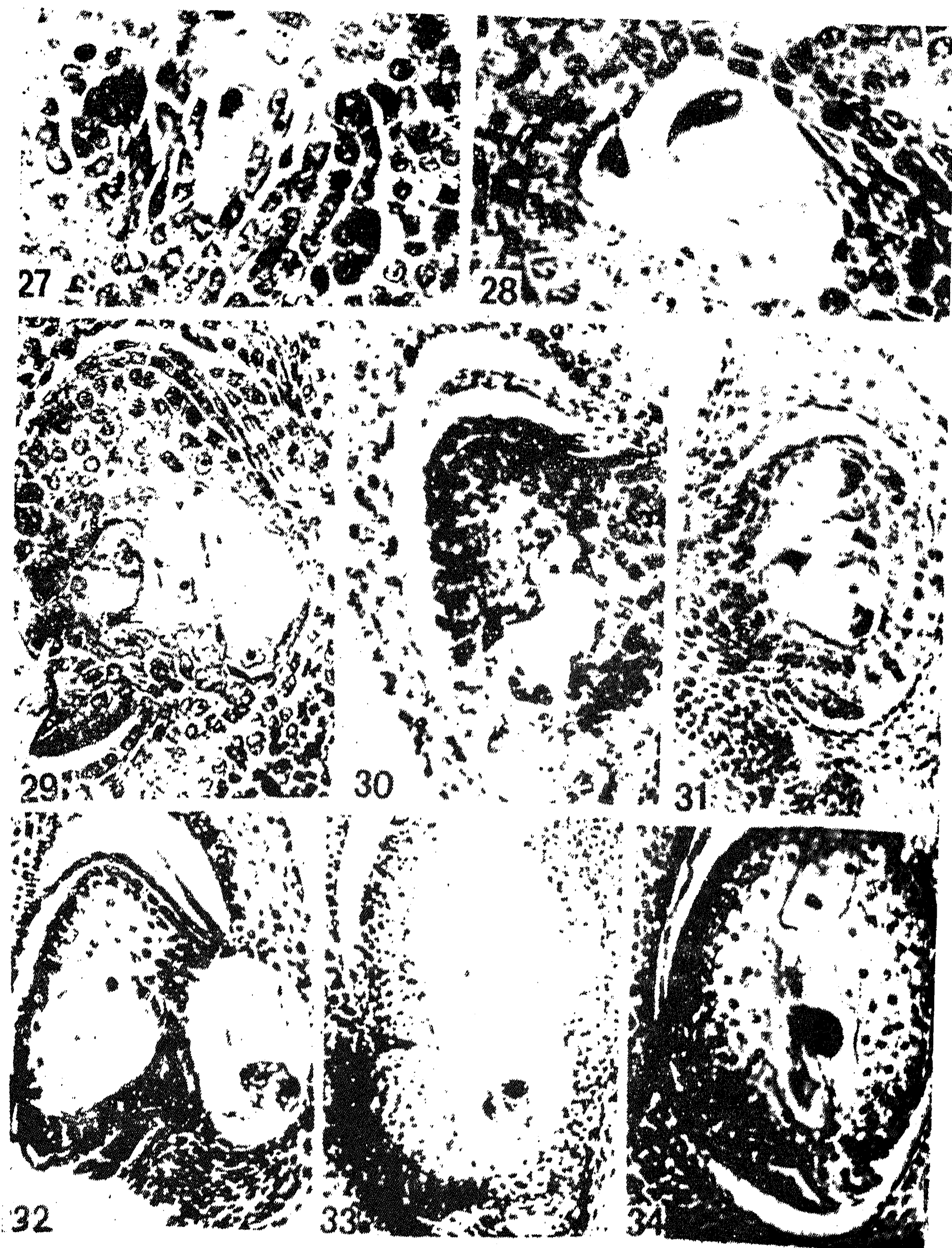
In addition to the aposporous cells 2 or 3 nucellar epidermal cells at the micropylar end become conspicuous with dense cytoplasm and enlarged nuclei (figure 30). In extreme cases aposporous embryosacs are found at the attachment of the ovule to the ovary wall (figure 32). The average value of embryosacs per ovule is indicated in table 1.

### 3·4. *Embryo and endosperm*

The association of embryo and endosperm varies in different embryosacs of the same ovule. Embryosacs containing well developed embryo and multinucleate endosperm occur along with embryosacs containing an undivided egg cell and a

**Table 1.** Average value of embryosacs per ovule.

| | No. of embryosacs per ovule | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| No. of of ovules examined | 15 | 16 | 30 | 15 | 18 | 16 | 10 | 10 |




**Figures 27–34.** Megasporogenesis, aposporous embryosac development and embryo and endosperm relationship, **27.** MMC, **28.** 2-binucleate aposporous embryosac, **29.** 3-uninucleate aposporous embryo sacs, **30.** 4-nucleate aposporous embryosac, **31.** organized aposporous embryosac, **32.** aposporous embryosac developing at the attachment portion of the ovule to the ovary wall and one of the embryosac showing an egg and a polar nucleus, **33.** many celled embryo with a polar towards one side of the embryo, **34.** an embryo showing disturbed polarity (magnification × 150).

single polar nucleus in the same ovule (figure 23). Embryosacs containing embryo with a single polar nucleus coexist with embryosacs containing undivided egg cell with endosperm in the same ovule (figure 24). Sometimes embryos lack normal polarity (figures 26, 34) and the polar nucleus is found lateral to the embryo. Precocious development of the embryo is frequently encountered as in figure 33 and a polar nucleus is found towards one side. Although, the number of embryos varies from 3 to 4, twin seedlings are only 4%. These seedlings also reproduced apomictically and triple seedlings were absent.

## 4. Discussion

*Cenchrus glaucus* is an obligate pentaploid apomict with chromosome number $2n = 45$. Microsporogenesis proceeds with chromosomal irregularities resembling those found in *Cenchrus* ciliaris (Fisher *et al* 1954) resulting in about 51% sterile pollen. The presence of high percentage pollen viability in *C. glaucus* may indicate pseudogamous seed formation. The chromosomal abnormalities in this taxon are similar to *Pennisetum setaceum, P. villosum* (Narayan 1951) which are also obligate apomicts. According to Weimarck (1972) such aberrations in *Hierochloe* species seem to be associated with the occurrence of apomixis.

Megasporogenesis sometimes occurs and embryosac formation is rarely encountered. In aposporous apomicts irregularity of meiosis at the time of megaspore formation may affect the ability of the embryosac formation. Degeneration of all the megaspores sometimes observed in *C. glaucus* may be due to irregular meiosis as reported in *Hieracium aurantiacum* (Skalinska 1971) and in *Poa pratensis* (Grazi *et al* 1961). However, 4-nucleate unreduced embryosac is of common occurrence. On the other hand, the megaspore mother cell or its products degenerate autonomoulsy even before the origin of aposporous initials. Such autonomous degeneration even before the origin of aposporous initials is reported in *Pennisetum villosum, P. setaceum* (Narayan 1951), *Paspalum secans* (Snyder 1957), *Poa granitica* (Skalinska 1959), *Heteropogon contortus* (Emery and Brown 1958), *Bothriochloa schaenum* (Brown and Emery 1957), *Pennisetum ciliare* (Snyder *et al* 1955), *Panicum maximum* (Warmke 1954) and in *Boutelouva curtipendula* (Mohamed and Gould 1966).

The presence of 4-nucleate aposporous embryosac in this genus was reported first in *Cenchrus ciliaris* (Snyder *et al* 1955). The 4-nucleate embryosac development has been described in detail in nearly 14 species in Poaceae. Brown and Emery (1958) have reported the presence of 4-nucleate aposporous embryosacs in 43 of 153 species.

A striking feature of this species, however, is apart from embryosacs present in the nucellar portion, embryosacs are also found in such extra-nucellar places, the broad base of the ovule where it is attached to the pericarp wall. Such nucellar embryosacs have been reported in *Pennisetum villosum* and *P. setaceum* (Narayan 1951), *P. mezianum* (Shanthamma 1974), *Themeda triandra* (Brown and Emery 1957), *Hierochloe odorata* (Weimarck 1967), *Hieracium aurantiacum* (Skalinska 1971) and *Hieracium pratense* (Skalinska and Kubien 1972).

Embryos in *C. glaucus* arise exclusively from unreduced aposporous embryosacs. Polyembryony as studied by Norstog (1957) is high in Poaceae. Armstrong

(1937) reported 42% polyembryony in a particular strain of *Poa*. Snyder *et al* (1955) reported 20% of polyembryony in *Pennisetum ciliare*, but in *Cenchrus glaucus* only 4% of the seeds produced twin seedlings. (All the seedlings reporduced apomictically. Triple seedlings were totally absent although in ovular sections apparently 2 to 3 embryos per ovule were commonly observed). The low frequency of twin seedling produced in *C. glaucus* may be attributed to the lack of endosperm formation in embryosacs of a single ovule.

## Acknowledgement

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Figures **12–26.** Megasporogenesis, aposporous embryosac development, and embryo and endosperm relationship. **12.** LS of nucellus showing megaspore mother cell and 3 conspicuous nucellar aposporous cells (× 3000), **13.** MMC at diakinesis and 2 aposporous cells (× 3000), **14.** metaphase I of MMC with a single aposporous cell initial (× 3000), **15.** dyad and 3 prominent nucellar cells (× 3000), **16, 17.** degenerated dyad and tetrad with nucellar cells (adjoining) prominent (× 2000), **18.** a group of 4 aposporous (uninucleate) embryosacs (× 2000), **19.** a group of 3 aposporous embryosacs, 2 embryosacs at binucleate stage and the other one is at metaphase, **20.** 3 aposporous embryosacs, one at 4-nucleate stage while the third one at anaphase (× 2000), **21.** a group of 3 aposporous embryosacs at different stages of development and one of the embryosacs is at 4-nucleate stage (× 1200), **22.** 3 aposporous embryosacs showing 2 degenerated synergids, an egg and a polar nucleus ( × 2000), **23.** two aposporous embryosacs, one showing many celled embryo and free nuclear endosperm and the other embryosac showing an egg and a polar nucleus (× 1200), **24.** ovule showing 2 embryosacs, one with many celled embryo and a single polar nucleus while the other embryosac with a single egg cell and 4 endosperm nuclei (× 1200), **25.** a group of 4-aposporous embryosacs, 3 embryosacs are binucleate and the remaining one showing unorganized 6-nucleate condition (× 1200), **26.** an aposporous embryosac with disturbed polarity of embryo (× 1200).

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# Regeneration of plantlets from callus of *Elettaria cardamomum* Maton

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**Abstract.** Embryo callus and callus of rootstocks of *in vitro*-raised seedlings of *Elettaria cardamomum* were grown on MS medium supplemented with CW + 2, 4-D + BAP. Differentiation of shoot buds, roots and leaves leading to the development of plantlets could be induced in callus by withdrawing 2, 4-D or substituting it by IAA or NAA in low concentrations.

**Keywords.** Callus culture ; cardamom ; regeneration.

## 1. Introduction

Reports on induction of shoot buds and whole plants from tissue cultures of both monocotyledonous and dicotyledonous plants have been numerous in recent years as evident from the spate of publications on the subject (see reviews by Murashige 1974 ; Narayanaswamy 1977). Clonal propagation through tissue culture has been successful with many spice and condiment plants such as *Foeniculum vulgare* (Maheshwari and Gupta 1965), *Anethum graveolens* (Ratnamba and Chopra 1974), *Carum corvi* (Ammirato 1974) and *Capsicum annuum* (Gunay and Rao 1978). Spectacular rate of multiplication of turmeric (*Curcuma longa*) plants have been reported in cultures of young vegetative buds isolated from the root stock (Nadaguada *et al* 1978). This prompted us to investigate the potential for organogenesis in tissue cultures of the cardamom (*Elettaria cardamomum* Maton of Zingiberaceae) widely used as a condiment. This paper reports the successful regeneration of shootbuds and plantlets from seedling callus of the herbaceous perennial species.

## 2. Methodology and results

### 2·1. *Seed germination*

Dry seeds of cardamom were surface sterilised by 0·1% mercuric chloride solution to which a few drops of the detergent Teepol had been added. After washing

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thoroughly in sterilized water, the seeds were sown on White's (1963) nutri agar. Slender seedlings were obtained in three weeks on incubation at 26° Whole seedlings bearing the first sheathing leaf and plumule were transferred Murashige and Skoog's (1962) medium (MS) to which auxins, cytokinins a coconut water (CW) as specified (table 1) and sucrose (2%) had been added. EDTA was used as the iron source. Each treatment comprised 12 replica Embryo callus was also obtained directly from seeds sown on the medium (figure containing an auxin such as 2, 4-D (2,4-dichlorophyenoxy acetic acid).

## 2·2. *Callus induction*

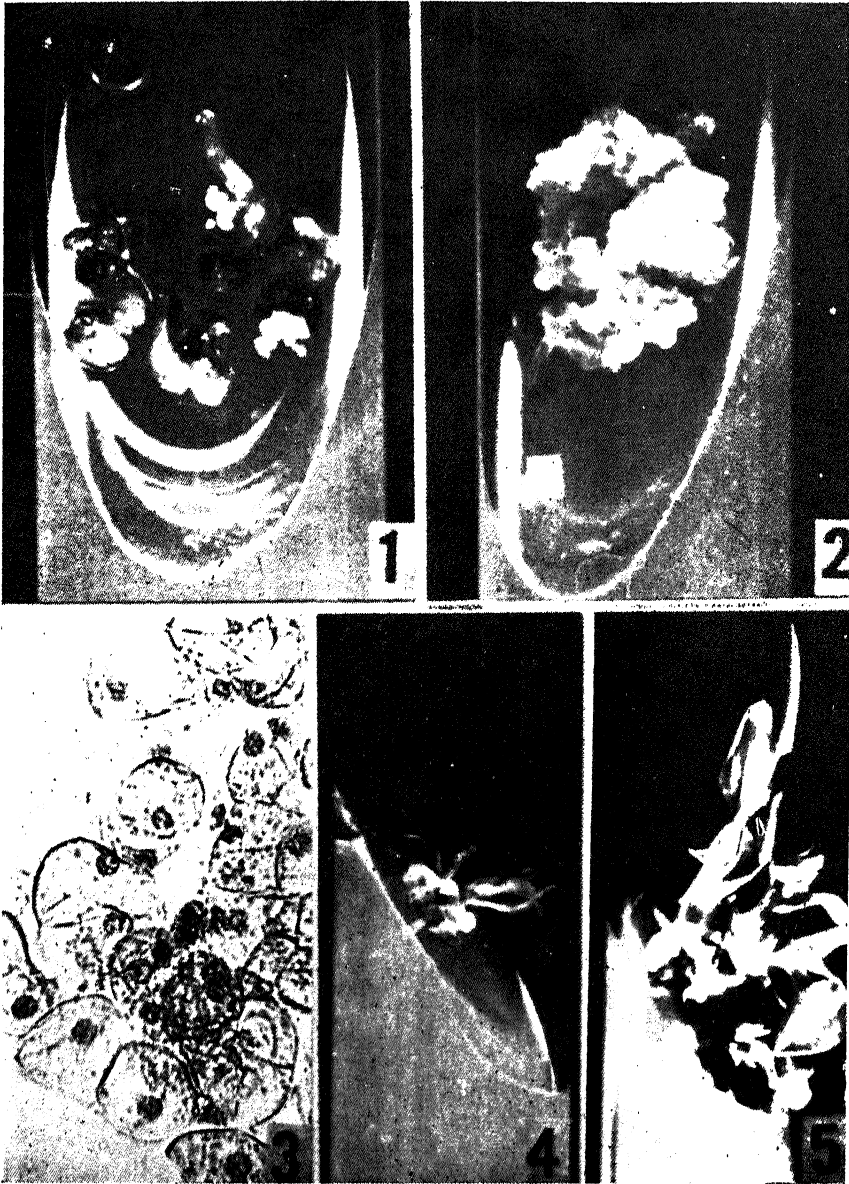
Five-week-old 3 cm long seedlings bearing the first sheathing leaf and plum were transferred to MS medium to which CW (18% v/v) + 2, 4-D(2mg/l) indole-3-butyric acid (IBA 2mg/l) or naphthalene acetic acid (NAA 2mg/l) a benzylaminopurine (BAP 2mg/l) had been added in combinations as need Proliferation of cells from the rootstock was observed in 75% of the cultures res ting in the formation of exuberant callus in 6 weeks after incubation (figure MS supplemented with CW (18% v/v) + 2, 4-D (2mg/l) + BAP (0·5mg/l) v conducive for callus initiation and growth which could be augmented by the ad tion of casein hydrolysate (CH, 1gm/l) in the medium. Neither yeast extr 250mg/l nor malt extract (250mg/l) proved favourable for callus growth; 2, 4 could, however, be replaced by IBA for callusing. Propionocarmine squas of the proliferating callus showed cells of diverse sizes and shapes (figure Tracheidal differentiation of cells was marked.

## 2·3. *Regeneration of shoot buds*

Callus growing on MS + CW + 2, 4-D was subcultured on medium devoid 2, 4-D but containing 1AA (2mg/l) or NAA (1mg/l). Three weeks after trans green nodular structures developed in the callus indicating the initiation of organis growth centres. Callus grown on medium with higher concentrations of the au became friable and was not conducive for shoot bud induction. But inducti

**Table 1.** Response of cardamom calli to growth regulators *in vitro* on sequen transfer.

| Sl. No. | Media composition (Hormone concentrations in mg/l) | Nature of response |
|---|---|---|
| 1. | MS + CW (18% v/v) + 2, 4-D (2) | Callusing good |
| 2. | MS + CW (18% v/v) + 2, 4-D (2) + CH (1g/l) + BAP (0·5) | Callusing exuberant |
| 3. | MS + CW (18% v/v) + 1AA or 1BA (1) + NAA (2) | Callus grew as vascular nodul |
| 4. | MS + CW (18% v/v) + 1AA (1) + BAP (2) | Shoot bud initiation in call (80%) |
| 5. | MS + CW (10% v/v) + BAP (2–5) + 1AA (1) | 4–6 shoot buds per subcultu |



Figures 1–5. Callus induction and shoot bud regeneration in cardamom. 1. Callusing of seedlings *in vitro* on MS + CW (18 %v/v) + 2,4-D (2 mg/l) +BAP (0·5 mg/l) 6 weeks after incubation, × 1·5. 2. Nodular callus derived from root stock of seedling transferred to MS medium + IAA (1 mg/l) + NAA (2 mg/l) × 1·5. 3. Propionocarmine squash preparation from root-stock callus piece showing free cells and cell aggregates × 800. 4. Regeneration of a shoot bud in primary callus grown on MS + BAP (2 mg/l) + IAA (mg/l), × 1·5. 5. Cluster of shoot buds regenerated from callus subcultures on MS + CW (10 %v/v) + BAP (2 mg/l) + IAA (1 mg/l) × 1·5.

ccurred if MS medium was supplemented with CW (10% v/v) + BAP 2-5 mg/l) with or without the addition of IAA and incubated for 6 weeks under 00 lux (figures 4,5). Four to six shoot buds could be obtained from each callus ubculture of uniform size. Rooting occurred at the base of individual shoot uds on prolonged incubation in the same medium (aged cultures) or when indivi-ual shootlets were isolated and grown on White's medium to which NAA (2mg/l) nd sucrose (1%) had been added.

## . Discussion

egeneration of plantlets in callus culture is an alternate means of propagation n cardamom. Callus subcultures could develop 4-6 regenerants, each of which as capable of rooting, when isolated and grown, forming a whole plant. Plant-ts propagated thus might not conform to parent genotype, having been obtained om seedling calli. Nevertheless, tissue culture provides a method by which a large umber of strains could be obtained for selection of desirable variants. Also, it ffers a method of rapid multiplication of elite varieties through multiple shoot duction. Preliminary studies have shown that test tube plants could be success-lly transferred to soil.

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# Studies in Cyperaceae : XVII Novelties in *Fimbristylis* (L.) Vahl and their vegetative anatomy

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**Abstract.** Two novelties of *Fimbristylis* collected from Tamil Nadu (Madras State) are described and illustrated. One of them belongs to the section *Fuscae* and the other one to *Cymosae*. The vegetative anatomical characters of these two novelties not only fall within the ambit of the range of anatomical variability of the genus as a whole but their respective anatomical features in combination appear to be characteristic and different from those of the species already known anatomically.

**Keywords.** Novelties in *Fimbristylis* ; vegetative anatomy.

***Fimbristylis scabrisquama*** **Govind** ***sp. nov.*****—Sect.** ***Fuscae*** **Ohwi (figure 1)**

*Perennis*. *Rhizoma* brevissima, lignosa. *Culmi* 1–2, pentaquetri, valde costati, sulcati, glabri, rigidi, erecti, straminei–atrobrunnei, scabridi in dimidio superiore, tecta ovato-lanceolatis squamis et denique fibrosis squamis, 15–25 cm × 0·6–0·8 mm. *Folia* multa, spiraliter disposita, canaliculata cum margine incurva recurva, brunnea–atrobrunnea, demum evadentia nigra, eligulata, acuminata (acuta), incrassata et in margine dimidio superiore scabrida, culmo quam angustiora, 10–15 cm × 1–2 mm ; vaginae omnes laminiferae, glabrae, oblique truncatae, nitideae vel pallidae, brunnae aliquando evadens nigrae, plusminusve corneae. *Inflorescentia* simplex, contracta, capitata, consistens (2–) 3–8 (–10) spiculis, 1·2–1·8 cm longa et lata. *Bracteae* anguste ovatae, aristatae, plusminusve scabridae in margine aristae, glabrae, inflorescentia multo breviores, 0·5–1·0 cm longae. *Radii primari* O, raro cum unus radius adest, triquetrous, laeves, usque 1 cm longus. *Spiculae* elliptico ovatae, acutae, castaneo-brunnae, plerumque fasiculatae, aliquando solitariae, sessiles, subteretes, 10–16 florae, 6–7 × 2–3 mm. *Glumae* late ovatae, acutae–subacutae, distichae in dimidio inferiore vel plus minusve per totum proprie oblique et lineariter scabridae in nudatis partibus, anguste scariosae ad marginem, plerumque ciliolatae in dimidio superiore margine cum lateribus inervis, cymbiformes, charatceae, nitidae, adpressae, mucronatae, 4·0–4·2 (incluso mucrone) × 3·0–3·2 mm ; carina aliquando valida 3 (–5) nervia, nervis excurrentibus in mucronem ; mucro plerumque recurvata, 0·5 mm longa ; nervis lateralis levior. *Rhachilla* pannose alata, excavata. *Stamina* 3 ; filamenta longe flexuosa, tenues, tortuosa, 2·5–2·8 mm longa ; anthera brunnea, apiculata

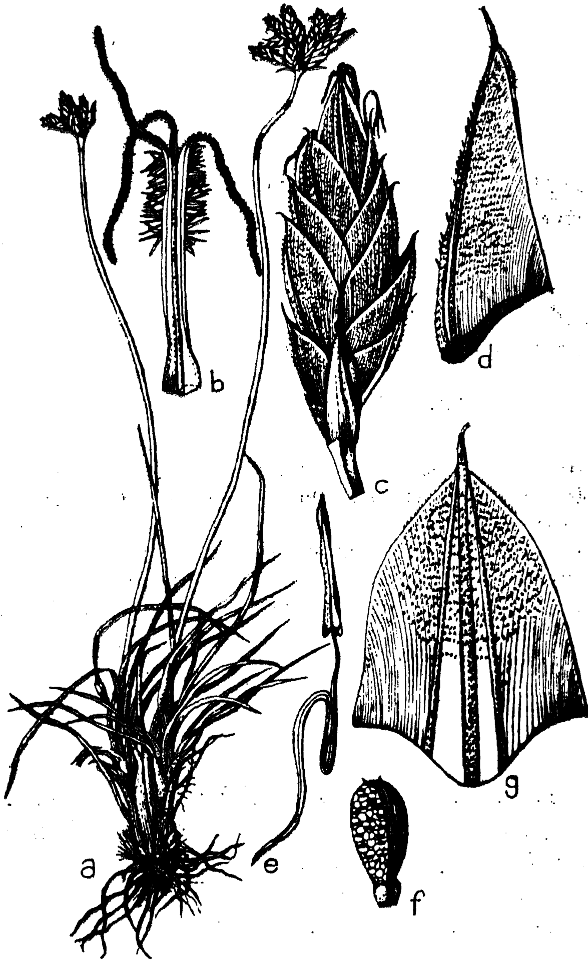


**Figure 1a-g.** *Fimbristylis scabrisquama* Govind. sp. nov. **a.** habit × ½; **b.** style and stigma × 11; **c.** spikelet × 5; **d.** glume, lateral view × 10; **e.** stamen × 8; **f.** nut × 23; **g.** glume, spread out × 10; (from Govindarajalu, 12,110, *type*).

cum plus minusve apice setacea, lineares, distincte calcarata ad basim, 1·9–2·0 mm longa. *Stylus* triqueter, vix dilatata ad basim, distincte fimbriata in superiore tertia parte vel dimidia, 2·4–2·5 mm longus ; stigmata 3, brevis ciliata per totum, stylo breviora, 1·7–1·8 mm longa. *Nux* angusta oblongo ovata, triquetra, tricostulata, albo straminea cum leviter lateralis convexa, stipitata, non umbonulata cum triangulariter apice plana, aliquantulum aspera ob minutas transverse elongates tuberculas in dimidio superiore, 0·6–0·8 × 0·3–0·4 mm ; cellulae epicarpicae in dimidio superiore circulares (plus minusve hexagonales), minutae, reticulatae ; stipes 0·12–0·15 mm longae.

*Perennial. Rhizome* short, woody. *Culms* 1–2, pentaquetrous, strongly ribbed, sulcate, glabrous, rigid, erect, stramineous-dark brown, scabrid in upper half,

covered by ovate–lanceolate scales and ultimately by the fibrous remains of the scales, 15–25 cm × 0·6–0·8 mm. *Leaves* many, spirally arranged, canaliculate with incurved margin, recurved, brown–dark brown finally tending to become black, eligulate, acuminate (acute), thickened and scabrid in upper half margin, shorter than culms, 10–15 cm × 1–2 mm; sheaths all laminiferous, glabrous, obliquely truncate, shining or dull, brown sometimes becoming black, more or less horny. *Inflorescence* simple, contracted, capitate consisting of (2–) 3–8 (–10) spikelets, 1·2–1·8 cm long and broad. *Bracts* narrowly ovate, aristate, more or less scabrid in the aristate margin, glabrous, much shorter than inflorescence, 0·5–1·0 cm long. *Primary rays* O, rarely when 1 present triquetrous, smooth up to 1 cm long. *Spikelets* elliptic ovate, acute, castaneous brown, usually clustered, sometimes solitary, sessile, subterete, 10–16 flowered, 6–7 × 2–3 mm. *Glumes* broadly ovate, acute–subacute, distichous in lower half or more or less throughout, characteristically obliquely and linearly scabrid in uncovered parts, narrowlys carious at margin, ususlly ciliolate in upper half margin with nerveless sides, cymbiform, chartaceous, shining, adpressed, mucronate, 4·0–4·2 (incl. mucro) × 3·0–3·2 mm; keel rather strong, 3 (–5) nerved, nerves excurrent into mucro; mucro usually recurved, 0·5 mm long; lateral nerves rather faint. *Rhachilla* raggedly winged, excavated. *Stamens* 3; filaments long, flexuous, slender, tortuous, 2·5–2·8 mm long; anther brown, apiculate with somewhat setaceous apex, linear, distinctly spurred at base, 1·9–2·0 mm long. *Style* triquetrous, hardly dilated at base, distinctly fimbriately hairy in upper 1/3 or 1/2, 2·4–2·5 mm long; stigma 3, shortly hairy throughout, shorter than style, 1·7–1·8 mm long. *Nut* narrowly oblong ovate, triquetrous, tricostulate, whitish stramineous with slightly convex sides, stipitate, non umbonulate with triangularly flat apex, somewhat rough due to minute transversely elongated tubercles in upper half, 0·6–0·8 × 0·3–0·4 mm; epicarpic cells in upper half circular (somewhat hexagonal), minute, reticulate; stipe 0·12–0·15 mm long.

Govindarajalu 12,110, Venniyar to Varaiyattumottai, Highwavys Mts., Madurai Dt., Tamilnadu occurring in open grassy slopes (type : PCM); Isotypes: 12,110 A (CAL); 12,110 B (MH); 12,110 C (PCM).

Related to *F. eragrostis* (Nees) Hance but differs in having culms covered at the base by ovate lanceolate scales and ultimately by their fibrous remains, much narrower canaliculate acuminate (acute) recurved leaves, simple contracted capitate short inflorescence consisting of lesser number of spikelets, primary rays when present triquetrous, clustered smaller spikelets, obliquely and linearly scabrid glumes, longer recurved mucro, slender tortuous very long staminal filaments, anthers with somewhat setulose apex, distinctly fimbriately hairy style, stigmas shorter than style, narrowly oblong ovate triquetrous tricostulate non umbonulate smaller nuts with triangular flat apex and rough surface marked by somewhat transversely elongated tubercles in upper half and reticulately arranged circular epicarpic cells.

*Note*.: Easily observable field and herbarium characters of this novelty are the presence of ovate lanceolate scales at the base of culms which are ultimately becoming fibrous, involute canaliculate leaves recurving towards the soil with a tendency

to become ultimately black, contracted capitate inflorescence and scabrid sub-distichous-distichous glumes. Not common in the said locality. This is named after its characteristic obliquely and linearly present scabrid condition of the glumes.

***Fimbristylis tortifolia*** **Govind.** ***sp. nov.*****—Sect.** ***Cymosae*** **Ohwi—(figure 2)**

*Perennis. Rhizoma* crassa, lignosa, horizontaliter vel oblique reptans (interdum verticaliter crescens) sine ullis obviis internodis. *Culmi* aliquantum tenues, pentagoni, incrassati et foliati ad basim, plerumque flexuosi (erecti), solitari, laeves, glabri, costati, sulcati, dense tectis multis basibus foliatis et demum fibrosis filamentis, plerumque curvati ad basim (15-) 20-35 cm × 0·75-1·0 mm. *Folia*

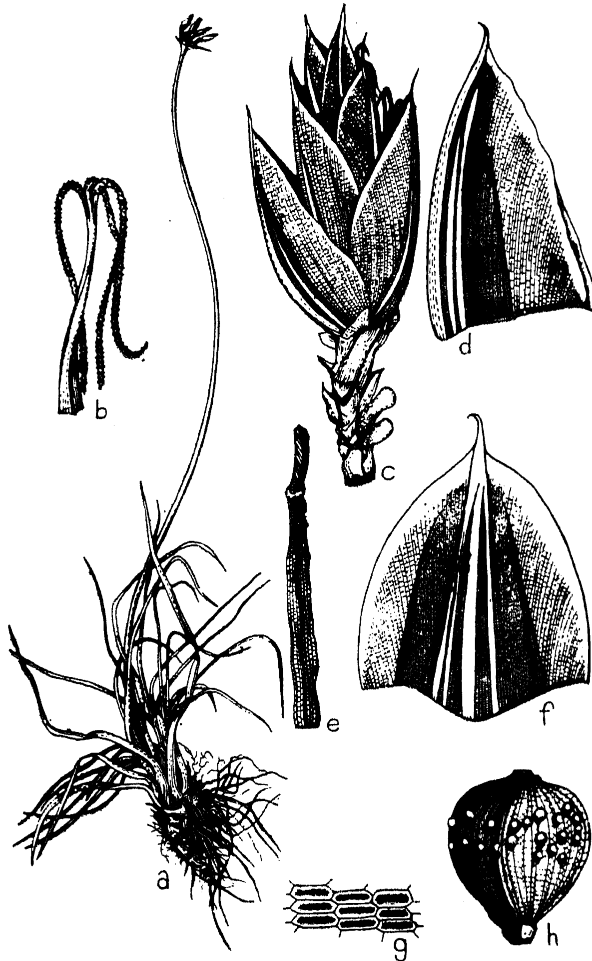


**Figure 2 a–h.** *Fimbristylis tortifolia* Govind. sp. nov. **a.** habit × ½; **b.** style and stigma × 11; **c.** spikelet × 5; **d.** glume, lateral view × 6; **e.** stamen × 10; **f.** glume, spread out × 6; **g.** outer epicarpic cells, diagrammatic; *h.* nut × 15; (from Govindarajalu 12,000, *type*).

multa, flexuosa, omnes recurva vel varie tortuosa vel saltem infima folia recurva, glabra, canaliculata, involuta, longinque et aliquantulum scabrida, laevis ad infimum dimidium marginem eligulata, leviter lata, acuta, culmo breviora, multinervia, non carinata, 8–15 cm × 1·0–1·5 mm. *Vaginae* brunnae, valde nerviae, glabrae, omnes laminiferae, infimae denique evadentes filamenta fibrosa, non corneae cum lateribus angustis membranaceis stramineis, oblique truncatae. *Inflorescentia* simplex, capitata (raro cum uno radio addita), consistens 3–8 (-10) spiculis, 8–10 cm longa et lata. *Bracteae* late ovato lanceolatae, atrobrunneae, late ad basim cum arista aliquantulum scabrida, rigidae, erectae–oblique erectae, aequilongae vel leviter longiores, inflorescentia 8–15 mm longae. *Radii* O. *Spiculae* ovato lanceolatae, acutae, teretes, castaneo brunnae, multiflorae, sessiles, 6–9 × 2·5–3·0 mm. *Glumae* late ovatae, acutae, glabrae et laeves ad apicem, patens cum distincte margine hyalina et cum lateribus uno nervis in dimidio una quoque, eglandulosae, non nitidae, distincte mucronatae, 4·5–5·5 (incluso mucrone) × 4·0 mm ; carina distincta, 5 nervia, excurrentes in mucronem ; mucro recurvata vel erecta, 0·6–0·7 mm longa. *Rhachilla* pannose alata, excavata. *Stamina* 3 ; anthera linearo oblonga, lutea ad apicem rotundata, non setacea, ad basim calcarata (lobata), 0·75–0·8 mm longa. *Stylus* triqueter cum leviter dilatata pyramidali basi, glaber vel leviter fimbriatus post trifurcationem, 1·75–2·0 mm longus ; stigmata 3, papillata, stylo longiora (aequilonga), 2·25–2·5 mm longa. *Nux* late obovata triquetra, tricostulata cum lateribus convexis, atrobrunnea demum evadens nigra, distincte stipitata, umbonulata, distincte tuberculata in dimidio superiore 1·1–1·25 (inclusis stipitibus) × 0·75–0·8 mm ; stipes 0·1–0·2 mm longus ; cellulae epicarpicae extimae in dimidio superiore transverse elongatae, hexagonales, tessellatae, occurrentes in 10–12 regularibus verticalibus seriebus in una quoque facie.

*Perennial. Rhizome* thick, woody, horizontally or obliquely creeping (sometimes vertically growing) without any obvious internodes. *Culms* rather slender, pentagonous, thickened and leafy at base, usually flexuous (erect), solitary, smooth, glabrous, ribbed, sulcate, densely covered by many leaf bases and ultimately by their fibrous strands, usually curved at base (15–) 20–35 cm × 0·75–1·0 mm. *Leaves* many, flexuous, all recurved or variously tortuous or at least the lowermost leaves recurved, glabrous, canaliculate, involute, distinctly and somewhat scabrid in the margins, smooth in lower half margins, eligulate, slightly broad, acute, shorter than culms, many nerved, non keeled, 8–15 cm × 1·0–1·5 mm; *Sheaths* brown, strongly nerved, glabrous, all laminiferous, lowermost ultimately becoming fibrous strands, non horny with narrow membranous stramineous sides, obliquely truncate. *Inflorescence* simple, capitate (rarely 1 ray added), consisting of 3–8 (–10) spikelets, 8–10 mm long and broad. *Bracts* broadly ovate lanceolate, dark brown, broad at base with somewhat scabrid arista, stiff, erect–obliquely erect, as long as or slightly longer than inflorescence, 8–15 mm long. *Rays* O. *Spikelets* ovate lanceolate, acute, terete, castaneous brown, many flowered, sessile, 6–9 × 2·5–3·0 mm. *Glumes* broadly ovate, acute, glabrous and smooth at apex, patent with distinct hyaline margin and 1 lateral nerves in each half, eglandular, not shining, distinctly mucronate, 4·5–5·5 (incl. mucro) × 4·0 mm ; keel distinct, 5 nerved, excurrent into mucro ; mucro recurved or erect, 0·6–0·7 mm long. *Rhachilla*

raggedly winged, excavated. *Stamens* 3 ; anther linear, oblong, yellow, rounded at apex without bristles, spurred at base (lobed), 0·75–0·8 mm long. *Style* triquetrous with slightly dilated pyramidal base, glabrous or slightly hairy behind trifurcation, 1·75–2·0 mm long ; stigma 3, papillose, (as long as) longer than style, 2·25–2·5 mm long. *Nut* broadly obovoid, triquetrous, tricostulate with convex sides, dark brown ultimately tending to become black, distinctly stipitate, umbonulate, distinctly tubercled in upper half, 1·1–1·25 (incl. stipe) × 0·75–0·8 mm ; stipe 0·1–0·2 mm long ; outer epicarpic cells in upper half transversely elongated hexagonal, tessellated, occurring in 10–12 regular vertical rows in each face.

Govindarajalu 12,000, Kulikad, Highwavys Mts., Madurai Dt., Tamilnadu, commonly occurring in wet open grassy rocks (*type*: PCM); *Isotypes* : 12,000 A (CAL) ; 12,000 B (MH) ; 12,000 C (BLAT) ; 12,000 D (BSI ; 12,000 E–V (PCM).

Related to *F. rigidiuscula* Govind. (sect. Cymosae Ohwi) but differs in having usually flexuous culms usually covered at base ultimately by fibrous strands of leaf bases, usually all or at least the lowermost leaves flexuous recurved or variously becoming tortuous, less scabrid or more or less smoooth margined leaves, slightly broader non keeled many nerved acute leaves, non horny brown sheaths with stramineous sides, ovate lanceolate spikelets, broadly ovate patent acute distinctly mucronate and distinctly hyaline margined glumes with 1 lateral nerve in each half, long recurved or erect mucro, shorter anthers with non bristly rounded apex, shorter style, stigma usually longer than style, broadly obovoid triquetrous tricostulate distinctly stipitate smaller nuts with distinct tubercles in upper half and transversely elongated hexagonal epicarpic cells occurring in lesser number of rows in each face.

*Note* : The following are the outstanding characters of this novelty that can easily be recognized in the field as well as in the herbarium. The flexuous culms are curving at the base and covered by fibrous strands of several leaf bases ; horizontally or obliquely creeping rhizomes ; flexuous or variously tortuous non keeled leaves with more or less smooth margins ; simple capitate inflorescence consisting of 3–6 ovate lanceolate spikelets. The name is based on the tortuous condition of the leaves.

## Vegetative anatomy

### *Materials and methods*

The materials for the anatomical investigation were taken from their respective isotypes. The methods that were followed in earlier works are adopted here (Govindarajalu 1966, 1968 a,b, 1975 *et seq.*). Most of the descriptive terms used here are those that have been recommended by Metcalfe and Gregory (1964). The typological terminologies proposed by Chaedle and Uhl (1948 a,b) for designating the types of vascular bundles and metaphloem are followed.

### *Fimbristylis scabrisquama Govind*

Leaf–*Abaxial surface* : Intercostal cells moderately long, axially elongated ; cell walls thin, smooth ; end walls straight. Stomata (L. 45–49·5 μm ; W. 27·0–

·5 μm), elliptic oblong, thick-walled ; subsidiary cells low dome-shaped ; inter-omatal cells long with concave ends. Silica cells short, broad, thin-walled curring in 3 continuous rows, each one of them containing 2–3 cone-shaped lica bodies surrounded by satellites.

*Adaxial surface* : Cells isodiametric, somewhat hexagonal ; cell walls thin, nooth. Stomata wanting. Other details as in alaxial surface.

*T.S. lamina* : Lamina examined 2·2 mm wide. Outline 'W' shaped, assymm-rical (figure 3,d). Keel inconspicuous. Margins dissimilar, one margin acute d the other one somewhat rounded (figure 3,d). Cuticle uniformly thick. ulliform cells not differentiated. Adaxial epidermal cells uniform in size and ape and larger than those of the abaxial. Sclerenchyma strands : adaxial 3 It. 72–90 μm ; W. 45–54 μm), pentagonal ; abaxial associated with each vb It. and W. 54-90 μm), rounded (pulviniform) ; submarginal strands (Ht. 36 μm ; . 157·5 μm), pulviniform. Adaxial hypodermis consisting of 2–3 layers of in-ted radially elongated or rounded colorless perenchyma cells. Chlorenchyma diating restricted to a axial one half of lamina. Vascular bundles *c.* 20 in mber comprising large, medium and small vb's and not showing any regular ernation with each other ; large vb's (type III A) oval in outline while medium d small vb's rounded (type I) ; metaxylem vessel elements (D. 27 μm in dia-eter) ; metaphloem of 'regular type'. Bundle sheaths 3 layered ; M.S. in l vb/s fibrous, complete ; in large vb's O.S. and I.S. parenchymatous, the former mplete while the latter incomplete and in the case of small vb's both complete. rcumvascular sclerenchyma absent. Tannin idioblasts common.

Culm-*Epidermis, surface view* : Cells axially elongated with thin somewhat uous walls. Stomata (L. 31·5–36·0 μm ; W. 27·0–31·5 μm, more or less borbicular, thick-walled ; subsidiary cells dome-shaped ; interstomatal cells long th concave ends. Silica cells short, rather narrow, thin-walled, occurring in more or less continuous rows and each cell containing usually 2-3 cone-shaped ica bodies surrounded by satellites. Silica particles very commonly and charac-istically present in cells in between the ribs and interstomatal cells ; likewise unded silica bodies characteristically present in subsidiary cells (figure 3,f).

*T.S. Culm* ; Long diameter of culm examined 0·8 mm. Outline subcircular th ribs and furrows (figure 3,e). Cuticle uniformly thick (thickness 13·5 μm) roughout. Epidermal cells thin-walled, similar in size and shape except at the s. Stomata in line with epidermis ; guard cells with outer ledges only ; sub-matal chamber very narrow and small. Hypodermis consisting of 3–4 (–5) yers of radiating chlorenchyma cells, the continuity of which interrupted by peri-eral ring of vb's (figure 3,e). Sclerenchyma strands (Ht. 45·0–67·5 μm ; W. ·0–45·0 μm), variable, pulviniform, rounded, triangular. Ground tissue made of large parenchymatous cells having intercellular spaces. vascular bundles 28 in number comprising both large and small vb's, arranged in two peripheral gs, the former forming the inner ring and the latter outer ring (figure 3,e) ; ge vb's 6 in number, oval shaped (type III B), the remainder rounded (type I) ;

large vb's containing protoxylem lacunae ; metaxylem vessel elements (D.*c.* 22·5 μm in diameter) ; metaphloem of 'regular type'. Bundle sheaths, see lamina. Circumvascular-selerenchyma little or rot developed. Tannin idioblasts abundant in hypodermis.

Rhizome. *Transverse section* : Diameter of the rhizome examined 3·2 mm. Epidermal cells thick-walled, isodiametric. Cortex very broad, organized by thick-walled parenchyma cells ; cells compactly arranged without intercellular spaces and containing abundant starch. Subepidermal fibrous strands (Ht. 174·0–290·0 μm ; W. 92·8–174·0 μm), triangular. Endodermoid layer made up of single layer of cells with 'U' shaped thickenings and forming a wavy limiting layer. Stele central containing many vb's more or less rounded in outline, amphivasal (type V) ; metaxylem elements (D. *c.* 9·0 μm in diameter). Central ground tissue similar to that of cortex. Tannin idioblasts large, very common throughout ground tissue.

Root. *Transverse section* : (figure 3,c). Diameter of the root examined 0·7 mm. Exodermis single layered consisting of cells similar in size and shape with suberized cell walls. Cortex : recognizable into 2 zones ; outer zone narrow consisting of 4–5 layers of compactly arranged parenchyma cells ; inner broad, lacunose due to concentrically arranged air-cavities ; air-cavities separated by radiating rows of parenchyma cells. Endodermis single layered consisting of cells with 'U' shaped thickenings and broad lumina. Pericycle of single layer of thick-walled cells. Metaxylem vessel central, solitary, rounded in outline (D. *c.* 45·0 μm in diameter). Protoxylem units 7–8 in number. Metaphloem not easily distinguishable. Central ground tissue parenchymatous.

*Fimbristylis tortifolia* Govind.

Leaf-*Abaxial surface* : Intercostal cells axially elongated, thin-walled, smooth ; end walls straight. Stomata (L. 45–54 μm ; W. 18·0–22·5 μm), narrowly oblong ; subsidiary cells low dome-shaped ; interstomatal cells short with concave ends. Silica cells rather broad, short, moderately thick-walled, present in 2–3 continuous rows ; each cell possessing (1–) 2–3 cone-shaped silica bodies with satellites.

*Adaxial surface* : Cells broad, isodiametric ; cellwalls thin, smooth, end walls straight Stomata absent. Silica cells occurring in a single more or less dis-ontinuous row ; other details see abaxialc surface.

*T.S. lamina* : Lamina examined 1·6 mm wide. Outline flatly triangular (figure 3, a), asymmetrical with abaxial ribs and furrows. Keel slightly developed, somewhat rounded or bilobed (figure 3,a). Margins dissimilar ; one margin sloping towards abaxial side, the other more or less truncate with depression (figure 3,a). Cuticle thick on either surface. Adaxial epidermal cells slightly larger than those of the abaxial. Bulliform cells not distinctly differentiated. (figure 3,a). Sclerenchyma strands : adaxial 5 in number (Ht. 40·5–45·0 μm ; W. 54·0–67·5 μm), pentangular ; abaxial strands (Ht. and W. 36·0–49·5 μm),

more or less rounded or pulviniform and associated with vb's (figure 3,a). Adaxial hypodermis consisting of 4–5 layers of compactly arranged inflated translucent polygonal parenchyma cells. Chlorenchyma restricted to abaxial one third of lamina. Air-cavities absent except at substomatal cavities. Vascular bundles 18–20 in number of two different sizes out of which 3 large and the rest small; both belonging to type III A and arranged without any regular alternation; median vb. adaxially capped by radiating rows of large translucent parenchyma cells (figure 3,a); metaxylem vessel elements (D. 18 μm in diameter); metaphloem of 'regular type'. Bundle sheaths in all vb's 3 layered; O.S. parenchymatous, complete; M.S. fibrous, complete; I.S. parenchymatous, incomplete in all vb's . Tannin idioblasts not observed.

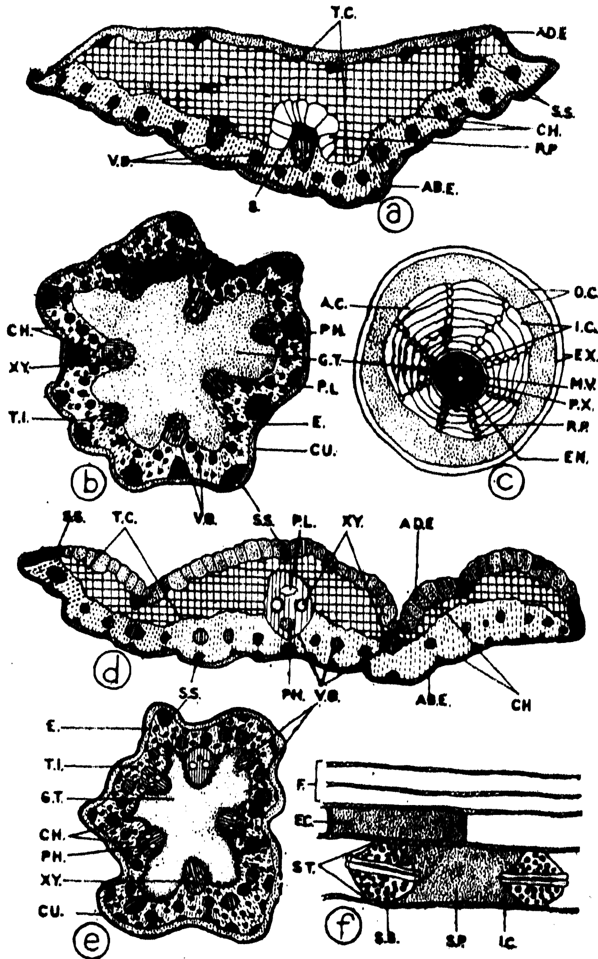


**Figure 3 a–f.** **a, b.** *Fimbristylis tortifolia* Govind. **a.** transection of lamina × 44; **b.** transection of culm × 33; *c–f. F. scabrisquama* Govind. **c.** Transection of root × 44; **d.** transection of lamina × 33; **e.** transection of culm × 44; **f.** surface view of epidermal cells (note presence of silica bodies in subsidiary cells and silica particles in epidermal and interstomatal cells) × 226. (All based on their respective *isotypes*).

Culm. *Epidermis, surface view* : Cells axially elongated ; cell walls thin, smooth. Stomata (L. 38·0 μm ; W. 27·0–28·8 μm), oblong elliptic ; subsidiary cells low dome-shaped ; interstomatal cells long with concave ends. Silica cells present in a single discontinuous row ; cells long, and each one of them containing 5–6 cone-shaped silica bodies without satellites.

*T.S. Culm* : Long diameter of culm examined 1·2 mm. Outine polygonal or somewhat subhexagonal with ribs and furrows (figure 3,b). Cuticle uniformly thick (thickness *c*. 9 μm) throughout. Epidermal cells thin-walled, similar in size and shape. Hypodermis consisting of 6–8 layers of non radiating chlorenchyma cells interrupted by fibrous strands and vb's (figure 3,b). Air-cavities represented by substomatal spaces. Sclerenchyma strands (Ht. 54·0–112·5 μm ; W. 99·0–135·0 μm), usually pulviniform (triangular ovate). Ground tissue consisting of large parenchyma cells arranged with intercellular spaces. Vascular bundles *c*. 43 in number comprising both large (type III A) and small vb's (type I) large vb's 7 in number somewhat oval or subcircular in outline containing protoxylem lacunae ; small vb's circular in outline and arranged peripherally in the form of undulating ring following the contour of the culm while large vb's forming the inner ring (figure 3,b) ; metaxylem vessel elements (D. 18·0–22·5 μm in diameter) ; metaphloem of 'regular type'. Bundle sheaths in all vb's 3 layered ; O.S. and I.S. parenchymatous, the former complete and the latter incomplete ; M.S. fibrous, complete in all vb's.

Root. *Transverse section* : Diameter of the root examined 0·6 mm. Exodermis : single layered consisting of cells uniform in size and shape ; cell walls suberized. Cortex : recognizable into two zones, the outer zone narrow consisting of 3 layers of compactly arranged thick-walled parenchyma cells ; inner cortex rather broad, lacunose ; air-cavities concentrically arranged and separated by radiating rows of parenchyma. Endodermis of single layer made up of slightly tangentially elongated cells with 'U' shaped thickenings. Pericycle consisting of a single layer of thick-walled cells. Metaxylem vessel solitary, central, circular in outline, thick-walled (D. 67·5 μm in diameter) ; protoxylem units 8 in number alternating with as many metaphloem units ; each unit of the metaphloem consisting of a group of 4 cells of which 2 being large sieve tube elements and 2 companion cells. Ground tissue made up of thick-walled parenchyma.

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*Key to figure lettering and text abbreviations* :

| | |
|---|---|
| A.B.E. | abaxial epidermis |
| A.C. | air-cavity |
| AD.E. | adaxial epidermis |
| CH. | chlorenchyma |
| CU. | cuticle |
| E. | epidermis |
| E.C. | epidermal cell |
| EN. | endodermis |
| EX. | exodermis |
| F. | fibres |
| G.T. | ground tissue |
| I.C. | interstomatal cell |
| I.CO. | inner cortex |
| M.V. | metaxylem vessel |
| O.C. | outer cortex |
| PH. | metaphloem |
| P.L. | protoxylem lacuna |
| P.X. | protoxylem |
| R.P. | radiating parenchyma |
| S. | sclerenchyma |
| S.B. | silica body |
| S.P. | silica particle |
| S.S. | sclerenchyma strand |
| ST. | stoma |
| T.C. | translucent cell |
| T.I. | tannin idioblast |
| V.B. | vascular bundle |
| XY. | metaxylem |

*Text abbreviations* :

| | |
|---|---|
| *c.* | *circa* |
| D. | diameter |
| Ht. | height |
| I.S. | inner sheath |
| L. | length |
| M.S. | middle sheath |
| O.S. | outer sheath |
| vb's | vascular bundles (sing. vb.) |
| W. | width |

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# Embryological studies in three species of *Cymbopogon* Spreng (Poaceae)

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**Abstract.** The embryology of *Cymbopogon nardus* var. *confertiflorus*, *C. martinii* var. *Motia* and *C. parkeri* has been studied. *C. nardus* and *C. martinii* have been observed to be seed-sterile owing to failure of fertilization while in *C. parkeri*, the seed-setting is only about 8·00%.

**Keywords.** Seed-sterility ; Cymbopogon ; Andropogoneae ; Poaceae.

## 1. Introduction

The genus Cymbopogon belongs to the tribe Andropogoneae of the subfamily Panicoideae and it is represented by 24 species in the Indian sub-continent (Bor 1960). A number of species of *Cymbopogon* yield essential oils and are used in perfumery. The embryology of *C. martinii* and *C. nervatus* has been worked out by Brown and Emery (1958) although of a preliminary nature. The present paper deals with the embryology of *Cymbopogon nardus* (L.) Rendle var. *Confertiflorus* (Steud.) Stapf ex Bor, *C. martinii* (Roxb.) Wats var. *Motia* and *C. parkeri* Stapf, to find the nature of seed sterility observed in different species.

## 2. Materials and methods

The material of *C. nardus* and *C. martinii* was collected from the Botanical Gardens of the Panjab University while that of *C. parkeri* from Shiwalik hills in the months August to November. Conventional methods of dehydration and embedding were used. The sections were cut at 5–10 $\mu$m and stained with safranin and fast green. For studying the growth of the pollen tube in the style, the method given by Khoshoo and Vij (1963) has been followed. For this purpose the gynoecia were fixed in 1 : 3 acetic alcohol, after 3, 6, 9 and 24 hrs of pollination. After half an hour the styles were transferred to 30% ethyl alcohol for preservation. The ovaries were transferred to 1% solution of acid fuchsin for about 20 min. Subsequently, the styles were cleared in lactic acid at 60° C and the whole mounts were made in pure lactic acid.

## 3. Observations and discussion

### 3.1. *Microsporogenesis and male gametophyte*

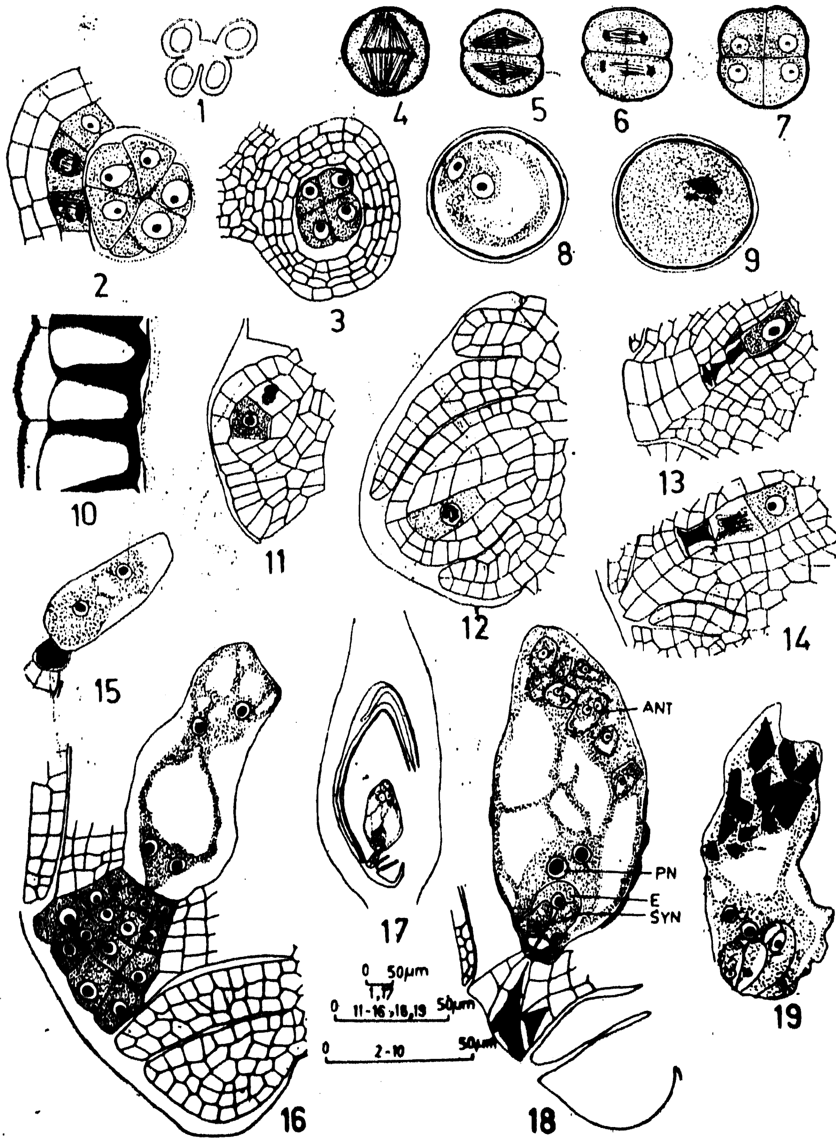
Anthers are tetrasporangiate (figure 1) and the anther wall consists of an epidermis, followed on the inner side by the endothecial layer, a single middle layer and the tapetum (figures 2, 3). The epidermal cells in mature anthers show deposition of oil droplets in *C. nardus* and endothecial cells develop fibrous thickenings (figure 10). The middle layer is ephemeral. The tapetum is of the glandular type and its cells become binucleate in *C. nardus* while they remain uninucleate in *C. martinii* and *C. parkeri*. As seen in transections the MMC are disposed in 4 or 5 rows (figures 2, 3). Meiosis in MMC is normal and it is of the successive type leading to the formation of isobilateral microspore tetrads (figures 4–7). The development of the male gametophyte occurs as described in other members of the family and the pollen grains are shed at the 3-celled stage (figures 8, 9). The pollen grains are monocolpate with a thick smooth exine and slightly thinner intine. The pollen fertility is about 90% in *C. nardus* and *C. parkeri* and about 70% in *C. martinii*.

### 3.2. *Ovary and ovule*

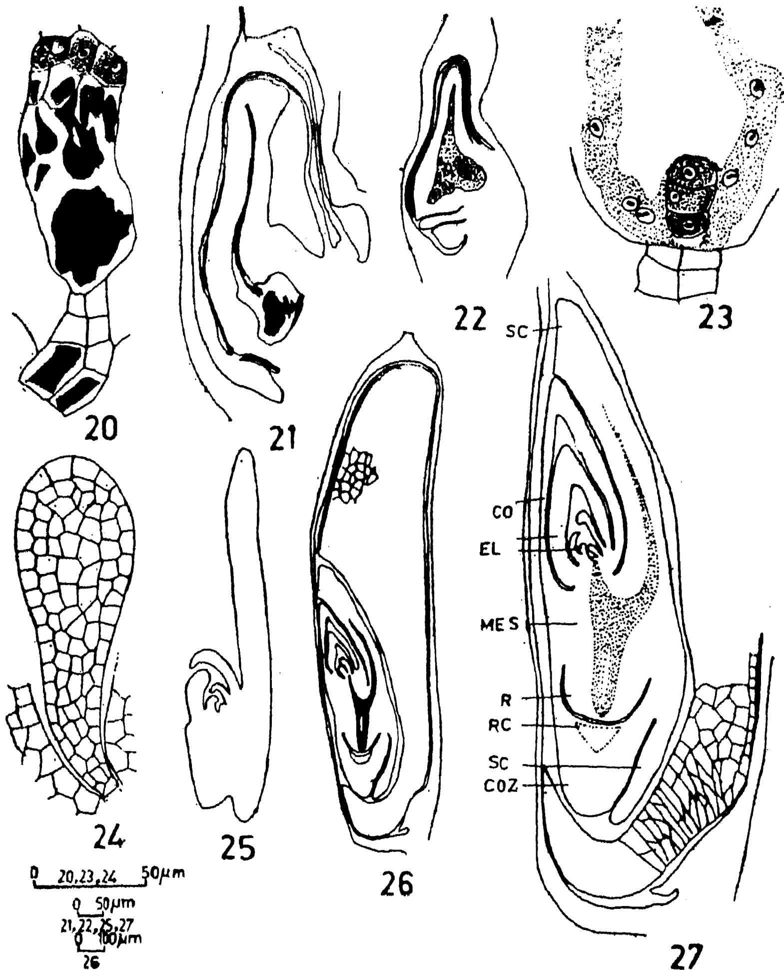
The ovary contains a sessile, bitegmic, pseudocrassinucellar hemianatropous ovule (figures 16, 17). The inner integument is composed of cells 2–3 layers thick but it usually fails to grow over the nucellus so that a definite micropyle is lacking (figure 16). In *Pennisetum typhoideum* (Narayanaswami 1953) and *Capillipedium huegelii* (Choda and Bhanwra 1980) the inner integument does not grow over the nucellus but in most other species of the family the micropyle is formed by the inner integument (Narayanaswami 1954, 1955, 1956). In *Cymbopogon parkeri* where some seed-setting is observed, the inner integument is found to degenerate after fertilization unlike that in *Saccharum officinarum* (Artschwager *et al* 1929) and *Sorghum vulgare* (Artschwager and McGuire 1949), belonging to Andropogoneae where they persist in mature caryopsis.

The outer integument on the upper side of the ovule is 2–3 cell layered but it covers only about a third of the ovule (figure 17), a feature characteristic of the subfamily Panicoideae (Chandra 1963; Venkateswarlu and Devi 1964). The outer integument on the lower side of the ovule shows about the same growth as the inner integument and is 2–5 cell layered (figures 16–18).

The nucellar epidermis undergoes 2–3 periclinal divisions in the region of the micropyle (figures 16, 18) by the time the megaspore tetrads are formed. In *C. nardus*, the nucellar cells in this region become conspicuous owing to their large size, dense contents and prominent nuclei (figure 16). The formation of a parietal tissue due to periclinal divisions in the nucellar epidermis near the micropyle has been reported in many species belonging to the subfamily Panicoideae (Chandra 1963; Venkateswarlu and Devi 1964). The nucellar tissue is absorbed by the developing embryo and endosperm and is hardly traceable in the mature caryopsis of *C. parkeri* (figures 26, 27).




**Figures 1–19.** Microsporangium, microsporogenesis, male gametophyte, megasporogenesis and female gametophyte. **1, 3, 11, 16–19.** *Cymbopogon nardus.* **2, 12, 14, 15.** *C. martinii.* **4–10, 13.** *C. parkeri.* **1.** TS of the anther, **2.** TS anther lobe showing periclinal division in the inner secondary parietal layer, **3.** TS anther lobe showing wall layers and sporogenous cells, **4–7.** Stages in microsporogenesis, **8, 9.** 2- and 3-celled pollen grains respectively, **10.** Portion of wall layers showing epidermis and fibrous thickenings of the endothecium, **11.** LS ovule primordium at archesporial cells stage, **12.** LS ovule at megaspore mother cell stage, **13, 14.** Megasporogenesis, **15, 16.** 2- and 4-nucleate embryo sac stages respectively, **17.** VS of the ovary and ovule, **18.** Embryo sac showing egg cell, two synergids, two polar nuclei and antipodal complex of several cells, **19.** Embryo sac showing egg apparatus, polar nuclei and degenerating antipodal cells. (*See explanation of abbreviation in p.* 58).




**Figures 20–27.** Post-pollination development. **20, 22.** *C. nardus*; **21, 23–27.** *C. parkeri*. **20–22.** Degenerating embryo sac and shrivelled ovule; **23–27.** Some stages in caryopsis development.

ANT—antipodal; CO—coleoptile; COZ—coleorhiza; E—egg; EL—embryonic leaf; END—endodermis; EPI—epidermis; IP—inner parietal layer; MES—mesocotyle; PN—polar nucleus; R—radicle; RC—root cap; S—sporogenous tissue; SC—scutellum; SYN—synergid.

### 3.3. *Embryo sac development*

The single hypodermal archesporial cell is differentiated in the nucellus, which increases in size and functions as the megaspore mother cell (figures 11, 12). It divides meiotically so as to form a linear tetrad of megaspores (figure 13). In *C. martinii*, however, the upper dyad cell degenerates without undergoing division (figure 14). The chalazal megaspore functions and develops into the polygonum type of embryo sac having an egg cell, two synergids, a central cell with its two

polar nuclei and three antipodal cells. The latter proliferate further and form 9–26 cells in *C. nardus*, 12–15 cells in *C. martinii* and 9–16 cells in *C. parkeri*. The multiplication of the 3 antipodal cells is commonly reported in grasses (Venkateswarlu and Devi 1964; Maze and Bohm 1973).

In *C. nardus* and *C. martinii*, the pollen grains germinate on the stigmatic hairs but the pollen tubes fail to reach the embryo sac due to some unknown factor. The embryo sac and the ovule eventually shrivel and undergo disintegration (figures 20–22). Occasionally the ovules become enlarged and they contain endosperm nuclei formed probably due to autonomous divisions of the secondary nucleus, but there is no embryo formation. In *C. parkeri*, however, about 8% seed-set has been noticed. The primary endosperm nucleus starts dividing earlier than the zygote. The endosperm is of the nuclear type (figure 23) as is reported in other grasses. The endosperm becomes completely cellular at globular stage of the proembryo. Figures 23–27 show some of the stages in the development of embryo in the species. The sequence of early development of the embryo could not be traced but the structure of the mature embryo is similar to that described by Reeder (1957) in other members of the tribe Andropogoneae. Seed-sterility in family Poaceae has been previously reported in *Helaria belangeri* and *H. mutica* by Brown and Coe (1951) and in *Digitaria decumbens* by Sheth *et al* (1956).

In *H. belangeri* and *H. mutica*, the degeneration of the female gametophyte may occur any time after megaspore formation and this has been suggested to be the cause of seed sterility. In the present species, however, the degeneration of the female gametophyte occurs only after it has attained maturity.

Poor seed-set as in *C. parkeri* and complete sterility as exhibited by *C. nardus* and *C. martinii* appear to be compensated by the predominance of vegetative propagation as a means of survival; consequent on perturbation of sexuality.

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# Reproductive efficiency of secondary successional herbaceous populations subsequent to slash and burn of sub-tropical humid forests in north-eastern India

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**Abstract.** Three categories of secondary successional herbaceous communities subsequent to slash and burn, viz., early successional non-sprouting, early successional sprouting and late successional populations were investigated for their reproductive efficiency considering leaf component since it is the chief organ of photosynthesis. Early successional non-sprouting populations were found to be reproductively the most efficient whereas the early successional sprouting populations allocated more to vegetatively reproducing organs. While the high reproductive potential of early successional non-sprouting species was associated with vigour and production efficiency of the species, this relationship was stronger with the latter characteristic. On the other hand, early successional sprouting populations showed inverse relationship between vegetative and sexual reproductive effort. The strategy of late successional species seems to be to maximize vegetative growth in a closed habitat. The significance of these strategies is discussed in the paper.

**Keywords.** Growth strategies ; leaf area ratio ; reproductive effort ; successional communities ; adaptation.

## 1. Introduction

Slash and burn agriculture, locally known as 'Jhum', is the most prevalent form of cropping in the hill regions of north-eastern India. The early successional herbaceous communities constitute an important phase in the fallow development during secondary succession subsequent to cropping. This community, which holds the ground for about 5–6 years, often gets arrested at this stage due to the shortening of the Jhum cycle, the intervening period before the cropping is done on the same site (Ramakrishnan *et al* 1981).

Optimization of reproductive output in plants is attained through a favourable partitioning of the available resources for various life-activities such as maintenance, growth and reproduction (Abrahamson and Gadgil 1973). Much is known about the resource allocation pattern in relation to the reproductive strategy of different plant species in an attempt to explain the ecological success of a species in a given environment (Harper and Ogden 1970 ; Ogden 1974 ; Abrahamson 1975, 1979 ; Newell and Tramer 1978). While such an approach has yielded

valuable information, little effort has been made to relate the reproductive growth strategy with the leaf growth (McNaughton 1975; Bazzaz and Harper 1977; Primack 1979). This approach for evaluating the reproductive strategy of plants is more relevant because, leaf as an organ is the chief region of photosynthetic activity. In the present paper, early successional non-sprouting and sprouting, and late successional herbaceous populations have been compared for their growth and reproductive characteristics considering leaf as the sole organ responsible for energy capture and its overall distribution. The non-sprouting species, obviously, are all established through seeds alone. Sprouting species, though they may also come through seeds, are those that are established through sprouts alone.

## 2. Study area and climate

The study was carried out in Burnihat (26° N latitude and 91·5° E longitude) in the Khasi Hills about 90 km north of Shillong, on precambrian rocks which are represented by gneiss, schists and granites. The soil is red sandy loam and of laterite origin. The pH ranges from 5 to 7. The angle of slope generally ranges from 20° to 40°.

Climatically the year can be divided into three distinct seasons ; the dry summer runs from mid-February to May and the rainy season extends from May to September with an annual rainfall of 2200 mm. The latter is a warm period with high humidity. The mild winter which is practically rainless except for a few showers, extends from November to February. The annual maximum and minimum temperatures are 33° C and 7° C respectively (figure 1).

## 3. Methods of study

Four fallows which were slashed in January 1978 and freshly burnt in March 1978 and two 40 year old forested fallows were selected for this study. While

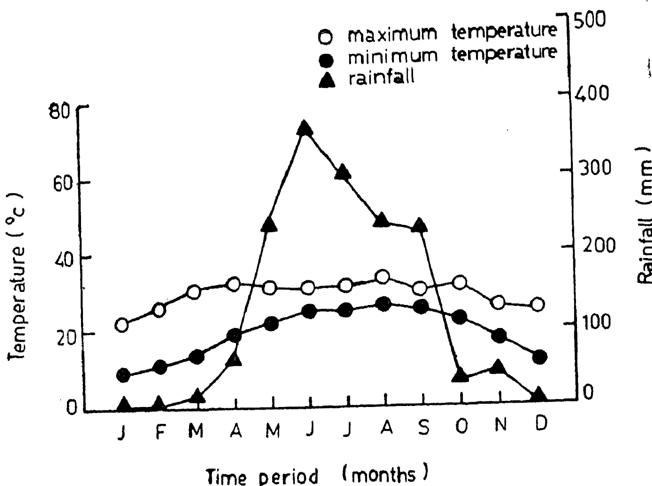


Figure 1. Ombrothermic diagram of the study area (1978).

mixed cropping is done normally at least for one year (Ramakrishnan *et al* 1981), for the purpose of the present study, the site was left directly as a fallow after the burn instead of being cultivated. The fire was a high intensity burn, since the slash burnt was derived from a 20 year regrowth. For each herbaceous species, phenological observations were made throughout the year using three permanent quadrats (50 cm × 50 cm) which were harvested at the fruiting stage. Those species which did not flower, were harvested at the end of the growing season. The samples were taken from uniformly monospecific patches of each species in order to minimize the variation due to micro-environment. Sample size ranged from 7 to 25 individuals. Below ground parts were carefully washed and leaf blade and seed components were detached. In situations where senescence started at flowering or fruiting stage, the fallen leaves and fruits or seeds were also included. Different components were dried at 80 ± 5° C in a hot air oven, for 48 hours and then weighed.

Leaf area (by planimeter) and leaf dry weight were estimated using three replicates ith 20 leaves per replicate. Total leaf area per plant was obtained by dividing total leaf biomass by dry weight per unit leaf area. Leaf area ratio was calculated as leaf area ($cm^2$) per unit (g) biomass.

Density values for different species were estimated by using 50 randomly placed 1 $m^2$ quadrats in each fallow (Misra 1968). Twenty quadrats at random were harvested for estimating the average biomass per plant. This alongwith the density values were used for calculating biomass per $m^2$. Each shoot was considered as a separate individual, in the case of the rhizomatous species. Community analysis was done at the end of the growing season.

## 4. Results

Table 1 shows the density and biomass values of different species in the early and late successional communities. Amongst the early successional annuals, *Erigeron linifolius* was numerically the most dominant followed by *Panicum maximum* and *Cassia tora*. However, *C. tora* having the lowest density, contributed maximum to the herbaceous biomass. Six other annuals were present in a small proportion and therefore are considered together. Amongst the early successional non-sprouting perennials, *Eupatorium odoratum* was the most dominant. About 64% of the herbaceous biomass was contributed by the sprouting species in the early successional communities. *Thysanolaena maxima*, though having higher relative density than *Saccharum arundinaceum*, contributed lesser in terms of biomass compared to the latter. In the late successional herbaceous communities, *Oplismenus compositus* was the most dominant component.

Species like *Erigeron linifolius*, *Eupatorium odoratum*, *Saccharum arundinaceum* and *Thysanolaena maxima* which were the most dominant component in the early successional communities, had lower leaf area ratio compared to the less frequent species like *Euphorbia hirta*, *Borreria articularis*, *Digitaria adscendens* and *Mimosa pudica*. Leaf area ratio of the late successional species was generally much higher than the early successional species. Even *E. odoratum* and *P. maximum*, which are common in the early and late successional stages, exhibited higher leaf area ratio in the late successional communities. Reproductive effort which was

Table 1. Density and biomass of different species in the early and late successional herbaceous communities.

| | Density (individuals/ m²) | Relative density (%) | Biomass (g/m²) | Biomass contribution (%) |
|---|---|---|---|---|
| *Early successional non-sprouting populations :* | | | | |
| *Annuals :* | | | | |
| Erigeron linifolius | 3·45 | 10·11 | 6·38 | 0·19 |
| Panicum maximum | 1·96 | 5·75 | 13·52 | 0·40 |
| Cassia tora | 1·10 | 3·22 | 16·61 | 0·49 |
| Others ($n = 6$) | 2·40 | 7·05 | 7·05 | 0·50 |
| *Perennials :* | | | | |
| Eupatorium odoratum | 3·75 | 10·99 | 1087·35 | 32·03 |
| Panicum khasianum | 2·45 | 7·18 | 15·44 | 0·45 |
| Others ($n = 4$) | 1·60 | 5·57 | 6·35 | 0·19 |
| *Early successional sprouting populations* | | | | |
| Thysanolaena maxima | 9·05 | 26·53 | 728·53 | 21·46 |
| Saccharum arundinaceum | 6·20 | 18·18 | 1305·10 | 38·45 |
| Imperata cylindrica | 1·50 | 4·40 | 87·38 | 2·58 |
| Others ($n = 2$) | 0·35 | 1·02 | 111·61 | 3·26 |
| *Late successional populations* | | | | |
| *Annuals :* | | | | |
| Panicum maximum | 5·62 | 16·11 | 3·37 | 3·59 |
| Oryza granulata | 1·85 | 5·30 | 12·21 | 13·02 |
| *Perennials :* | | | | |
| Oplismenus compositus | 21·46 | 61·53 | 27·84 | 28·82 |
| Centotheca lappacea | 2·35 | 6·74 | 11·16 | 11·90 |
| Others ($n = 7$) | 3·60 | 10·32 | 40·07 | 42·67 |

$n$ is the number of species.

worked out in relation to leaf growth (seed (mg)/10 cm² leaf) indicates much higher values for species like *E. odoratum* and *E. linifolius* in early successional communities. Amongst the early successional sprouting perennials, *Imperata cylindrica* and *Grewia elastica* did not flower in the first post fire year and *M. pudica* had much higher reproductive effort than the others. The late successional species, on the other hand, had comparatively very low values for reproductive effort; in *Hedychium coccineum* and *Curculigo recurvata* flowering was not observed during the year of study (figure 2).

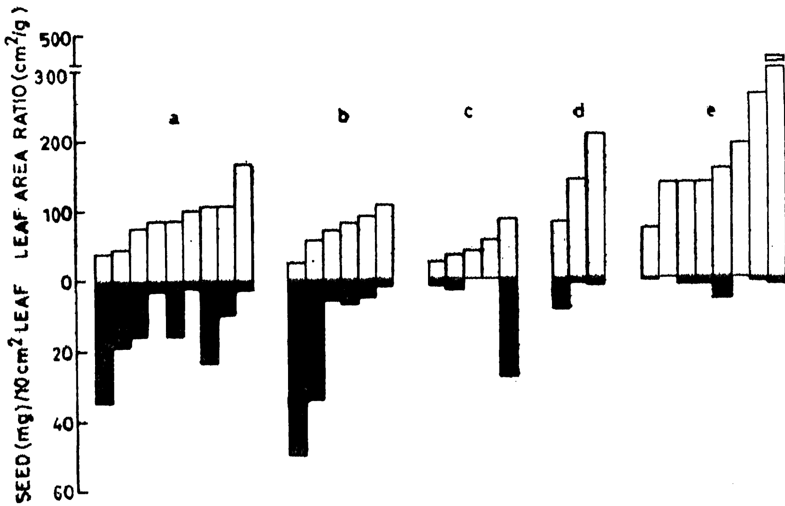


Figure 2. Leaf area ratio and reproductive effort (seed mg/10 cm² leaf) of secondary successional herbaceous populations. Non-sprouting early successional populations. (a) Annuals, (b) perennials, (c) sprouting early successional perennials, late successional populations, (d) annuals. (e) perennials. From left to right the different columns are: *Erigeron limifolius* Willd., *Rottboelia goalparensis* Bor., *Cassia tora* L., *Crossocephalum crepidioides* (Benth.) S., *Brachiaris distachya* (L.) Stapf., *Panicum maximum* Facq., *Euphorbia hirta* L., *Borreira articularis* (L.f.) Wild., *Mollugo stricta* L., *Eupatorium odoratum* L., *Setaria palmifolia* (Koen.) Stapf.. *Paspalidium punctatum* (Burm.) A. Camus., *Panicum khasianum* Munro., *Manisuria granularis* L.f., *Digitaria adscendens* (H.B.K.) Henr., *Saccharum arundinaceum* Hook f., *Thysanolaena maxima* (Roxb.) O. Ketze., *Grewia elastica* Royle, *Imperata cylindrica* Beauv., *Mimosa pudica* L., *Oryza granulata* Nees et Arn., *Panicum maximum* Facq., *Rumex nepalensis* Spreng., *Eupatorium odoratum* L., *Hedychium coccineum* Ham. *Carex cruciata* Wahl., *Oplismenus compositus* Beauv., *Centotheca lappacea* Deov., *Curculigo recurvata* Dryand., *Cyperus globosus* Allioni., *Chlorophytum arundinaceum* Baker.

These population characteristics were compared to assess their ecological mportance. Pairwise comparison was made by Mann–Whitney's two sample rank test. Early successional non-sprouting category had significantly ($P < 0 \cdot 05$) lower leaf area ratio but higher reproductive effort than the late successional category. Early successional sprouting species showed significantly ($P < 0 \cdot 05$) lower leaf area ratio than those of the early successional non-sprouting and late successional types. However, its reproductive effort was not significantly different ($P > 0 \cdot 05$) from both the categories.

Regression analysis showed that while, reproductive effort was negatively correlated with leaf area ratio in the early successional non-sprouting category ($r = -0 \cdot 72$, $P < 0 \cdot 01$), positive correlation existed in the early successional sprouting category ($r = 0 \cdot 86$, $P < 0 \cdot 05$). Further, ir the early successional non-sprouting category only, reproductive effort was positively correlated with leaf area per plant (figure 3). In the late successional category, no significant relationship could be detected between leaf characteristics and reproductive effort.

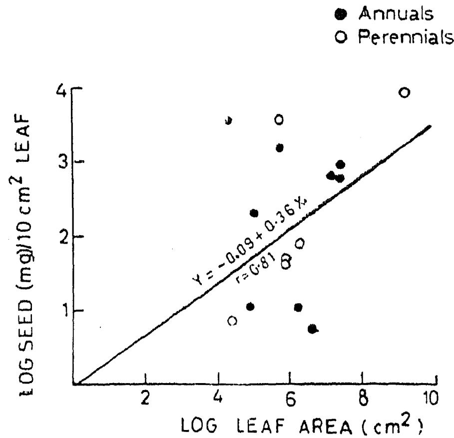


**Figure 3.** Relationship between leaf area ($cm^2$)/plant and reproductive effort (seed mg/10 $cm^2$ leaf) in the non-sprouting early successional category.

Early successional non-sprouting populations when considered separately as annuals and perennials did not differ significantly between themselves for their leaf area ratio or reproductive effort. However, the negative correlation obtained for these categories, between leaf area ratio and reproductive effort, was more significant for the perennials ($r = 0 \cdot 93$, $P < 0 \cdot 01$) compared to the annuals ($r = 0 \cdot 65$, $P < 0 \cdot 05$). Reproductive effort was found to be positively correlated with absolute leaf area in the perennials only ($r = 0 \cdot 80$, $P < 0 \cdot 05$) and not in the annuals (figure 3).

## 5. Discussion

Leaf area ratio is an important structural concept as it expresses the proportion of assimilatory surface to respiratory mass (Evans 1972). Though the different species exhibit a range of variation, late successional populations which occupy the habitat of a low light regime, have higher leaf area ratio than the early successional ones as the adaptation in the former is to synthesize and maintain the maximum light interception surface whereas the latter occupying a productive and open environment divert their resource budget to other life purposes as growth and reproduction. Higher leaf area ratio in shaded environments compared to that in the open was also reported by Myerscough and Whitehead (1977) and Bazzaz and Harper (1977). Lower leaf area ratio of early successional sprouting species compared to that of the early successional non-sprouting types may be accounted as due to the preferential allocation of photosynthates to the underground organs of the former. The somewhat lower reproductive effort of the early successional sprouting populations compared to the non-sprouting ones, though not statistically significant ($P > 0 \cdot 05$), might have evolved due to the failure of their regeneration through seedlings (Wilson 1971).

Absolute leaf area of a plant gives an idea about its capacity of light interception and vigour while leaf area ratio, the ratio of light interception surface and total biomass ($cm^2$ leaf area/g biomass) indicates the efficiency of dry matter production on leaf area basis. Significantly positive correlation of leaf area and negative correlation of leaf area ratio with reproductive effort in the early successional non-sprouting category show that high reproductive potential is associated with the vigour and also the production efficiency of the species. Comparatively stronger correlation of leaf area ratio with reproductive effort tlan that of the absolute leaf area with the reproductive effort in the early successional non-sprouting species indicates that reproductive success here depends more upon the production efficiency rather than the overall vigour of the plant. In contrast, early successional sprouting populations exhibited positive correlation of leaf area ratio with sexual reproductive effort. Thus a species like *Mimosa pudica* which has a high leaf area ratio allocates more for sexual reproductive effort. Also it so happens that this species is less vigorous in its vegetative regeneration compared to others like *Saccharum arundinaceum* and *Thysanolaena maxima*, and thus compensates more through sexual reproduction. Complete paucity of flowering in the first post-fire year, as in *Imperata cylindrica* and *Grewia elastica* has been shown for a number of shrub species (Gill 1975). This aspect of the problem is receiving our attention. Within the late successional group no significan ($P > 0{\cdot}05$) relationship between leaf characteristics and reproductive effort wa found, suggesting that sexual reproduction is not related with production efficiency or vigour of the plant ; the strategy seems to be to maximize vegetative-growth in a closed habitat.

Considering the annuals and perennials of the early successional non-sprouting category separately, certain differences are apparent. While reproductive effort seems to be dependent upon production efficiency in both the cases, it was positively correlated with absolute leaf area in the case of perennials alone indicating that the vigour of the plant is less critical for the annuals having a single possibility of flowering during their life-span.

MacArthur and Wilson (1967) pointed out that organisms in open environments are selected for greater reproductive capacity (*r*-strategy) while those in closed environments are selected for greater ability to compete for resources, though at the cost of lower reproductive potential (*K*-strategy). Grime (1974, 1977) has extended this argument by describing three primary strategies in plants which are related to their ability to withstand competition, stress and disturbance. Here, ruderal and stress tolerant strategies correspond to the extreme of *r*- and *K*-selection while highly competitive species of productive environments occupy an intermediate position. The findings of the present study clearly indicate that early successional non-sprouting populations are of ruderal type as they are equipped with the strategy to maximize seed production in order to colonize a disturbed habitat whereas early successional sprouting and late successional populations direct their synthetic capacity for competition and stress tolerance respectively by economizing on the reproductive growth. Vegetative reproduction has been looked merely as a growth in a horizontal plane (Harper 1977) and, therefore, is not considered in the present study.

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# Vesicular arbuscular mycorrhiza in subtropical aquatic and marshy plant communities

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**Abstract.** Occurrence of vesicular arbuscular mycorrhiza in five subtropical ponds, i.e., eutrophic (*P*1, *P*2 and *P*3), running water (*P*4), oligotrophic lake (*P*5) and marshy plant community (*M*) was studied. It was observed that the plants growing in *P*1, *P*5 and *M* habitats exhibited the vesicular arbuscular mycorrhizal association, whereas the fungal association was lacking in plants of *P*2, *P*3 and *P*4 ponds. The endogonaceous spore population was estimated from water and sediments of the different ponds and it was found that endophytes in sediments are less in terrestrial habitats and completely absent from water samples. The bioassay studies revealed that plants without mycorrhizal association grew poorly and all the endophytes isolated could establish vesicular arbuscular mycorrhizal associations in pot cultures.

**Keywords.** Vesicular arbuscular mycorrhiza ; subtropical aquatic community ; endophytes ; bioassay.

## 1. Introduction

Vesicular arbuscular mycorrhiza (VAM) is universal in occurrence (Nicolson 1967; Mosse 1973) and is useful to the host plant in various ways ; in enhancing the uptake of nutrients (Hayman 1975) and water (Safir *et al* 1972), in resisting against pathogen (Marx 1975) and in increasing the effective absorption surface of roots (Hayman and Mosse 1971). Most of the rushes and sedges, however, are reported to be non-mycorrhizal (Powell 1974 ; Khan 1974 ; Harley 1969 ; Gerdemann 1975). Recently, a few temperate (Sondergåard and Laegåard 1977) and tropical (Bagyaraj *et al* 1979) aquatic species were reported to be mycorrhizal. In the present study five aquatic and one marshy sub-tropical plant community were examined for occurrence of VAM and endophytic fungi. To test the ability and efficiency of these endophytes a bioassay was also developed to test its potential use in propagation of VAM in successiona communities of aquatic systems.

## 2. Materials and methods

### 2.1. *Site selection*

Five fresh water bodies and one marshy habitat in Shillong (altitude 1450 m, latitude 25·34° N and longitude 91·56° E) were selected for the present study.

The ponds are designated as *P1*, *P2*, *P3*, *P4*, *P5* and *M* (marshy habitat). *P1*, *P2* and *P3* are eutrophic ponds, *P4* is a running stream and *P5* is an oligotrophic lake. *P1* remains dry in summer and receives water during the rainy season.

### 2.2. *Collection of root samples and assessment of VAM*

Intact plants with roots from different localities were collected in containers. The roots were washed with tap water and cut into segments of approximately 1 cm in length (100 segments from five plants). Further, the root segments were processed and stained for VAM infection by the Phillips and Hayman (1970) technique. Percentage of root infection was calculated in the presence of either vesicle, arbuscules or both by counting the infected segments by the slide method (Mishra *et al* 1981).

### 2.3. *Estimation of endogonaceous spores*

Fifty ml of water and 30 g of sediment were collected by water sampler from each site in five replicates. The sediment was wet sieved and decanted (Gerdemann and Nicolson 1963) and water was filtered through Whatman No. 1. filter paper. The spores retained on the sieves and filter paper were examined under a binocular microscope.

### 2.4. *Bioassay study for infection efficiency of endogonaceous spores in pot cultures*

Sterilized maize seeds were germinated in sterilized moist chambers. Five seedlings (2 cm radicle stage) were transplanted to pots (11 × 10″) containing sterilized soil (soil + sand in equal amount w/w). The plants were inoculated with endophytes isolated from the sediments ; uninoculated pots received soil with microflora but were devoid of mycorrhizal propagules. All the pots were regularly watered. Plants were harvested 15, 30 and 45 days after transplanting. Root infection, shoot height, dry weight and leaf production were recorded at each harvesting.

### 2.5. *Physico-chemical analysis of water and soil*

pH of soil and water was measured by electronic digital pH meter. Organic carbon (Walkey method), nitrate (phenol disulphonic method) and phosphorus (Bray's method) were estimated as outlined by Jackson (1967).

## 3. Results

Five subtropical aquatic species, *viz. Rotala rotundifolia*, *Paspalum dilatatum*; *Polygonum hydropiper*, *Nymphaea alba* and *Hydrilla verticellata* have been observed for the first time as mycorrhizal (table 1, figures 1-4). Vesicular arbuscular mycorrhiza (VAM) was observed in *P1*, *P5* and *M* plant communities. Plants growing in *P2*, *P3* and *P4* ponds were devoid of VAM infection. Percentage infection was highest in *P5* plants followed by *P1* and was least in plants from the marshy (*M*) habitat (table 2). Vesicles and hyphae were regularly observed. The population of endogonaceous spores in general was low in all the sediments from different sites and the number did not differ significantly (table 2). No

**Table 1.** Percentage occurrence of vesicular arbuscular mycorrhiza in roots of different plant species of sub-tropical aquatic and marshy communities.

| Aquatic community | Plant species | VAM (%) | Marshy community | Plant species | VAM % (root) |
|---|---|---|---|---|---|
| $P_1$ | *Rotala rotundifolia* | 10·00 | | *Impatiens chinensis* | 100·00 |
| | *Paspalum dilatatum* | 60·00 | | *Drosera* sp. | 64·00 |
| | *Polygonum hydropiper* | 56·00 | | *Utricularia* sp. | 73·00 |
| | *Cyperus distans* | 0·00 | | *Sonchus* sp. | 52·00 |
| | | | | *Polygonum capitatum* | 51·00 |
| $P_2$ | *Cardamine hirsuta* | 0·00 | | *Drymaria cordata* | 33·00 |
| | *C. macrophylla* | 0·00 | | *Plantago major* | 19·00 |
| | *Scirpus articulatus* | 0·00 | | *Nasturtium indica* | 0·00 |
| | *S. juncoides* | 0·00 | | *Anemone rivalaris* | 0·00 |
| | *Eleocharis congesta* | 0·00 | | *Steudnera colocasioides* | 0·00 |
| $P_3$ | *Rotala rotundifolia* | 0·00 | | *Oenothera javinaca* | 0·00 |
| | *Cyperus distans* | 0·00 | | *Brassica juncea* | 12·00 |
| | *Eleocharis congesta* | 0·00 | | *Rumex nepalensis* | 28·00 |
| | *Spargonium ramosum* | 0·00 | | *Galium rotundifolium* | 16·00 |
| | | | | *Panicum brevifolium* | 46·00 |
| $P_4$ | *Hydrilla verticillata* | 0·00 | | | |
| | *Alternanthera philoxeroides* | 0·00 | | | |
| | *Monochoria hastata* | 0·00 | | | |
| | *Hydrocotyle sibthorpioides* | 0·00 | | | |
| | *Lasia spinosa* | 0·00 | | | |
| $P_5$ | *Rotala rotundifolia* | 3·00 | | | |
| | *Hydrilla verticillata* | 16·00 | | | |
| | *Nymphaea alba* | 12·00 | | | |

**Table 2.** Endogonaceous spore population and frequency of VAM mycorrhizal plants in different localities.

| Sites | Mean spore population/30 g/ml soil/water | | Frequency (%) of mycorrhiza |
|---|---|---|---|
| | Water (per 30 ml) | Soil (per 30 g) | |
| $P_1$ | 0 | 27 | 75 |
| $P_2$ | 0 | 33 | 0 |
| $P_3$ | 0 | 6 | 0 |
| $P_4$ | 0 | 7 | 0 |
| $P_5$ | 0 | 11 | 100 |
| $M_6$ | 0 | 34 | 71·3 |

endogonaceous spores were found in water. The pot culture studies on infection efficiency of endogonaceous spores from different sites revealed that they may infect and establish in roots growing in soil and that their efficiency may differ (table 3). Uninoculated seedlings did not grow well, whereas the seedlings with mycorrhizal association grew better.

Soil and water from all the sites were acidic in nature. Organic carbon was very low in water samples and highest in soil of marshy land. Nitrate and phosphate were highest in *P*2 and *P*1 and lowest in *P*5 and *M* sites (table 4).

## 4. Discussion

The study reveals that VAM occur rarely in aquatic subtropical plant communities, but they are not completely absent. The results are, therefore, contrary to the views of Harley (1969), Gerdemann (1975), Powell (1974) and Khan (1974), but support the findings of Sondergåard and Laegåard (1977) and Bagyaraj *et al* (1979). Apart from environmental factors like light, temperature and aeration, the occurrence of VAM and its intensity may be regulated by the nutrient status of the aquatic system. Gerdemann (1968) observed that mycorrhiza may be low in rich soil. The amount of infection in a few species, *i.e.*, *Rotala rotundifolia* and *Hydrilla verticellata* differed insignificantly in different water systems depending on the nutrient status and other physical factors of these systems. In general, *P*1, *P*5 and *M* sites favoured VAM establishment but *P*2, *P*3 and *P*4 ponds did not possess any mycorrhizal association. It seems that temporary drying of *P*1 and *M* habitats in summer may initiate the VAM establishment. A similar trend was also observed in other environmental conditions (Read *et al* 1976). The high percentage occurrence of VAM in *P*5 may be attributed to its oligotrophic nature (Sondergåard and Laegåard 1977), as *Hydrilla verticellata* was mycorrhizal in *P*5 community but not in *P*4. Besides the root system, the shoot also helps the aquatic plants in absorbing the nutrients from water and this may be one of the reasons for the absence or less frequent mycorrhizal association in certain plants. Sutcliffe (1962) suggested that in aquatic plants, the roots primarily act as anchors ; on the other hand, Denny (1972) concluded that the nutrients may enter through roots and shoots. Therefore, it seems that phosphorus uptake may depend on the efficiency of the root system, *i.e.*, some root systems may be adaptive enough to draw the phosphate at low level even in the absence of VAM association (Powell 1975). VAM may not be of much importance in plants growing in rich medium (*P*2, *P*3 and *P*4 system) but it may help the hosts in absorption of the nutrients growing in low level nutrient systems (*P*5). Therefore, two conditions, *i.e.*, temporary drying of the aquatic system and the oligotrophic nature seem to be more favourable for VAM development. The presence of endophyte spores in all aquatic systems suggests that these spores probably enter into the water system through run off from terrestrial ecosystem, their subsequent development, however, is governed by water regime, light, aeration and other factors (Mosse 1973). The presence of endophyte spore in *P*2 community may further indicate that either these species are not capable of causing infection or plants may not be susceptible in such systems (Reeves *et al* 1979 ; Miller 1979). The bio-

**Figures 1–4.** 1. Spore, *Glomus* sp. infecting root tissue of *Panicum brevifolium* (× 1,000), 2. Vesicles and hyphae in root tissue of *Impatiens balsamia* (× 100), 3. Obovate vesicles of *Glomus* sp. in the root tissue of *Rotala rotundifolia* (× 400), 4. Round vesicles with oil globule and degenerating stage of hypha in root tissue of *Nymphaea alba* (× 400).

Table 3. Development and effect of VAM in maize inoculated with endophytes from different habitats.

| Endophyte inoculation from different sites | 15 days | | | | 30 days | | | | 45 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf No. | Stem height (cm) | Shoot dry weight (g) | VAM | Leaf No. | Stem height (cm) | Shoot dry weight | VAM | Leaf No. | Stem height (cm) | Shoot dry weight (g) | VAM |
| $P_1$ | 5 | 5·7 | 80·0 | .. | 5 | 16·9 | 120·0 | + | 6 | 20·4 | 160·0 | + |
| $P_2$ | 5 | 5·0 | 125·0 | .. | 7 | 19·5 | 200·0 | + | 8 | 27·5 | 245·0 | + |
| $P_3$ | 4 | 4·1 | 65·0 | .. | 5 | 15·5 | 80·0 | – | 5 | 17·6 | 92·0 | + |
| $P_4$ | 5 | 5·3 | 90·0 | .. | 6 | 17·8 | 180·0 | – | 6 | 22·8 | 108·0 | + |
| $P_5$ | 4 | 3·9 | 53·0 | .. | 5 | 16·2 | 96·0 | – | 7 | 24·4 | 102·0 | + |
| $M_6$ | 5 | 5·6 | 130·0 | .. | 7 | 18·9 | 210·9 | + | 8 | 28·6 | 265·0 | + |
| Uninoculated | 3 | 3·6 | 45·0 | .. | 4 | 12·0 | 60·0 | – | 4 | 14·2 | 70·1 | – |

Table 4. Physico-chemical characters of soil and water in different water bodies.

| Site | pH | | Org. Carbon (%) | | Nitrate (ppm) | | Phosphate (ppm) | |
|---|---|---|---|---|---|---|---|---|
| | water | soil | water | soil | soil | water | water | soil |
| $P_1$ | 6·5 | 8·3 | 0·05 | 4·9 | 2·2 | 0·20 | 3·6 | 6·62 |
| $P_2$ | 6·4 | 6·3 | 0·07 | 7·6 | 1·6 | 0·17 | 3·8 | 7·8 |
| $P_3$ | 6·0 | 6·3 | 0·05 | 5·3 | 1·6 | 0·07 | 1·07 | 3·18 |
| $P_4$ | 6·6 | 6·5 | 0·06 | 5·6 | 1·8 | 0·09 | 0·1 | 2·01 |
| $P_5$ | 6·1 | 6·3 | 0·04 | 3·9 | 1·4 | 0·05 | 0·01 | 1·96 |
| $M$ | .. | 6·2 | 0·00 | 8·0 | 0·1 | .. | .. | 1·42 |

assay studies using maize (*Zea mays*) as test plant suggested that these endophytes may develop VAM and enhance the growth of plants, when placed into sterilized soil. It is of great ecological importance to study the establishment, germination and entrance of these endophytes into the hosts under diverse environmental conditions, in understanding of the developmental pattern of aquatic and terrestrial communities. Baylis (1959) suggested that VAM play a significant role in the evolution of plant communities. Therefore, studies in controlled conditions in aquatic habitats on the esta lishment of VAM will provide information towards further understanding of succession in aquatic systems and would aid management of aquacultural systems.

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# *Chandrasekharania* : A new genus of Poaceae from Kerala, India

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**Abstract.** A new genus, *Chandrasekharania* and a new species *Chandrasekharania keralensis* under it are being described from Cannanore District, Kerala.

**Keywords.** *Chandrasekharania keralensis,* Poaceae ; new genus; new species.

## 1. Introduction

Intensive explorations are being conducted in various parts of Kerala to clearly understand the flora of this botanically rich area. During these tours a number of very interesting plants were collected. One of these belonging to the family Poaceae, on critical study with reference to available literature (see Bor 1960 ; Ved Prakash *et al* 1978) and the specimens available in the Herbarium of Southern Circle, Botanical Survey of India, Coimbatore (MH) and the Central National Herbarium, Calcutta (CAL), turned out to be quite distinct. So a specimen along with a detailed description and analytical sketches was sent to Dr Thomas A Cope, Herbarium, Royal Botanic Gardens, Kew, for expert opinion. He confirmed that our collection belongs to a new genus. This along with its type species is described here.

## 2. Latin description

***Chandrasekharania*** V J Nair, V S Ramachandran *et* P V Sreekumar *gen. nov.* Gramen annuum. Culmi erecti vel decumbentes. Folia lanceolata vel ovato-lanceolata, basibus cordatis. Ligulae obscurae. Inflorescentia contracta, racemiflora, spiculae omnibus similaribus, lateraliter compressis, floribus duobus, pedicellatis, pedicellis numquam articulatis. Flosculi similes, hermaphroditi. Glumae inaequales, coriaceae, ovatae vel ovatolanceolatae, 7-nervatae, aristatae, aristis curtis et rectis. Glumae infernae breviores in dorsi parte superiore pilis basi tuberculatis. Lemmata 5-nervatae, coriacea, elliptico-lanceolata, apicibus breviter emarginatis, aristatis ad sinus. Aristis curtis et rectis. Paleae ovato-ellipticae, hyalinae, binervatae, bicarinatae, carinis ciliatis, apicibus bilobatis. Ovarium glabrum. Styli 2, distincti. Stigmata plumosa. Stamina 3, filamentis brevissimis. Lodiculae 2. Grana ellipsoidea.

Species typica sequens :

***Chandrasekharania keralensis*** V J Nair, V S Ramachandran *et* P V Sreekumar *sp. nov.*

Gramen annuum usque 40 cm altum. Culmi graciles, striati leaves et glabri. Nodis inferioribus radicantes. Folia 2–4·5 cm longa, 5–8 mm lata, marginibus et paginis ambabus pilis densis vel sparsis, basi tuberculatis. Vaginae striatae, glabrae, internodiis breviores. Inflorescentia contracta, ovoidea vel oblonga, spiciformis, racemiflora, 1·5–2·5 cm longa, 1–1·5 cm lata. Spiculae 5–6 mm longae. Pedicelli 0·5–1·5 mm longi. Glumae infernae 7-nervatae, nervi duo alternati et mediani prominentes, nervi cateri inconspicui, ovatae, 3·5–4 mm longae, aristatae, arista *ca.* 3 mm longa, costa et arista scabridus, margines ciliolati, dimidia supera paginarum dorsalia dense tecta pilis, basibus tubercularibus. Glumae superae 5·5–6 mm longae, ovato-lanceolatae acuminatae, aristatae, 7-nervatae, margines ciliolati nervi scaberuli, arista scabrida, *ca.* 3 mm longa. Lemmata elliptico-lanceolata, 4–5 mm longa, apicibus breviter emarginatis, aristatis ad sinus, coriacea, glabra praeter scaberula costas, arista scabrida, 1·5–2 mm longa. Paleae *ca.* 4 mm longae, subtiles hyalinae, binervatae, dorsiis dimidiis infernis pilis longiore, cariniis ciliatis, apicibus bilobatis, lobi acuti. Gynoecium *ca.* 2 mm longum. Ovarium 0·5 mm longum, oblongum. Antherae *ca.* 1·25 mm longae. Fila *ca.* 0·25 mm longa. Lodiculae cuneatae, apicibus emarginatis. Grana ellipsoidea, *ca.* 1 mm longa, fusca et extremis distalibus maculae ateris.

Holotypus : Kerala, Cannanore District, Kannoth, ± 175 m, 18–2–1978, *V S Ramachandran* 54064 (CAL). Isotypi in K et MH.

## 3. Description

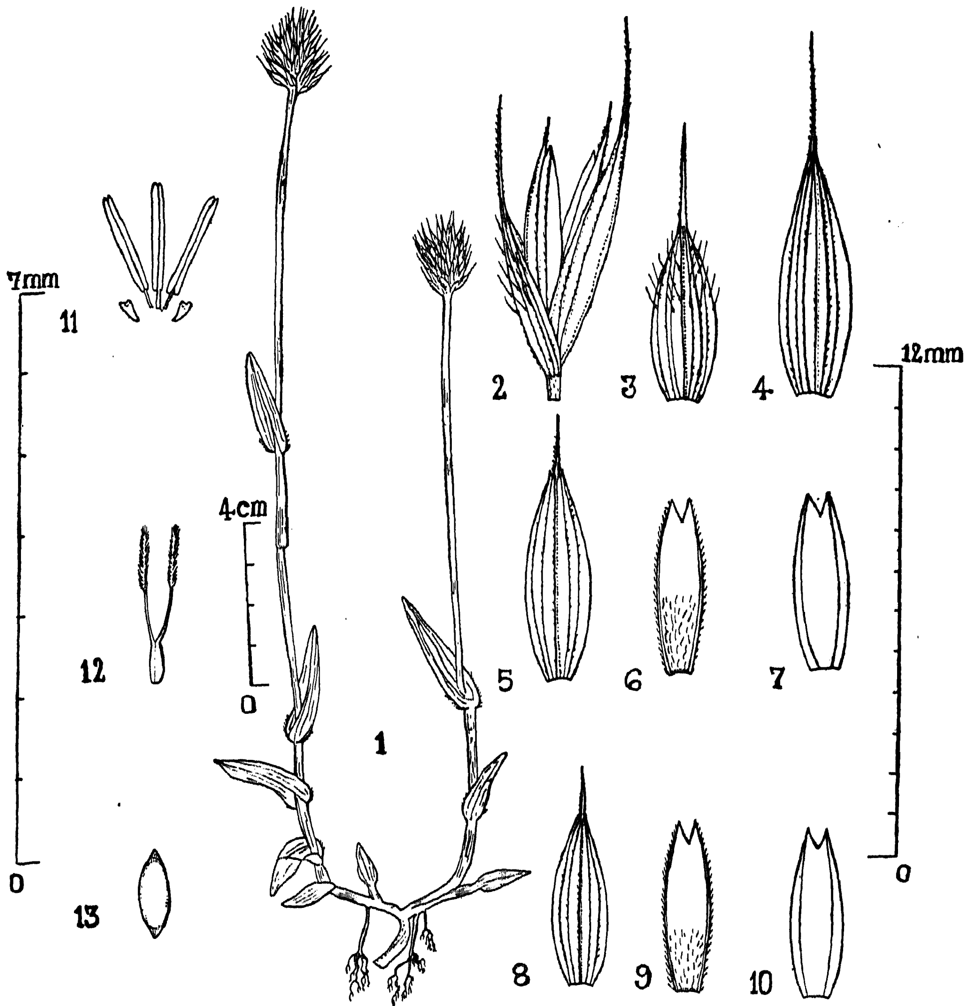
***Chandrasekharania*** V J Nair, V S Ramachandran *et* P V Sreekumar *gen. nov.*

Annual grass. Culms erect or decumbent. Leaves flat, lanceolate or ovate-lanceolate, base cordate. Ligules obscure. Inflorescence terminal, solitary, contracted, racemose. Spikelets all alike, laterally compressed, two flowered, pedicelled, pedicels never jointed. Florets similar, bisexual. Glumes unequal, coriaceous, ovate or ovate-lanceolate, 7-nerved, awned, awns short and straight. Lower glume smaller with the dorsal surface with tubercle based hairs in the upper half. Lemma 5-nerved, coriaceous, elliptic-lanceolate, minutely notched at the apex with an awn in the sinus. Awns short and straight. Palea ovate-elliptic, hyaline, 2-nerved, 2-keeled, keels ciliate, apex two lobed. Ovary glabrous. Styles 2, distinct. Stigmas feathery. Stamens 3. Filaments very short. Lodicules 2. Grain ellipsoid.

Type species follows :

***Chandrasekharania keralensis*** V J Nair, V S Ramachandran *et* P V Sreekumar *sp nov.*

Annual grass up to 40 cm tall. Culms rooting at the lower nodes, slender, striate, smooth, glabrous. Leaves 2–4.5 cm long, 5–8 mm broad, both surfaces and margins with dense or sparse tubercle based hairs. Sheaths shorter than the




**Figures 1–13.** *Chandrasekharania keralensis gen. et sp. nov.* **1.** Habit. **2.** Spikelet. **3.** Lower glume. **4.** Upper glume. **5.** Lower lemma. **6.** Lower palea—dorsal view. **7.** Same—ventral view, **8.** Upper lemma **9.** Upper palea—dorsal view. **10.** Same—ventral view. **11.** Lodicules and stamens. **12.** Gynoecium. **13.** Grain.

internodes, striate, glabrous. Inflorescence a contracted ovoid or oblong spikate raceme, 1·5–2·5 cm long, 1–1·5 cm broad. Spikelets 5–6 mm long. Pedicels 0·5–1·5 mm long, scabrid. Lower glume 3·5–4 mm long, ovate, awned, awn *ca.* 3 mm long, densely hairy on the upper half of the dorsal surface with tubercle based hairs, the median and the alternating two nerves prominent, the midrib and the awn scabrid, margins ciliolate. Upper glume 5·5–6 mm long, ovate-lanceolate, acuminate, the tip with an awn *ca.* 3 mm long, scaberulous on the nerves, scabrid on the arista, margins ciliolate. Lemma elliptic-lanceolate, 4-5 mm long, shortly notched at apex with an awn in the sinus, coriaceous, glabrous except for the scaberulous midrib, awn scabrid, 1·5–2 mm long. Palea *ca.* 4 mm

long, delicate, hyaline, long-ciliate on the lower half of the outer surface, keels ciliate, apex bilobed, lobes acute. Gynoecium *ca.* 2 mm in length ; ovary *ca.* 0·5 mm long, oblong. Anthers *ca.* 1·25 mm long, filaments *ca.* 0·25 mm long. Lodicules cuneate and shallowly notched at apex. Grains ellipsoid, *ca.* 1 mm long, brown coloured with a black spot at the distal end.

Holotype : Kerala, Cannanore District, Kannoth, ± 175 m, 18–2–1978, *V S Ramachandran* 54064 (CAL). Isotypes in K and MH.

## 4. Affinities

Affinities of this new genus are not very clear. According to Dr T A Cope, Kew Herbarium, anatomical studies are required to clearly understand its affinities (personal communication).

## 5. Etymology

The genus is named after Dr N Chandrasekharan Nair, the first author's teacher and present Joint Director, Botanical Survey of India, Coimbatore, in recognition of his outstanding contributions to Indian botany. The specific epithet is after 'Kerala' the state from where the plant has been collected.

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# Chromosome relationships of spinous solanums

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**Abstract.** Chromosome pairing was studied in reciprocal hybrids of *S. integrifolium* and *S. indicum* and the $F_1$ *S. integrifolium* × *S. surattense*. Pairing was generally close and meiosis regular with higher chromosome associations. All hybrids were highly sterile. Such sterility could be due to the formation of unbalanced gametes following pairing and exchange between partially homeologous chromosomes. *S. integrifolium*, *S. indicum*, *S. surattense* along with *S. melongena* and its wild forms form a closely related group of taxa.

**Keywords.** *Solanum indicum*; *S. surattense*; *S. integrifolium*; $F_1$ hybrids; sterility; chromosome relationships.

## 1. Introduction

Chromosomal studies on species hybrids are of considerable importance in elucidating species relationships and evolutionary trends. Such studies on spinous Solanums, which are important medicinally as well as vegetables, were meagre because of the difficulty in producing interspecific hybrids. Magoon *et al* (1962) cited the earliest attempts of species hybridization in solanums including those of Sarvayya (1936), Hagiwara and Iida (1939) and Tatebe (1939). Bhadhuri (1951) first discussed the interrelationships of spinous solanums including the origin of *S. melongena*. Further attempts in this direction were made by Zutshi (1967), Rajasekaran (1969, 1970a, b, 1971), Rajasekaran and Sivasubramanian (1971), Rangaswamy and Kadambavanasundaram (1974), Veerabhadra Rao (1977), Veerabhadra Rao and Rao (1977b, c), Kirti and Rao (1978, 1981a, b, c). Most of the cultivars of the brinjal are susceptible to various diseases and pests (Krishnaiah and Vijay 1975). Wild species such as *S. integrifolium* and *S. indicum* are resistant to some of the diseases and pests. However, these species cannot be utilized in egg plant improvement unless the interrelationships of the whole group are unravelled. First attempts of crossing *S. integrifolium* and *S. melongena* were made by Hagiwara and Iida (1939), Tatebe (1939), Khan *et al* (1978). Kirti and Rao (1981c) discussed at length the chromosome relationships between these two species as well as *S. integrifolium* and

*S. indicum* var. *multiflora*. The chromosome relationships *S. integrifolium*, *S. indicum*, *S. surattense* and other species are discussed in some detail here.

## 2. Materials and methods

Seeds of *S. indicum* L. and *S. surattense* Burm. F. (= *S. xanthocarpum* Schrad and Wendi) were collected from plants occurring wild while seeds of *S. integrifolium* were kindly provided by Prof P V Bhiravamurthy of the Andhra University, who obtained them from Denmark.

Method of crossing was the one employed by Veerabhadra Rao and Rao (1977a).

Standard propionic carmine schedule was used for PMC smears.

## 3. Results and discussion

### 3.1. *Crossability*

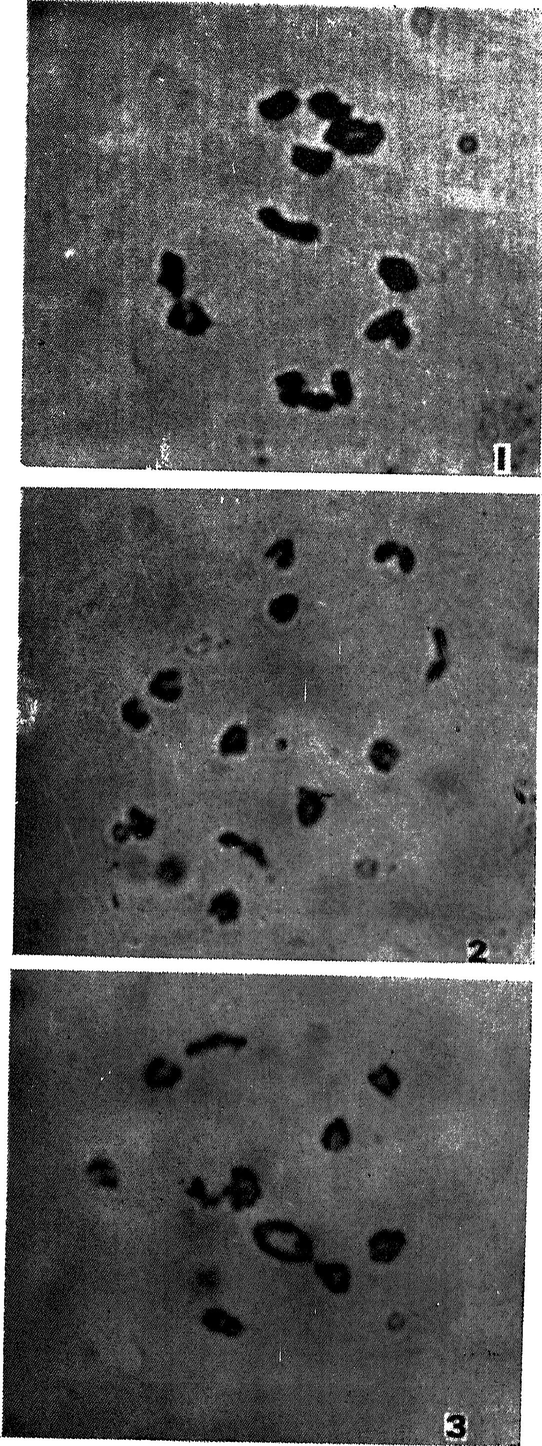
An on-the field screening for functional pistillate flowers was done since stylar heteromorphism is prevalent in spinous solanums. Only flowers with long and exserted styles can be used as pistillate flowers in crossing experiments. Rao and Veerabhadra Rao (1976) have studied this phenomenon in some detail in *S. surattense*. Shamim Baksh *et al* (1978) described this in *S. integrifolium*. In *S. indicum*, the inflorescence is a 3–4 flowered cyme and only the basal most flower having the long and exserted style sets fruit. Others are generally with shorter and inserted styles and do not set fruit. So this phenomenon should be taken as an important criterion in the consideration of crossability.

The ease with which any two species are crossed reflects to some extent the nearness of the species concerned with the absence of prezygotic incompatibility barriers. In the present study, *S. integrifolium* and *S. indicum* could be crossed in reciprocal directions and the percentage of hybrid seed obtained was very high (table 1). *S. integrifolium* could be crossed with *S. surattense* in only one direction and only a solitary hybrid could be realised. Thus it seems *S. integrifolium* is more closely related to *S. indicum* on the basis of this observation than to *S. surattense* even though other factors are to be taken into consideration.

### 3.2. *Morphology of parental species and hybrids*

*S. indicum* and *S. surattense* conformed to the classical descriptions of Gamble (1957). *S. integrifolium* is an erect herb with small white flowers, and scarlet red coloured and lobed berries.

Reciprocal hybrids of *S. integrifolium* and *S. indicum* resembled each other and were intermediate between the parents in some features and resembling either of the parents in some respects. Similarly the $F_1$ *S. integrifolium* × *S. surattense* was intermediate.




**Figures 1–3.** Cytology of hybrids ; Diakinesis-Metaphase-I in *S. indicum* × *S. integrifolium* **1.** $10_{II} + 1_{IV}$. **2.** $12_{II}$. **3.** $10_{II} + 1_{IV}$.

**Table 1.** Results on crossability relationships of *S. integrifolium*, *S. indicum* and *S. surattense.*

| | *S. integrifolium* × *S. indicum* | *S. indicum* × *S. integrifolium* | *S. integrifolium* × *S. surattense* |
|---|---|---|---|
| umber of pollinations made | 15 | 70 | 105 |
| umber of fruits set | 1 | 1 | 1 |
| umber of healthy seeds per fruit | 165 | 174 | 1 |
| rcentage of seed germination | 97·6 | 94·2 | 100 |
| rcentage of hybrid seed obtained in relation to the number of 'target' ovules* per ovary | 78·9 | 56·5 | 0·48 |

Approximated from the average number of seeds per fruit in parental species, 209 in *integrifolium* and 308 in *S. indicum.*

**Table 2.** Frequencies of nuclei with different chromosome associations observed in hybrids of *S. integrifolium*, *S. indicum* and *S. surattense.*

| *integrifolium* *S. surattense* requency of nuclei | *S. integrifolium* × *S. indicum* Frequency of nuclei | | Kinds of chromosome associations per nucleus | | | | *S. indicum* × *S. integrifolium* Frequency of nuclei | |
|---|---|---|---|---|---|---|---|---|
| )iakinesis | Diakinesis | Metaphase-I | IV | III | II | I | Diakinesis | Metaphase-I |
| 9 (40·9) | 27 (44·3) | 15 (50·0) | 1 | .. | 10 | .. | 68 (63·0) | 28 (73·7) |
| 3 (13·6) | 7 (11·5) | .. | 1 | .. | 9 | 2 | 5 (4·6) | .. |
| .. | .. | .. | 1 | .. | 8 | 4 | 2 (1·8) | .. |
| .. | 4 (6·6) | 2 (6·7) | .. | 1 | 10 | 1 | 4 (3·7) | 1 (2·6) |
| 2 (9·1) | .. | .. | .. | 1 | 9 | 3 | 1 (0·9) | .. |
| 4 (19·2) | 18 (29·5) | 12 (40·0) | .. | .. | 12 | .. | 23 (21·3) | 8 (21·1) |
| 4 (18·2) | 5 (8·2) | 1 (3·3) | .. | .. | 11 | 2 | 5 (4·6) | 1 (2·6) |
| 22 | 61 | 30 | Total number of nuclei analysed | | | | 108 | 38 |

gures in parentheses indicate percentage frequency).
Metaphase-I data not available.

### 3·3. *Cytological observations*

Meiosis proceeds regularly in the parents with the formation of twelve bivalents ($2n = 24$) and regular chromosome separation at anaphase I and II.

Various types of chromosome associations (figures 1–3) and their frequencies observed in the hybrids are summarised in table 2. Higher chromosome associations in hybrids indicate chromosomal structural repatterning in the divergence of the concerned taxa.

Mean frequencies of chiasmata in parents and hybrids are summarized in table 3. Mean values in hybrids were significantly lower than in parents indicating somewhat reduced chromosome homeologies.

Later stages of meiosis followed normally with regular chromosome segregation at anaphases I and II. Laggards, bridges without fragments and micronuclei were rarely encountered. Despite regular meiotic divisions, all the hybrids were over 95% pollen sterile. Stebbins (1950) advocated that cryptic chromosomal structural differences are responsible for sterility in hybrids with regular meiotic events. It is also possible that segregational events, following pairing and exchange of segments between partially homeologous chromosomes of the two genomes leading to the formation of unbalanced gametes, eventually lead to lethality (Kirti 1978).

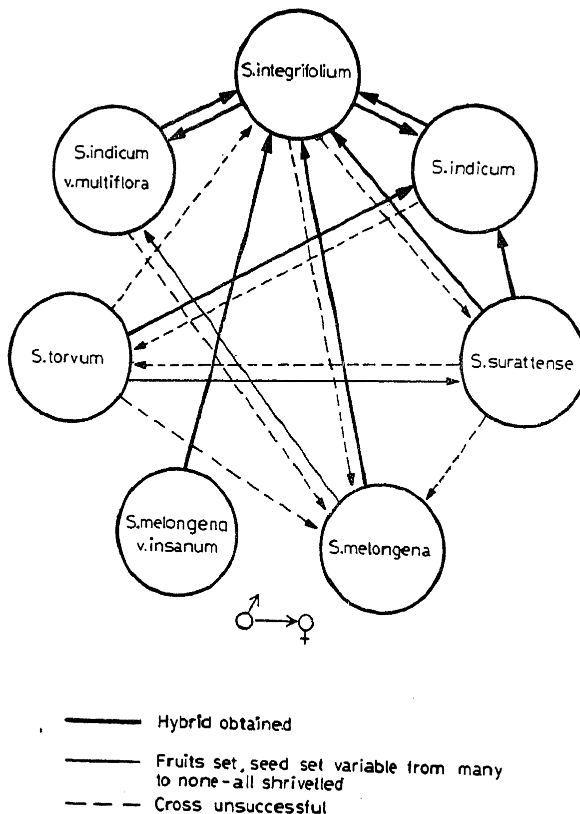



Figure 4. Crossability relationships of some spinous solanums.

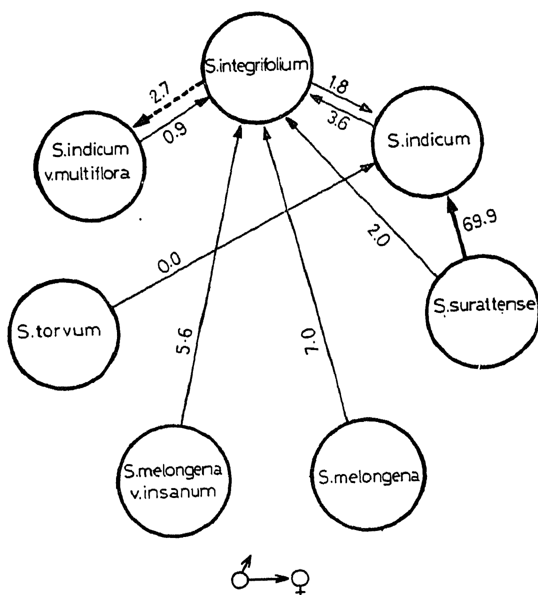



**Figure 5.** Fertility relationships of some spinous solanums.

**Table 3.** Average chiasma frequencies observed in *S. surattense*, *S. integrifolium*, *S. indicum* and their hybrids.

| | *S. surattense* | *S. integrifolium* | | *S. indicum* | | *S. integrifolium* × *S. indicum* | | *S. indicum* × *S. integrifolium* | | *S. integrifolium* × *S. surattense* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Diak* | Diak | Meta-I | Diak | Meta-I | Diak | Meta-I | Diak | Meta-I | Diak* |
| Number of PMCs analysed | 30 | 41 | | 32 | 27 | 61 | 30 | 108 | 38 | 22 |
| Average chiasma frequency per nucleus | 18·17 ±0·20 | 19·24 ±0·37 | 19·04 ±0·61 | 20·06 ±0·23 | 19·90 ±0·40 | 17·65 ±0·16 | 17·56 ±0·39 | 17·83 ±0·20 | 17·20 ±0·34 | 16·41 ±0·26 |
| Per bivalent | 1·51 | 1·60 | 1·59 | 1·67 | 1·66 | 1·47 | 1·46 | 1·49 | 1·43 | 1·32 |

Diak = Diakinesis Meta-I = Metaphase-I
* Metaphase-I data not available.

### 3.4. *Species relationships*

Thus on the basis of the above cytogenetic observations on crossability, chromosome pairing and meiotic events, *S. integrifolium* is closely related to *S. indicum* and *S. surattense* (figures 4 and 5). The relationship with the former species seems to be much closer since they can be crossed in reciprocal directions and the recombination potential of the reciprocal hybrids is approximately the same as in the parents. The hybrid, *S. integrifolium* × *S. surattense*, could not be maintained for long and extensive analyses on this hybrid could not be done. But to the extent possible, it was observed that univalents were more frequent and mean chiasma frequencies were comparatively lower. Also the cross was achieved with greater difficulty and only a solitary hybrid could be realised. The reciprocal cross was unsuccessful. But as far as possible, *S. integrifolium* and *S. surattense* are also closely related. While full bivalent pairing has been reported in hybrids of *S. surattense* and *S. indicum*, even though the hybrids are sterile (Bhadhuri 1951 ; Rajasekaran 1969), Raju *et al* (1981) observed higher chromosome associations and a good amount of fertility in the hybrid, *S. indicum* × *S. surattense*. Thus it can be concluded that the three species *S. integrifolium*, *S. indicum* and *S. surattense* form a closely related group. With the reports of hybridization in spinous solanums of Bhadhuri (1951), Zutshi (1967), Rajasekaran (1969, 1970a, b, 1971), Rangaswamy and Kadambavanasundaram (1974), Veerabhadra Rao and Rao (1977b), Khan *et al* (1978), Kirti and Rao (1982), *S. integrifolium*, *S. melongena* and its wild forms, *S. surattense*, *S. indicum* form a group of closely related taxa.

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# Groundnut rust—its survival and carry-over in India*

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**Abstract.** Groundnut rust has become an important disease in India, particularly in the South, probably because of extensive and continuous cultivation of the crop. Uredospores present on crop debris in the field, and on pods or seeds in storage at ambient temperatures, lost viability within 6 weeks. They retained viability for long periods when stored at − 16° C. Neither teliospores nor any collateral or alternate hosts were found. Seeds heavily contaminated with viable uredospores and sown in sterile soil gave rise to disease-free seedlings. There should be no risk of spread of rust from properly treated seed samples.

**Keywords.** Groundnut rust; survival; carry-over; *Puccinia arachidis* Speg.; *Arachis hypogaea* L.

## 1. Introduction

Rust of groundnut (*Arachis hypogaea* L.), caused by *Puccinia arachidis* Speg., was reported from Punjab, India, in 1969 (Chahal and Chohan 1971) and now occurs in most groundnut-growing Indian States (Subrahmanyam *et al* 1979). The disease has become particularly important in South India, where groundnuts are grown for much of the year and where conditions favour development and spread of the pathogen. This paper deals with the survival of the rust fungus and presents results of investigations on possible carry-over of the disease in crop debris, on seeds, and on weeds. The biology of the fungus is discussed in relation to distribution of rust and groundnut cropping seasons.

## 2. Materials and methods

### 2.1. *Survival of uredospores in crop debris*

Dried haulms of groundnut collected from rust-infected rain-fed and irrigated crops (cv. TMV-2) during 1976–78 were immediately exposed to weather by spreading them in shallow layers in the field at ICRISAT Centre farm. At intervals, uredospores were collected from the crop debris (dried haulms), suspended in sterile distilled water on glass slides, and incubated in the dark at 25° C. After 6 hr, 1000 spores were checked for germination.

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### 2.2. *Effect of temperature on uredospore longevity*

Uredospores, freshly collectee from infected plants, were placed in glass vials and stored at temperatures of − 16, 6, 25 and 40° C. At intervals, they were sampled and tested for viability as described above.

### 2.3. *Presence of uredospores on pods and seeds*

Pods were collected from a crop with severe rust and separated into those with no shell damage and those with shells broken during threshing. Undamaged pods were shaken in distilled water to which Tween 80 (1 : 1000) had been added, and washings were centrifuged at 2,000 rev/min for 1 hr. The pellet obtained was examined microscopically for uredospores. Damaged pods were carefully opened and seeds were removed with minimal contact with the outside of the shells. The seeds were washed and the washings examined as described for undamaged pods.

### 2.4. *Longevity of uredospores on stored seed*

Seeds were dusted with freshly collected uredospores and stored in cloth bags in the laboratory at 25 to 30° C. Samples were removed at 5-day intervals and uredospores washed off the seeds and their viability tested as described above.

### 2.5. *Carry-over of rust on seed*

Seeds of rust-susceptible cultivar TMV-2 were surface-sterilised by immersion for 5 min in a 0·1% aqueous solution of mercuric chloride to which a small amount of Tween-80 had been added. They were then washed in repeated changes of sterile tap water. Isolation plant propagators (Burkard Manufacturing Company, England) were prepared containing steam-sterilised garden soil in pots which could be watered from below with sterile tap water. Into the pots in one unit, 200 seeds were aseptically sown. In another unit, 200 seeds liberally coated with freshly collected uredospores were sown. A further 200 seeds were aseptically sown in a third unit, and after germination, the seedlings were dusted with freshly collected uredospores. Seedlings were checked for rust infection.

### 2.6. *Germination of uredospores on germinating seeds*

Two-day-old seedlings of the cultivar TMV-2 were carefully washed, testas removed, and 100 cotyledons and 50 radicles excised. These organs were surface-inoculated with a suspension of uredospores and placed in moist chambers for incubation in the dark at 25° C. Samples were removed after 24 hr, stained, and examined under the microscope. In another test, artificially-contaminated seeds were sown in sterile soil, and resulting seedlings were carefully removed and examined at intervals.

### 2.7. *Search for teliospores and collateral hosts*

A large number of specimens of rust-infected groundnut from different parts of the country were examined for the presence of teliospores. Some 2,000 entries from the ICRISAT groundnut germplasm collection were also examined at various

stages of development under severe rust infection. Attempts were also made to induce telial production by growing rust-infected plants of the susceptible TMV-2 cultivar under the following combinations of temperature and day length in plant growth chambers.

| Treatment | Day temperature (° C) | Night temperature (° C) | Day length (hr) |
|---|---|---|---|
| 1 | 20 | 10 | 8 |
| 2 | 30 | 10 | 8 |
| 3 | 30 | 20 | 10 |
| 4 | 35 | 25 | 12 |
| 5 | 40 | 30 | 12 |
| 6 | 25 | 25 | 12 |
| 7 | 15 | 15 | 12 |

Various common crop and weed plants growing in or near fields of rust-infected groundnuts on the ICRISAT farm and farmers' fields were examined for rust. Some were also subjected to inoculation with uredospores in greenhouse tests ; the groundnut cultivar TMV-2 was used as a susceptible check.

## 3. Results and discussion

### 3.1. *Survival of uredospores in crop debris*

The high initial viability of uredospores decreased rapidly with exposure to weather (table 1). This was most marked in uredospores from irrigated crops, probably because of the higher temperatures experienced in May than in the November-to-January period following the rain-fed crop. Invariably, uredospores on exposed crop debris lost all viability within 30 days. Similar work in other parts of India also indicates that uredospores are short-lived in crop debris under field conditions (Lingaraju *et al* 1979 ; Mallaiah and Rao, personal communication).

### 3.2. *Effect of temperature on uredospore viability*

Spores remained viable for several months when stored at low temperature (– 16° C) while at 40° C they lost viability within 5 days (table 2). At the intermediate temperatures, viability decreased with time of storage and was completely lost within about 2 months. Mallaiah and Rao (personal communication) found that uredospores remained viable for up to 4 weeks when stored at temperatures below 30° C but lost viability within 2 weeks when stored at temperatures above 35° C. It would thus appear that temperature is an important factor influencing viability and longevity of rust uredospores.

### 3.3. *Carry-over and distribution on seed*

Carry-over and dissemination of uredospores on groundnut pods and seeds have been suggested. Peregrine (1971) indicated that movement of contaminated

**Table 1.** Viability of uredospores after various periods of exposure to weather on infected crop debris.

| Period of exposure (d) | Percentage of uredospores viable* | | | |
|---|---|---|---|---|
| | Rainy-season crops | | Post-rainy-season crops | |
| | 1976 | 1977 | 1976–77 | 1977–78 |
| 0 | 65 | 90 | 82 | 89 |
| 6 | 36 | 74 | 9 | 0 |
| 14 | 1 | 42 | 1 | 1 |
| 20 | 0 | 26 | 0 | 0 |
| 22 | 0 | 10 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 |
| Period of test | 13–12–1976 to 7–1 –1977 | 7–11–1977 to 2–12–1977 | 4–5–1977 to 30–5–1977 | 2–5–1978 to 28–5–1978 |
| RH% 0714 hr | 80·7 | 83·5 | 60·7 | 60·7 |
| 1414 hr | 26·0 | 46·6 | 26·9 | 23·9 |
| Temp. °C : Max. | 28·3 | 28·0 | 37·6 | 39·7 |
| Min. | 13·4 | 19·5 | 24·9 | 25·6 |

* 1,000 spores per sample. Figures to nearest whole number.

**Table 2.** Effects of storage temperature on viability of uredospores.

| Storage temperature (°C) | Percentage* of uredospores viable after storage for | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | | | | | | | | | | |
| | 5 | 13 | 28 | 40 | 48 | 60 | 70 | 78 | 99 | 110 | 120 |
| −16 | 88 | 82 | 89 | 90 | 98 | 88 | 92 | 93 | 92 | 94 | 93 |
| 6 | 84 | 85 | 82 | 35 | 15 | 4 | 0 | 0 | 0 | ... | ... |
| 25 | 81 | 88 | 80 | 24 | 0 | 0 | 0 | 0 | 0 | ... | ... |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ... | ... |

* 1,000 spores per sample. Figures to nearest whole number.

seed may have been involved in the spread of rust to Brunei. Pods from a rust-infected crop would be contaminated with spores during threshing and any damage to shells could well lead to contamination of seeds. Seeds could also be contaminated during shelling. Examination of pods from a severely rusted crop showed presence of uredospores on the shells. Where shells were broken, uredospores were found on the seed surfaces.

**Table 3.** Effects of storage at room temperature (25–30° C) on viability of uredospores.

| Percentage* of uredospores viable after storage for : Days | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 |
| 95 | 72 | 30 | 28 | 25 | 28 | 30 | 29 | 39 | 10 | 0 | 0 |

*1,000 spores per sample. Figures to nearest whole number.

The viability of uredospores on seed stored at room temperature for varying engths of time is shown in table 3. Viability decreased rapidly with storage ime from an initial 95% to zero after 45 days.

Surface-sterilised seeds of cultivar TMV-2 sown in sterile soil in isolation plant ropagators gave rust-free seedlings. Seeds similarly treated, but coated with iable uredospores prior to sowing, also gave rise to rust-free seedlings. A 'check' reatment where the foliage of seedlings was dusted with uredospores resulted in evere rust disease within 25 days of sowing. This supports the argument that urface contamination of seeds with uredospores is unlikely to result in rust nfection of seedlings.

When excised cotyledons and radicles of germinating seedlings were surface-noculated with uredospores and incubated in the dark, the spores germinated nd appressoria were produced, but there was no development of disease. Exami-ation of seedlings from seeds heavily contaminated with uredospores and sown n sterile soil again showed germinated uredospores with appressoria, but no rust leveloped.

There would appear to be little danger of rust disease developing, from uredo-pores carried on sown seed. Also, there is no authenticated report of the ust fungus being internally seed-borne.

Although rust has spread rapidly to most parts of the world in recent years Hammons 1977 ; Subrahmanyam *et al* 1979), there are still some groundnut-rowing areas where it is not present. Plant quarantine authorities and those oncerned with distribution of groundnut germplasm are understandably concerned vith the possible spread of the disease to these areas through contaminated seed amples. However, the practice of dressing seed with fungicides, the rapid loss of viability of uredospores at ordinary temperatures and their inability to infect eeds or germinating seedlings below ground all indicate that disease spread hrough properly treated and handled seed samples is extremely unlikely. To btain successful spread, viable uredospores would have to be carried to the urface of foliage of the susceptible plant under environmental conditions onducive to infection. This is more likely to happen due to long-distance air lispersal or contamination on clothes and baggage of air travellers than on roperly treated seed samples.

### 3.4. *Biology of the rust fungus*

The pathogen is known almost exclusively by its uredial stage. There are a few ecords of the occurrence of the telial stage on cultivated *Arachis hypogaea* in

South America (Spegazzini 1884 ; Hennen *et al* 1976) and on wild *Arachis* spp. (Guarch 1941 ; Bromfield 1971). In India, Chahal and Chohan (1971) recorded the occurrence of teliospores on groundnut leaves but gave no details of spore morphology and the disease has not recurred in Punjab. There has been no other authenticated report of the occurrence of teliospores of groundnut rust.

We have examined many specimens of rust-infected groundnuts from different parts of India but have found only uredospores. Some 2,000 entries from the ICRISAT groundnut germplasm collection were examined at various stages of development under severe rust infection, but again only the uredial stage of the rust was found.

Attempts were made to induce teliospore production by growing rust-infected plants under various combinations of temperature and day length but were unsuccessful. It is not known if the fungus can produce pycnia and aecia or if any alternate host is involved in the life cycle. It would appear that uredospores are the main, if not the only, means of dissemination of the groundnut rust fungus.

**Table 4.** Plant species examined as possible collateral hosts of rust.

| *Leguminous crop plants* | *Non-legumes* |
|---|---|
| *Cajanus cajan* (L.) Millsp. | *Acanthospermum hispidum* DC. |
| *Canavalia gladiata* DC. | *Achyranthes aspera* L. |
| *Cicer arietinum* L. | *Aerva monsoniae* (L.F.) Mart. |
| *Crotalaria juncea* L. | *Amaranthus viridis* L. |
| *Cyamopsis tetragonoloba* (L.) Taub. | *Anisomeles indica* (L.) O. Ktze. |
| *Glycine max* (L.) Merr. | *Boerhaavia diffusa* L. |
| *Lablab purpureus* (L.) Sweet | *Catharanthus pusillus* (Murr.) G. Don |
| *Lens culinaris* Medik. | *Corchorus aestuans* L. |
| *Phaseolus lunatus* L. | *Cyperus compressus* L. |
| *P. vulgaris* L. | *C. rotundus* L. |
| *Sesbania* sp. | *Dactyloctenium aegyptium* (L.) Beauv. |
| *Vicia faba* L. | *Digitaria ciliaris* (Retz.) Koeler |
| *Vigna mungo* (L.) Hepper | *Eclipta alba* (L.) Hassk. |
| *V. radiata* (L.) Wilcz. | *Euphorbia hirta* L. |
| | *Evolvulus alsinoides* (L.) L. |
| *Leguminous weeds* | *Ipomoea tridentata* Roth |
| | *Lactuca hastata* DC. |
| *Aeschynomene aspera* L. | *Lagascea mollis* Cav. |
| *A. indica* L. | *Leucas lavandulifolia* Sm. |
| *Alysicarpus monilifer* (L.) DC. | *Micrococca mercurialis* Bth. |
| *Cassia tora* L. | *Mollugo pentaphylla* L. |
| *Indigofera hirsuta* L. | *Ocimum americanum* L. |
| *Stylosanthes fruticosa* (Retz.) Alston | *Panicum* sp. |
| *Tephrosia hirta* Ham. | *Phyllanthus niruri* L. |
| *T. purpurea* (L.) Pers. | *Portulaca oleracea* L. |
| *Zornia diphylla* (L.) Pers. | *P. quadrifida* L. |
| | *Sida* sp. |
| | *Trianthema portulacastrum* L. |
| | *Tridax procumbens* L. |

There is no record of the occurrence of any collateral hosts of groundnut rust outside the genus *Arachis*, and in India wild *Arachis* spp. occur only in research centres and can hardly be involved in perpetuation of the disease. The possible occurrence of other hosts was considered, and various common crop and weed plants growing close to or within fields of rust-infected groundnuts (table 4) were regularly examined for the presence of rust, but no case of infection was found Some of these plants were also subjected to inoculation with rust uredospores in greenhouse tests, but again no case of infection was recorded.

### 3.5. *Cropping seasons and rust survival and spread*

There is no uniform groundnut growing season in India. In some of the southern states, particularly Andhra Pradesh, Tamil Nadu and Karnataka, groundnuts are grown in some areas throughout the year (figure 1), presenting excellent opportunity for survival of rust. About 90% of the crop is grown in the rainy season, most of the rest is grown in the post-rainy dry season under irrigation. In some places a summer crop is grown.

Rust attack is most severe on the rainy-season crops but can still be noticeable on dry-season crops. The disease has been seen on the summer crop in parts of Andhra Pradesh, but pustules developed very slowly and did not sporulate until the coming of the monsoon rains, when the disease developed rapidly on the maturing crop.

On the rainy-season crop, the disease appears in July and August in South India, in September in Central India, and in October in North India (Mayee *et al* 1977). In Central and North India normally only a rainy-season crop is grown, and it is thought that the groudnut crops in South India may act as a reservoir of rust disease from which spores are carried by the monsoon winds to infect the crops in the north. The present trend towards increased cultivation of groundnuts in southern India, particularly the irrigated dry-season crops, could result in more effective carry-over and spread of rust disease within the country.

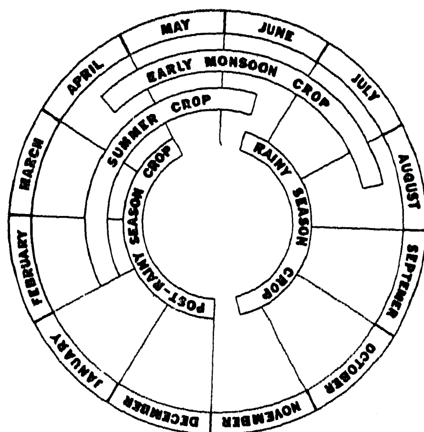


**Figure 1.** Groundnut cropping seasons in India.

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# Correlated promotion of ray-floret growth in chrysanthemum by potassium chloride, gibberellic acid and sucrose

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**Abstract.** The role of $10^{-2}$ M, $2 \times 10^{-2}$ M, $4 \times 10^{-2}$ M potassium chloride, gibberellic acid ($10^{-5}$ M; $GA_3$) and sucrose ($5 \times 10^{-2}$ M) (used individually and in various combinations) in the elongation growth of excised ray-florets of *Chrysanthemum morifolium* var. Jyothsna, was investigated. KCl ($10^{-2}$ M) caused 33·3% increase in elongation as compared to control (16·7%). With $GA_3$ and sucrose the percentage of elongation recorded was 39·8 and 28·9 respectively. Maximal growth response (82·8%) was recorded in KCl ($4 \times 10^{-2}$ M) + $GA_3$ ($10^{-5}$ M) + sucrose ($5 \times 10^{-2}$ M). When used in combination either with $GA_3$ or sucrose, KCl showed an almost additive effect, whereas in the presence of both it acted synergistically. It is inferred that the increased turgor resulting from sucrose-promoted potassium uptake along with $GA_3$-caused tissue extensibility accounts for enhanced floret growth.

**Keywords.** *Chrysanthemum*; cell elongation; flower growth; gibberellic acid; potassium chloride; sucrose.

## 1. Introduction

Studies on the opening of flowers harvested at the immature stages of development (bud-cut flowers) have gained importance owing to several commercial advantages (Marousky 1971; Halevy and Mayak 1974). Sucrose and gibberellins have been used for promoting flower bud opening in chrysanthemums and gladioli (Marousky 1971, 1972; Bravdo *et al* 1974; Rao and Mohan Ram 1979). In several bud-cut flowers, however, further opening is a serious problem. It is envisaged that the difficulties faced in causing the opening of bud-cut flowers can be overcome after the basic processes controlling petal growth have been understood.

There has been a good amount of physiological work on flower initiation and senescence. In comparison, literature on flower growth is scanty. The investigations on cell and organ expansion have been largely confined to vegetative parts. These events are known to be under the control of turgor pressure, viscoelastic properties of the wall and cell wall synthesis. Ions such as $K^+$ are crucial in regulating osmotic potential of the cell sap (Haschke and Lüttge 1975; Parrish and Davies 1977; Stuart and Jones 1977, 1978). Sugars have been shown to be involved in the synthesis of wall precursors, besides regulating osmotic potential and providing energy (Siegelman *et al* 1958). Gibberellins regulate the viscoelastic properties of cell wall (Kamisaka *et al* 1972; Adams *et al* 1975; Nakamura

*et al* 1975 ; Kawamura *et al* 1976 ; Coartney and Morre 1980) besides promoting the influx of solutes (Katsumi and Kazama 1978). With this information in the background, a study of the role of KCl, gibberellic acid and sucrose on ray-floret elongation in chrysanthemums was taken up.

## 2. Material and methods

Stocks of *Chrysanthemum morifolium* var. " Jyothsna " (Asteraceae) were procured from the National Botanical Research Institute, Lucknow, and were grown in the Botanical Garden of the Department. The plants flowered profusely in December. Capitula (measuring approximately 14 mm in diameter) in which the ray-floret would become visible the next day were used for experimentation. Ray-florets belonging to the outer two whorls and measuring 9 or 9·5 mm were excised from the capitula. For each treatment 20 ray-florets, in groups of five, were floated in petri plates containing 30 ml of the test solution and kept under continuous light (cool-white fluorescent tubes ; 1200 Lux) at 20 ± 2° C. The test solutions consisted of distilled water (control) ; potassium chloride (KCl) at $10^{-2}$ M, $2 \times 10^{-2}$ M and $4 \times 10^{-2}$ M ; gibberellic acid ($GA_3$) at $10^{-5}$ M ; sucrose (S) at $5 \times 10^{-2}$ M ; KCl ($10^{-2}$ M) + S ($5 \times 10^{-2}$ M) ; KCl ($2 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) ; KCl ($4 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) ; KCl ($10^{-2}$ M) + $GA_3$ ($10^{-5}$ M) ; KCl ($2 \times 10^{-2}$ M) + $GA_3$ ($10^{-5}$ M) ; KCl ($4 \times 10^{-2}$ M) + $GA_3$ ($10^{-5}$ M) ; KCl ($10^{-2}$ M) + S ($5 \times 10^{-2}$ M) + $GA_3$ ($10^{-5}$ M) ; KCl ($2 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) + $GA_3$ ($10^{-5}$ M) ; KCl ($4 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) + $GA_3$ ($10^{-5}$ M). Streptomycin (25 ppm) was added to all the solutions to prevent microbial infection. The length of the floret was measured every 24 hr to the nearest 0·1 mm. Each experiment lasted 10 days.

## 3. Observations

In all the treatments (including the control) the florets showed an increase in length with time (table 1). Among the three concentrations of KCl used, the florets showed the maximum response with $10^{-2}$ M (33·3 %), followed by $2 \times 10^{-2}$ M (22·2 %) and $4 \times 10^{-2}$ M (20·0 %) by day 5.

The floret length increased by 28·9 % with sucrose and by 49·8 % with $GA_3$, the maximum length having been attained on day 6. When KCl was used in combination with $GA_3$ or sucrose, greater elongation of the florets than in the treatments with only KCl, sucrose or $GA_3$ was recorded. When KCl (all the three concentrations) and sucrose were present together, the elongation ranged from 46·2 % to 49·5 %. In the presence of $GA_3$, KCl ($2 \times 10^{-2}$ M) showed as high as 57 % increase in length. With KCl ($10^{-2}$ M) + $GA_3$ and KCl ($4 \times 10^{-2}$ M) + $GA_3$ the corresponding values were 50·0 and 47·3 % respectively.

The florets exhibited highest elongation when they were kept in a mixture containing KCl, sucrose and $GA_3$. The increase in length observed over the control was 82·8 % when the concentration of KCl in the combination was $4 \times 10^{-2}$ M. However, $10^{-2}$ M KCl and $2 \times 10^{-2}$ M KCl also showed large increases up to 65·6 and 72 %, respectively. With the exception of KCl ($10^{-2}$ M)

**Table 1.** Increase in the length of the ray-florets over the initial length (in mm).

| Treatments \ Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0·5±·03 | 0·9±·03 | 1·2±·04 | 1·3±·05 | 1·4±·05 | 1·5±·06 | 1·5±·06 | 1·5±·06 | 1·5±·06 |
| K ($10^{-2}$ M) | 0·6±·03 | 1·1±·05 | 1·7±·08 | 2·4±·08 | 3·0±·09 | 3·0±·09 | 3·0±·09 | .. | .. |
| K ($2 \times 10^{-2}$ M) | 0·5±·04 | 1·1±·06 | 1·6±·05 | 1·8±·04 | 2·0±·03 | 2·0±·03 | 2·0±·03 | .. | .. |
| K ($4 \times 10^{-2}$ M) | 0·5±·04 | 1·0±·04 | 1·3±·04 | 1·5±·04 | 1·8±·05 | 1·8±·05 | 1·8±·05 | .. | .. |
| Sucrose ($5 \times 10^{-2}$ M) | 0·9±·04 | 1·1±·06 | 1·5±·06 | 1·8±·07 | 2·1±·09 | 2·6±·11 | 2·6±·11 | 2·6±·11 | .. |
| GA ($10^{-5}$ M) | 1·2±·05 | 1·7±·05 | 2·2±·06 | 2·9±·08 | 3·5±·07 | 3·7±·07 | 3·7±·07 | 3·7±·07 | .. |
| K ($10^{-2}$ M) + S ($5 \times 10^{-2}$ M) | 0·8±·05 | 1·2±·04 | 1·6±·04 | 1·9±·06 | 2·7±·10 | 3·4±·09 | 3·8±·09 | 4·1±·08 | 4·3±·09 |
| K ($2 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) | 0·8±·04 | 1·3±·05 | 1·7±·07 | 2·1±·07 | 2·9±·07 | 3·6±·08 | 4·3±·09 | 4·4±·08 | 4·4±·08 |
| K ($4 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) | 0·7±·05 | 1·5±·06 | 2·0±·06 | 2·6±·09 | 3·3±·09 | 3·9±·06 | 4·2±·08 | 4·5±·09 | 4·6±·10 |
| K ($10^{-2}$ M) + GA ($10^{-5}$ M) | 1·0±·03 | 1·7±·05 | 2·5±·06 | 3·0±·08 | 3·7±·06 | 4·2±·08 | 4·5±·09 | 4·7±·08 | 4·7±·08 |
| K ($2 \times 10^{-2}$ M) + GA ($10^{-5}$ M) | 1·1±·04 | 1·8±·07 | 2·5±·08 | 3·2±·09 | 4·0±·10 | 4·6±·10 | 5·0±·11 | 5·2±·10 | 5·3±·11 |
| K ($4 \times 10^{-2}$ M) + GA ($10^{-5}$ M) | 0·9±·04 | 1·7±·06 | 2·4±·06 | 2·9±·06 | 3·3±·06 | 3·7±·09 | 4·0±·08 | 4·3±·07 | 4·4±·06 |
| K ($10^{-2}$ M) + S ($5 \times 10^{-2}$ M) + GA ($10^{-5}$ M) | 1·0±·05 | 1·7±·05 | 2·2±·07 | 3·2±·12 | 4·2±·09 | 5·1±·08 | 5·5±·08 | 5·9±·10 | 6·1±·12 |
| K ($2 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) + GA ($10^{-5}$ M) | 1·1±·03 | 1·8±·04 | 2·4±·06 | 3·4±·05 | 4·5±·11 | 5·4±·11 | 6·1±·13 | 6·5±·11 | 6·7±·11 |
| K ($4 \times 10^{-2}$ M) + S ($5 \times 10^{-2}$ M) + GA ($10^{-5}$ M) | 0·9±·02 | 1·6±·04 | 2·3±·06 | 4·0±·06 | 5·6±·08 | 6·5±·08 | 7·0±·09 | 7·4±·11 | 7·7±·11 |

K = Potassium chloride ; S = Sucrose ; GA = Gibberellic acid ; .. = Experiment terminated ; Initial length of ray-floret = 9·0 or 9·5 mm.

+ $GA_3$, in which the florets showed an increase in length till day 8, in the other combinations floret elongation continued until the termination of the experiment.

It is interesting that the effect elicited by KCl in combination with either sucrose or $GA_3$ was nearly additive and that in combination with both was synergistic.

## 4. Discussion

In the present work it was noted that KCl caused greater elongation at low rather than high concentration. Potassium has been shown to play an important role in the elongation of vegetative tissues by altering the osmotic potential (Stuart and Jones 1977, 1978), by effecting wall loosening (Tagawa and Bonner 1957 ; Haschke and Lüttge 1975), by acidification of the incubation medium (Thimann and Schneider 1938 ; Ordin *et al* 1956 ; Tagawa and Bonner 1957 ; Haschke and Lüttge 1975) or by acting as a co-factor for stimulating certain enzymes (Mähler 1961 ; Purves 1966).

In the presence of sucrose the florets showed 12·2% higher increase in length than that observed in the control. Besides acting as a source of energy and in providing building blocks for cell wall synthesis, sucrose is probably involved in osmoregulation, providing the necessary force for the maintenance of turgidity in the elongating ray-florets of chrysanthemums (present work) as has been shown for other flowers (Winkenbach and Matile 1970 ; Dilley and Carpenter 1975). Additionally, in the present investigation, sucrose showed better response in combination with KCl. There is evidence that the energy for $K^+$ ion uptake and for the probable production of carbon skeletons for organic anions that move with $K^+$ ions is derived from sucrose (Satter *et al* 1976). Satter *et al* (1976) have suggested that the rhythmically controlled sucrose permeation in *Samanea* pulvini could regulate sucrose-$H^+$ transport and thus, in order, membrane potential, salt flux and water flux resulting in increased turgor.

The enhanced ray-floret elongation observed in the present study in the presence of $GA_3$ could emanate from its effect on the viscoelastic properties of the cell (i.e., cell wall extensibility) (Kamisaka *et al* 1972 ; Adams *et al* 1975 ; Coartney and Morre 1980), osmoregulation (Kazama and Katsumi 1973) or the synthesis of cell wall material (McComb 1966 ; Srivastava *et al* 1975) as demonstrated in several other systems.

In the present work a greater elongation of the ray-florets was noted in response to KCl + sucrose + $GA_3$ over that with KCl + $GA_3$ or KCl + sucrose. When used in combination either with $GA_3$ or sucrose, KCl showed an almost additive effect, whereas in the presence of both it acted synergistically. A combined effect of sucrose and $GA_3$ has also been noted in the stimulation of elongation of hypocotyl segments (Purves and Hillman 1958 ; Kazama and Katsumi 1973), in the linear growth of staminal filaments (Murakami 1973) and in flower growth and opening (Rao and Mohan Ram 1979).

$GA_3$ perhaps enhances ATPase activity. This could regulate $K^+$ ion and sucrose influx (Katsumi and Kazama 1978) thereby regulating osmotic potential and turgor pressure leading to ray-floret elongation in chrysanthemum.

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# Nuclear behaviour during heartwood formation in *Acacia auriculiformis* A. Cann.

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**Abstract.** Nuclear behaviour is studied in *Acacia auriculiformis* A. Cann. with reference to aging in both axial and ray parenchyma cells (contiguous to vessels and away from the vessels). The size of the nucleus and nucleolus reduces gradually towards the inner sapwood and at last they disappear at the heartwood boundary. Nuclei show lobing, fissuring, fragmentation and contraction in this zone prior to their disintegration. Thus a gradual loss of vitality of parenchyma cells during aging is noted. The parenchyma cells contiguous to vessels seem to be more actively involved in formation of heartwood extractives.

**Keywords.** Nuclear disintegration ; nucleolus ; nucleus ; slenderness ratio ; transition wood ; *Acacia auriculiformis.*

## 1. Introduction

The transformation of sapwood into heartwood during aging involves loss of protoplasm of living cells and concomitant accumulation of the extractives. The parenchyma cells of the sapwood are known to play a major role in heartwood formation as in the majority of the species they are the only living cells in the sapwood (exceptionally living fibres occur in the sapwood). The physiological status of the living parenchyma cells during different stages of aging is still a matter of controversy. Evaluation of the vitality of the parenchyma cells and the rate of metabolic activities is very important and can be done only by adequate methods. Bosshard (1966) considers that the form and dimensions of the nuclei are very valuable indications for tracing the activity of a cell. Alteration of nuclear configurations, in conifers, as a part of necrobiosis of storage tissue can be of great use in studying the heartwood formation. This can be studied in two ways : by determining the degree of slenderness ratio, and by calculating the nuclear surfaces (Bosshard 1965). Although nuclear behaviour in ray cells has been studied in detail, information on changes in shape and size of the nucleus in the parenchyma contiguous to the vessels is not available. As there is a remarkable difference in the nuclear size and shape in the cells contiguous to vessels and those away from the vessels, the nuclear behaviour is studied in relation to the above situations in both axial and ray parenchyma cells of *Acacia auriculiformis* A. Cann. The nuclear disintegration has also been studied.

## 2. Material and methods

The wood material of *A. auriculiformis* A. Cann. was collected from the University botanical garden and was fixed in FAA (Sass 1958). The fixed material was divided into blocks of suitable size for microtomy. No intermediate block was discarded so that continuity was maintained from cambium to heartwood. The radial strips of wood were divided into outer sapwood, middle sapwood, inner sapwood and sapwood-heartwood boundary or transition. Radial sections of 15 $\mu$m thickness were cut from each of the blocks and stained with toluidine blue 'O' (O'brien *et al* 1964), and pyronin-methyl green (Brachet 1953). Camera lucida drawings of 100 nuclei each from axial and ray parenchyma cells (contiguous as well as away from the vessels) from each zone of the wood were made on a piece of paper using an oil immersion object. The length, width and sectional area of the nucleus were measured using area calculating device (ACD) (Chavan *et al* 1979). The slenderness ratio of the nuclei was obtained by dividing the average length by average width of the nucleus.

## 3. Observations

The ray and axial parenchyma cells of sapwood contain nuclei but in fibres the nuclei disappear in the outermost sapwood. For brevity we avoid the terms axial and ray parenchyma cells in this paper, instead they are referred to as cells at many places except where the two types of cells are to be specifically mentioned. The chromaticity of the nucleus is comparatively higher in parenchyma cells contiguous to vessels (figures 1–3, 7–11) as compared to those away from the vessels (figures 4–6, 12–14). However, the chromaticity of the nuclei in general increases in the cells of the inner sapwood (figures 3, 6, 11, 14). At the heartwood boundary the nuclei show the highest chromaticity (figures 15–25).

Usually the nuclei are uninucleolate, but occasionally binucleolate nuclei are also observed (figure 1). The nucleolus is very prominent in parenchyma of the outer and middle sapwood, especially in the cells contiguous to vessels (figures 1, 7–10). The size and distinctness of the nucleolus are reduced towards the inner sapwood (figures 3, 6, 11, 14), and it becomes totally indistinct in the cells of heartwood boundary (figures 15–25). The nucleolus is larger in the nuclei of cells contiguous to vessels (figures 1, 2, 7–10) than those of the cells which are not contiguous to vessels (figures 4, 5, 12, 13).

The nuclei of the cells contiguous to vessels and those away from the vessels show a great difference in their cross-sectional area and slenderness ratio (SR) during various stages of transformation of sapwood into heartwood.

### 3.1. *Nuclear area*

The size of the nucleus as revealed by its sectional area differs from zone to zone. Cells contiguous to vessels possess larger nuclei (figures 1–3, 7–11) than the cells away from the vessel (figures 4–6, 12–14).

**Figures 1–14. 1–3.** Nucleus in ray cells contiguous to vessels. **4–6.** Nuclei in ray cells away from vessels. **7–11.** Nuclei in axial parenchyma cells contiguous to vessels. **12–14.** Nuclei in axial parenchyma cells away from vessels. **1, 4, 7, 12.** Outer sapwood. **2, 5, 8–10, 13.** Middle sapwood. **3, 6, 11, 14.** Inner sapwood. Inserted scale, 12·5 microns.

**Figures 15–25.** Nuclei at the heartwood boundary. **15–22.** In ray cells. **23–25.** In axial parenchyma cells. **15–18.** Lobed nuclei. **19, 20.** Fissuring of the nucleus. **21.** Condensed nuclei. **22.** Nuclear fragments. **23.** Lobing of the nucleus. **24.** Nuclear fragmentation. **25.** Contracted nucleus. Inserted scale, 12·5 microns.

3.1a. *Nuclear area in parenchyma cells contiguous to vessels* : Among the cells contiguous to vessels the nuclei are larger in axial parenchyma (figures 7–10) than those in the ray cells (figures 1, 2; table 1). There is a little and gradual reduction in the nuclear size of ray cells as traced from outer sapwood to the middle sapwood, but then the nuclei show sharp reduction in their size as traced towards the inner sapwood (table 1). Further reduction in nuclear size from inner sapwood to heartwood boundary is very little. On the other hand the nuclear size in axial parenchyma gets reduced slightly as traced towards the middle sapwood, and from middle sapwood to heartwood boundary through the inner sapwood the nuclear size diminishes rapidly and rather uniformly (table 1).

3.1b. *Nuclear area in parenchyma away from vessels* : The changes in the nuclear size are not prominent in these cells (table 1). However, as traced towards the heartwood boundary after a slight enlargement of the nucleus in ray cells from outer to middle sapwood a slight reduction in its size follows from middle to inner sapwood. Again at the heartwood boundary a slight increase in the nuclear size is observed (table 1). In the ray cells of the heartwood boundary region the nuclei are much lobed and slightly enlarged (figures 15–18). On the other hand the size of the nucleus in axial parenchyma cells decreases from outer sapwood to middle sapwood. As traced from middle to inner sapwood the axial parenchyma cells do not show appreciable reduction in their nuclear size, but from inner sapwood to heartwood boundary there is appreciable reduction in nuclear size (table 1).

Thus, reduction in the nuclear size is observed from outer sapwood to heartwood boundary except in ray cells away from the vessels. But, invariably there is a reduction in the size of the nuclei from middle to inner sapwood.

### 3.2. *The slenderness ratio (SR)*

The nuclear SR (length/width) differs with the increasing radial distance from the cambial zone. Slenderness ratio serves as an index of the changes in nuclear shape occurring simultaneously with the changes in nuclear size.

**Table 1.** Average sectional area (NA) in $\mu m^2$ and slenderness ratio (SR) of nucleus in ray (RP) and axial parenchyma (AP) of various zones of wood.

| Zone of wood | Parenchyma contiguous to vessels | | | | Parenchyma not contiguous to vessels | | | |
|---|---|---|---|---|---|---|---|---|
| | Axial parenchyma | | Ray parenchyma | | Axial parenchyma | | Ray parenchyma | |
| | NA | SR | NA | SR | NA | SR | NA | SR |
| OS | 78 | 3·0 | 70 | 3·2 | 23 | 2·7 | 16 | 4·0 |
| MS | 75 | 4·3 | 58 | 4·4 | 19 | 2·2 | 20 | 3·9 |
| IS | 51 | 5·0 | 40 | 4·2 | 17 | 2·7 | 15 | 3·7 |
| HB | 32 | 4·9 | 37 | 4·2 | 13 | 3·0 | 20 | 3·4 |

HB, heartwood boundary ; IS, inner sapwood ; MS, middle sapwood ; OS, outer sapwood. Data based on measurements of 8,000 nuclei.

3.2a. *SR of nuclei in cells contiguous to vessels* : A gradual elongation accompanied with a reduction in width is noted in the nuclei of ray (figures 1, 2) and axial parenchyma (figures 7–10) from outer to middle sapwood. Hence, the SR increases from outer to middle sapwood in ray and axial parenchyma cells (table 1). The SR in ray cells does not change appreciably thereafter, but in axial parenchyma it continues to increase up to the inner sapwood and then remains more or less constant (table 1).

3.2b. *SR in parenchyma cells away from the vessels* : The SR of nuclei in ray parenchyma cells gets reduced gradually towards the heartwood boundary (table 1). Nonetheless, in the axial parenchyma the SR of the nucleus reduces abruptly as traced from outer to middle sapwood, and from middle sapwood to heartwood boundary it increases in uniform and moderately steep manner (table 1).

3.3. *Nuclear disintegration*

In both ray and axial parenchyma cells the nuclei disintegrate at the heartwood boundary. The nuclei in axial parenchyma disappear slightly earlier than those of the ray cells. Prior to the disintegration the nuclei show some morphological variations. In some ray cells they appear variously lobed (figures 15–18). Constrictions appear along the length of the nucleus at one (figures 15, 16) or more loci (figures 17, 18). Thus, the nuclei appear enlarged and convoluted. Nuclei in some ray cells show longitudinal fissuring (figures 19, 20). whereas in others they appear dense, spherical and highly contracted (figure 21). A few cells at the heartwood boundary contain only fragments of nuclear material (figure 22).

The nuclei in axial parenchyma rarely show lobing prior to their disintegration (figure 23). Fragmentation of the nucleus can be observed in some cells (figure 24). In a few axial parenchyma cells the nuclei appear very small and highly wrinkled (figure 25).

## 4. Discussion

The cells with high activity and high vitality possess large nuclei and nucleoli (Bosshard 1966). The nuclei in sapwood near cambium have larger surface than in the sapwood adjacent to the heartwood (Bosshard 1965). Earlier studies on nuclei of storage tissue of wood have considered slenderness ratio of the nucleus and nuclear surfaces in cells in relation to their radial position from the cambium (Frey-Wyssling and Bosshard 1959; Bosshard 1965, 1966). But, these are not studied in relation to their radial position from cambium, as well as in relation to their contiguity to the vessels. The present study clearly indicates that the nuclei of the cells contiguous to vessels are quite different in their size and morphology from those belonging to the cells away from the vessels. The axial and ray parenchyma cells which are contiguous to vessels in the investigated species possess larger nuclei and nucleoli as compared to those which are away from the vessels. Further the chromaticity of these larger nuclei in cells contiguous to vessels is comparatively high. A similar situation was found in other angiosperm species investigate dearlier (Bhat and Patel 1980). It reveals that the cells in the contiguity of vessels are more active than other living cells of the wood.

From outer sapwood to the heartwood boundary through the middle and inner sapwood a gradual reduction in the size of the nuclei is observed, except in ray cells away from the vessels. The reduction in nuclear size is more prominent in the cells contiguous to vessels. The reduction in nuclear size is also accompanied by a gradual reduction in nucleolar size and loss of its distinctness. These facts suggest that the activity and vitality of the cells gradually diminish during aging. In exceptional cases nuclear enlargement in ray cells away from the vessels at the heartwood boundary is not accompanied by a nucleolar enlargement. Bosshard (1966) considered the dimension of nucleolus as one of the indications of cell vitality and reported increase in dimension of nucleolus in the transition zone between the sapwood and heartwood. Fukazawa and Higuchi (1966) observed a gradual reduction in the RNA content from cambium to negligible amount at the intermediate wood. As we could not find either increase in nucleolar size or RNA content (as judged by staining reaction) at the heartwood boundary we consider the nucleolar enlargement at the heartwood boundary in ray cells away from the vessels to be merely a stage of its disorganisation and it is attributed to the intensive lobing of the nucleus. Enlargement of the nucleus at the heartwood boundary, prior to nuclear disorganization, was observed by us earlier in *Ougeinia oojeinensis* and *Garuga pinnata* (Bhat and Patel 1980).

The data show that the degree of slenderness varies in different zones of the wood with the decreasing size of the nucleus. A more prominent increase in the slenderness ratio is observed with the decreasing nuclear size in cells contiguous to vessels, while in cells away from the vessels the change in the SR was not very prominent. In the ray cells away from the vessels the SR gradually decreases. In our earlier observations also we had noted that the SR either increases or decreases towards the heartwood boundary (Bhat and Patel 1980). The form of the nucleus does not appear to be significant for tracing the activity of the cell. It should be regarded together with the size (Bosshard 1966). Higuchi *et al* (1967) found a gradual elongation of the nucleus in conifers with the increasing distance from the cambial zone, and in angiosperms the variation in the shape of the nucleus was found to be more or less dependent on the species. In the present species variation in nuclear shape appears to depend on the type of the cell and/or contiguity of the cell to the vessels.

Histochemical observations in other angiosperm species have revealed that the extractives are formed earlier in the cells contiguous to vessels than the others (Bhat 1981). Thus, it appears probable from the data that the cells in contiguity with the vessels are more actively involved in the metabolic activities involved in the formation of heartwood extractives.

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# Identity of *Ficus macrocarpa* Wt. *ex* King (= *F. amplocarpa nom. nov.*) and *F. guttata* (Wt.) King—A reinvestigation with anatomical evidence

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**Abstract.** The two interesting and endemic but hitherto taxonomically indistinguishable and confused south Indian species of *Ficus* (*F. guttata* and *F. macrocarpa*) were reinvestigated both exomorphologically and anatomically. With the help of data thus obtained their original specific status instead of recently reduced ranks has been restored, better and dependable distinguishing characters have been blocked out and the existing confusions, inaccuracies and inconsistencies in literature have all been rectified. Revised descriptions, illustrations and a workable key are presented. Vegetative anatomy and the descriptions and illustrations of male flowers are given for the first time. *F. amplocarpa* is proposed as a new name for *F. macrocarpa* in view of its being a later homonym. It is established that these two taxa belong to section *Neomorphe* King and not to *Rhizocladus* Endl. Anatomically *F. amplocarpa* is considered to be less specialized than *F. guttata.*

**Keywords.** *Ficus guttata*; *Ficus amplocarpa*; morphology; anatomy; revised descriptions; taxonomy.

## 1. Introduction

Among the south Indian species of *Ficus*, *F. guttata* and *F. macrocarpa* are two interesting taxa which are rather uncommon and restricted in distribution even in the localities of their occurrence. They resemble each other so closely that their respective identity and taxonomic status have hitherto been confused and misunderstood. This unfortunate situation in the first place seems to have stemmed from the circumstances that the establishment of these two taxa was originally based on just one or two complete, incomplete or badly preserved specimens eventually resulting in recording wrong observations and conclusions by earlier authors. Secondly the subsequent treatments of these taxa in all Indian *Floras* [King, Ann. R. Bot. Gard. Calc. 1 (1888) 166, 167 pl. 208, 209; *et* in Hook. *f.* Fl. Br. Ind. 5 (1888) 534; Brandis, Ind. Tr. (1921) 621; Fischer in Gamble's Fl. Pres. Madras 3 (1928) 951; Fyson, Fl. s. Ind. Hill Stn. 1 (1932) 541 *et* 2 (1932) *t.* 473] perpetuate what is contained in the protologue without adding any new points for further improvement. Thirdly the illustrations as provided by Wight [Ic. 6 (1853) *t.* 1965, 1966] and King (*l.c.*) reflect not only few inaccuracies in respect of the delineation of certain characters but both their illus-

trations are not comparable. Lastly the differentiating key characters between these two taxa have not been sufficiently emphasized but instead more number of overlapping and common characters camouflaging their respective identity is repeatedly given.

King (*l.c.*) has placed these two taxa under his section *Neomorphe*. Corner [Gard. Bull. Sing. 18 (1960) 7, 32] on the other hand has transferred them to altogether a different section *Rhizocladus* Endl. to which *F. laevis* belongs as they happen to be root climbers and possessing leaves similar to those of *F. laevis* but differing from the latter by the cauliflorous condition only. Furthermore he has reduced *F. macrocarpa* as a variety of *F. laevis* [*F. laevis* var. *macrocarpa* (Miq.) Corner] and *F. guttata* as synonym of the variety.

The aim of the present investigation in the first place is to wipe out all the existing wrong and inconsistent details of these two species and to restore their original specific status and section utilizing new and overlooked exomorphic characters combined with anatomical data hitherto unavailable, and secondly to provide revised descriptions and illustrations based on more and better samples and liquid preserved materials.

The information on general and wood anatomy is meagre in general for the family as a whole and still less in particular for this large tropical genus *Ficus* (Solereder 1898 ; Metcalfe and Chalk 1950). Furthermore no anatomical information is available on *F. amplocarpa* and *F. guttata*. In the present work the vegetative anatomy of both these species is undertaken from this standpoint and also to apply the anatomical data thus obtained as supplementary evidence towards the elucidation of the taxonomical problems mentioned above.

## 2. Materials and methods

For exomorphological studies both liquid preserved and herbarium materials cited under each species were used. Likewise both dried and materials pickled in FAA were used for the anatomical investigations. The dried materials were revived by boiling in 2 : 1 mixture of glycerol and 'Det' which is a commercial reagent (Govindarajalu 1966). After washing in water the revived materials were stored in FAA. Epidermal peelings were prepared by treating the laminal bits taken from the midregions of the lamina with 5% Jeffrey's maceration reagent for about 6 hr at room temperature. When the appropriate stage is reached the abaxial and adaxial epidermal layers were separated carefully and the unwanted mesophyll cells gently removed with the help of a fine brush. Finally they were stained with safranin and mounted in glycerine. Serial microtome sections were prepared following the conventional methods of dehydration, clearing and embedding (Johanson 1940). The old internodes were repeatedly boiled in water and later softened with hydrofluoric acid of commercial strength for 4 hrs. After thorough washing in running water the sections at a thickness of 14 $\mu$m were taken with the sledge microtome and they were stained with safranin and permanent slides prepared following the customary methods (Johanson 1940). The early secondary xylem was macerated using Jeffrey's maceration reagent (10%) for about 3 hrs at 60° C for taking the measurements of vessels, libriform and septate fibres.

## 3. Observations

### 3.1. *Descriptions*

*Ficus amplocarpa, nom. nov.*

*F. macrocarpa* Wt. *ex* King, Ann. R. Bot. Gard. Calc. 1 (1888) 166, pl. 208 *et* in Hook. *f.* Fl. Br. Ind. 5 (1888) 534; Brandis, Ind. Tr. (1921) 610; Fischer in Gamble's Fl. Madras 3 (1928) 951, 955; Fyson, Fl. s. Ind. Hill Stn. 1 (1932) 541 *et* 2 (1932) *t.* 473; *non* Bl. Cat. Gewass Buitenz. 36 (1823) *et* Beijdr. (1825) 459, *nec* Lévillé and Vaniot in Mem. Acad. Barcelona 6 (1907) 152—*Pogonotrophe macrocarpa* Miq. in Hook. Lond. J. Bot. 7 (1848) 74; Wight, Ic. 6 (1853) *t.* 1965; King in Hook. *f.* Fl. Br. Ind. 5 (1888) 534; Brandis, Ind. Tr. (1921) 610; Fischer in Gamble's Fl. Pres. Madras 3 (1928) 955.—*Ficus vagans* var. *macrocarpa* Miq. Ann. Mus. Bot. Lugd. Bat. 3 (1867) 293.—*F. laevis* var. *macrocarpa* (Miq.) Corner, Gard. Bull. Sing. 18 (1960) 7 [excl. *Covellia guttata* Wt. and *F. guttata* (Wt.) King]—figure 1 N, 2 A–G.

Tall scandent tree. *Twigs* of first few internodes densely hairy, fistular, rooting at nodes. *Leaves* broadly elliptic ovate, rounded at base with entire margin, membranous (subcoriaceous), usually trinerved (quintinerved), densely or sparsely tomentose beneath, glabrous above, abruptly acuminate, 10–15 × (5–) 7–9 cm; tomentum over the veins brown or rusty brown and the remainder colourless; lateral veins 3 (–4) pairs, slightly raised beneath, curvipinnate, arcuate; petiole subterete, tomentose, ultimately becoming glabrous, 3·0–4·5 cm long; stipules ovate lanceolate, acute (glabrous) 1·5–2·0 cm long. *Receptacle* ramiflorous, in fascicles on naked pendent and/or horizontal cable like branches, globose, purplish red with white streaks and patches, densely pubescent, later becoming glabrous containing all 3 kinds of flowers without hispid hairs in the interior, 3·0–6·5 cm across; basal bracts absent; peduncles up to 1 cm long with few small bracts at base. *Male flowers* dark brown, orange yellow (when fresh), 4-gonous, pedicellate, closely arranged behind ostiolar scales in 4 rows, 1·8–2·0 mm long; pedicels flattened, membraneously winged, conspicuously 1 nerved, widening towards and hairy at base, up to 2 mm long; tepals 4–5, tannin punctate, dissimilar in size and shape, concave; outer pair narrowly elliptic oblong, 2·4–2·5 × 1·4 mm; inner pair elliptic ovate, 2·0–2·4 × 1·0–1·4 mm; stamens 2, free, almost sessile, surrounded by a dense tuft of stiff erect, brownish hairs, up to 1·5 mm long; anthers fleshy, more or less falcate or erect, mucronate, distinctly 4 lobed, trigonous, more or less basifixed or basally adnate, up to 1·3 mm long; filament 0·2 mm long. *Female flowers* few, intermingled with male, 2·2 mm long (excl. pedicel, incl. style), pedicellate, rare; pedicel nearly 1·5 mm long, hairy at base and top, membraneously winged, flattened, strongly 1 nerved; tepals 4, elliptic ovate, inflated, purplish red, obtuse, tannin punctate, equalling or shorter than ovary, concave, 1·5–1·6 × 1 mm; ovary subglobose, up to 1·5 mm long; style excentric, exserted, glabrous, slightly widening towards top, 0·5-0·6 mm long; stigma slightly dilated. *Gall flowers* abundant, 2·5 mm long (excl. pedicel, incl. style), pedicellate; pedicel hairy at base, 3·0-6·5 mm long; tepals 6 as long as or longer than ovary, black, narrowly spathulate, attenuating towards base, acute-subacute, 2·8–3·0 × 0·5 mm; ovary subglobose containing pupa, 2 mm long;

style short, lateral, included, erect, adherent with ovary wall, black, 0·5 mm long, widening into discoid stigma. *Achene* subspherical.

Specimens examined : Govindarajalu 8161, on the way to Thandikudi, Kodaikanal, Madurai Dt. ; Govindarajalu 15894, on the river banks of Arungallar, Pannaikadu, Kodaikanal, Madurai Dt., alt. 1300–1400 m (PCM) ; Joseph 12312, Waverly estate, Anamalai, Coimbatore Dt. ; Vajravelu 37026, Kanakarai R.F., Nilgiris Dt. (MH).

Distribution : Coonoor ; Lamb's Rock Road, Pulneys ; Sholas on Church Cliff, Kodaikanal. Seems to be an endemic to south Indian Hill stations.

*F. guttata* (Wt.) King

*F. guttata* (Wt.) King, Ann. R. Bot. Gard. Calc. 1 (1888) pl. 209 ; King in Hook. *f.* Fl. Br. Ind. 5 (1888) 534 (*sub F. guttata* Kurz, *sphalm.*) ; Fischer in Gamble's Fl. Pres. Madras 3 (1928) 951 and 955 ; Brandis, Ind. Tr. (1921) 610 ; Fyson, Fl. s. Ind. Hill Stn. 1 (1932) 542; *Covellia guttata* Wt., Ic. 6 (1853) 8, *t.* 1966, figures 1 A–M.

Tall scandent tree. *Twigs* densely hairy, sometimes glabrous, solid, rooting at nodes. *Leaves* coriaceous, (subcoriaceous), petiolate, broadly cordiformis, cordate or subcordate at base with entire margin, usually trinerved (quintinerved), densely tomentose throughout beneath and glabrous above, abruptly acuminate, (8–) 10–15 × 6–12 cm; lateral veins 3 (–4) pairs, raised beneath, curvipinnate, arcuate ; petiole subterete, tomentose ultimately becoming more or less glabrous, 2–4 (–8) cm long; stipule ovate-lanceolate, acute, rusty brown tomentose, 1·0–1·5 cm long. *Receptacle* short peduncled occurring in fascicles from the tubercles on the branches of main stem, globose-subglobose, densely rusty tomentose later becoming glabrous, 2·5–3·5 cm across ; interior of receptacles with hispid hairs ; basal bracts 3, broadly ovate. *Male flowers* dark brown, trigonous, tannin punctate, sessile, closely arranged behind ostiolar scales more or less in 2 rows, 2·0 × 1·5–1·6 mm ; tepals brownish yellow, 2 + 2, similar, elliptic ovate, 2·0 × 1·8–2·0 mm. *Stamen* 2, basally united, up to 2 mm long; anthers fleshy, obcuneate, trigonous, erect, mucronate, basally surrounded by a dense tuft of dark reddish brown stiff erect hairs, 1·5 × 0·8–0·9 mm ; filament 0·5 mm long. *Female flowers* few, intermixed with male, 2·7–3·0 (incl. pedicel) × 1·5 mm; pedicellate; pedicel 0·8–1·0 mm long, hairy at base, flattened ; tepals 5–6 (–7), narrowly elliptic ovate or oblong, purplish red, subequal, obtuse, shining, longer than ovary, concave, tannin punctate, 1·5–1·6 × 0·6–0·7 mm ; ovary obovoid, up to 1 mm long ; style excentric, erect, distantly hairy behind stigma, exserted, 1·0–1·2 mm long ; stigma peltate, or discoid with crenulate margin (sometimes oblique). *Gall flowers* abundant, pedi, cellate, 2·5 mm long (excl. pedicel, incl. style); pedicel hairy at base, 6·5–7·0 mm long, rest as in female flowers ; tepals 6, dissimilar, narrowly obovate or spathulate attenuating towards base, acute-subacute, 2·5–3·0 × 0·6–0·8 mm; ovary sessile globose-subglobose containing pupa, 2·0–2·2 mm long; style short, glabrous, included, lateral, curved, adherent with ovary ending in dilated stigma, 0·5–0·6 mm long.

Specimens examined : Fyson 6448, *sine loco* ; Fyson, *s.n.*, Shembaganur, Kodaikanal, Madurai Dt. ; Fischer, *s.n.*, Coonoor, Nilgiris ; Govindarajalu 15720, Tiger shola, Kodaikanal, Madurai Dt. *et* 15930, Thandikudi, Kodaikanal, Madurai Dt. (all PCM) ; Joseph 12312, Waverly estate, Anamalai, Coimbatore Dt.

(1333 m) ; Sebastine 18343, Lockhart gap, Kottayam Dt. (1700 m) *et* 24988, Perumalmalai, Kodaikanal, Madurai Dt. (1700 m) ; Vajravelu 26131, Silent Valley R.F., Palghat Dt. (950 m) *et* 35010, Curzon estate, Nilgiris (1925 m) *et* 37026, Konakarai R.F., Nilgiris ; Shetty 37613, Avalanche, Nilgiris (2000 m) ; Vajravelu 39645, Kodanad, Nilgiris (1850 m)—all MH.

Distribution : Districts of Coimbatore, Madurai, Nilgiris and Palghat seems to be restricted to south Indian Hill stations.

### 3.2. *Anatomy*

*Ficus amplocarpa*

Lamina—Abaxial surface : Cells hexagonal, variable in size and shape ; cell walls thin, straight (undulate). Stomata (L. 18–22·5 $\mu$m ; W. 9–13·5 $\mu$m), thin-walled, anomocytic, elliptic (figure 2 M). Trichomes (L. 348–512 $\mu$m), unicellular (2–3 celled), erect or reclinate, acute (figure 2 I), the basal cells of which containing colourless nodular deposits abundant throughout (figures 3 B ; 4 G) ; unicellular microhairs (figure 4 B) and bicellular microhairs (L. 18·0–22·5 $\mu$m) with basal rosette of cells common in the interveinal areas as in *F. guttata* (figure 3 E) ; shortly stalked multicellular capitate or peltate glandular trichomes common over the veins (figure 2 J) ; erect, unbranched, pointed, multicellular hairs (L. 232–406 $\mu$m) abundant (figure 3 C). Idioblasts containing deposits of calcium carbonate of variable shapes and sizes usually occurring in groups of few excessively thick-walled cells commonly present as in *F. guttata* (figure 2 K, L).

Adaxial surface : Cell walls excessively thick-walled, straight. Stomata, calcium carbonate containing idioblasts absent. Other details, see abaxial surface.

T.S. Lamina : Width of lamina examined 0·4–0·45 mm. Cuticle thin over adaxial and thick with undulations over abaxial surface. Keel 'U'-shaped, adaxially grooved in the midrib ; margin subacute sloping downwards containing 3–4 layers of collenchyma and one small submarginal vb. Adaxial epidermal cells isodiametric with a tendency to become subdivided in certain places. Abaxial epidermal cells tangentially elongated, dissimilar in size, some of them containing granular materials in the form of nodules. Palisade 2–3 layered ; spongy mesophyll conspicuously lacunose with large intercellular spaces and abundant tannin ; mesophyll cells tangentially elongated, lobed, reticulately arranged. Cystoliths common beneath abaxial epidermis (figure 4 B). Abaxial hypodermis 4–5 layered, collenchymatous. Midrib : vascular strands deeply crescentiform adaxially closed by strap-shaped single strand (figure 3 G) ; central ground tissue parenchymatous containing few phloem bundles ; vascular strands subtended by 4–5 discontinuous layers of gelatinous fibres (figures 2 H ; 3 G) ; nests of sclereids with rounded outline and gelatinous wall layers belonging to convolute type (Type 'A', Höster and Liese, 1966) less common. Girders adaxial, incomplete, collenchymatous, 8–10 celled in height and 4–5 celled in width present for all vb's. Laminal vb's somewhat circular in outline surrounded by a single layer of parenchymatous bundle sheath. Tannin abundant throughout.

T.S. Petiole (Distal) ; Outline circular with adaxial median groove (figure 4 A). Diameter of petiole examined 2·8–3·0 mm. Epidermal cells isodiametric containing tannin. Hypodermis single layer of isodiametric tannin free cells. Outer cortex consisting of 10–12 layers of lamellar collenchyma ; inner cortex

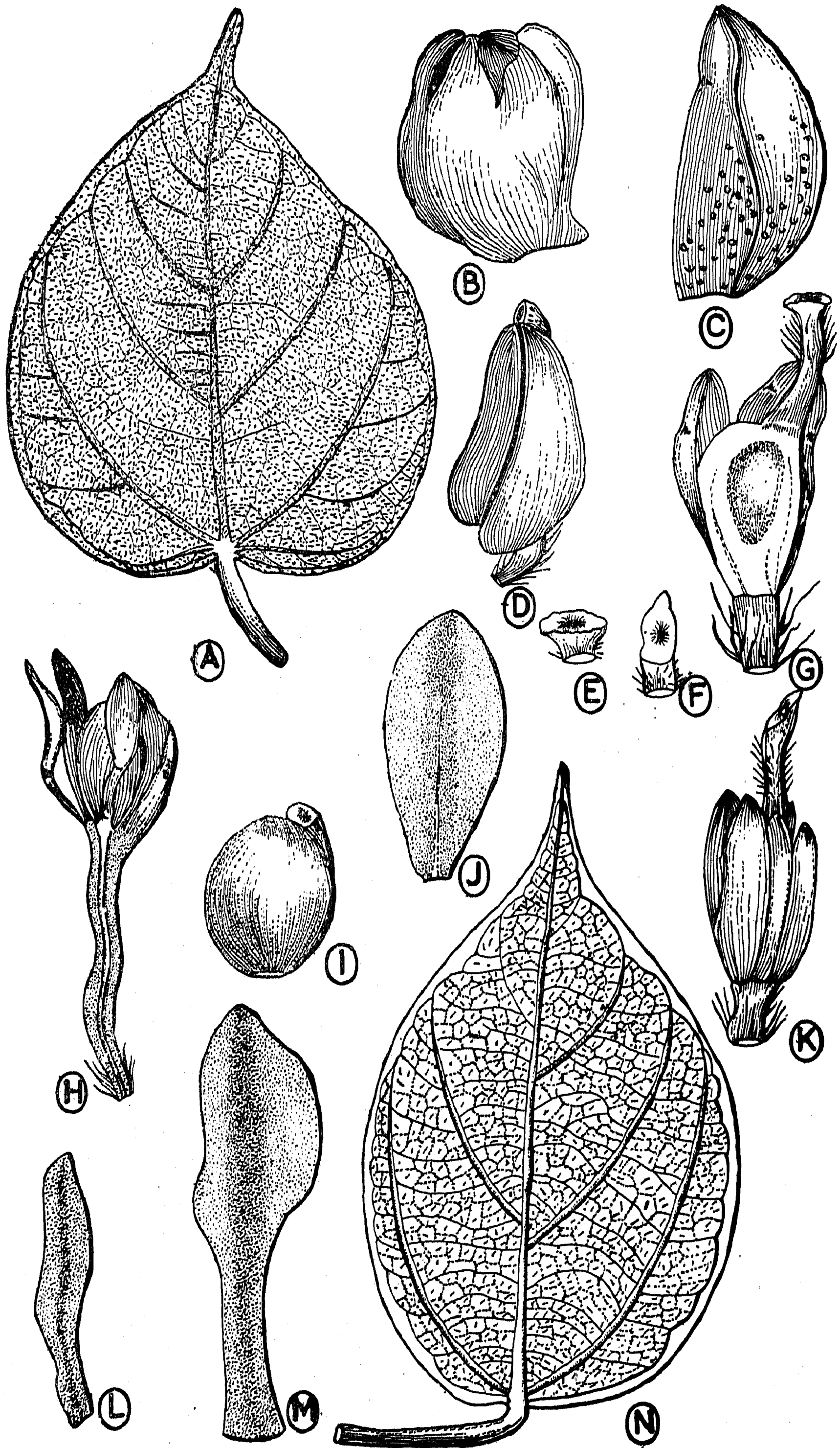



Figure 1. See for caption page 128.

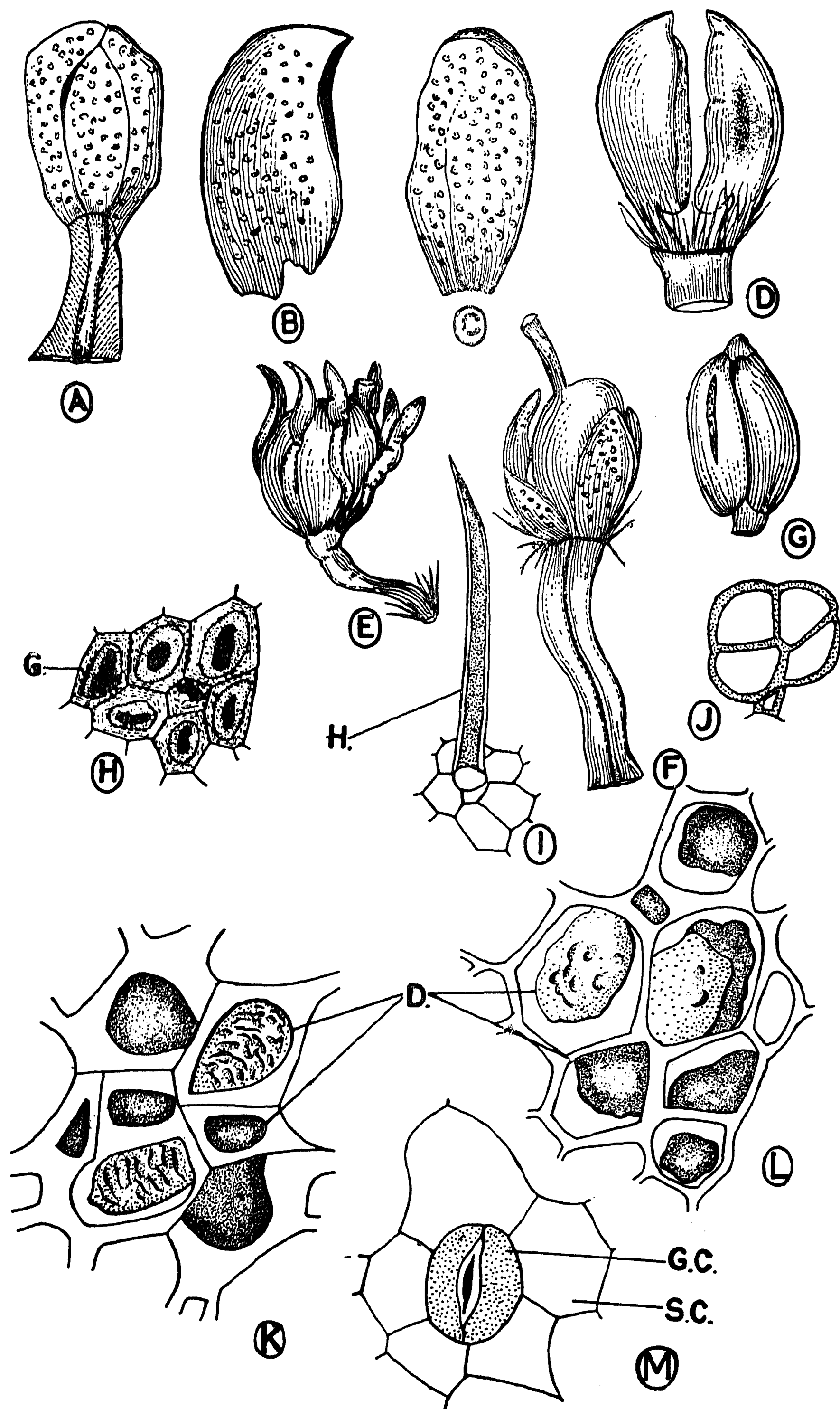



Figure 2. See for caption page 128.

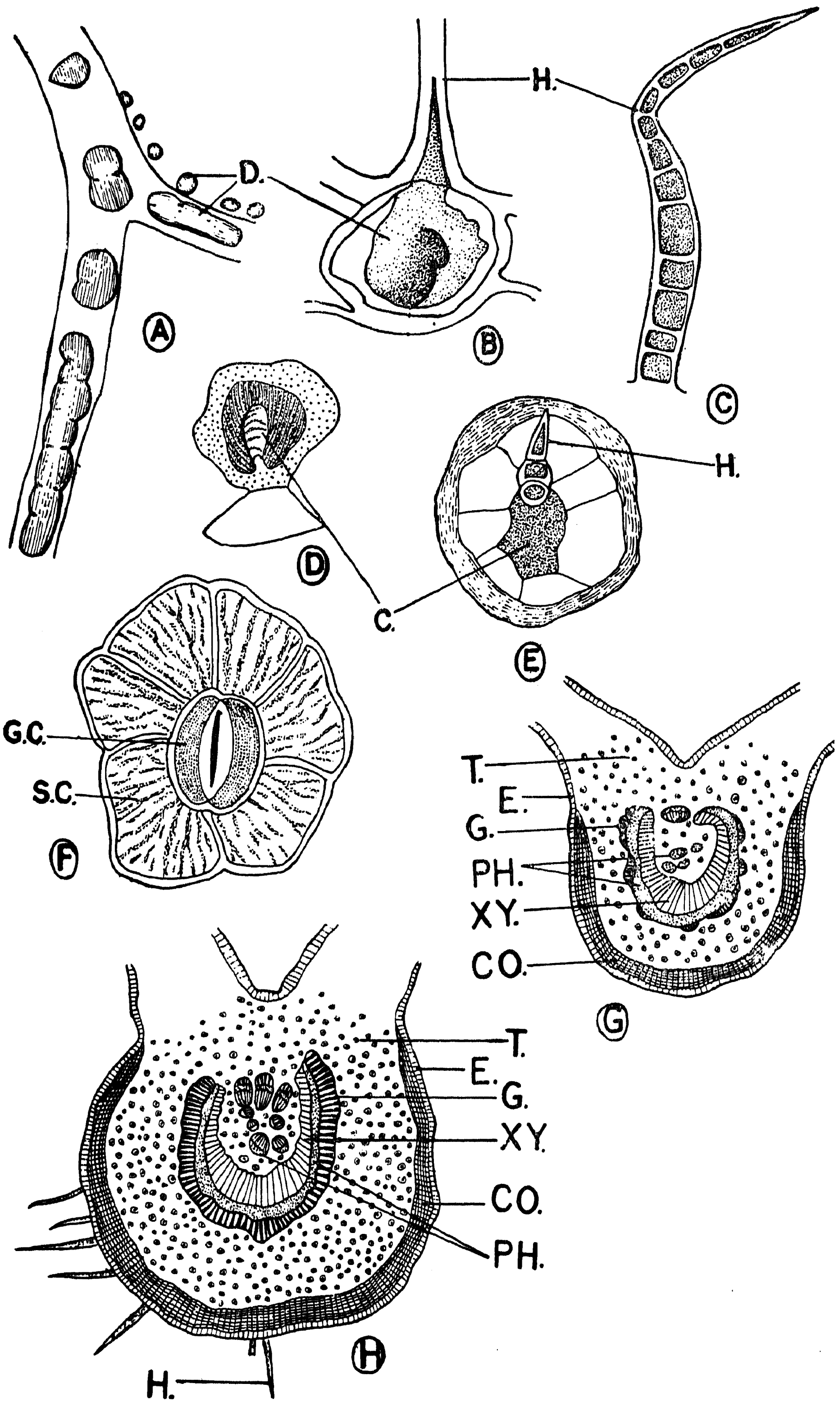
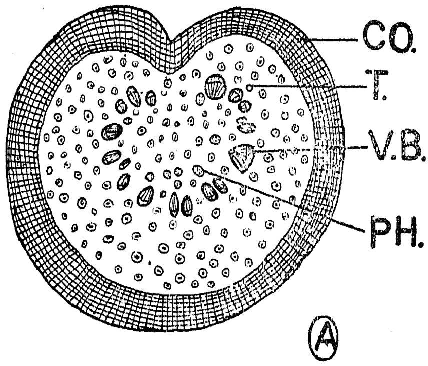
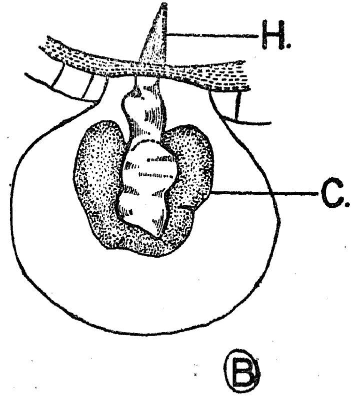
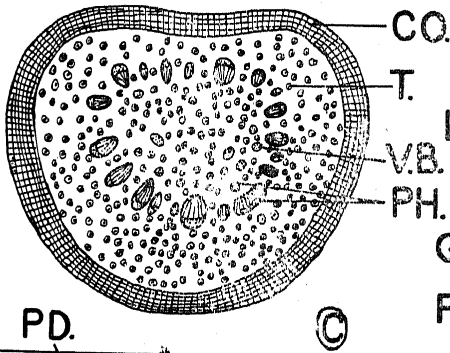
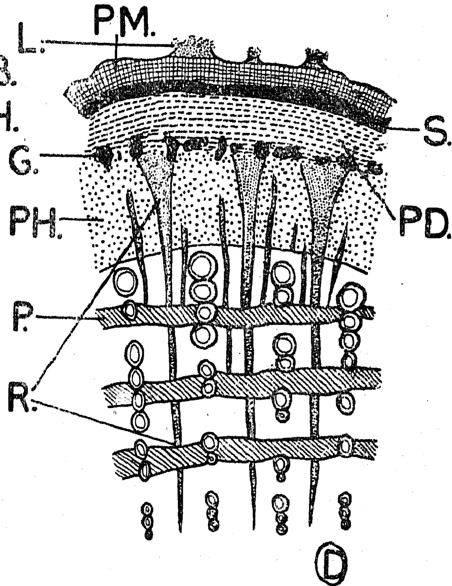


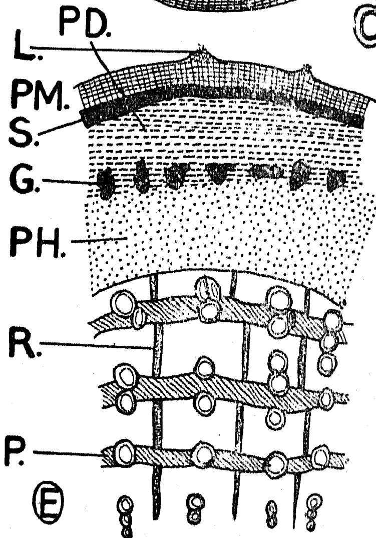

Figure 3. See for caption page 128.

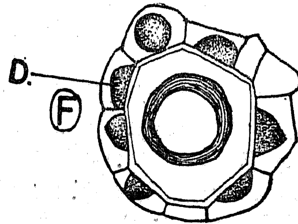


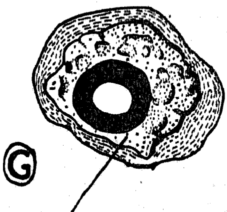



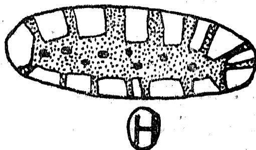




Figure 4. See for caption page 129.

broad consisting of thin-walled polygonal parenchyma cells arranged with intercellular spaces. Vb's 16–18 in number of different sizes, discrete arranged in a ring with a gap confronting adaxial groove (figure 4 A). Central ground tissue parenchymatous containing 7–8 phloem bundles in abaxial half (figure 4 A). Trichomes (L. 348–928 μm), erect, multicellular, brown (due to tannin), unbranched, pointed, thick-walled present ; club shaped unicellular trichomes (L. 58–116 μm), excessively thick-walled containing tannin also present ; peltate or spherical multicellular, shortly stalked glandular trichomes present and all of them intermixed. Cubical crystals and irregularly shaped crystalline bodies less common in ground and cortical parenchyma cells. Basal and mid-regions of petiole differing by containing 25 vb's subtended by discrete units of gelatinous fibres (Type A). Basal and midregions of petiole, see distal region of petiole.

T.S. Node : Pentalacunar, each one of three adaxial gaps confronted by a single trace except two abaxial gaps containing split traces in each (Howard 1974).

T.S. Internode (early secondary xylem) : Diameter of internode examined 7–8 mm. Phellem superficial. Phelloderm broad. Sclereids tangentially elongated or elliptic with lumen and ramiform pits (figure 4 H), 2–3 layered forming a continuous ring present beneath phellem ; brachysclereids with reduced lumina and gelatinous wall layers occurring in several tangential units throughout secondary cortex and secondary phloem (figure 4 E). Growth rings present. Libriform fibres thin-walled (L. 1160–1508 μm) ; septate fibres not common (L. 580–812 μm). Parenchyma paratracheal banded, 2–3 layered (figure 4 E). Pores solitary, circular-oval or in short radial multiples of 2–3. Vessels (L. 348–580 μm), oblique porous (transverse) with alternate intervascular pittings, non-storied. Tannin rare in secondary cortex. Starch grains abundant in cortex, xylem parenchyma and rays.

*F. guttata*

Lamina—Abaxial surface : Cells polygonal, thin-walled ; cell walls straight. Stomata (L. 21·2–26·5 μm ; W. 15·9–21·2 μm), thick-walled, anomocytic, broadly elliptic (figure 3 F). Trichomes (L. 337·5–540 μm), unicellular, erect or reclinate, thick-walled, pointed, mounted on dilated calcified base abundant over veins as in *F. amplocarpa* (figures 3 B, 4 C) ; trichomes surrounded by 1 concentric row of cells, the latter usually containing smooth (figure 4 F) or unevenly striated calcareous deposits and groups of such cells variable in number and arrangement abundantly present independent of trichomes also (figures 2 K, L). Calcareous deposits usually of elongated form (smaller rounded ones) occurring in discontinuous rows over larger veins also present (figure 3 A) ; shortly stalked multicellular capitate or peltate glandular trichomes common as in *F. amplocarpa* (figure 2 J) ; multicellular trichomes (L. 348–928 μm), see *F. amplocarpa* ; prickles (L. 22·5–27·0 μm), 2 celled, excessively thick-walled, pointed, present usually in crypts opposite to cystoliths (figure 3 E).

Adaxial surface : Cells variable in size with moderately thick walls. Trichomes less common. Other details, see abaxial surface.

T.S. Lamina : Width of lamina examined 0·4–0·47 mm. Adaxial epidermis usually tending to become 2–3 layered due to subdivisions. Midrib vasculature as in *F. amplocarpa* but adaxially confronted by (2–) 3 (–4) discrete strands and enclosing within variously oriented 3–5 additional strands (figure 3 H). Sclereids

with gelatinous wall layers as in *F. amplocarpa* (figure 2 H) characteristically and abundantly present in regular radial rows subtending the strands (figure 3 H). Central ground tissue heterogeneous containing parenchyma, sclerenchyma and few groups of gelatinous fibres. Phloem bundles absent. Cubical crystals present in phloem parenchyma and in central ground tissue. Other details as in *F. amplocarpa*.

T.S. Petiole (distal) : Diameter of petiole examined *c.* 4 mm. Vb's 18–20 in number of different sizes, discrete, arranged in a ring without adaxial gap, enclosing within phloem bundles (figure 4 C). Trichomes (L. 290–464 μm), 1–2 celled, erect, thick-walled, unbranched, pointed, containing tannin abundant ; trichomes of other types found in *F. amplocarpa* absent. Basal and mid regions differing by way of reduction in the number of phloem bundles and for other details see distal. Other details as in *F. amplocarpa*.

T.S. Node : See *F. amplocarpa*.

T.S. Internode (early secondary xylem) : Pores usually in radial multiples of (2–) 3–4. Vessels (L. 174–324 μm), storied. Tyloses common. Secondary phloem rather broad and radially traversed by dilated rays (figure 4 D). Other details, see *F. amplocarpa*.

## 4. Discussion

The present study clearly indicates that *Ficus amplocarpa* and *F. guttata* are two distinct species and in this respect the former is characterized by usually membranous broadly elliptic ovate leaves with rounded base, fistular internodes, ramiflorous larger receptacles on naked pendent and/or horizontal cable like branches, ebracteate receptacles tetragonous pedicellate male flowers, flattened membranously winged 1 nerved pedicel widening and hairy towards base, unequal tepals in male flowers, almost sessile free stamens with somewhat falcate anthers, smaller female flowers with longer pedicels and 4 tepalled perianth, subglobose ovary, glabrous style slightly widening towards top with slightly dilated stigma, gall flowers with elliptic linear tepals and discoid stigma. With reference to the above mentioned characters *F. guttata* differs from *F. amplocarpa* by coriaceous (subcoriaceous) leaves, broadly cordiform solid internodes, usually smaller receptacles developing from tubercles of stem and branches, bracteate receptacles, sessile trigonous male flowers with similar tepals, stamens more or less united at base with longer filaments, obcuneate anthers, larger female flowers with shorter pedicels and perianth of 5–6 (–7) tepals, obovoid ovary, style distinctly hairy behind stigma, peltate or infundibuliform stigma with crenulate margin, gall flowers with narrowly obovate or spathulate tepals and dilated stigma.

Wight (1853) has given the illustrations of both *F. amplocarpa* ( = *F. macrocarpa*) and *F. guttata* under *Pogonotrophe macrocarpa* Miq. (Ic. *t.* 1965) and *Covellia guttata* Wt. (Ic. *t.* 1966) respectively, the former without the analysis of floral parts and the latter accompanied by them. Furthermore the green colour of the receptacles, glabrous condition of young twigs, petioles and stipules, emarginate, subacute or attenuating base mentioned by Wight (1853) for *F. macrocarpa* are all quite contrary to the present observations.

King (Ann. Roy. Bot. Gard. Calc. 2 (1888) pl. 209) has adopted the figures of female flowers of *F. guttata* as given by Wight (*l.c.*) with some modifications. But the figures of vegetative organs and the receptacles as given by Wight on the one hand and those of King on the other show no agreement at all. Wight has shown the infundibuliform stigma to be hairy at the margin and also said that the receptacles do not contain any male flowers which are not correct.

The present observations which are based on more number of herbarium specimens and liquid preserved materials differ from those of King (1888) in respect of *F. amplocarpa* ( = *F. macrocarpa*). King reports the absence of male and gall flowers in the receptacles but in fact the former are present in fewer numbers just behind the ostiolar scales and the latter are abundant in the rest of the receptacles. The perianth of female flowers are said to be made up of 6 tepals instead of only 4 tannin punctate tepals. He says fertile female flowers are sessile or pedicellate and the style hairy. On the contrary they are always pedicellate and style glabrous. The stigma is distinctly entire and dilated and not bilobed as mentioned by King (1888). The ovary is not stipitate as shown in pl. 208, f. 5, 7 and 9. *Ficus macrocarpa* as illustrated by King shows more number of primary lateral nerves, glabrous twigs, leaves and stipules, receptacles without white patches, hairy style, bilobed stigma and elliptic ovate tepals which prove to be otherwise according to our observations. In the legend for figure 1 it is stated that it is part of fascicle of receptacle from the stem below the leaves but actually they are arranged in an elongated cable like pendent and/or horizontal branches. It is shown that fertile female flowers are pedicellate (figures 5 and 7) and sessile (figure 6) but the present observation reveals only pedicellate condition.

King (Ann. Roy. Bot. Gard. Calc. 2 (1888) 167) has said that in *F. guttata* he could not observe the male flowers in the only receptacle that was available to him and also as in the case of *F. macrocarpa* he could not come across any gall flowers since all the flowers in the single receptacle appeared to be fertile female flowers. In fact, the receptacles are found to contain all the three kinds of flowers. According to King (*l.c.*) the fertile female flowers are said to be sessile which is contrary not only with reference to his own figure (pl. 209, f. 6) but our observations. Each tepal is shown to be strongly medianly 1 nerved instead of the nerveless condition. The ovary is obovate and the style is distinctly hairy behind stigma unlike those of the figures in which the former is shown as subglobose and the latter perfectly glabrous. Another interesting situation in this respect is that Wight's figure 4 (Ic. *t* 1966) representing the female flowers of *F. guttata* ( = *Covellia guttata*) is given as such with slight enlargement by King (*l.c.* pl. 209, figure 6) but the stigmatic margin shown to be hairy (Wight's figure 4) has been shown as perfectly glabrous (King's figure 6). Furthermore King's figures 6 and 7 (pl. 209) are labelled as female flowers under different stages of development but they all look so different that one has got nothing to do with the other.

Corner (Gard. Bull. Sing. 18 (1960) 7) has reduced *F. macrocarpa* as a variety of *F. laevis* Bl. under sect. *Rhizocladus* Endl. and *F. guttata* as a synonym of this variety. The reasons given by him for this procedure are the existence of root climbing habit and the shape of the leaves which are similar to those of *F. laevis*. Nevertheless *F. laevis* differs from *F. amplocarpa* (= *F. macrocarpa*) by its climbing habit, dentate margin and deeply cordate base of the leaf, axillary solitary smaller

greenish yellow receptacles on long peduncles subtended by 3 basal bracts with densely hispid hairy interior, 5 linear lanceolate tepals in male and female flowers, glabrous staminal filament, dorsifixed nonfleshy anthers with subsagittate base and acute apex, terminal style equalling the length of achene, bifid stigma and elongated ovoid achene. Furthermore when the characters of both *F. amplocarpa* and *F. guttata* agree well with those of the section *Neomorphe* King in that the flowers are unisexual, male and gall flowers in one set of receptacle, fertile female flowers in another set of receptacle, male flowers with 2 stamens, inflated perianth with 3 or 4 membranous tepals, fertile female flowers smaller than male or gall flowers, receptacles often very large in fascicles from tubercles on the stem and longer branches, trees rarely scandent shrubs, never epiphytal, they are allowed to retain their berth in the sect. *Neomorphe* itself. Furthermore as the specific differences between *F. amplocarpa* and *F. guttata* are many and clear cut (see above) and each one of them in turn widely differs from *F. laevis* in respect of several characters the original specific status of the former two taxa is restored.

As mentioned by King (Ann. Roy. Bot. Gard. Calc. 2 (1888) 129), Miquel (Ann. Mus. Lugd. Bat. 3 (1867) 278) has considered *Pogonotrophe macrocarpa* (Wt. Ic. *t.* 1965) as referable to *F. vagans* Roxb. but Roxburgh's manuscript drawing of the latter in *Hb. Cal.* shows that it is clearly identical with authentic specimens of *F. laevis* Bl.

Fyson (Fl. s. Ind. Hill. stn. i. (1932) 541) distinguishes *F. amplocarpa* from *F. guttata* only by the glabrous young parts of the former and the hairy young parts of the latter and not on the basis of any other characters. Although the peduncle bearing the receptacle and leafy branch as illustrated by Fyson *l.c.* 2 (1932) appears to be satisfactory the shape of the leaves has greater resemblance to *F. guttata* than to *F. amplocarpa.* Likewise the female flowers illustrated by him differ from the present observations.

The anatomical differences between *F. amplocarpa* and *F. guttata* are so significant and convincing that these two taxa can be maintained undoubtedly as two distinct species on this ground also. *F. amplocarpa* differs from *F. guttata* by the absence of 2-celled thick-walled prickles in the leaf, midrib with crescentiform vascular strand adaxially closed by single strap-shaped strand, occurrence of gelatinous fibres in 4–5 discontinuous layers subtending the midrib vascular strands, central ground tissue of midrib containing phloem bundles, less common occurrence of sclereids with gelatinous wall layers subtending the midrib strands, presence of club shaped and peltate or spherical glandular trichomes in the petioles, non-storied vessels, longer vessels with oblique porous perforations, absence of tyloses, nondevelopment of radially traversing dilated rays in the secondary phloem, longer libriform and septate fibres. Incidentally it is also interesting to observe that *F. amplocarpa* is found to be less specialized than *F. guttata* since the vessels of the former are longer with oblique porous perforations and non-storied, and septate and libriform fibres are longer than those of the latter.

Leaves broadly elliptic, membranous, rounded at base; receptacle large 3·0–6·5 cm across with white blotches, ebracteate and without hispid hairs in the interior; stamens free with somewhat falcate anthers; tepals of female flowers 4; equalling or shorter than ovary; ovary subglobose; style glabrous, stigma dilated..............................*F. amplocarpa* (= *F. macrocarpa*)

Leaves broadly cordiform, coriaceous, cordate or subcordate at base; receptacles small, 2·5–3·5 cm across without white blotches, bracteate with hispid hairs in the interior; stamens basally united with obcuneate anthers; tepals of female flowers 5–6–(–7); longer than ovary; ovary obovoid; style hairy behind stigma; stigma usually peltate or discoid........................*F. guttata.*

## Acknowledgement

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**Figures 1 A–N.** *Ficus guttata.* **A.** abaxial surface of leaf × 1/5; **B.** male flower bud × 8; **C.** tepal of male flower × 14; **D.** stamen × 16; **E.** and **F.** different forms of stigma (diagrammatic); **G.** female flower with tepals removed showing one form of stigma × 12; **H.** gall flower × 7; **I.** ovary of gall flower × 8; **J.** tepal of female flower × 16; **K.** female flower (entire) with another form of stigma × 11; **L.** and **M.** tepals of gall flower (two types) × 13; **N.** *F. amplocarpa* abaxial surface of leaf × ¼.

**Figures 2 A–M. A–J.** *F. amplocarpa.* **A.** male flower bud × 8; **B.** outer tepal of male flower × 11; **C.** inner tepal of male flower × 13; **D.** staminal pair of a single male flower × 16; **E.** gall flower × 5; **F.** female flower × 10; **G.** another type of stamen × 14; **H.** cortical gelatinous fibres (type A) × 170; **I.** unicellular hair with a basal rosette of cells × 107; **J.** peltate glandular hair × 170; **K.** and **L.** *F. guttata* leaf epidermal cells containing different forms of calcareous deposits × 170; **M.** *F. amplocarpa* surface view of stoma × 450.

**Figures 3 A–H. A.** *Ficus guttata* portion of lateral vein showing different forms of calcareous deposits × 170; **B.** and **C.** *F. amplocarpa*: **B.** part of leaf epidermal hairs showing calcareous deposit containing basal cell × 170; **C.** multicellular hair over veins × 100; **D–F.** *F. guttata*: **D.** cystolith containing idioblast × 170; **E.** 2-celled microhair with cystolith containing basal idioblast × 450; **F.** surface view of stoma × 450; **G.** *F. amplocarpa* transection of midrib × 22; **H.** *F. guttata* transection of midrib × 22.

**Figures 4 A–H.** A. and **B.** *Ficus amplocarpa* : **A.** transection of petiole at distal end (semidiagrammatic) ; **B.** cystolith opposed by 1-celled microhair × 170 ; C. and **D.** *F. guttata*: C. transection of petiole at distal end (semidiagrammatic); **D.** transection of old internode (semidiagrammatic) ; **E.** *F. amplocarpa* transection of old internode (semidiagrammatic) ; **F.** *F. guttata* leaf epidermal hair base encircled by calcareous deposit containing cells × 170; G. and **H.** *F. amplocarpa* : **G.** surface view of cystolith containing hair base × 170 ; **H.** brachysclereid from cortex × 65.

*Abbreviations* : C. cystolith CO. cortex; D. calcareous deposit; E. epidermis ; G. gelatinous fibres G.C. guard cell ; H. hair; L. lenticel; P. xylem parenchyma; PD. phelloderm; PH. phloem ; PM. phellem ; R. rays ; S. sclereids ; T. tannin idioblast ; VB. vascular bundle ; XY. xylem ; *c. circa* ; D. diameter ; Ht. height ; L. length ; vb. vascular bundle (pl. vb's) ; W. width.

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# The genus *Jackiella* in South India

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**Abstract.** *Jackiella ceylanica* Schiffn. *ex* St. and *J. javanica* var. *cordifolia* Schiffn. are being reported for the first time from India. The taxonomic details of the two taxa together with a discussion of the tenability of two varieties of *J. javanica* Schiffn. namely var. *cordifolia* and var. *cavifolia* have been given.

**Keywords.** Bryophyta; hepaticae; Genus *Jackiella*; South India; taxonomic details.

## 1. Introduction

In a recent contribution (Udar and Kumar 1981) the range of distribution and diagnostic characteristics of the marsupial genus *Jackiella* together with the taxonomic details of *J. javanica* Schiffn. from eastern India had been given. However, during a survey of south Indian liverworts, which have not yet received proper attention, plants resembling *J. javanica* in all characters, except in habit of plant, size and shape of leaf, degree of development of trigones in cells of leaf and stem and in structure of capsule wall, were discovered. Among all these characters, the development of trigones seems to be ecologically influenced but the habit of the plant, the shape of the leaves and the structure of capsule wall show more or less stabilised features which merit recognition of varietal ranks for the east Indian and south Indian plants. Schiffner (1900) had already recognised two varieties of *Jackiella javanica* Schiffn. primarily based on vegetative characters viz., *J. javanica* var. *cavifolia* Schiffn. from Java, Sumatra and *J. javanica* var. *cordifolia* Schiffn. from Java (see also Bonner 1966). The former variety is stated to be characterised by small and creeping plants with leaves broader than long and the latter by more elongated, uprising to almost erect plants with leaves longer than broad. The plants of east Indian territory could be referred to as *J. javanica* var. *cavifolia* and those from south Indian territory as *J. javanica* var. *cordifolia*. Our study based on east Indian (from Sikkim and Shillong) and south Indian (from Wellington and Dodabetta) plants reveals that sporophytic differences are also present between these two varieties hitherto not recognised.

A survey of plants from Kodaikanal (Palni Hills) in the Indian peninsular region, nearer to Ceylon, revealed plants which resemble *J. ceylanica* Schiffn. *ex* St.—a taxon which has so far been considered endemic to Ceylon. The discovery of this species from India constitutes a trans-oceanic disjunct distribution for this taxon

In the present state of our knowledge the genus *Jackiella* is represented in India by *J. ceylanica, J. javanica* var. *cavifolia* and *J. javanica* var. *cordifolia*. The taxonomic details of *J. ceylanica* and *J. javanica* var. *cordifolia*, occurring in south India, are being described in the present paper. The remaining taxon from eastern India has already been reported elsewhere (Udar and Kumar 1981).

## 2. Materials and methods

The plants of *J. javanica* var. *cordifolia* were collected from Wellington and Dodabetta and *J. ceylanica* from Kodaikanal. The taxonomic details of the former taxon are based on plants collected from Wellington except those relating to the capsule wall which are drawn from the plants of Dodabetta. The plants were stretched in water and placed in incubator for 24 hr at 40° C. The slides were prepared in 70% glycerine.

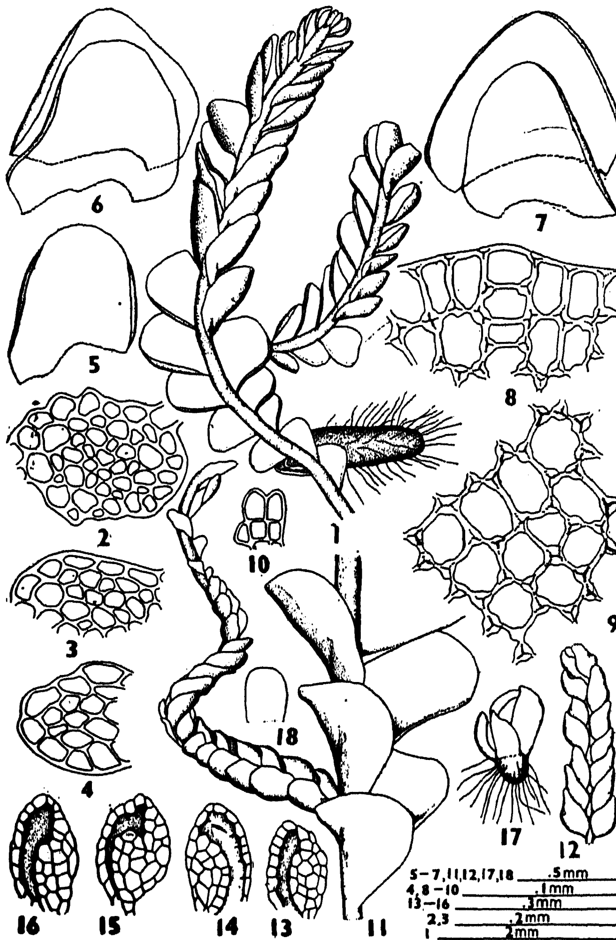
## 3. Key to the Indian species of *Jackiella*

1. Plants erect, deep brown. Leaves with curved margin. Male inflorescence with 7-15 (or more) pairs of male bracts ; male bracts oblong with flat dorsal lobe.................................................*J. ceylanica*
1. Plants prostrate-suberect, light green. Margin of leaves not curved. Male inflorescence with 3-10 pairs of male bracts; male bracts globose-subglobose with saccate dorsal lobe.....................*J. javanica*.............2
2. Plants prostrate. Leaves broader than long, concave, with decurrent antical margin. Inner layer of capsule wall in surface view with incomplete thickening bands..............................*J. javanica* var. *cavifolia*
2. Plants suberect. Leaves longer than broad, without decurrent antical margin. Inner layer of capsule wall usually with complete thickening bands ..........................................*J. javanica* var. *cordifolia*

## 4. Observation

4.1. *Jackiella ceylanica Schiffn. ex St. Species Hepaticarum* 3 : 272, (1908). *Figures* 1-18.

Plants dioecious, dark brown, up to 15 mm long, rigid, erect, in dense mat. Branches ventral-intercalary, large, ascending upward, brownish green to deep brown. Stem up to 8(-10) cells across, 140-210 $\mu m$ in diam., cortical cells isodiametric or variable deep brown, thick-walled, 18-33 × 18-25 $\mu m$ ; medullary cells usually isodiametric, yellowish brown, thin to thick-walled, 12-33 × 18-25 $\mu m$. Leaves succubous, entire, ovate to oblong, usually broader than long, or occasionally longer than broad, 0·65-0·97 (-1·2) × 0·8-1·0 mm, deep brown, sub-opposite, insertion oblique, apex obtuse, base broad ; antical margin slightly decurrent, curved ; postical margin arched, sometimes curved, marginal cells longer than broad, broader than long or as long as broad, 15-30 × 15-30 $\mu m$ ; middle and basal cells longer than broad, occasionally broader than long, (18-




**Figures 1–18.** *Jackiella ceylanica* **1.** Female plant with marsupium. **2–4.** T. S. of axes (**2, 3** axes of vegetative plant ; **4.** axis of male inflorescence). **5–7.** Leaves: **8.** Marginal cells of leaf. **9.** Middle cells of leaf. **10.** Underleaf. **11.** Portion of male plant with male inflorescence. **12.** Male inflorescence. **13–16.** Male bracts. **17.** Young marsupium. **18.** Female bract.

21–44 (–51) × 18–44 µm ; cell walls thick, sometimes thin in younger leaves, trigones prominent and bulging. Gemmae not seen. Underleaves highly reduced, only near apex of stem, (1–) 2–3 cells long, 2–3 cells broad, connate at base with the leaf of one side, with 1-celled marginal teeth ; cells thin-walled, trigones feebly developed. Rhizoids scarcely present on axis, usually restricted at base of underleaves, with swollen tips harbouring mycorrhiza. Male plant with short, spicate, ventral branches, androecia terminal or intercalary consisting of 7–15 or more pairs of male bracts ; male inflorescence usually with 5-celled thick axis having indistinct cortical and medullary regions ; male bracts bilobed, oblong, 180–260 × 105–155 µm : ventral lobe oblong, comparatively larger, saccate : dorsal lobe oblong, smaller, flat, enclosing single antheridium ; male bracteoles restricted

only at base of male inflorescence. Female plant with 2 or more short, ventral, female inflorescence having 1–2 pairs of female bracts at mouth of marsupium ; female bracts saccate, apex obtuse, sometimes subacute to acute; perianth absent ; marsupium deep brown, cylindrical, 0·5–1·0 mm long, with numerous rhizoids on its surface ; archegonia up to 3–5 at mouth of the marsupium with short neck.

4·1a. *Specimens examined* : LWU 79/66, 95/66 ; Coll. R. Udar and S. C. Srivastava ; Loc. Kodaikanal (Palni Hills, South India), alt. ca 1200 m ; Date January 6, 1966 ; Det. R. Udar and A. Kumar.

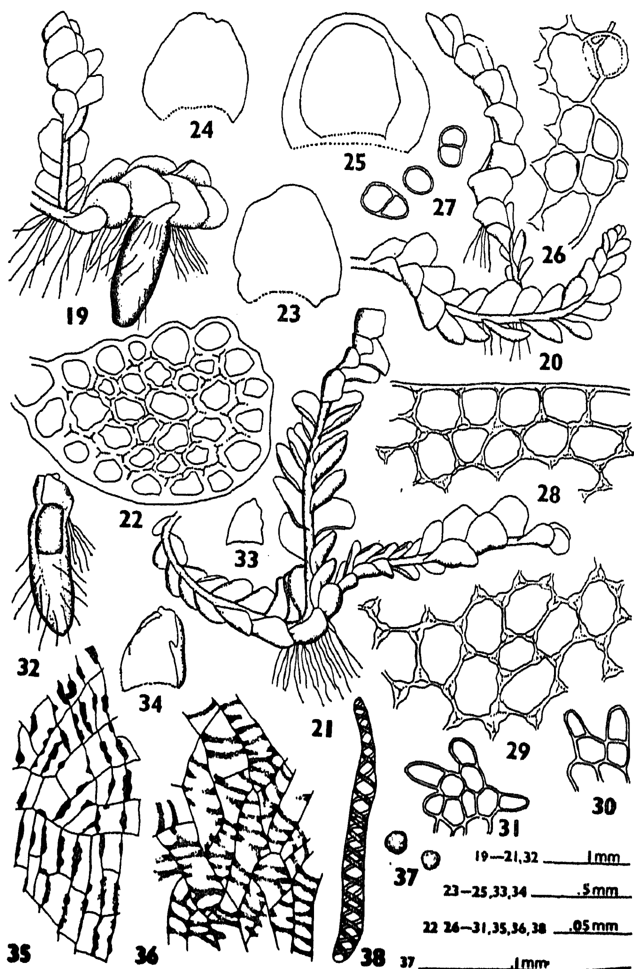
4·1b. *Ecology* : The plants grow on rocky soil in association with *Pogonatum* sp. on moist and exposed surface.

4·1c. *Discussion* : *J. ceylanica* Schiffn. *ex* St. was originally described by Stephani (1906–1909) from Ceylon, who provided a short Latin diagnosis only along with some illustrations in his unpublished Icones No. 2024. Later Abeywickrama (1959) reported it from the same island with a meagre description and wrong illustration (see fig. 16c, p. 48). The adequate taxonomic details of this plant do not seem to have been published so far. The characters of this species from Kodaikanal reveal a great deal of diversity and plasticity in vegetative and reproductive characters which tend to approach *J. javanica.* However, *J. ceylanica* differs from the latter in larger, ascending to erect and deep brown plants, and with curved margins in leaves. The androecia are very characteristic in being terminal or intercalary and consist of 7–15 or more pairs of oblong male bracts with flat dorsal lobe. In *J. javanica,* however, the plants are smaller, prostrate and light green with more or less flat leaves. The androecia are terminal with 3–10 pairs of male bracts which are globose to subglobose with saccate dorsal lobe.

Considerable variation occurs in the leaves in *J. ceylanica* which may be ovate to oblong, broader than long (figures 6, 7) or longer than broad (figure 5) but characteristically with curved margin. The underleaves are highly reduced (figure 10) and apparently confined near the apex of the young shoot. The rhizoidal tips are swollen and harbour mycorrhiza. They may perform similar function of nitrogen fixation as reported for the east Indian plants by Udar and Kumar (1981).

4.2. *Jackiella javanica* var. *cordifolia* Schiffn.

Denkscher. Mat. Nat. Cl. Kais. Acad. Wiss. Wien 70 : 217 ( : 115), (1900). Figures 19–38. Plants dioecious, light green, occasionally yellowish brown, up to 10 mm long, delicate, prostrate to suberect, in dense mat. Branches ventral intercalary, small, ascending, light green. Stem prostrate or with ascending apex, up to 6–7 cells across, 100–160 μm in diam., cortical cells isodiametric, light to deep brown, thick-walled, 7–28 × 7–28 μm ; medullary cells more or less isodiametric, light brown to yellowish brown, thick to thin-walled, trigonous, 10–25 × 10–23 μm. Leaves succubous, entire, ovate or cordate or subquadrate to slightly oblong, usually longer than broad or as long as broad or occasionally broader than long, light green to light brown, 0·45–0·62 × 0·44–0·54 mm at middle ; at plant apex usually broader than long, light green, (0·5–) 0·64–0·75 × (0·6–) 0·75–0·9 (–1·0) mm, sub-opposite, insertion oblique, apex obtuse, occasionally slightly retuse, margin entire (to wavy), not decurrent ; marginal cells usually as long as broad,




**Figures 19–38.** *Jackiella javanica* var. *cordifolia*. **19.** Female plant with marsupium. **20, 21.** Vegetative plants. **22.** T.S. of axis. **23–25.** Leaves. **26.** Marginal cells of leaf showing attached gemmae. **27.** Gemmae. **28.** Marginal cells of leaf. **29.** Middle cells of leaf. **30, 31.** Underleaves. **32.** Marsupium with young sporophyte. **33, 34.** Female bracts. **35.** Outer layer of capsule wall. **36.** Inner layer of capsule wall. **37.** Spores. **38.** Elater.

longer than broad or broader than long, 18–28 (–38) × 18–28 (–33) $\mu$m ; middle and basal cells isodiametric, more or less longer than broad, 20–44 × 15–30 $\mu$m ; cell walls thin, trigones prominent, bulging ; base broader in younger leaves. Gemmae occasional, at apical margin of young leaf, hyaline to yellowish brown, 1-celled, spherical, 10–15 $\mu$m in diam., 2-celled, 18–35 × 12–15 $\mu$m. Underleaves reduced, up to 4–5 cells long, 4–5 cells broad, bifid, lobes uniseriate, margin dentate, connate at base with leaves of one side, cell walls thin, trigones feebly developed. Rhizoids numerous on prostrate axis, particularly near the base of underleaves, with swollen tips harbouring mycorrhiza. Male plants absent,

Female plant with 1–2 short, ventral, female inflorescence having 1–2 pairs of female bracts at mouth of marsupium ; female bracts saccate, apex obtuse to subacute with entire to wavy margin; perianth absent ; marsupium light to deep brown, cylindrical to sickel-shaped, narrowed at base, 1–2 mm long, 0·4–0·55 mm wide, with some rhizoids at surface, archegonia up to 6 at mouth of marsupium with short neck. Sporophyte young, enclosed within marsupium. Seta small. Capsule cylindrical, deep reddish brown, 540–760 μm long, 215–325 μm wide, with obtuse apex on 1-celled thick capsular disc, dehiscence in two valves each with a small cleft at apex; wall bis-tratose ; cells of outer layer quadrate to subquadrate to elongated, 23–44 × 12–24 μm with nodular to confluent thickenings on radial walls, occasionally also on transverse walls ; cells of inner layer subquadrate to elongated, 25–50 (–60) × 20–25 μm with complete, sometimes incomplete thickening bands on inner tangential wall, sometimes semiannular bands bifurcate on inner tangential wall. Spores spherical, light yellowish-brown, 6–9 μm in diam., exine with small papillae. Elaters 75–210 μm long, 11–16 μm broad at middle, reddish brown, bispiral.

4·2a. *Specimens examined*: LWU 375/71 ; Coll. R. Udar and party ; Loc. Wellington (Nilgiri Hills, South India), alt. ca 1350 m ; Date December 31, 1971. LWU 184/72 ; Coll. R. Udar and party ; Loc. Dodabetta (Nilgiri Hills, South India), alt. ca 2670 m ; Date January 5, 1972 ; Det. R. Udar and A. Kumar.

4·2b. *Ecology* : The plants grow on laterite soil on rock surface in dense mats at moist places in association with *Jungermannia* sp. and *Pogonatum* sp.

4·2c. *Discussion* : *J. javanica* var. *cordifolia* Schiffn. was instituted by Schiffner (1900) from Java and is being described from the bryoflora of Wellington and Dodabetta (South India) for the first time from India. The plants differ from *J. javanica* var. *cavifolia* in habit and in shape of leaves (see Schiffner 1900). However, the capsule wall structrue—a sporophytic character, earlier not known, also provides additional differentiating features for the two varieties. The former variety has prostrate plants with ascending apex (figure 21) or ascending branches (figure 20) which are prostrate in the latter. The leaves in var. *cordifolia*, similar to *J. ceylanica*, show plasticity in size and shape as they are longer than broad, flat, hardly decurrent and comparatively smaller at middle part of the axis whereas in var. *cavifolia* these are broader than long, concave and antically decurrent. In addition to these vegetative features, the development of thickening bands on inner tangential wall of the inner layer of capsule wall is usually complete in var. *cordifolia* but normally incomplete in var. *cavifolia.*

The leaves show a great deal of variation in their size and shape. These are longer than broad and cordate to ovate or sometimes subquadrate to slightly oblong at middle and basal part of the axis but broader than long towards apex. The margin may be entire or wavy with obtuse to retuse apex (figure 24) which is due to formation of gemmae. The gemmae are 1–2 celled (figure 27) and formed exogenously from the marginal cells of the leaves (figure 26). The rhizoids are mostly restricted on prostrate axis and also on the surface of marsupium (figure 19) with swollen tips harbouring mycorrhiza. The marsupium is either cylindrical or sickel-

shaped and the female bracts are with truncate to obtuse apex and entire margin (figure 32) or with acute to subacute apex and wavy to dentate margin (figures 33, 34).

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# *Geocalyx* Nees—a rare marsupial genus from India

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**Abstract.** Taxonomic details of *Geocalyx graveolens* Nees recently discovered from the Valley of Flowers in western Himalayas (altitude ca 4670 meters) have been provided. The discovery of this genus in the above area not only extends its range of distribution in the Himalayas but also constitutes a new record of this taxon in Indian bryoflora. The plants are monoecious and characterized by undulating stem, bifid leaves which are flat or ascending, and bifid underleaves. The androecial and gynoecial branches are ventral and axillary. The marsupium arises initially as tuber-like structure (often 2–8 in number on the ventral surface of a plant) and, at maturity becomes cylindrical with numerous rhizoids studded on its surface.

**Keywords.** Bryophyta; Hepaticae; Jungermanniales; Geocalycaceae; *Geocalyx*; new record for India.

## 1. Introduction

*Geocalyx* Nees is a small genus represented by only seven species, viz., *G. caledonicus* St. from New Caledonia, *G. contortuplicatus* Mont. *et* Nees from San Domingo, *G. graveolens* (Schrad.) Nees from Europe, Siberia, Japan and North America, *G. orientalis* Besch. *et* Spr., *G. borbonicus* St. (c.f. *orientalis*) from Reunion, *G. novazelandiae* Herz. from New Zealand and *G. yakusimensis* Hatt. from Japan (Stephani 1906-1909; Macvicar 1926; Herzog 1935; Hattori 1948; Hodgson 1958; Bonner 1965).

This genus is remarkable in having the young sporophyte deeply sunk in a fleshy, cylindrical sac-like, pendulous, marsupium arising from the postical surface of the stem. Although several marsupial taxa are known from Indian sub-continent but the details of the marsupium are available only in *Jackiella javanica* Schiffn. and *J. ceylanica* Schiffn. luxuriantly distributed in eastern Himalayas and south India (Udar and Kumar 1981, 1982). The present paper provides details of *Geocalyx graveolens*, another marsupial taxon, recently collected from the Valley of Flowers. The discovery of this genus in the above area not only extends its range of distribution in the Himalayas but also constitutes a new record of this taxon in Indian flora. Evans (1939) treated this genus under the family Harpanthaceae (see also Müller 1951-1958; Hodgson 1958). Schuster (1972), on the other hand, erected a new suborder Geocalycinae to accommodate Geocalycaceae to which this plant belongs, along with other families.

## 2. Taxonomic description

*Geocalyx graveolens* (Schrad.) Nees, Eur. Lab. II. p. 397 1836 (figures 1–25)

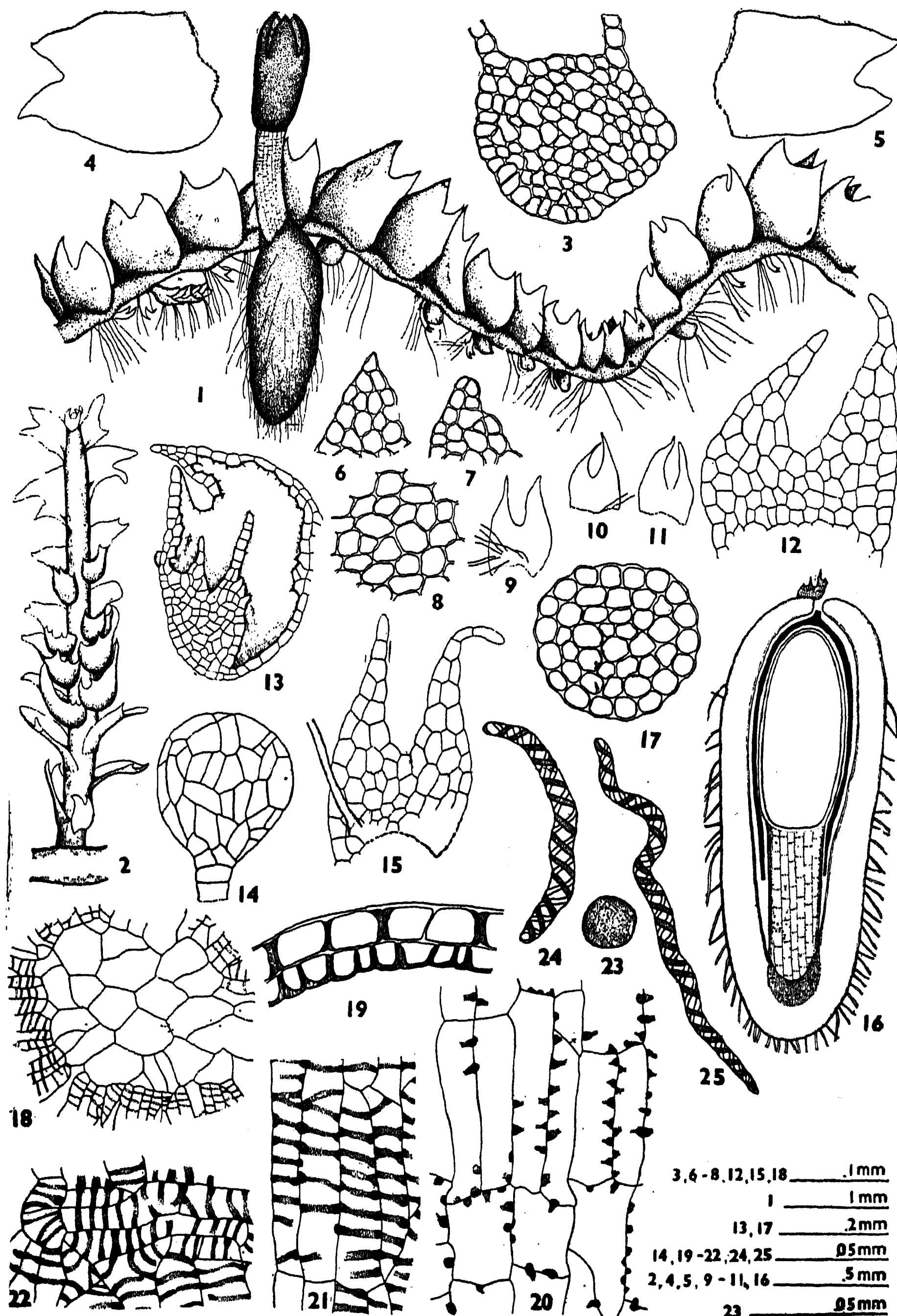
Plants yellowish-green to green, sparingly branched, creeping with distinct undulations. Stem 1–1·5(–2) cm long, 9–11 cells across, cortical and medullary cells alike. Leaves succubous, sub-opposite or alternate, obliquely inserted, flat or ascending, lamina up to 24 cells broad, bifid, occasionally tri-tetrafid, sinus broad, extending 1/4 to 1/3 of leaf length, lobes ovate, 10–14 cells long, 9–14 cells broad, 682–770 × 561–605 μm, apex acute to subacute (1-celled or 2-celled long), antical margin decurrent, postical margin straight, leaf cells polygonal with inconspicuous trigones, 15–23 × 10–18 μm at the margins, 19–29 (39) × 21–33 (45)μm towards the middle and the basal region. Underleaves bifid, with narrow sinus extending to 1/2 or more of length, lobes 5–10 cells long and 5–7 cells broad, 275–407 (605) × 198–275 μm, with smooth margin, apex acuminate, usually uniseriate, cells polygonal, without conspicuous trigones. Rhizoids numerous, usually in fascicles at the bases of the underleaves and also scattered on the stem ventrally. Monoecious. Antheridial shoot ventral, in the axil of underleaf, 495–715 μm long, spikate occasionally showing proliferation; antheridial bracts in 4–6 pairs, imbricate, shortly bilobed, saccate, ventral lobe with laciniate margin, incurved ; bracteoles bifid, one per pair of bracts, usually 1/2 bifid with 3-celled uniseriate acuminate apices ; antheridium single in the axil of each bract, body sub-globose, 56–67 (141) × 64–67 (147)μm with irregularly arranged jacket cells, stalk biseriate, 21–33 μm long. Archegonial branches ventral, in the axil of underleaves, archegonia 3–4 at the apex surrounded by small perichaetial leaves. Marsupium initially small, tuber like, later becoming fleshy and cylindrical, 2–2·5 mm long, with narrow base, studded with numerous rhizoids. Sporophyte with an anchor-shaped multicellular foot buried at the base of the marsupium ; seta cylindrical, 7–8 cells across, up to 2 cm long, hyaline ; capsule cylindrical, deep brown, dehiscing in four equal valves extending up to the base of the capsule, capsule wall bistratose, outer layer of cells quadrate to elongated, with 2-phase development, thickenings nodulose on the radial walls, extending slightly on the tangential walls, marginal cells of each valve lacking any such thickenings ; inner layer of quadrate to elongated cells with complete–incomplete bands on the inner tangential walls usually connecting the radial bands at both ends. Spores yellowish to reddish-brown, globose to sub-globose 8–12 μm in diameter, exine minutely papillose. Elaters reddish-brown, 96–192 μm long, 7–8 μm broad, bispiral to occasionally trispiral with blunt to tapering ends.

### 2.1. *Distribution*

Europe, America, Russia, Japan and India.

### 2.2. *Specimens examined*:

LWU 4279, 4390 Coll. S. C. Srivastava, Dinesh Kumar and D. K. Singh: Loc: On way to Hemkund from Ghangaria (Valley of Flowers), alt. ca 4670 meters Date 22 May 1980. Det. R. Udar, S. C. Srivastava and D. Kumar.




**Figures 1–25.** *Geocalyx graveolens.* **1.** Plant showing a male branch, a mature marsupium (with exserted sporophyte) and several tuberous young marsupia. **2.** A male branch showing proliferation. **3.** T.S. stem. **4, 5.** Bifid leaves. **6, 7.** Apices of the leaf lobes. **8.** Leaf cells from middle and base. **9–12.** Underleaves. **13.** Male bract. **14.** Antheridium. **15.** Male bracteole. **16.** L.S. marsupium with sporophyte. **17.** T.S. seta. **18.** Base of capsule. **19.** T.S. capsule wall. **20.** Cells of the outer layer of capsule wall. **21, 22.** Cells of the inner layer of capsule wall. **23.** Spore. **24, 25.** Elaters.

### 3. Remarks

This taxon grows in the alpine or subalpine zone of the western Himalayas (ca 4670 m) with low temperature and long seasonal periods of snow cover. It exhibits a variety of habitat preferences as well as liverwort associates because of considerable variation in the microclimate where these plants grow. Growth of the plants is favourable under diffused sunlight on soil covered rocks, on decaying forest litter or at the base of small bushy trees at different locations along with *Lophocolea minor* Nees, *Lophozia incisa* (Schrad.) Dum., *Haplomitrium hookeri* (Sm.) Nees, *Blepharostoma trichophyllum* (L.) Dum., *Jungermannia* (Plectocolea) *limbatifolia* Amak. and species of *Calypogeia* Raddi, *Plagiochila* Dum. and *Jamesoniella* (Spruce) Schiffn. A more or less similar habitat preference as well as the liverwort associates at the generic level have been reported by Schuster (1953) in American population. *Haplomitrium hookeri* has been found for the first time growing in association with *Geocalyx graveolens.*

The stem, bearing a number of marsupia on the ventral surface, is highly undulated and internally undifferentiated (figures 1, 2). Succubously arranged leaves are obliquely inserted and slightly raised above the substratum forming a continuous gutter (channel) over the dorsal surface rather characteristic of this taxon. Both leaves and underleaves are typically bifid and the margin is usually entire except in few underleaves where a tendency of developing additional lobe on one or both sides is present.

The sexual branches are usually short and arise from the postical surface in the axil of underleaves (figure 1) as also in *Jackiella javanica.* The antheridial branches sometimes proliferate (figure 2). The bracteoles are similar to amphigastria and are relatively smaller, sometimes bearing rhizoidal outgrowth (figure 15). Owing to the absence of perianth the archegonia having short necks are surrounded by small perichaetial leaves which persist till the capsule remains embedded within the marsupium. In the early stage of development there is a rapid elongation of the lower side of the stem of the archegonial branch and eventually the apex bends upwards. After fertilization the tissue beneath the archegonial group undergoes rapid meristematic activity causing the formation of initially a small ventral tuber-like marsupium (figure 1). According to Schuster (1966) the development of the marsupium takes place "....due to an auxin secreted by the embryo or at least by something derived from the embryo—the embryo factor." However, there is no experimental proof regarding this contention. Simultaneously with the formation of marsupium the development of sporophyte also takes place. As the growth continues further the marsupium becomes pendulous and cylindrical, enclosing the developing sporophyte. The marsupium grows downwards into the substratum and remains studded with dense rhizoids of the simple type. The sporophyte even up to a late stage of development remains embedded within the marsupium. When the sporophyte is mature the seta elongates considerably and the capsule is protruded out of the calyptra as well as the marsupium. The foot is multicellular, anchor-shaped and remains embedded in the basal tissue of the marsupium (figure 16) unlike that of *Jackiella* which has a large haustorial collar consisting of numerous uniseriate septate filaments (connate at base) arising from the junction of foot and seta. The outer layer of capsule wall shows biphasic development with nodulose thickenings on radial walls slightly extending over the tangential walls

(figure 20). The cells of the inner layer have incomplete or semiannular bands formed by the fusion of thickenings on the radial walls and the tangential walls (figure 21). Such thickenings appear 'I'-shaped or very rarely 'L'-shaped in the outer layer and 'U'-shaped in the inner layer of cells in a transverse section (figure 19).

The plant has been discovered from high altitude zone of the western Himalayas —the liverwort flora of which still remains unexplored. A cursory survey of the entire collection of liverworts revealed that the area is extremely rich in rare taxa which are either poorly known or still not described in Indian bryology.

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# Ontogeny of the paracytic stoma: Variations and modifications

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**Abstract.** It was generally believed that the topography of the cells surrounding the guard cells in the mature condition indicate their mode of development. However, it has now been established that more than one ontogenetic type may correspond to a single mature type, or it may lead to the development of varied stomatal types. The paracytic stoma was studied from this viewpoint. It was found that it may be formed through one of at least eight different modes. These are classified and reviewed. The need to undertake studies on the ontogeny of this type of stoma in various groups of plants has been emphasized.

**Keywords.** Paracytic ; stoma ; ontogenetic types ; variations.

## 1. Introduction

Metcalfe and Chalk (1950) defined the paracytic stoma as a stoma "accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells ". Earlier, Vesque (1881, 1889) named such stomata as " Rubiaceous " and cited the family Rubiaceae as a typical example. Florin (1931, 1933, 1934), based on his studies of gymnosperms alone, proposed the term " syndetocheilic " for such stomata, but the name also implied a mesogenous development of the subsidiary cells. Developmental studies on stomata of the Rubiaceae by Tognini (1897) and Pant and Mehra (1965) confirmed that the parallel subsidiaries in these plants were formed from the same mother cell as the guard cells.

It was, therefore, generally believed that paracytic stomata always developed in a mesogenous or syndetocheilic manner. However, it has been well established that the developmental type should not be inferred from a study of the mature stomatal complex alone but actual developmental studies should be carried out.

Recent work (Stebbins and Jain 1960 ; Stebbins and Khush 1961 ; Pant and Kidwai 1966 ; Tomlinson 1974) on monocotyledons has brought to light the fact that here the paracytic stomata develop perigenously and the subsidiary cells are formed from lateral cells adjacent to the guard cells.

Indeed, the paracytic stomata, formed by these two entirely different modes, viz, mesogenous and perigenous, look so similar that it has aroused a great deal of

controversy and various authors have described their formation by different ontogenetic pathways in one and the same plant. In *Equisetum*, Duval-Jouve (1864) and Chatterjee (1964) mentioned that the stomatal meristemoid first divides by a periclinal wall, each of these segments then divides by an anticlinal wall to form two overlapping subsidiaries from the outer cell and two sunken guard cells from the inner cell. However, other authors (Strasburger 1866–1867 ; Johnson 1933 ; Hauke 1957 ; Pant and Mehra 1964a ; Pant and Kidwai 1968) found no periclinal division and the stomata according to them are of the ordinary paramesogenous type.

In *Gnetum*, the development of stomata was described as haplocheilic or perigenous by some authors (Maheshwari and Vasil 1961a, b ; Inamdar and Bhatt 1972) whereas, others on the basis of division figures have concluded that the two parallel subsidiary cells are formed mesogenously (Takeda 1913b : Kaushik 1974 ; Nautiyal *et al* 1976 and others), but as they fall short of the poles, the stomata may even be termed mesoperigenous.

The paracytic stoma is perhaps the most common among plants, occurring in pteridophytes, gymnosperms, monocotyledons and dicotyledons. Dilcher (1974) has pointed out that " more than one ontogenetic type may correspond to a single mature stomatal type ". This is perhaps nowhere better illustrated than in the paracytic stoma and a detailed study of its development based on published literature and illustrations has brought to light a number of different ontogenetic pathways which ultimately result in the formation of a paracytic stoma in the adult condition, the three main types being the perigenous, the mesoperigenous and the mesogenous types. Some of these types have not actually been reported but as the possibility of their discovery at a later date remains, they have been included here. Some types have been reported only as variations of other predominant types and not as characteristic of any particular taxon. The terminology according to any particular author is indicated by appropriate reference. A few new terms are also being introduced for the first time here. The following main categories have been found (figure 1).

## 2. Ontogenetic pathways leading to the formation of paracytic stomata

### 2·1. *Perigenous type* (Pant and Mehra 1964b)

Here a protoderm cell becomes directly converted into the guard cell mother cell and divides once to form the two guard cells. The parallel subsidiary cells are formed from cells on the side of the guard cells (figure 1·1).

(a) *Hemipara-perigenous type* : Only one lateral subsidiary cell is formed from a perigene cell on the side of a guard cell and the stoma is of the hemiparacytic type in the mature condition (figure 1·1a). Fryns Classens and Van Cotthem (1973) named it the mono-perigenous type but here it has been termed the hemipara-perigenous type in keeping with their hemipara mesoperigenous type. Although this type has not been described so far, Fryns Classens and Van Cotthem (1973) suggested that some stomata on *Polygonum lanigerum* (Inamdar 1969b) may follow this developmental pathway.

| | | ONTOGENETIC MODES OF PARACYTIC STOMA FORMATION | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MERISTEMOID | FIRST DIVISION | SECOND DIVISION | THIRD DIVISION | FOURTH DIVISION | FIFTH DIVISION | MATURE STOMA |
| 1·1 | *a* | PERIGENOUS | HEMI-PARA- | | | | | | | |
| | *b* | | PARA- | | | | | | | |
| 1·2 | *a* | MESO PERIGENOUS | HEMI-PARA- | | | | | | | |
| | *b* | | PARA- (TYPE 1) | | | | | | | |
| | *c* | | PARA- (TYPE 2) | | | | | | | |
| 1·3 | *a* | MESOGENOUS | PARA- | | | | | | | |
| | *b* | | HEMI PARALLELO- | | | | | | | |
| | *c* | | PARALLELO- | | | | | | | |

**Figure 1.** Diagrammatic representation of the different ontogenetic modes of paracytic stoma formation. The perigene cells and their divisions are represented by dotted lines. **1·1.** Perigenous. **a.** Hemi-para perigenous. **b.** Paraperigenous. **1·2.** Mesoperigenous. **a.** Hemi-para-mesoperigenous. **b.** Para-mesoperigenous (Type I). **c.** Para-mesoperigenous (Type II). **1·3.** Mesogenous. **a.** Para-mesogenous. **b.** Hemi-parallelo-mesogenous. **c.** Parallelo-mesogenous.

(b) *Para-perigenous type* : The stoma is formed in the perigenous manner and the two parallel subsidiaries are formed by asymmetric divisions in the cells lying on the two lateral sides of the guard cells (figure 1.1b). This has been termed the biperigenous (Paliwal 1969) or the diperigenous (Fryns Classens and Van Cotthem 1973) type but for the sake of uniformity the term paraperigenous is preferred here. This type is common in the monocotyledons and has been described in the Musaceae, Gramineae, Juncaceae, Cyperaceae, Alismataceae, Marantaceae (Strasburger 1866–1867 ; Porterfield 1937 ; Flint and Moreland 1946; Stebbins and Jain 1960 ; Metcalfe 1960), twenty-four families of monocotyledons (Stebbins and Khush 1961), Araceae (Pant and Kidwai 1966), Commelinaceae, Zingiberaceae (Tomlinson 1969), Centrolepidaceae and Phydraceae (Paliwal 1969) and others.

## 2·2. *Mesoperigenous type* (*Pant and Mehra* 1964*b*)

The cells surrounding the guard cells in the adult condition have a dual origin, some being formed from the same meristemoid as the guard cells while the others are perigenous in origin (figure 1.2).

(a) *Hemipara-mesoperigenous type (Fryns Classens and Van Cotthem* 1973) : The single lateral subsidiary cell is formed from the stomatal meristemoid (figure 1·2a) before the formation of the two guard cells, *e.g.*, *Lophosoria* (Kondo and Toda 1956, 1959), some Rubiaceae (Pant and Mehra 1965), Nyctaginaceae (Inamdar 1968), *Bigonia* (Inamdar 1969a), *Crotalaria* (Shah and Gopal 1969), *Kalanchoe* (Inamdar and Patel 1970), Polemoniaceae, Boraginaceae and Solanaceae (Patel and Inamdar 1971) and some stomata of *Rauwolfia* and *Tabernae montana* (Trivedi and Upadhyay 1976) and *Annona* (unpublished observations).

(b) *Para-mesoperigenous type I or Para-eumesoperigenous type* : The adult paracytic stoma is surrounded by two parallel subsidiaries, one of which is formed from the stomatal meristemoid and the other by a division in the perigene cell on the other side of the guard cells (figure 1·2b). This type is the true mesoperigenous paracytic type (*cf* type 1·2c) and although it has not been described so far, it has been included here as the possibility of its occurrence exists. According to Payne (1970), some stomata in *Liriodendron tulipidera* may be formed in this manner.

(c) *Para-mesoperigenous type II or Para-pseudomesoperigenous type* : The adult stoma is paracytic and its two lateral subsidiary cells are formed from the same initial as the guard cells (mesogenous). However, they do not meet at one or both poles of the guard cells and the neighbouring perigene cells abut on the polar ends of the guard cells (figure 1·2c). Due to this falling short of the mesogene lateral subsidiaries towards the poles, the stoma is considered as mesoperigenous. This view has been expressed by Pant and Mehra (1964), Pant (1965), Fryns Classens and Van Cotthem (1973) and Nautiyal *et al* (1976), *e.g.*, *Welwitschia* (Takeda 1913a), *Gnetum* (Takeda 1913b; Nautiyal *et al* 1976), *Drimys* (Bondeson 1952), *Linum* (Paliwal 1961), *Borreria* and *Oldenlandia* (Pant and Mehra 1965), *Bignonia* (Inamdar 1969a), Polygonaceae (Inamdar 1969b), *Jasminum* (Inamdar *et al* 1970), *Claytonia* (Payne 1970), *Casuarina* (Pant *et al* 1975), *Xylonymus* and *Brassianthus* (Den Hartog and Baas 1978), and some stomata in *Zornia* (Kannabiran 1975a), *Abrus precatorius* (Kannabiran 1975b), *Dipteria* (Khare 1978) and *Annona* (unpublished observations).

If the mesogene subsidiaries meet at only one of the poles, the stoma is frequently surrounded by three cells and appears aniso- or tricytic, *eg*. some stomata in *Rumex* (Verma 1975), *Zornia* (Kannabiran 1975a), *Brassiantha* (Den Hartog and Baas 1978) and *Annona* (unpublished observations).

2·3. *Mesogenous type (Pant and Mehra* 1964*b*)

The lateral subsidiary cells are mesogenous in origin and they completely flank the guard cells so that no other cells immediately surround the guard cells (figure 1·3).

(a) *Para-mesogenous type (Fryns Classens and Van Cotthem* 1973) : There are only two lateral subsidiary cells, parallel to the guard cells and these are mesogenous (figure 1·3a). Recorded from Cheiropleuriaceae, Dipteridaceae and Dicksoniaceae, *Phorbitis*, *Basella* and *Opuntia* (Strasburger 1866–1867), *Convolvulus*, Euphorbiaceae, *Impatiens* and *Coffea* (Tognini 1897). Magnoliaceae (Paliwal

and Bhandari 1962 ; Pant and Gupta 1966), *Cheiroplueria* and *Cibotium* (Kondo and Toda 1959), Convolvulaceae (Pant and Banerjee 1965), Rubiaceae (Pant and Mehra 1965), *Strapleonema* (Stace 1965), Portulaceceae, *Trianthema* (Ramayya and Rajagopal 1968), Dicksoniaceae, Dipteridaceae and Lophosoriaceae (Van Cotthem 1970), Nyctaginaceae, Polygonales, Centrospermae, Zygophyllaceae, Simerubaceae, Salvadoraceae, *Bigonia* and *Ipomea* (Inamdar 1968, 1969a, b, c, d, e), Leguminosae (Shah and Gopal 1970; Bora and Baruah 1979; Farooqui 1979), *Zornia* (Kannabiran 1975a), *Abrus precatorius* (Kannabiran 1975b), *Rauwolfia* and *Chatharanthus* (Trivedi and Upadhyay 1973, 1976, 1977), *Bhesa* sp. and *Hedraianthera* (Den Hartog and Baas 1978), *Dipteris* (Khare 1978) and many others.

(b) *Hemiparallelo-mesogenous type* : Three cells are cut off from the stomatal meristemoid in an alternate fashion so that the stoma is surrounded by 2 inner subsidiary cells and an outer encircling cell (figure 1·3b). This was first described by Payne (1970) under the parallelocytic type. However, as only one additional cell is formed, it has been separated here, *e.g.*, some stomata of *Cinchona succirubra* (Pant and Mehra 1965), Magnoliaceae (Pant and Gupta 1966) and *Rauwolfia serpentina* (Trivedi and Upadhyay 1976).

(c) *Parallelo-mesogenous type* (*Fryns Classens and Van Cotthem* 1973): The meristemoid, instead of forming only two parallel subsidiary cells divides further in the same alternate manner and forms two or more additional cells (figure 1·3c) which surround the subsidiaries and are parallel to them (encircling cells), *e.g.*, Cactaceae, Convolvulaceae, Euphorbiaceae, Leguminosae, Portulacaceae, Rubiaceae and Umbelliferae (Payne 1970), *Euonymus globularia* (Den Hartog and Baas 1978).

The paracytic stoma may also show variations by further divisions in its subsidiary cells. If one of the subsidiary cells divides the stoma may appear tricytic or anisocytic in the adult condition. If both the subsidiaries divide, the stoma may appear tetracytic or anomocytic. All these variations have been noticed in *Strychnos* (unpublished observations). Therefore, although their adult form may differ, they begin their development in essentially the same manner.

## 3. Discussion

The paracytic stoma may be formed by many different ontogenetic pathways. It is, therefore, essential that actual developmental studies should be carried out before their ontogeny can be determined.

Of the eight categories described, the most common are the para-perigenous, para-pseudomesoperigenous and the para-mesogenous types. The others represent variations of these and their occurrence is limited or not reported so far. A study of development in varied groups of plants may lead to their discovery at a later date.

Metcalfe and Chalk (1950) have listed 105 dicotyledonous families in which the paracytic type of stoma is predominant. In addition a number of monocotyledons' pteridophytes and gymnosperms also have the paracytic stoma. There is an urgent need to work out the exact mode of stomatal development in all these taxa.

There are other paracytic stomata, like those of the Cycadeodales, whose development will probably never be determined because they occur only as fossils.

According to Takhtajan (1969) the paracytic type of stoma is the most likely basic type of stomatal apparatus in the evolution of flowering plants. Its occurrence in such diverse groups as the pteridophytes, gymnosperms, monocotyledons and dicotyledons may support such an assumption.

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# Growth response of some thermophilous fungi at different incubation temperatures

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**Abstract.** Growth response of 21 thermophilous fungi at 10 different temperatures from 15–62° C has been studied. These fungi could be categorized into three groups, i.e., microthermophiles, thermotolerant and true thermophiles. The temperature relations of 6 thermophilous fungi namely *Aspergillus tamarii*, *A. terreus* var. *aureus* (microthermophiles), *A. nidulans* var. *echinulatus*, *A. viridi-nutans*, *A. fumigatus* var. *ellipticus* and *A. caespitosus* (thermotolerant) are being reported for the first time. The growth rates of different fungi varied from 0·19–1·25 mm/hr at their optimum temperatures. *Penicillium* sp. the slowest and *Thermoascus* sp. were the fast growing fungi.

**Keywords.** Microthermophiles; thermotolerant; thermophiles; temperature; growth rates.

## 1. Introduction

Thermophilous fungi are those which show temperature optima in the range of 25 to 55° C (Apinis 1963). These fungi play a significant role in degradation of plant material, composting and humification (Fergus 1971 ; Cailleux 1973 ; Tansey and Brock 1978 ; Jain *et al* 1979). The occurrence of thermophilous fungi is now known to be ubiquitous (Tansey and Brock 1978). In India these fungi have also been reported from various substrates like compost (Maheshwari 1968 ; Qureshi and Johri 1972), stored grains (Mehrotra and Basu 1975), coal mine soils (Thakre and Johri 1976), nesting materials of birds (Satyanarayana *et al* 1977) and from air (Thakur 1977). Recently, Sandhu *et al* (1980) and Sandhu and Singh (1981) studied the occurrence and some ecological features of thermophilous fungi associated with decomposing sugarcane bagasse, and forest soils. The present study describes the effect of different incubation temperatures on the growth rates and development of these fungi.

## 2. Materials and methods

Twenty-one fungi isolated from bagasse and forest soils were studied for their response to different temperatures. The culture media used for the identification

of aspergilli and *Penicillium* sp. were czapek's agar, malt extract agar and yeast glucose agar respectively (Raper and Fennell 1973). Yeast starch agar (Cooney and Emerson 1964) was used for the remaining fungi belonging to mucorales, ascomycetes and hyphomycetes. Mycelial discs of 3 mm diameter from the periphery of growing colonies were used as the inoculum and the plates were incubated at 15° C, 20° C, 27° C and 32–62° C with 5° C interval. The petri plates kept at 42–62° C were placed in polythene bags and a beaker of water was also placed in each of these incubators to avoid desiccation. The bags were opened twice daily to aerate the cultures. Growth response was recorded in terms of colony diameter daily. Sporulation and cleistothecia production were noted visually and confirmed by observing under the binocular and by slide preparations. The growth rate of each fungus was calculated on yeast starch agar as follows :

$$\text{Growth rate} = \frac{\text{Average diameter in mm of four petri plates}}{\text{Total time period in } h}.$$

## 3. Results and discussion

Based on colony diameter the growth response of 21 fungi to 10 different incubation temperatures yielded 2 microthermophiles, 15 thermotolerant and 4 true thermophiles (table 1). A comparison of our results with the literature revealed that the temperature relations of 6 thermophilous fungi namely *Aspergillus tamarii*, *A. terreus* var. *aureus* (microthermophile), *A. nidulans* var. *echinulatus*, *A. viridinutans*, *A. fumigatus* var. *ellipticus* and *A. caespitosus* are being reported for the first time. The temperature relations of the other fungi studied, in general, fall within the range described by Tansey and Brock (1978). Temperature responses of *Thermomyces lanuginosus*, *Humicola grisea* var. *thermoidea* and *Mucor pusillus* are more close to those reported by Fergus (1964) and Evans (1971) respectively than Apinis (1963) and Qureshi and Johri (1972). However, *Humicola grisea* var. *thermoidea* differed from Evans (1971) since our isolates could not grow at the minimum temperature of 20° C. The cardinal temperatures of *Aspergillus fumigatus* and *Sporotrichum thermophile* varied widely from Qureshi and Johri (1972) and almost fell within the range given by Evans (1971) and Tansey and Brock (1978). In the case of *Aspergillus nidulans* and *A. terreus* the cardinal temperatures were similar to those reported by Evans (1971) but had more variation of minimum temperature than maximum. This may be due to the fact that in our study minimum temperature was recorded up to 15° C only. The temperature variations reported above may be due to strain variations, nutritional requirements and temperature study at wider temperature intervals.

There was also variation in temperature regarding the optimum and the range among the species and their varieties, e.g., *Aspergillus fumigatus* and *A. fumigatus* var. *ellipticus* had their optimum temperature at 37° C and 42° C respectively (figure 1). In the case of *A. terreus* and *A. terreus* var. *aureus* both had their optimum temperature at 37° C. The former could grow up to 47° C while the limit for the latter was at 42° C (figure 1 and table 1). There was a variation in optimum temperature for the development of the perfect and imperfect stage as in the case of *A. nidulans* and *A. nidulans* var. *echinulatus*, maximum cleistothecia formation was observed at 42° C while the sporulation was maximum at 37° C,

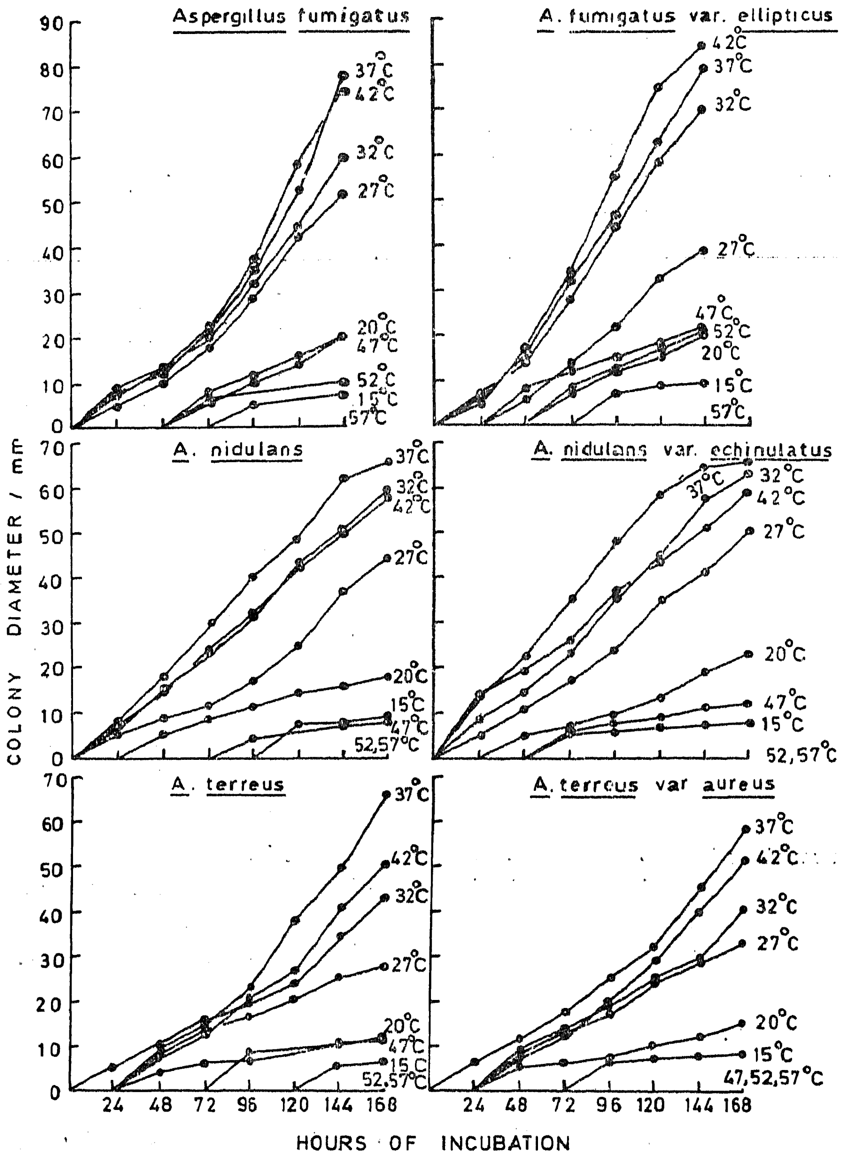


**Figure 1.** Growth response of one microthermophile (*Aspergillus terreus* var. *aureus*) and 5 thermotolerant fungi at different temperatures.

Similarly, abundant superficial cleistothecia like bodies of *A. caespitosus* were seen at 42° C while the optimum temperature for sporulation was 37° C. The temperature range for sporulation and reproductive structures in all the groups was narrower than that for mycelial growth.

The growth rates of optimum temperature of 21 fungi varied from 0·19–1·25 mm/hr (figure 2). *Penicillium* sp. was the slowest and *Thermoascus* sp. the fastest growing species. The fungi having growth rates of 0·19–0·39, 0·40–0·75

**Table 1.** Optimum temperature and range for growth and sporulation of 21 thermophilous fungi.

| Group | | Temperature range (°C) for mycelial growth | Temperature range (°C) for sporulation | Optimum temperature (° C) | Fungi |
|---|---|---|---|---|---|
| Microthermophile | | 15–42 | 20–37 | 32 | *Aspergillus tamarii* (S*) |
| | | | | 37 | *A. terreus* var. *aureus* (B) |
| Thermotolerant (psychrotolerant) | I | 15–47 | 27–42 | 32 | *Paecilomyces varioti* (B) |
| | | | | 37 | *Aspergillus nidulans* (S), *A. nidulans* var. *echinulatus* (S), *A. terreus* (S), *A. caespitosus* (B), *Acrophialophora fusispora* (B), *Penicillium* sp. (B) |
| | | | | 42 | *Thielavia sepedonium* (S), *Absidia corymbifera* (B) |
| | II | 15–52 | 27–47 | 37 | *Aspergillus fumigatus* (B), *A. viridinutans* (S), *Sporotrichum thermophile* (B) |
| | | | | 42 | *Aspergillus fumigatus* var. *ellipticus* (S), *Absidia spinosa* (B), *Rhizopus microsporus* (S) |
| True thermophiles | I | 20–52 | 27–47 | 42 | *Mucor pusillus* (B, S) |
| | II | 27–52 | 32–47 | 47 | *Humicola grisea* var. *thermoidea* (B) |
| | III | 32–52 | 37–47 | 42–52 | *Thermoascus* sp. (S) |
| | IV | 32–57 | 37–52 | 47 | *Thermomyces lanuginosus* (B, S) |

* Source of fungi. B, bagasse and S, forest soil.

and 0·86–1·25 mm/hr were categorized as slow, moderate and fast growing respectively. Comparison of growth rates at the optimum temperatures of these fungi show that 8 are slow growing and out of these 2 are microthermophiles and 6 thermotolerant. Nine species belong to moderately growing group, 7 of which are thermotolerant and 2 true thermophiles. In the fast growing group 2 are true thermophiles and 2 thermotolerant.

The growth response of these fungi to different temperatures was determined on the basis of colony diameter. According to Hawker (1950) and Cochrane (1958) the colony diameter alone does not account for density height and depth of the colony. However, it has been justified for studies in which only one environmental variable is studied for example temperature (Brancato and Golding 1953 ; Cochrane 1958 ; Evans 1971 ; Trinci 1971 ; Tansey 1972).

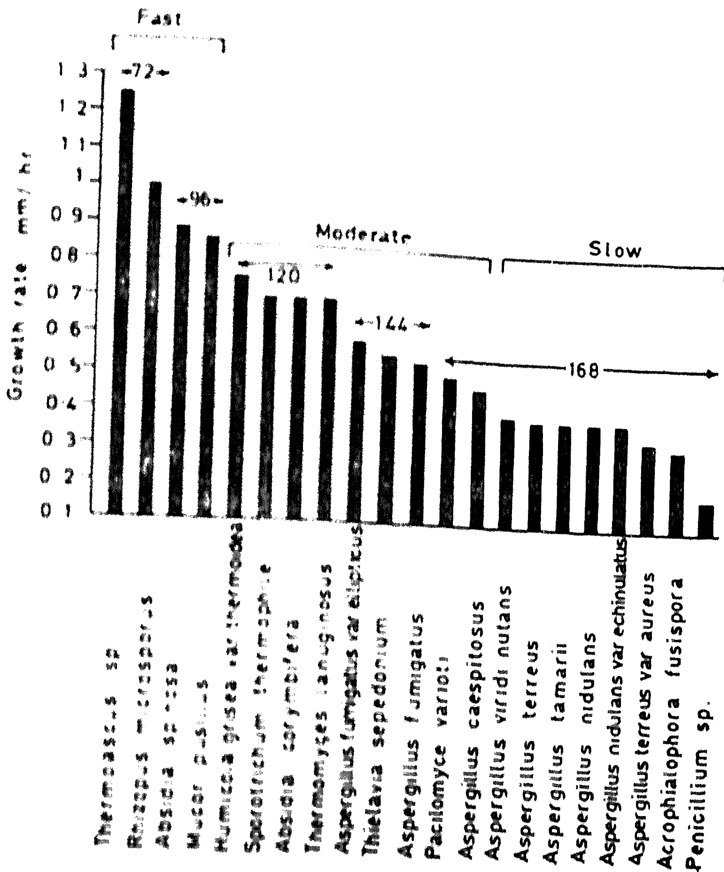


**Figure 2.** Comparative maximum growth rates at optimum temperatures of 21 thermophilous fungi indicating fast, moderate and slow growing groups. Figures above the bars indicate period of incubation in hours.

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# Studies on *Beggiatoa* : Distribution and growth in aquatic habitats of Visakhapatnam

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**Abstract.** *Beggiatoa* is distributed in 11 of the 12 polluted aquatic habitats occurring on the sediment and decaying leaves as a thick white scum at 2, in detectable population at 6, and in very less population at 3 habitats where $H_2S$ necessary for its growth is produced. The *Beggiatoa* spp. present in these habitats are considered as belonging to the 6 species described in Bergey's manual. *B. minima* with less than 1·0, *B. leptomitiformis* with 1·66, *B. alba* with 3·32 and 4·98 μ wide trichomes are distributed in 2, 6, 11 and 2 habitats respectively, which are fresh water, brackish and marine habitats. *B. arachnoidea* with 9·96, *B. mirabilis* with 17·0 and *B. gigantea* with 28·22 μ wide trichomes are present in only one brackish habitat. *B. alba* (3·32 μ) occurs alone at 2 habitats, and in association with either one or more of the other *Beggiatoa* species at the remaining 9 habitats. Except *B. mirabilis* and *B. gigantea*, the others distributed in the 11 habitats could multiply in enrichment culture media that contained extracted rice straw pieces and waters from their respective habitats, and aggregate into visual white colonies, or loose clumps in a thick film on the surface of medium, rice straw pieces and glass. *B. mirabilis* and *B. gigantea* seem to be halophilic, truly autotrophic and more exacting than the other species that exhibited differences in salt tolerance when grown in crude enrichment culture media of differing salt content. It appears that physiological strains or groups differing in salt tolerance may be existing in these species of *Beggiatoa*.

**Keywords.** *Beggiatoa* ; distribution ; growth ; aquatic habitats ; Visakhapatnam ; enrichment culture medium.

## 1. Introduction

*Beggiatoa* is a large, colourless, filamentous, gliding bacterium, and easily recognizable. It is usually considered as carrying on chemolithotrophic nutrition, oxidizing $H_2S$, and depositing sulphur intracellularly as droplets. It is widespread in distribution, occurring in lake bottoms, stream beds, ponds, sulphur springs, brackish and marine habitats; even in the rice rhizosphere (Pitts *et al* 1972) and water-saturated soils of rice fields (Joshi and Hollis 1977), playing an important role, the detoxification of $H_2S$ formed due to putrefaction, and activities of anaerobic organisms such as *Desulfovibrio* spp. It is associated with, and also

often described as indicative of strongly polluted polytrophic habitats. Ever since the investigations, and the concept of chemolithotrophy by Winogradsky (1887, 1888, 1889) attempts have been made (Keil 1912 ; Cataldi 1940 ; Faust and Wolfe 1961 ; Lackey 1961 ; Scotten and Stokes 1962 ; Pringsheim 1964 ; Kowallik and Pringsheim 1966 ; Jorgensen 1977) to increase the small number of *Beggiatoa* trichomes present in samples from natural habitats into a rich population by reliable enrichment culture techniques for isolation and cultivation in pure culture media ; to study its distribution, morphology, growth and nutrition. Pringsheim (1964) studied in detail the species differentiation of *Beggiatoa*, and pointed out among other problems that the number and types of forms found under a variety of ecological conditions are obscure.

The review of the literature showed the need for detecting and isolating as many forms of *Beggiatoa* as possible for taxonomic differentiation, and studying its nutrition ; and more so because there are no reports from India. Since Visakhapatnam abounds with a variety of aquatic habitats that are continuously polluted with urban refuse, sewage and industrial effluents, detailed investigations are undertaken to study the distribution, growth, nutrition and species differentiation of *Beggiatoa*. This paper presents the observations and experimental results on distribution and growth of *Beggiatoa* isolated from natural " polluted " habitats, for the first time from India.

## 2. Materials and methods

Twelve sites of different aquatic habitats in Visakhapatnam were surveyed for *Beggiatoa*. Their location and some relevant features are as follows :

*Site* 1 : The edge of a shallow one feet wide canal of a septic tank near the Botany department garden, Andhra University campus. The entire edge is covered with a thick white scum. The decaying leaves present in the canal and at the edge are also covered with a white scum. The sediment is black silt. pH of water : 7, salinity : 0·58 ppt.

*Site* 2 : The edge of a shallow sewage canal near Rama Krishna Mission, Maharanipet. The sediment is fine sand. pH of water : 7, salinity : 0·72 ppt.

*Site* 3 : The edge of a shallow sewage canal near Visakha women's college, Maharanipet. The sediment is black silt. pH of water : 7, salinity : 1·07 ppt.

*Site* 4 : The edge of a stagnant pond receiving sewage water near " convent junction ". The sediment is black silt. pH of water : 7, salinity : 1·07 ppt.

*Site* 5 : The edge of a sewage stream near " naval coast battery ", Maharanipet. The sediment is light black fine sand. pH of water : 7, salinity : 1·08 ppt.

*Site* 6 : The edge of a sewage stream near the wooden bridge, ferry road-old post-office junction. The stream joins the harbour channel, and the sample site is at about 200 metres from the channel. During high tide there will be a back flow of sea water. The sediment is black silt. pH of water : 7, salinity : 1·4 ppt.

*Site* 7 : The edge of a stream adjacent to oil-loading tanks near naval headquarters. The bank is muddy, and covered with a thick asphalt-like layer mixed

with cakes and clumps of resin-like material, a conversion product of oil. pH of water : 7·5, salinity : 4·5 ppt.

*Site* 8 : The edge of the sea water canal carrying effluents from the Coromandel fertilizers factory. The site is located near the Hindustan Petroleum Oil Refineries entrance gate. The sediment is light red silt. pH of water : 5 and 2, salinity 19·0 ppt.

*Site* 9 : The edge of a stagnant brackish water pond near 'Chavulamadam'. The sediment is black silt. pH of water : 8·5, salinity : 20·0 ppt.

*Site* 10 : The edge of one of the Harbour channel terminals near sulphur-unloading berth of the harbour. The bank is fine sandy covered with a thick asphalt-like layer mixed with cakes and clumps of a resin-like material, a conversion product of oil. pH of water : 7·5, salinity : 28·0 ppt.

*Site* 11 : The edge of the sea water canal carrying effluents from Hindustan Petroleum Oil Refineries. The bank is muddy and covered with a thick asphalt-like layer mixed with cakes and clumps of a resin-like material like above, but covered with a thick white scum. pH of water : 7, salinity : 31·25 ppt.

*Site* 12 : The edge of the sea coast near the fishing outer harbour. The sediment is light black fine sand. pH of water 7, salinity : 34·0 ppt.

At these sites the sediment samples along with some water were collected with a thoroughly washed stainless steel spoon into 250 ml Erlenmayer flasks. Decaying leaves, or decaying leaves covered with white scum were also collected into separate flasks. The surface water samples were collected into 500 ml flasks. The flasks were plugged with cotton.

The pH of surface water samples was noted using pH indicator papers (BDH) supplied by the Chemicals Division, Glaxo Laboratories (India) Ltd., Bombay.

The salintiy of surface water samples was estimated after centrifuging at 4000 r.p.m. for 15 min by conductivity method using direct reading conductivity meter-303 (Systronics, Ahmedabad), and conductivity of different concentrations of common salt solution as standard.

To detect the occurrence of *Beggiatoa*, the sediment, decaying leaf and water samples on arrival at the laboratory, immediately and also after 24 hr incubation in darkness at room temperature, *ca* 28° C, were repeatedly examined microscopically.

To ascertain the distribution of *Beggiatoa* the samples were subjected to crude enrichment culture technique described by Faust and Wolfe (1961) with some modifications. Instead of seasoned roadside winter grass, seasoned rice straw was used. The procedure to extract and dry rice straw pieces of 2 cm in size was the same as that of Faust and Wolfe. The enrichment medium for sample of each site consisted simply of 0·6 g of extracted rice straw bits and 70 ml of surface water collected at the site. The surface water used is referred to as site water. The media were taken into 150 ml flasks, and plugged with cotton to prevent evaporation. The media were not sterilized, because sterilization in the initial studies proved to be less effective in increasing the population of *Beggiatoa* trichomes. The unsterilized media were inoculated with sediment of about 1 ml in volume, or 2 decaying leaf bits of about 1 cm² in size, or sediment plus decaying leaf bits. One set was left without adding either sediment or decaying leaf bits,

They were incubated at room temperature, *ca* 28° C, for 30 days in darkness.

To study their growth, and salt tolerance the same crude enrichment culture technique was followed with some alterations. The enrichment medium for *Beggiatoa* of each site consisted of the same quantity of rice straw bits, and 70 ml of either respective unfiltered site water or filtered site water, or filtered and stored sea water (pH 7·0 ; salinity 35·0 ppt), or tap water (pH 6·8 ; salinity 0·31 ppt), or metal distilled water (pH 6·5 ; salinity 0·016 ppt). The media were taken in 150 ml flasks. These were also not sterilized. They were inoculated with sediment only, and incubated as above. The site and sea waters were filtered through Whatman No. 1 filter paper.

The crude enrichment cultures were observed directly, and also examined microscopically. The amount of growth was estimated visually because of the tendency of the organisms to form aggregations of trichomes, fragmentation of trichomes, and due to certain practical difficulties in estimating the exact amount of growth by other methods in vogue

To test sulphur, the trichomes were transferred on to microscope slides, and treated with aceto-carmine (Ellis 1932).

To identify the species of *Beggiatoa*, the width of the trichomes, which was uniform, was considered and measured at × 970 magnification.

To observe the gliding movement on agar surface, the method of Faust and Wolfe (1961) was followed using their modified Cataldi's agar medium dried at 60° C for about 10 min.

## 3. Results and discussion

The species differentiation in *Beggiatoa* is mainly based on the width of trichomes. According to the usual nomenclature of *Beggiatoa* forms (Buchanan and Gibbons 1974), the forms with 1$\mu$ or less wide are called *B. minima* Winogradsky ; those between 1 and 2 $\mu$ wide trichomes are called *B. leptomitiformis* Trevisan ; those between 2·5 and 5 $\mu$ wide as *B. alba* (Vaucher) Trevisan ; those between 5 and 14 $\mu$ wide as *B. arachnoidea* (Agardh) Rabenhorst ; those between 15 and 21 $\mu$ wide as *B. mirabilis* Cohn ; and those between 26 and 55$\mu$ wide as *B. gigantea* Klas. However, Pringsheim (1964) while discussing the species concept in *Beggiatoa* concluded that after dropping for taxonomic purposes the use of the arrangement of the sulphur droplets etc., one has to drop that of the width also, and there is no feature to replace it. In the present study as there is no other feature except width for species identification, width alone considered for identifying the species of *Beggiatoa*, and the usual nomenclature is followed in mentioning them.

In the present study, all the above mentioned 6 species of *Beggiatoa* are identified in the aquatic habitats of Visakhapatnam (figure 1). The trichomes of *B. minima* and *B. leptomitiformis* are less than 1·0 and 1·66 $\mu$ in width respectively. Of *B. alba*, two distinct kinds or strains of consistent and uniform width were observed. The trichomes of one are 3·32 $\mu$, and those of the other are 4·98 $\mu$ wide. The trichomes of *B. arachnoidea*, *B. mirabilis* and *B. gigantea* are 9·96, 17·0 and 28·22 $\mu$ wide respectively.

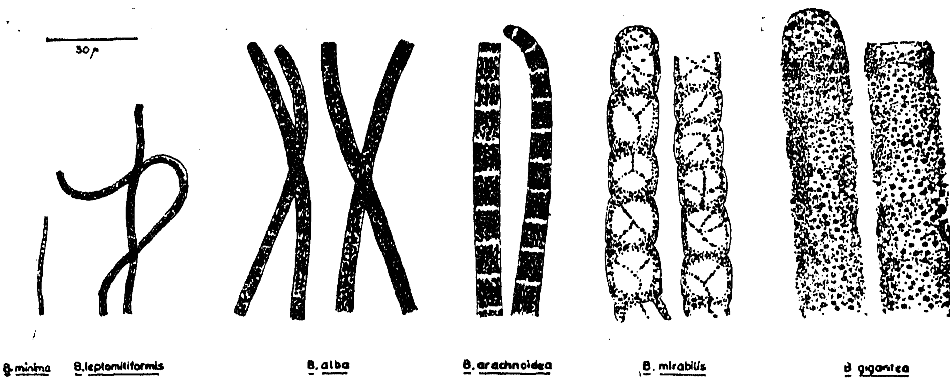



**Figure 1.** The *Beggiatoa* spp. occurring in the aquatic habitats of Visakhapatnam.

**Table 1.** Distribution of *Beggiatoa* spp. in the sites.

| Site No. | *B. minima* | *B. lepto-mitiformis* | *B. alba* | | *B. arach-noidea* | *B. mirabilis* | *B. gigantea* |
|---|---|---|---|---|---|---|---|
| | (1·0 μ) | (1·66μ) | (3·32μ) | (4·98μ) | (9·96μ) | (17·0μ) | (28·22μ) |
| 1. | + | + | + | − | − | − | − |
| 2. | − | − | + | − | − | − | − |
| 3. | − | + | + | − | − | − | − |
| 4. | − | − | + | + | − | − | − |
| 5. | − | + | + | − | − | − | − |
| 6. | − | − | + | − | − | − | − |
| 7. | − | − | + | + | − | − | − |
| 8. | − | − | − | − | − | − | − |
| 9. | − | + | + | − | − | − | − |
| 10. | − | + | + | − | + | + | + |
| 11. | − | + | + | − | − | − | − |
| 12. | + | − | + | − | − | − | − |

- indicates presence and — indicates absence of *Beggiatoa* group.

The distribution of these 6 species in the 12 sites given in table 1 shows that
*eggiatoa* is distributed in all the sites, except site 8 of the stream carrying
ffluents from Coromandel Fertilizers Factory. In the sediment, the decaying
af and the water samples of this site, which is at a distance of about 2 km
om the factory, except a few minute unicellular bacteria, neither *Beggiatoa* nor
ny other associated organisms were found even when the samples were subjected
enrichment culture technique (tables 1, 2, 3). Further, when the sediment was
isturbed for collection there was no odour of $H_2S$, indicating the absence of
ny kind of organisms responsible for $H_2S$ production. It appears that the high
cidity of water (pH 5 to 2) due to the effluents released is the main cause for the

**Table 2.** Growth of *Beggiatoa* spp. in crude enrichment culture media containing site waters.

| Site No. | *Beggiatoa* spp. | Culture medium and inoculum | | | |
|---|---|---|---|---|---|
| | | Site water | Site water + sediment | Site water + decaying leaf | Site water + sediment and decaying leaf |
| 1. | *B. minima* | ++ | +++ | +++ | +++ |
| | *B. leptomitiformis* | +++ | ++++ | ++++ | ++++ |
| | *B. alba* (3·32 μ) | ++ | +++ | +++ | +++ |
| 2. | *B. alba* (3·32 μ) | +++ | ++++ | ++++ | ++++ |
| 3. | *B. leptomitiformis* | ++++ | ++++ | ++++ | ++++ |
| | *B. alba* (3·32 μ) | +++ | +++ | +++ | +++ |
| 4. | *B. alba* (3·32 μ) | +++ | ++++ | ++++ | ++++ |
| | *B. alba* (4·98 μ) | + | ++ | ++ | ++ |
| 5. | *B. leptomitiformis* | ++++ | ++++ | ++++ | ++++ |
| | *B. alba* (3·32 μ) | +++ | +++ | +++ | +++ |
| 6. | *B. alba* (3·32 μ) | ++ | ++++ | ++++ | ++++ |
| 7. | *B. alba* (3·32 μ) | ++++ | ++++ | ++++ | ++++ |
| | *B. alba* (4·98 μ) | + | ++ | ++ | ++ |
| 8. | .. | – | – | – | – |
| 9. | *B. leptomitiformis* | ++++ | ++++ | ++++ | ++++ |
| | *B. alba* (3·32 μ) | +++ | +++ | +++ | +++ |
| 10. | *B. leptomitiformis* | ++++ | ++++ | ++++ | ++++ |
| | *B. alba* (3·32 μ) | ++++ | ++++ | ++++ | ++++ |
| | *B. arachnoidea* | ++++ | ++++ | ++++ | ++++ |
| | *B. mirabilis* | * | * | * | * |
| | *B. gigantea* | * | * | * | * |
| 11. | *B. leptomitiformis* | + | +++ | +++ | +++ |
| | *B. alba* (3·32 μ) | + | ++ | ++ | ++ |
| 12. | *B. minima* | ++ | +++ | +++ | +++ |
| | *B. alba* (3·32 μ) | + | ++ | ++ | ++ |

– = Nil, + = Very poor, ++ = Poor, +++ = Fair, ++++ = Good.

* No growth, but survived for 7 days after incubation.

**Table 3.** Growth of *Beggiatoa* spp. in enrichment culture media containing different waters.

| Site No. | Site water salinity (ppt) | *Beggiatoa* spp. | Culture media with | | | | |
|---|---|---|---|---|---|---|---|
| | | | Unfiltered site water | Filtered site water | Tap water (salinity 0·31 ppt pH 6·8) | Distilled water (salinity 0·016 ppt pH 6·5) | Filtered sea water (salinity 35·0 ppt pH 7-0) |
| 1. | 0·58 | *B. minima* | +++ | +++ | +++ | +++ | – |
| | | *B. leptomitiformis* | ++++ | ++++ | ++++ | +++ | – |
| | | *B. alba* (3·32 μ) | +++ | +++ | +++ | +++ | – |
| 2. | 0·72 | *B. alba* (3·32 μ) | ++++ | ++++ | ++++ | ++++ | – |
| 3. | 0·72 | *B. leptomitiformis* | ++++ | ++++ | +++ | +++ | – |
| | | *B. alba* (3·32 μ) | +++ | +++ | ++ | ++ | – |
| 4. | 1·07 | *B. alba* (3·32 μ) | ++++ | ++++ | +++ | – | – |
| | | *B. alba* (4·98 μ) | +++ | +++ | ++ | – | – |
| 5. | 1·08 | *B. leptomitiformis* | ++++ | ++++ | +++ | +++ | – |
| | | *B. alba* (3·32 μ) | +++ | +++ | ++ | ++ | – |
| 6. | 1·40 | *B. alba* (3·32 μ) | ++++ | ++++ | – | – | ++ |
| 7. | 4·50 | *B. alba* (3·32 μ) | ++++ | ++++ | ++ | ++ | – |
| | | *B. alba* (4·98 μ) | +++ | +++ | + | + | – |
| 8. | 19·0 | .. | – | – | – | – | – |
| 9. | 20·0 | *B. leptomitiformis* | ++++ | ++++ | ++++ | ++++ | – |
| | | *B. alba* (3·32 μ) | +++ | +++ | +++ | +++ | – |
| 10. | 28·0 | *B. leptomitiformis* | ++++ | ++++ | +++ | ++ | +++ |
| | | *B. alba* (3·32 μ) | ++++ | ++++ | +++ | ++ | +++ |
| | | *B. arachnoidea* | ++++ | ++++ | +++ | – | +++ |
| | | *B. mirabilis* | * | * | @ | @ | * |
| | | *B. gigantea* | * | * | @ | @ | * |
| 11. | 31·25 | *B. leptomitiformis* | +++ | ++++ | ++ | – | ++ |
| | | *B. alba* (3·32 μ) | ++ | +++ | ++ | – | ++ |
| 12. | 34·0 | *B. minima* | +++ | + | + | – | + |
| | | *B. alba* (3·32 μ) | ++ | + | + | – | + |

– = Nil, + = Very poor, ++ = Poor, +++ = Fair, ++++ = Good.
* No growth, but survived for 7 days after incubation.
@ Not survived for even 1 day after incubation.

absence of not only *Beggiatoa* but also other associated organisms. On the contrary, the samples of the site 11 of the nearby stream carrying effluents from Hindustan Petroleum Oil Refineries, when examined immediately or after 24 hr incubation on arrival at the laboratory, were teeming with trichomes of *B. leptomitiformis* followed, in number, by those of *B. alba* (3·32 $\mu$), in association with some protozoans, *Oscillatoria* filaments and even nematodes. Further, the entire bank of the stream is covered with a thick asphalt-like layer mixed with cakes or clumps of resin-like material, a conversion product of oil, which in turn are covered with a white scum in which the two *Beggiatoa* spp. are predominant. In addition, when the scum was disturbed for collection there was a strong odour of $H_2S$ also. The same is almost the case with site 1 of the narrow and shallow drain of a septic tank, where *B. leptomitiformis* trichomes followed, in population, by those of *B. alba* (3·32 $\mu$) and *B. minima* are present in association with *Thiospira* and some other minute bacteria, protozoans, *Oscillatoria* filaments, nematodes, and even insect worms. At the remaining sites, no such white scum was observed. However, in the samples of the sites 4, 5, 6, 7, 9 and 10 were found the *Beggiatoa* spp. listed against each site in table 1. These are also associated with some minute bacteria, protozoans, *Oscillatoria* filaments, diatoms, nematodes and insect worms. The samples of site 10 contained, in addition, aggregations of many actively gliding spiral trichomes of *Spirulina* sp. At these sites also, when the sediment was disturbed for collection there was a feeble odour of $H_2S$. On the other hand, in the samples of the sites 2, 3 and 12, *Beggiatoa* spp. alone were not detected in the initial microscopic examination. However, there was a feeble odour of $H_2S$ at the sites, when the sediment was disturbed for collection.

The trichomes of these *Baggiatoa* spp. present in the samples collected were full of intracellularly deposited sulphur globules, and in active gliding condition. In samples incubated for 24 hr in darkness, the trichomes present in the sediment, or on the decaying leaves, or in the white scum aggregated into loose white clumps on the surface of the glass, and as a white film at the surface of water. It appears that the trichomes had moved out of the sediment, or decaying leaves, or white scum, and aggregated on the surface of the glass, and water by gliding over the surface of glass. However, in samples from sites 2, 3, 8 and 12 no such aggregations were found. But, when the samples of these sites were subjected to enrichment culture technique, *B. minima*, *B. leptomitiformis* and *B. alba* (3·32 $\mu$) trichomes had cropped up in considerable population in the case of samples from sites 2, 3 and 12 only. This reveals that even a thorough direct microscopic examination of samples immediately and after 24 hr incubation on arrival at the laboratory will not suffice to detect *Beggiatoa*, if they occur in very small numbers between the detritus particles. However, this is not the case if they are subjected to a reliable enrichment culture technique, or inoculated into a suitable enrichment culture medium that is favourable for the small numbers of *Beggiatoa* trichomes to multiply into a rich population.

The avaliability of a reliable enrichment culture technique is an important step in dealing with any problem concerning *Beggiatoa*, either its natural distribution, or isolation and cultivation in pure culture media, or taxonomic differentiation, or nutrition, or tolerance to salinity, etc. Some of the techniques available are the *Butomus*-rhizome technique, the technique with decaying hay and sulphate, and the sulphur spring technique of Winogradsky (1887); the macerated hay and

sulphate technique of Cataldi (1940) ; for isolation of marine strains of *Beggiatoa* the hay medium with inorganic salts and artificial sea water technique of Pringsheim (1946) ; and the extracted roadside winter grass technique, a modification of the method of Cataldi, and the weathered Corncob technique of Faust and Wolfe (1961). Of these, as the extracted roadside winter grass technique of Faust and Wolfe is reported as a reliable and the most satisfactory method by Pringsheim (1964), and very simple, it was used to ascertain the occurrence of *Beggiatoa* spp. in the sites. However, as the dried roadside winter grass is not available in our place, dried roadside seasoned grass was used initially with either tap water or site waters as the enrichment culture medium, and inoculated with sediment, or decaying leaf bits. The media did not increase the population of *Beggiatoa* trichomes. And even when green or dried *Ulva* bits, or dried dicot leaf bits were used the result was same. As *Beggiatoa* occurs in rice rhizosphere (Pitts *et al* 1972) and water-saturated soils of rice fields (Joshi and Hollis 1977) seasoned rice straw was next used with site waters as the enrichment culture medium. It has promoted the growth of *Beggiatoa* fairly well (table 2).

Clear visual white colonies of the type described by Faust and Wolfe (1961) appeared on 6, 8, 10 and 15 days after incubation in the media with site waters, and inoculated with sediment or decaying leaf bits from the sites 1, 4, 6 and 7 respectively (figure 2). These colonies were of various sizes ; the average was about 1·0 mm in diameter. They appeared both on rice straw bits and on the surface of the flasks. Further, bundles of trichomes radiated from the centre of each colony producing a starlike appearance. On the other hand, in the remaining cultures of the samples from the other sites, instead of such colonies, a thick white film was formed at the water surface, and on the rice pieces in all the cultures, except in those of site of the stream carrying effluents from Coromandel Fertilizers Factory. The microscopic examination of the colonies or the white film revealed the *Beggiatoa* spp. listed in table 1. The amount of growth of species of each site, estimated visually, was also recognizable (table 2). In general, the trichomes of *B. leptomitiformis* were more in number than those of others present among them. Of the remaining, the trend in density of population was first those of *B. alba* (3·32 $\mu$), followed sequentially by those of *B. alba* (4·49 $\mu$), *B. minima, B. arachnoidea, B. gigantea* and *B. mirabilis,* when present in association with each other.

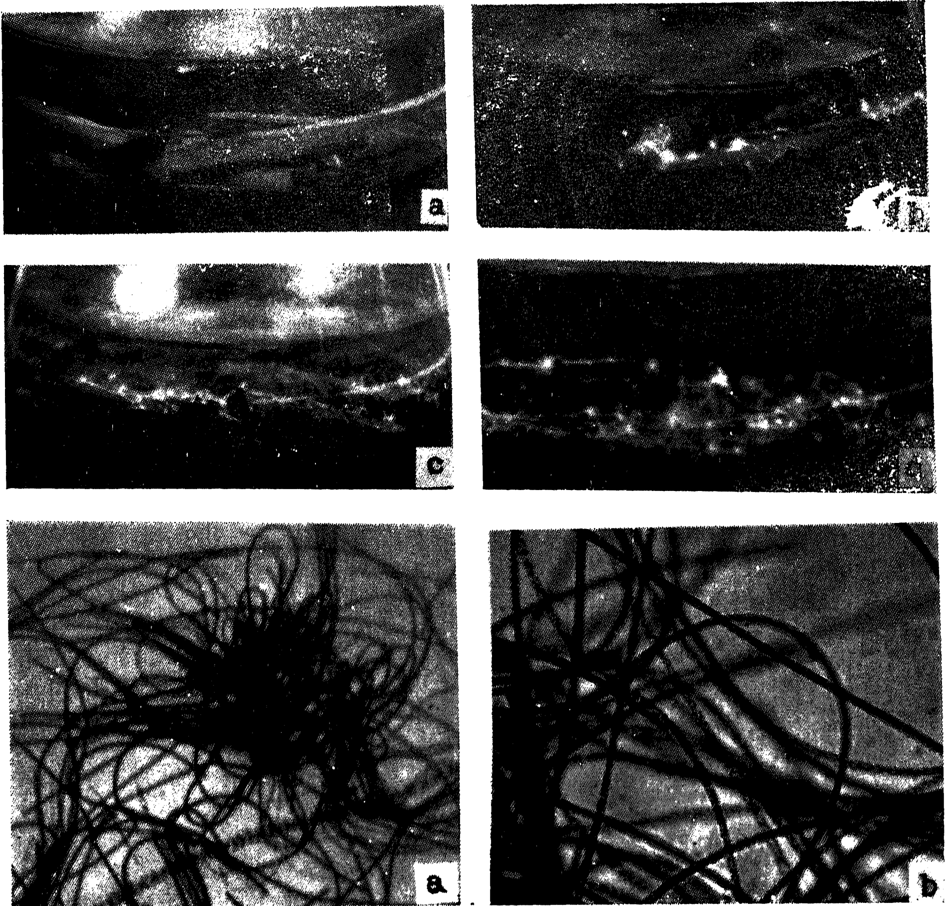
In cultures of samples from sites 1, 7, 9 and 11, recognizable amount of growth occurred within 5 to 8 days ; increased fairly well till 20 to 25 days after incubation, and declined thereafter. However, in cultures of site 7, there was total lysis of trichomes from the 24th day after incubation. On the contrary, in those of sites 2 to 6 and 12, recognizable amount of growth was evident from 10 to 15 days ; increased fairly well till 21 to 23 days after incubation, and declined thereafter. Total lysis of trichomes, however, occurred after 28 and 25 days after incubation in the case of cultures of sites 6 and 12 respectively. Whereas, in cultures of site 10, in which *B. alba* (3·32 $\mu$), *B. leptomitiformis, B. arachnoidea, B. mirabilis* and *B. gigantea* were present the growth of *B. alba* and *B. leptomitiformis* was evident from 14 days, and of *B. arachnoidea* from 7 days, and continued to increase till the 30th day after incubation. On the other hand, in *B. mirabilis* and *B. gigantea* even one day after incubation aggregations of trichomes as loose clumps appeared on the surface of glass, and in the film formed at the surface of

water. It appears that the trichomes present in the inocula had crept on to glass surface and into the film. Further, the trichomes started lysing from the 4th day, and completely dissolved by the 7th day after incubation revealing that they failed to multiply in the enrichment cultures.

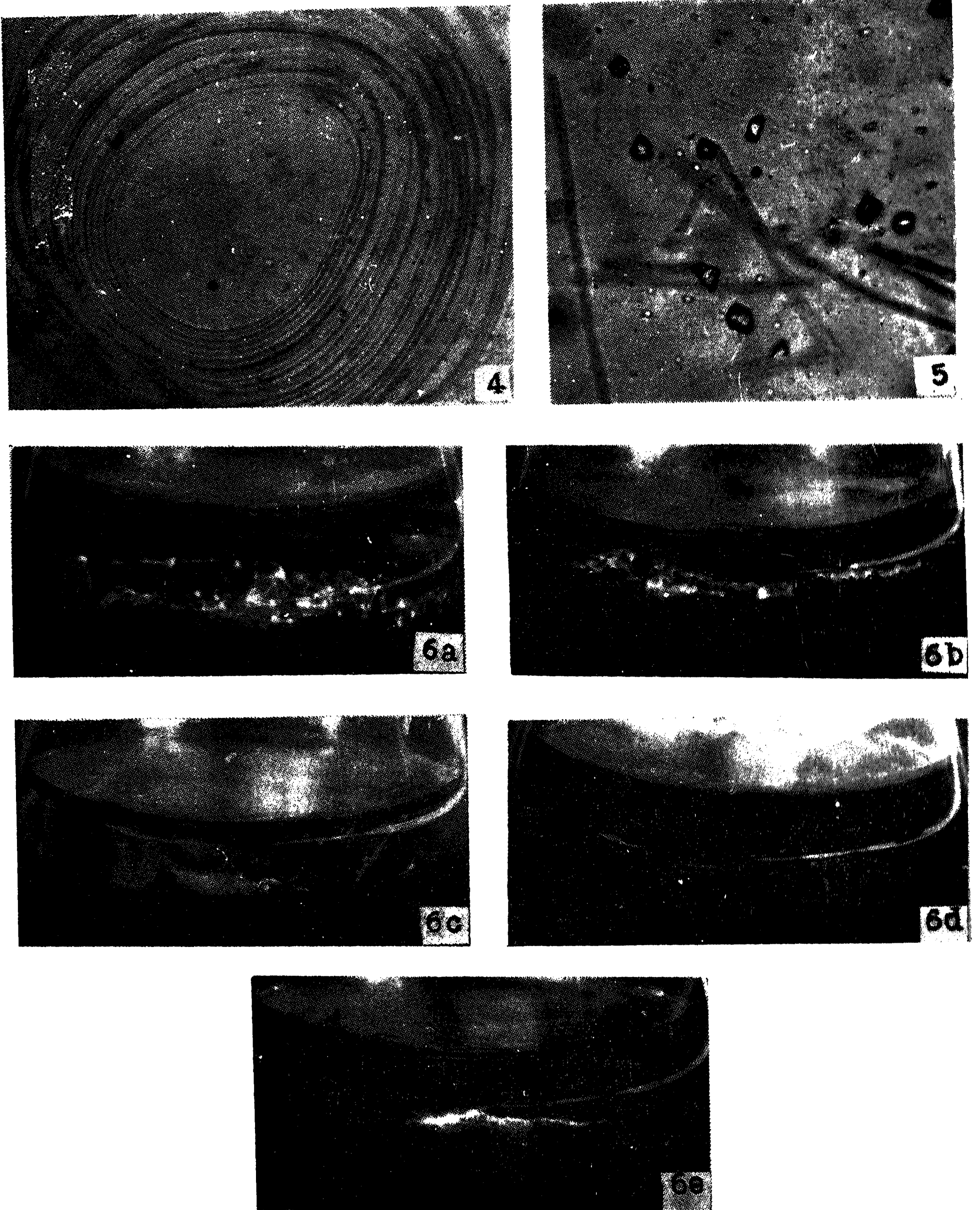
The microscopic examination of colonies, or loose clumps, or white film placed on microscope slides, clearly showed the gliding movement of the trichomes of all the species from the centre of the aggregations or colonies ; and folding of the trichomes back into the aggregate (figure 3). The dispersed trichomes of all the species also exhibited active gliding movement. The speed of movement, however, seems to be different in different species. The trichomes of *B. alba, B. leptomitiformis,* and *B. minima* seem to be fast moving, whereas those of *B. arachnoidea, B. mirabilis* and *B. gigantea* slow moving. The repeatedly washed colonies of site 9 cultures containing a mixed population of *B. alba* and *B. leptomitiformis* trichomes on Cataldi's agar medium solid surface exhibited not only the characteristic circular flow pattern (figure 4) but also other patterns that resembled rivers. Such patterns were also reported earlier by Faust and Wolfe (1961) and Pringsheim (1964).

The intracellular deposition of sulphur as droplets was also observed in the trichomes of all the species of *Beggiatoa* multiplied in the crude enrichment culture media. The treatment to trichomes with aceto-carmine resulted in dissolution of sulphur droplets, and crystallisation of sulphur into flat octohedra outside the trichomes (figure 5). The arrangement of sulphur droplets in the trichomes of the different species of *Beggiatoa* was, however, different. It appears that the arrangement of sulphur droplets is a characteristic feature of each species irrespective of its distribution. But in enrichment cultures, sulphur-free trichomes were also observed. These were in small numbers, and present in declining cultures only.

It is evident from the above observations made on the samples collected, and the growth of *Beggiatoa* spp. in enrichment culture media, that *Beggiatoa* is distributed in 11 sites out of the 12 investigated, and favourable conditions for its existence prevail at these sites, except at site 8. The pH of waters of these sites containing *Beggiatoa* is not much varied, about 7·0 or above, the optimum for most aquatic bacteria. The same was also observed earlier by Lackey (1961) in his studies on occurrence of *Beggiatoa* relative to pollution. However, with regard to salt content there are considerable differences between the waters of different sites, ranging between very low (0·58 ppt) and very high (34 ppt) salt content. Sites 1 to 5 with waters having 0·58, 0·72, 1·07, 1·07 and 1·08 ppt salt content respectively are fresh-water habitats. Sites 6 and 7 with waters having 1·4 and 4·5 ppt salt content respectively are soft brackish water habitats. Sites 9 and 10 with waters having 19 and 20 ppt salt content respectively are hard brackish water habitats, whereas sites 11 and 12 with waters having 31·25 and 34 ppt salt content respectively are truly marine habitats. As shown in tables 1 and 2, *Beggiatoa* of equal diameter exist in all these habitats. Further, there is no difference between them in appearance. If any difference existed, it would be physiological only, either in nutrition, or growth rate, or indifference to variation in salt content etc. To study the growth and tolerance to low or high salt content the sediment samples of all the 12 sites were, therefore, subjected to enrichment culture technique using the rice straw medium with respective site waters, or tap water of pH 6·8 and 0·31 ppt salinity, or metal distilled water of pH 6·5 and



Figures 2 and 3. 2. Visual colony growth of *Beggiatoa* spp. of site 7, in enrichment culture medium containing site water. **a** Site water only, **b** Inoculated with decaying leaf, **c** Inoculated with sediment + decaying leaf, **d** Inoculated with sediment (enlarged view). **3.** Aggregation of *Beggiatoa* trichomes. **a** The gliding movement of the trichomes from the centre of the aggregation, and folding of the trichomes back into the aggregate (× 700), **b** Edge of the aggregate showing trichomes filled with sulphur droplets also (× 860).




**Figures 4–6.** **4.** Circular gliding pattern of a mixed population of *B. alba* and *B. leptomitiformis* trichomes on modified Cataldi's agar medium surface (× 860). **5.** Octohedral crystals of sulphur outside the trichomes treated with aceto-caramine (× 1940). **6.** Visual colony growth of *Beggiatoa* spp. of site 7 in enrichment culture media containing **a** Unfiltered site water (good growth), **b** Filtered site water (good growth), **c** Tap water (poor growth), **d** Distilled water (poor growth), **e** Sea water (nil growth); the white scum contained no *Beggiatoa*.

0·016 ppt salinity, or sea water of pH 7 and 35 ppt salinity. The results are shown in table 3 and figure 6.

In these cultures of samples from site 8 *Beggiatoa* was not detected, confirming the above results that it is not occurring at this site. In the remaining cultures of samples from 11 sites, the *Beggiatoa* spp. listed in tables 1 and 2 were again observed. In general, by 6, 8 10 and 15 days after incubation clear visual white colonies described above also appeared in the culture media favourable for growth of *Beggiatoa* from the sites 1, 4, 6 and 7 respectively (figure 6). On the other hand, in the remaining cultures, whichever was favourable for growth, *Beggiatoa* trichomes except those of *B. mirabilis* and *B. gigantea* increased in population between 8 and 15 days after incubation and aggregated into loose clumps in the white film that was formed at the surface of water and glass. The microscopic examination of colonies or loose clumps showed actively gliding trichomes filled with sulphur droplets. It appears that enough $H_2S$ is produced in the media due to putrefaction of sulphur containing proteins that were left in the extracted rice straw pieces, and supported the growth of *Beggiatoa*. Further, oxidation of $H_2S$ must have taken place, and probably organic nutrients were also utilized for growth.

Regarding *B. mirabilis* and *B. gigantea*, the trichomes as in the previous enrichment cultures aggregated by one day after incubation on rice bits, surface of glass, and at the surface of medium with site waters or sea water only. They remained for 3 days in active gliding state ; thereafter started declining, and completely lysed and dissolved by 7th day after incubation revealing that they failed to grow in the media. Whereas in tap water and distilled water media the trichomes did not even appear either on rice bits, or on the surface of glass, or at the surface of water ; indicating that they lysed within one day after incubation. Further, when the trichomes of these two species were suspended in tap water, or distilled water, or sea water, or their site water for microscopic examination, those suspended in tap water, or distilled water have bulged and burst releasing colloidal mass of cytoplasm and sulphur droplets. This confirms the earlier observations by Lackey (1961), Pringsheim (1964) and others that they are restricted to brackish or marine habitats and appear to be strictly autotrophic and rather delicate and exacting. These two largest species, hence, have not yet been grown successfully either in crude enrichment cultures or in pure cultures by anybody.

The remaining *Beggiatoa* spp. of each site, as shown in table 3, could grow in enrichment culture media, but their amount of growth was different in different media, ranging between good to nil growth. Further, the lag period was also different. Where the amount of growth was good and fair, recognizable growth occurred by 5 to 8 days ; increased steadily till 20 to 25 days after incubation and thereafter declined. On the contrary, where it was poor and very poor recognizable growth appeared by 10 to 15 days ; increased very slowly till 20 to 25 days after incubation ; and afterwards declined. Thus, these differences in the growth of *Beggiatoa* spp. in different enrichment culture media suggest that strains or groups differing in salt tolerance may exist within a species. *B. minima* occurs in 2 sites, a freshwater site 1 and a marine site 12. The nil growth in sea water medium of that occurring in the fresh water habitat indicates that it has a low salt tolerance. Whereas the growth, though very poor, in filtered site water, sea water and tap water media of that occurring in the marine habitat shows that

it is indifferent to variation in salt content, and has a wide salt tolerance. Further, the nil growth of this marine form in distilled water medium may be due to lack of enough mineral salts for its growth in the medium.

*B. leptomitiformis* is distributed in 6 sites ; 3 freshwater sites (1, 3 and 5), 2 brackish water sites (9 and 10), and 1 marine water site (11). The forms occurring in freshwater habitats failed to grow in sea water medium, indicating that they have a low salt tolerance ; and seem to be restricted to freshwater habitats only. On the other hand, of the two occurring in brackish water habitats, the one from site 9 of a stagnant brackish water pond failed to grow in sea water medium, whereas the other from site 10 of one of the harbour channel terminals near sulphur-unloading berth could grow in all the media indicating that the former has a low salt tolerance and the latter has a wide salt tolerance. On the contrary, that occurring at site 11 of the sea water canal carrying effluents from the Hindustan Petroleum Oil Refineries by its good, fair, poor and nil growth in site waters, tap water, sea water and distilled water media respectively, shows that it is indifferent to variation in salt content, and can thrive well if enough mineral salts were available for its growth. Thus, these *B. leptomitiformis* forms, though similar in appearance and morphology, may be different physiologically ; the 3 occurring in sewage containing freshwater habitats formed one group ; the two occurring in sewage containing brackish water pond, and oil containing and probably sulphur-rich harbour channel terminal formed two distinct groups ; and the one occurring in sea water canal carrying effluents from the oil refineries formed another group.

*B. alba* of 3·32 μ wide is distributed in 11 sites, suggesting that it is ubiquitous in distribution. The forms occurring in freshwater habitats, sites 1 to 5, by their nil growth in sea water seem to be restricted to fresh water habitat, and have a low salt tolerance. It appears that physiologically these may belong to one group. The one from site 6 of a sewage stream that joins the harbour channel receiving sea water during high tides, by its nil growth in tap water or distilled water, and poor growth in sea water media seems to be possessing high salt tolerance. Further, it appears to belong to a separate group. On the other hand, those occurring in sites 7 and 9 exhibited nil growth in sea water medium only. It appears that these two could not tolerance high salt content and prefer lower levels, and may belong to one group physiologically. Whereas that occurring in site 10, a hard brackish water habitat, by its growth in all the media indicates that it is indifferent to variation in salt content, and has a wide salt tolerance. Most likely it may belong to another group. And those occurring in sites 11 and 12 truly of marine habitats, showed growth in all the media, except in distilled water medium. It shows that these two are also indifferent to variation in salt content with, however, a preference for at least some amount of mineral salts for growth, and may belong to one group. Thus, *B. alba* forms distributed in the 11 sites, though similar in appearance and morphology are different in salt tolerance, and may belong physiologically to 5 different groups.

The *B. alba* strain with 4·98 μ wide trichomes is distributed in two sites, 4 and 7. Their nil growth in sea water medium indicates that they have a low salt tolerance. Further, the poor and nil growth in tap water and distilled water respectively by the one from site 4, and very poor growth in tap water and distilled water by the other from site 7 indicate that the two may be different physiologically.

The remaining widest forms of *Beggiatoa*, viz., *B. arachnoidea*, *B. mirabilis* and *B. gigantea* are detected in only one site (10). Of these, *B. arachnoidea* alone could grow both in tap water and sea water indicating that it is indifferent to variation in salt content. However, its nil growth in distilled water may be due to lack of enough mineral salts in the medium. On the contrary, *B. mirabilis* and *B. gigantea* by their nil growth in any of the media seem to require more exacting conditions for growth in culture ; halophilic, and strictly autotrophic.

## 4. Conclusion

The present study reveals that *Beggiatoa* is widespread in distribution ; *B. minima*, *B. leptomitiformis* and *B. alba* are ubiquitous occurring in both freshwater and marine habitats ; *B. arachnoidea*, *B. mirabilis* and *B. gigantea* are restricted to brackish and marine habitats ; and conditions supporting the growth of these are prevailing in 11 sites, out of the 12 investigated. Further, the enrichment culture medium containing extracted rice straw bits and the respective site waters proves to be a reliable and satisfactory medium for increasing the small number of *Beggiatoa* trichomes into a rich population, which is a prerequisite for isolation and cultivation in pure cultures. Although the species of *Beggiatoa* identified are considered as belonging to the six species described in Bergey's manual according to the usual nomenclature, the occurrence of *Beggiatoa* of identical diameters both in freshwater and marine habitats ; and the differences in growth in rice straw medium with different waters of varying salt content suggest that width is not the sole feature to be taken into consideration for species differentiation, substantiating the views of Pringsheim (1964) and others. That physiological groups exist within the smaller forms of *Beggiatoa* is also confirmed, though based only on growth in crude enrichment culture media. However, there is every need for isolating and cultivating these forms in pure culture media for further detailed consideration of species differentiation, and their nutrition.

## Acknowledgements

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# Photoperiodic control of extension growth, bud dormancy and flowering of *Nerium indicum* Mill. and *Thevetia peruviana* Schum.

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MS received 12 November 1981

**Abstract.** Plants of *N. indicum* and *T. peruviana* grew taller and produced more leaves under LD than under ND condition. While *T. peruviana* plants were taller and had more leaves under ND than under SD, those of *N. indicum* did not differ under the two photoperiods. In both cases LDs delayed the onset of bud dormancy but hastened the initiation of floral buds. While in *T. peruviana* floral buds were not formed under SD condition, in *N. indicum* floral buds were formed but they did not develop into flowers. While in *N. indicum* more flowers were produced under LD than under ND condition, in *T. peruviana* the number produced was higher under ND than under LD condition.

**Keywords.** Photoperiod; extension growth; bud dormancy; flowering; *Nerium indicum*; *Thevetia peruviana*.

## 1. Introduction

Studies on the effect of photoperiod on extension growth, bud dormancy and flowering of woody species have shown that in general while long days prolong the period of extension growth and therefore delay the onset of dormancy, short days hasten the onset of rest (Nanda 1963; Whalley and Cockshull 1976; Bhatnagar and Talwar 1978; Singh and Nanda 1981). The floral response, however, varies with the plant species. Thus, the young seedlings of *Coffea arabica* produce floral buds under short day condition (Piringer and Borthwick 1955) while in *Hydrangea* (Piringer and Stuart 1955) and *Malus hupehensis* (Zimmermann 1971) floral buds are induced under long day conditions. Davidson and Hamner (1957) have reported that although long days induce floral buds in *Rhododendron catawbiense*, short days are needed for their development into flowers. In contrast to this, Mirov (1956) has reported that photoperiod does not affect the flowering response of 35 exotic pines. This paper deals with the effect of photoperiod on extension growth, bud dormancy and flowering of two garden plants, *Nerium indicum* and *Thevetia peruviana*.

## 2. Materials and methods

Plants of *Nerium indicum* Mill. were raised by planting one-year old stem cuttings (15 cm each), while those of *Thevetia peruviana* Schum. were raised from the seed collected locally from a healthy tree growing in the Panjab University Campus. The seed was sown on February 1, 1980 in 3:1 mixture of field soil and sand in earthenware pots (25 cm dia) under three photoperiodic regimes namely; long day (LD) – consisting of continuous illumination which was provided by supplementing the normal day-length by unfiltered 200 watt light from incandescent lamps which provided light intensity of about 3000 lux at the level of the plants; normal day ND - consisting of natural day-length conditions prevailing at Chandigarh (figure 1) and short day (SD) – consisting of 8 hr daily light alternating with 16 hr dark which was provided by covering the plants with thick tarpaulin sheets daily from 1700 hr to 900 hr which led to the rise in temperature in the range of 3-6°C throughout the course of experimentation. To ensure healthy growth, Hoagland's nutrient solution (Hoagland and Arnon 1939) was supplied to the plants twice a week during the course of experimentation from April 1980 to June 1981.

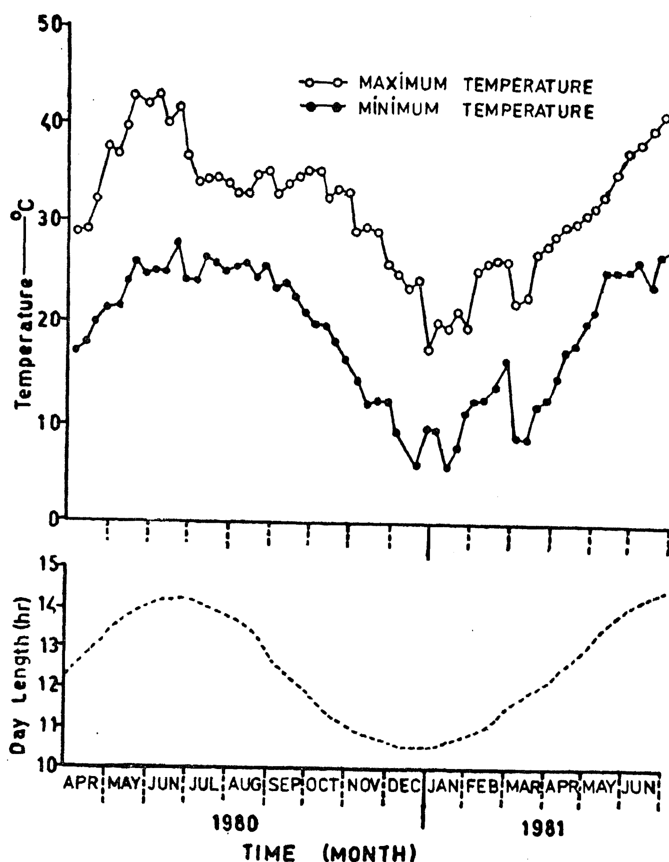


**Figure 1.** Temperature and day length conditions prevailing at Chandigarh during April 1980 to June 1981.

Observations of extension growth were recorded at 14-day intervals whereas the number of leaves was counted daily. Records were also maintained of the dates of initiation of floral buds. The number of floral buds produced and the time taken for them to open into flowers were also recorded. The 'period of dormancy' in this paper refers to the period during which the shoot apex remained apparently inactive and no new leaves were produced on the plant.

## 3. Observations

The results are presented diagrammatically in figures 2 and 3 and table 1.

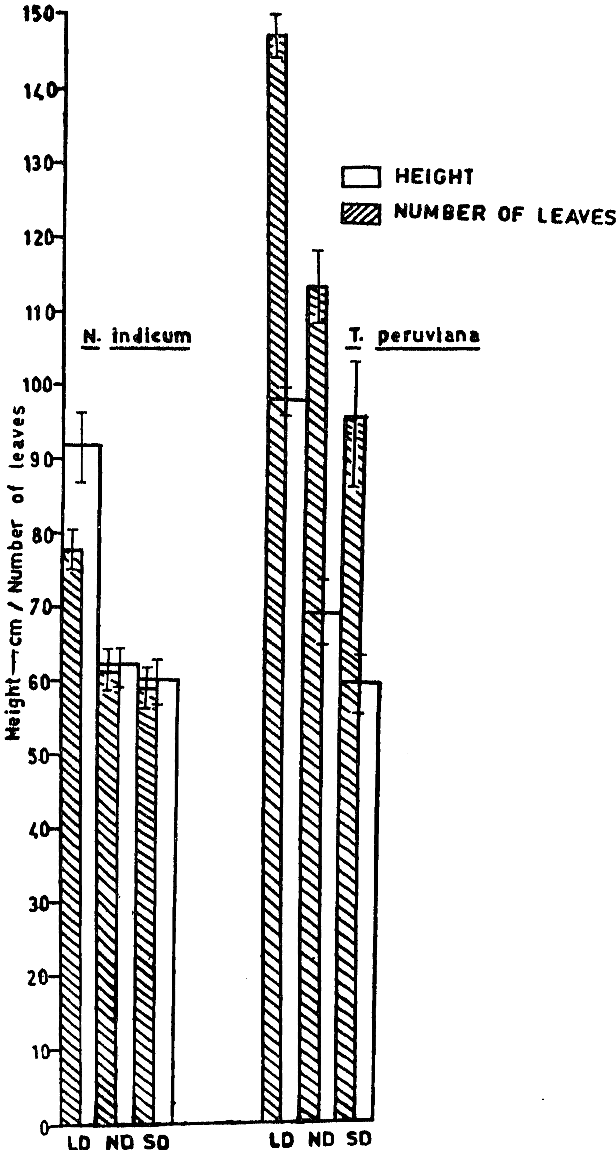


**Figure 2.** Effect of photoperiod on height of the main axis and number of leaves produced on *N. indicum* and *T. peruviana*.

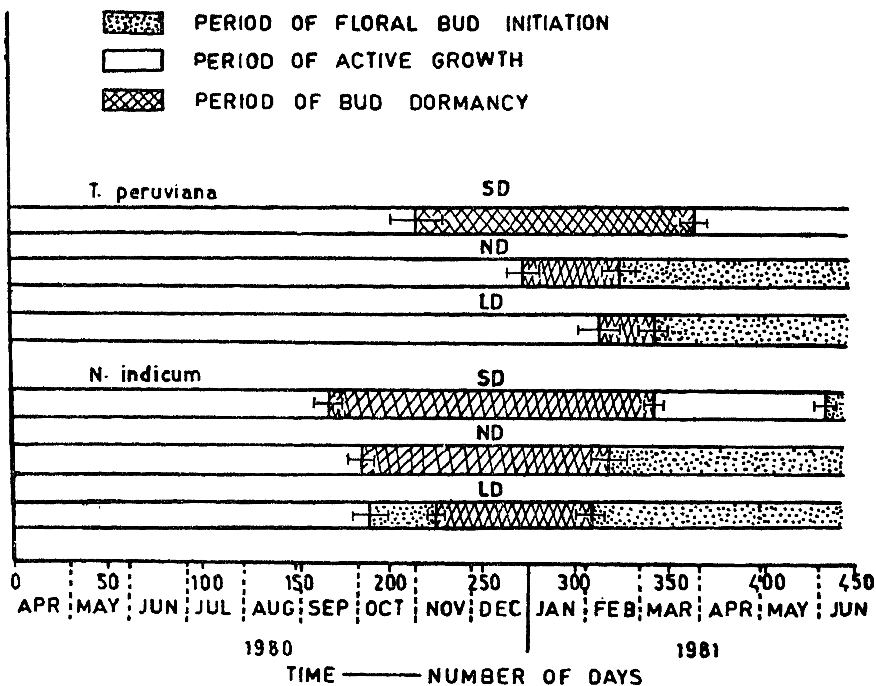


**Figure 3.** Effect of photoperiod on periods of active growth and bud dormancy in *N. indicum* and *T. peruviana*.

**Table 1.** Effect of photoperiod on flowering of *N. indicum* and *T. peruviana*.

| Photoperiod | Days to floral bud initiation | Number of floral buds | Days taken to flower opening | Number of flowers | Number of plants out of 10 that produced floral buds |
|---|---|---|---|---|---|
| | | | *N. indicum* | | |
| LD | 190.5±2.50 | 70.8±2.81 | 314.4±2.81 | 17.5±1.71 | 10 |
| ND | 315.2±3.40 | 35.6±3.10 | 356.7±4.31 | 10.5±3.81 | 10 |
| SD | 340.0±4.21 | 3.0±1.05 | — | — | 10 |
| | | | *T. peruviana* | | |
| LD | 340.5±3.42 | 6.1±2.40 | 360.8±4.21 | 4.5±2.85 | 10 |
| ND | 320.7±5.16 | 18.7±3.08 | 348.9±3.91 | 10.6±1.31 | 10 |
| SD | — | — | — | — | — |

± SE at 95% level of significance

### 3.1 *Extension growth and number of leaves*

Figure 2 shows that plants of both the species grew taller and produced more leaves under LD than under ND and SD conditions. While in *N. indicum* the height

and number of leaves produced under SD condition did not differ from that produced under ND condition, in *T. peruviana* plants remained significantly shorter and produced fewer leaves under SD than under ND condition.

### 3.2. *Periods of growth and bud dormancy*

In *N. indicum*, plants continued growth till mid November under LD as compared to that till early October and mid September under ND and SD conditions, respectively. The period of rest that followed also lasted till early February under LD but till mid February and early March under ND and SD conditions, respectively (figure 3).

In *T. peruviana*, growth continued till early February under LD condition but lasted till the end of December and October under ND and SD conditions, respectively. The period of rest that followed and which lasted till mid-February and early February under ND and LD conditions, respectively, continued till early March under SD condition.

It may be noted that in both the species the period of rest was shorter under LD but longer under SD than under ND condition (figure 3).

### 3.3. *Days to floral bud initiation*

In *N. indicum*, floral bud initiation in plants under LD condition started in early October prior to the onset of dormant phase. In contrast to this under ND condition floral buds were initiated in mid February. Floral bud formation under both these conditions continued till the end of June when the experiment was terminated. In contrast to this, the formation of floral buds under SD condition was delayed to early June. Floral bud initiation, thus, occurred earlier under LD but later under SD than under ND condition (figure 3).

In *T. peruviana* floral bud initiation was observed immediately after the period of rest in plants exposed to LD or ND conditions so that it occurred earlier under ND than under LD condition (table 1). Plants maintained under SD condition did not produce floral buds. It may be noted that the hastening effect of LDs was markedly more in *N. indicum* than in *T. peruviana*.

### 3.4. *Days to floral bud opening*

Although LDs hastened the initiation of floral buds, the initiated buds in *N. indicum* took longer to develop into flowers under LD than under ND condition. Floral buds produced under SD condition in this species did not develop into flowers at all. In contrast to this in *T. peruviana* while the initiation of floral buds occurred earlier under ND condition, their development was hastened under LD condition (table 1). As stated earlier, plants of this species did not produce floral buds under SD condition.

### 3.5. *Number of floral buds and flowers*

Table 1 shows that while in *N. indicum* the number of floral buds and flowers was higher under LD than under ND condition, in *T. peruviana* it was higher under ND than under LD condition. As stated earlier, in *T. peruviana* no floral buds were produced under SD condition and in *N. indicum* a few floral buds which were produced did not develop into flowers.

## 4. Discussion

The early onset of dormant phase under SD condition in plants of both the species reported in this paper is in accord with the results reported earlier in some other plants (Nanda 1963; Whalley and Cockshull 1976; Bhatnagar and Talwar 1978; Singh and Nanda 1981). Hastened onset of rest period in plants under SD condition may be due to accumulation of some growth inhibitory substance(s) (Wareing and Saunders 1971) or to a decrease in photosynthates due to reduced daily light period.

The fact that in *N. indicum* floral buds are produced even under SD condition, while in *T. peruviana* they are not produced under this photoperiod shows that while the former is quantitative, the latter is qualitative long day in its response to photoperiod.

But the more interesting point is that in *N. indicum* although floral buds are produced, they fail to develop into flowers showing that the photoperiodic requirement for the completion of these two phases *i. e.*, (i) induction of floral buds and (ii) their development into flowers, may not be the same. That the requirement of these two phases may vary is shown in soybean (Jindal and Nanda 1978) and *Bauhinia acuminata* (Singh and Nanda 1981). It cannot, however, be ruled out that failure of buds of this species to develop into flowers may be due to the limitation of photosynthates under SD condition. This is particularly in the light of work of Ramina *et al* (1979) and Even-Chen and Sachs (1980) who reported that photosynthetic availability influences the flowering intensity in *Bougainvillea.*

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# Interaction of kinetin with B group vitamins on the seedling growth of green gram (*Phaseolus radiatus* L.)

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MS received 17 November 1981 ; revised 13 May 1982

**Abstract.** Kinetin (50 and 100 mg $l^{-1}$) inhibited both the shoot and the root growth. Inhibition of root growth by kinetin is considered to be mainly due to inhibition of protein synthesis. Vitamins of the B group *viz.*, riboflavin, thiamin, niacin and pantothenic acid are found to be antagonistic to kinetin in reversing the inhibition of protein synthesis of the root. Vitamins, probably by acting as inducers of protein synthesis, antagonized the action of kinetin. The response of kinetin to shoot protein content is different from that of the root.

**Keywords.** Kinetin ; B-vitamins ; root protein ; green gram.

## 1. Introduction

Our knowledge of cytokinins and their possible functions in root growth is extremely limited. That cytokinins inhibit root growth was shown by Gaspar and Xhaufflaire (1967). But how they inhibit root growth was not categorically explained. Hussain *et al* (1980) working on nutsedge with vitamins of B group and kinetin have noticed that riboflavin and pyridoxin up to 100 mg $l^{-1}$ promoted root and shoot growth of the plantlets, whereas kinetin produced short, thick shoots and inhibited root growth, with increasing concentration.

The present study has been designed to understand the interaction of vitamins and cytokinins which might throw some light on the growth of root and shoot systems of intact seedlings. Only inhibitory concentrations (50 and 100 mg $l^{-1}$) of kinetin were used in order to find out the efficacy of vitamins to counteract the influence of kinetin. Earlier report by Gopala Rao *et al* (1975) indicated that riboflavin can effectively reverse the chloramphenicol inhibited growth of green gram seedlings. It is a well-known fact that chloramphenicol is a potent inhibitor of protein synthesis. Interaction of auxins and riboflavin in growth reactions in plants was studied by Artamonov (1974). The present study is intended to find out the capacity of vitamins to counteract the effect of kinetin in shoot or root growth inhibition.

## 2. Materials and Methods

Seeds of green gram (variety G.G 525) were surface sterilized with 0.1% mercuric chloride for 3 min, washed thoroughly with distilled water and allowed to grow

in petridishes. The seeds were subjected to presowing soaking for 24 hr with kinetin (50 and 100 mg $l^{-1}$) and vitamins of the B group *viz.*, riboflavin, thiamine, niacin and pantothenic acid, each at a concentration of 100 mg $l^{-1}$ since it was found to be effective in reversing the kinetin inhibited protein synthesis. Then the seeds were allowed to grow in distilled water in diffuse light in the laboratory up to 8 days. Growth in length of the seedlings was measured for shoot and root separately. Ten replications, each replications representing five seedlings were maintained for growth measurements. The protein content was estimated separately in the shoot and the root portions using the method of Lowry *et al* (1951) at 2 day intervals. Three replications were maintained for protein estimations.

## 3. Observations

### 3.1 *Extension growth*

With kinetin (50 and 100 mg $l^{-1}$) treatment both the root and shoot growth were inhibited (table 1). The results discussed pertain to 100 mg $l^{-1}$ concentration alone. On the eighth day, for example, the root growth of control seedlings was 8.0 cm while that of kinetin treated seedlings was 3 5 cm with 50 mg $l^{-1}$ and 1.03 cm with 100 mg $l^{-1}$ respectively. The shoot growth of control seedlings was 17.5 cm while that of kinetin treated seedlings was 16.1 cm with 50 and 0.8 cm with 100 mg $l^{-1}$ respectively (table 1).

**Table 1.** Effect of kinetin on growth (cm) and protein content (mg/g. dry wt.) of the seedlings.

| Treatment | Observation | Part of the seedling | Age of seedling | | | |
|---|---|---|---|---|---|---|
| | | | 2 days | 4 days | 6 days | 8 days |
| Control | Growth | Root | 2.55 ±0.05 | 6.46 ±0 91 | 760 ±0.66 | 8.00 ±0.65 |
| | | Shoot | 1.30 ±0.03 | 9.25 ±0.27 | 9.75 ±0.81 | 17.50 ±0.41 |
| | Protein | Root | 109.5 ±7.6 | 124.5 ±7.3 | 117.0 ±9.1 | 82.5 ±1.8 |
| | | Shoot | 124.5 ±1.9 | 172.5 ±3.8 | 127.0 ±1.8 | 90.0 ±2.6 |
| Kinetin (50 mg $l^{-1}$) | Growth | Root | 0.55 ±0.03 | 0.70 ±0.02 | 1.13 ±0.04 | 3.45 ±0.25 |
| | | Shoot | 0.63 ±0.03 | 2 53 ±0.07 | 2.63 ±0.03 | 16.15 ±0 29 |
| | Protein | Root | 153.0 ±1.63 | 96.5 ±1.51 | 90.0 ±0.87 | 70.5 ±0.88 |
| | | Shoot | 208.5 ±2.91 | 182.0 ±4.15 | 159.0 ±1.35 | 102.0 ±2.01 |
| Kinetin (100 mg $l^{-1}$) | Growth | Root | 0.43 ±0.06 | 0.6 ±0.08 | 0.8 ±0.09 | 1.03 ±0.04 |
| | | Shoot | 0.6 ±0.09 | 2.53 ±0.07 | 4 30 ±0.46 | 8.03 ±0.21 |
| | Protein | Root | 109.5 ±1.25 | 60.0 ±0.62 | 51.0 ±0.57 | 45.0 ±0.69 |
| | | Shoot | 187.5 ±3.56 | 185.5 ±2.25 | 163.5 ±1.79 | 79.5 ±1.27 |

### 3.2 *Protein content*

The root protein content was reduced with both concentrations of kinetin (50 and 100 mg $l^{-1}$). There was only an initial increase with 50 mg $l^{-1}$ kinetin on the second day of seedling growth. On the eighth day, for example, the root protein content of control seedlings was 82.5 mg while that of kinetin treated seedlings was 70.5 mg with 50 mg $l^{-1}$ and 45.0 mg with 100 mg $l^{-1}$ respectively (table 1).

It is tempting to note that the shoot protein content was enhanced with kinetin (100 mg $l^{-1}$) except on the eighth day (table 1).

### 3.3 *Interaction of kinetin (100 mg $l^{-1}$) with vitamins on protein content*

There was about 50% reduction in root protein content with kinetin treatment when compared to that of control seedlings (from 80 mg to 40 mg) on the eighth day. Although there was an initial raise with riboflavin treatment up to fourth day, it was followed by a steep fall during later stages. The interaction of kinetin with riboflavin enhanced protein content of the root from 40 mg to about 140 mg on the eighth day (250%) or a 3 fold increase. Thiamine, niacin and pantothenic acid could raise the protein level from 40 mg to 80 mg not exceeding the control level when they interacted with kinetin (figure 2).

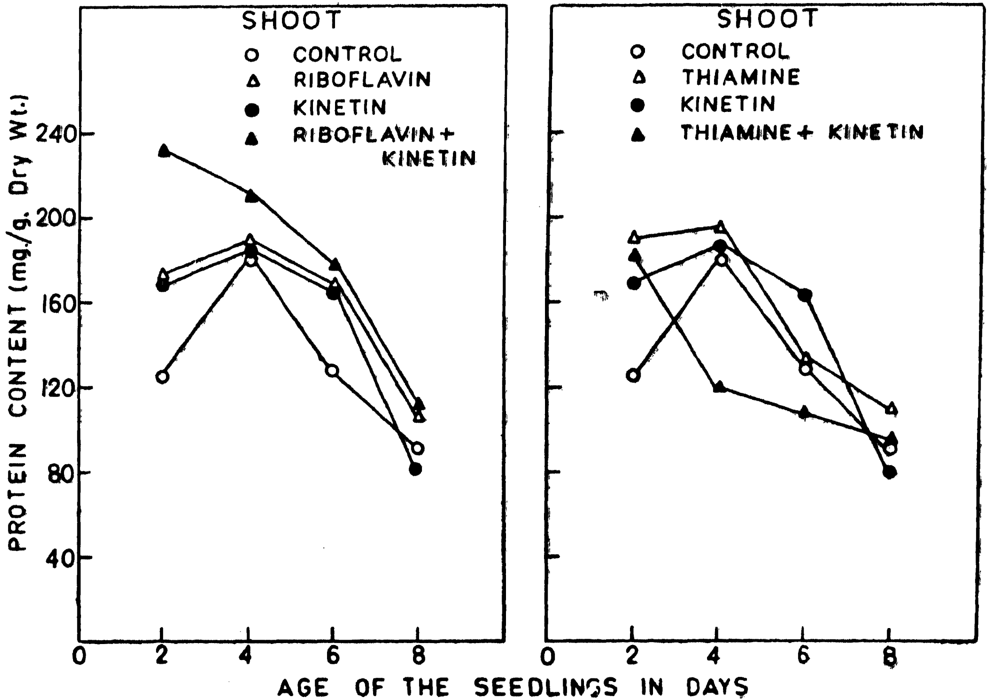


**Figure 1A.**

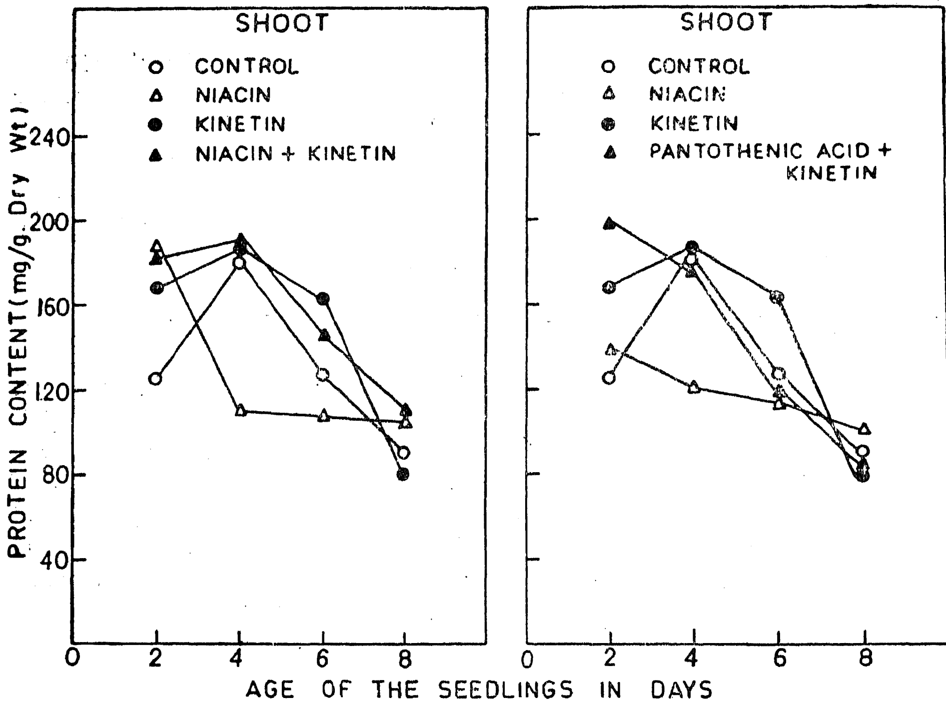


**Figure 1B.**

In the case of shoot, riboflavin (figure 1A) increased the protein content from a value of 124.5 mg to 172.5 mg (38%) on the second day and from a value of 90 mg to 105 mg (16%) on the eighth day. Thiamine also increased the protein content of the shoot. Niacin and pantothenic acid (figure 1B) could not increase the protein as effectively as the former two vitamins except on the second day. As opposed to the response of the root, shoot protein content was not reduced by kinetin, but instead, increased up to the sixth day at least. The interaction of riboflavin with kinetin caused a significant increase in protein content of the shoot. Thiamine interaction with kinetin caused an initial increase only, followed by a gradual reduction. Interaction of pantothenic acid with kinetin was quite similar to that of thiamine. Interaction of niacin with kinetin was almost similar to that of riboflavin in that it caused an additive effect in increasing the protein content of the shoot (figure 1B). The behaviour of riboflavin and niacin on the one hand and that of thiamine and pantothenic acid on the other hand were found to be similar in their interaction with kinetin.

## 4. Discussion

Kinetin at 50 and 100 mg $1^{-1}$ is inhibitory to both the shoot and the root growth. One of the main causes of root growth inhibition (table 1) by kinetin is the reduction in protein content. Since all the B vitamins used in the present study were able to effectively reverse the inhibition of protein synthesis by kinetin, it might be

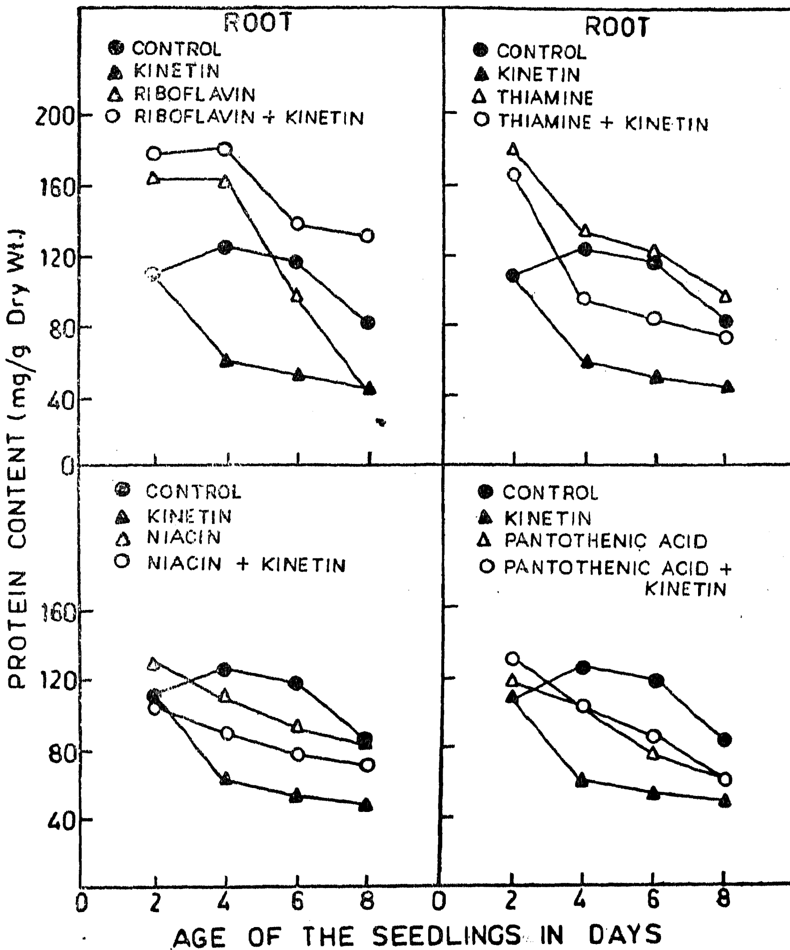


**Figure 2.**

assumed that vitamins increase protein synthesis by operating either at transcription or translation level. This assumption can be corroborated by the observation made by Srivastava (1967) that cytokinins can also inhibit RNA synthesis. A consequence of the inhibition of RNA synthesis is inhibition of protein synthesis. Gopala Rao *et al* (1975) earlier reported enhanced protein synthesis by B vitamins in green gram. It is a well-known fact that chloramphenicol is an effective inhibitor of protein synthesis. Gopala Rao *et al* (1976) observed that riboflavin can effectively reverse the chloramphenicol inhibited growth of green gram. Basing on these observations it can be assumed that a probable site of action of vitamins of the B group could be at transcription or translation level.

The present study also reveals that the response of kinetin to root protein might be different from that of shoot protein since the root protein content was significantly reduced and that of the shoot was significantly enhanced. The present study forms the basis for future work on the role of vitamins at molecular level.

## Acknowledgements

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# Leaf architecture of Apocynaceae

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**Abstract.** Leaf architecture including venation pattern has been studied in 19 genera and 29 species of the Apocynaceae. The leaves are simple, alternate, opposite or whorled with entire margin and a simple midrib. The major venation pattern conforms to pinnate camptodromous type with festooned brochidodromous secondaries. The qualitative and quantitative features are charted. The leaf size, areole size, number of vein endings entering the areoles and number of vein terminations entering the areoles vary from species to species even within the same species. The highest degree of vein order is observed up to 7°. Isolated tracheids, isolated vein endings, isolated free vein endings and tracheoidal elements are noticed. Bundle sheath cells ensheaths all category of veins.

**Keywords.** Anatomy ; leaf architecture ; venation pattern ; Apocynaceae.

## 1. Introduction

Only sporadic information is available on the venation in some members of the Apocynaceae (Chandra *et al* 1969, 1972; Kapoor *et al* 1969; Sharma *et al* 1970). The leaf architecture of the Apocynaceae has not been studied in detail. The present investigation has been undertaken to give a comprehensive and detailed account of leaf architecture of the Apocynaceae.

## 2. Materials and methods

The material of the 19 genera and 29 species of Apocynaceae was collected from different places in Gujarat, Karnataka (Lalbagh, Bangalore) and Kerala States. The mature leaves were cleared following the procedure of Rao *et al* (1980). Photomicrographs were taken with a Carl-Zeiss photomicroscope I using yellow filter and ORWO NP 15 film. Leaf size was measured using graph paper. The areole size, the number of veinlets entering in areole and the number of vein endings entering in areole were taken in five different fields of different leaves, and the average value was recorded. Terminologies to describe leaf architecture were adopted from Hickey (1973), Hickey and Wolfe (1975) and Melville (1976).

**Table 1.** Showing the qualitative features and numerical data on the venation pattern of some Apocynaceae.

| Sl. No. | Name of the taxa | Locality | Shape | Apex | Base | Margin | Texture |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1. | *Aganosma caryophyllata* G. Don. | local | ovate | acute | obtuse | entire | chartaceous |
| 2. | *Allamanda cathartica* Linn. | Bulsar, Gujarat | ovate | acuminate | obtuse | entire | coriaceous |
| 3. | *A. nerifolia* Hook | Lalbagh Bangalore | oblong | ,, | ,, | ,, | chartaceous |
| 4. | *A. violacea* Garden & Field | ,, | ovate | acute | acute | ,, | coriaceous |
| 5. | *Alstonia scholaris* R. Br. | Dangs, Gujarat | ,, | ,, | ,, | ,, | ,, |
| 6. | *Alyxia pubescens* Turril | ,, | ,, | ,, | obtuse | ,, | chartaceous |
| 7. | *Carissa carandas* Linn. | Kerala | ,, | ,, | acute | ,, | coriaceous |
| 8. | *C. congesta* Wight | Dangs, Gujarat | ,, | obtuse | obtuse | ,, | chartaceous |
| 9. | *C. spinarum* Linn. | ,, | ,, | ,, | ,, | ,, | ,, |
| 10. | *Catharanthes major* Linn. | Kerala | ,, | ,, | cordate | ,, | ,, |
| 11. | *C. pusillus* G Don. | Bulsar, Gujarat | ,, | acute | obtuse | ,, | ,, |
| 12. | *C. roseus* G. Don. | Local | ,, | obtuse | ,, | ,, | membranaceous |
| 13. | *C. variegata* | ,, | ,, | ,, | ,, | ,, | chartaceous |
| 14. | *Cerbera manghas* Linn. | Lalbagh, Bangalore | oblong | acute | acute | ,, | coriaceous |
| 15. | *C. odollum* Gaertn & Bunt | ,, | ,, | obtuse | ,, | ,, | ,, |
| 16. | *Chonemorpha macrophylla* G. Don. | Lalbagh, Bangalore | wide ovate | obtuse | obtuse | entire | coriaceous |
| 17. | *Holarrhena antidysenterica* G. Don. | Dangs, Gujarat | ovate | acute | obtuse | ,, | chartaceous |
| 18. | *Kopsia fruticosa* A. DC. | Kerala | elliptic | acuminate | acute | ,, | ,, |
| 19. | *Nerium indicum* Mill. | Local | narrow ovate | acute | ,, | ,, | coriaceous |
| 20. | *Parsonsia spiralis* Vidal | Kerala | ovate | acute | obtuse | ,, | chartaceous |
| 21. | *Plumeria rubra* Linn. | Local | obovate | obtuse | acute | ,, | coriaceous |
| 22. | *Rauwolfia serpentina* Benthe & Kurz | Lalbagh, Bangalore | ovate | acute | obtuse | ,, | chartaceous |
| 23. | *R. tetraphylla* Linn. | ,, | ,, | ,, | ,, | ,, | ,, |
| 24. | *Tabernamantana divaricata* R. Br. | Local | ,, | acuminate | ,, | ,, | ,, |
| 25. | *Thevetia peruviana* (Pers.) K. Schum. | ,, | needle-shaped | acute | acute | ,, | coriaceous |
| 26. | *Trachelospermum jasminoides* Lem. | Lalbagh, Bangalore | ovate | ,, | obtuse | ,, | chartaceous |
| 27. | *Vallaris solanacea* (Roth.) O. K. | Dangs, Gujarat | oblong | acuminate | ,, | ,, | ,, |
| 28. | *Wrightia tinctoria* R. Br. | Lalbagh, Bangalore | ovate | ,, | ,, | ,, | ,, |
| 29. | *W. tomentosa* Roem & Schutt | Dangs, Gujarat | ,, | acute | ,, | ,, | ,, |

| Predominant tertiary vein origin angle | Marginal ultimate venation | Leaf area in mm² | No. of 2° veins along one side midrib | Range of angle between 1° & 2° veins | No. of areoles/ mm² | No. of veinlets entering in areole/ mm² | vein ending termination/mm² |
|---|---|---|---|---|---|---|---|
| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| RR, RA | incomplete | 3870 | 8 - 12 | 50°-65° | 1 | 11 | 21 |
| AR, RO | incomplete | 3635 | 15 - 50 | 60°-85° | 1 | 8 | 32 |
| RA, OR, OO | ,, | 2830 | 12 - 15 | 50°-75° | 1 | 8 | 27 |
| RR, OR, RA | ,, | 5290 | 12 - 16 | 40°-65° | 1 | 8 | 56 |
| absent | ,, | 3775 | 35 - 38 | 65°-70° | absent | — | — |
| RA, RO, OR | Fimbriate | 2565 | 15 - 18 | 45°-60° | 1 | 11 | 18 |
| RO, AR, RR | incomplete | 125 | 8 - 10 | 25°-40° | 2 | 8 | 13 |
| AA, AR, RA | ,, | 1170 | 7 - 10 | 70°-85° | 1 | 22 | 49 |
| AR, RA, AO | ,, | 965 | 8 - 10 | 60°-80° | 1 | 20 | 37 |
| RR, RO, RA | ,, | 735 | 8 - 10 | 45°-75° | 1 | 9 | 13 |
| RO, RA, RR | ,, | 815 | 9 - 12 | 35°-55° | 1 | 15 | 22 |
| RR, RO | ,, | 935 | 7 - 10 | 25°-45° | — | — | — |
| RR, RO | ,, | 760 | 8 - 10 | 50°-75° | 1 | 10 | 9 |
| RR, RA, RO | ,, | 4210 | 15 - 18 | 45°-60° | 1 | 10 | 25 |
| RA, RO, RR | ,, | 4490 | 16 - 20 | 40°-55° | 1 | 9 | 22 |
| RR, RA | incomplete | 12875 | 20 - 25 | 70°-85° | 2 | 9 | 19 |
| AR, RR, RA | ,, | 3180 | 12 - 16 | 45°-65° | 2 | 6 | 19 |
| RA, AA, OA | fimbriate | 8850 | 18 - 22 | 55°-75° | 3 | 11 | 45 |
| RR, RO, RA | ,, | 2460 | 110 - 125 | 70°-85° | 6 | 3 | 1 |
| RR, OR | incomplete | 5135 | 14 - 16 | 65°-80° | 1 | 6 | 31 |
| RO, RR, RA | ,, | 12015 | 30 - 35 | 35°-55° | 1 | 9 | 29 |
| OR, RR, AR | ,, | 2615 | 12 - 15 | 45°-70° | 1 | 10 | 40 |
| RA, RR | ,, | 765 | 9 - 12 | 65°-75° | 1 | 12 | 41 |
| RR, AR, RO | ,, | 2135 | 10 - 14 | 35°-65° | 1 | 14 | 42 |
| AR, AO | ,, | 775 | 15 - 20 | 30°-45° | 2 | 14 | 10 |
| RA, OR, RR | ,, | 715 | 6 - 9 | 60°-80° | 2 | 5 | 26 |
| RO, RR, RA | ,, | 2850 | 12 - 15 | 70°-80° | 1 | 9 | 33 |
| OR, RA, RR | ,, | 5365 | 12 - 15 | 70°-85° | 2 | 8 | 17 |
| OR, RR, RA | ,, | 5530 | 11 - 15 | 60°-80° | 1 | 9 | 21 |

## 3. Observations

### 3.1 *Morphological description*

*Leaves* simple, alternate, opposite or whorled. *Shape* ovate, narrow to w ovate, obovate, oblong (figure 1 A), elliptic. *Margin* entire (figures 1 A, B, I *Apex* acute obtuse (figure 1 A) or rounded. *Base* acute, obtuse (figure 1 A) cordate. Texture chartaceous, membranaceous or coriaceous. The qualitative l features of the species studied are given in table 1.

### 3.2 *Major venation pattern*

The venation pattern conforms to pinnate camptodromous type with festoon brochidodromous secondaries in which secondaries do not merge at the mar but upturn and join together in a series of prominent arches forming brochic dromous secondaries having a set of secondary loops outside the main brochic dromous and form "festooned brochidodromous" type of Hickey and Wolfe (19 or multiarcuate wherein secondary veins form a coarcuate infra-marginal v and breaking up into a series of small arching loops forming a zone between infra-marginal vein and the margin (Melville 1976) in all the species, except *Catharanthus roseus* where the venation pattern seems to be eucamptodromo In eucamptodromous venation pattern, secondaries upturned and gradua diminishing apically inside the margin, connected to the super adjacent secon ries by a series of cross veins without forming prominent marginal loo (Hickey 1973) or simple curvipinnate. Here, secondaries curve gradually towa the margin and often form marginal or submarginal veins (Melville 1976). T primary vein or midrib is the thickest vein of the leaf and after its departure fr petiole it traverses straight or markedly curved. The thickness of the prima vein gradually decreases towards the apex. The next smaller size class of ve are the secondary veins (2° veins) whose origin may be on either side of primary vein in an alternate or sub-opposite fashion. The number of 2° veins either side of the primary vein varies from species to species irrespective of leaf size. The secondary veins do not merge into the margin but turn upwa and form arches with super adjacent secondaries with acute, right angle or obt angle. Composite intersecondary veins are observed in all species. Intramargi vein is observed in *Nerium indicum.* Intramarginal vein closely paralleling leaf margins and into which the secondary veins are fused; probably the result the fusion and straightening of the exmedial brochidodromous secondary a segments into what appears to be an independent vein (Hickey 1973) or margi vein simple and linear which is situated close to the leaf edge and without a other veins extending beyond it formed by linking the ends of all of the excurr veins at the margin (Melville 1976) (figure 1 B).

The tertiary veins arise from the secondaries having no definite patterns of an of origin. Predominant tertiary vein angles of origin are right angle right an (RR), right angle acute (RA), right angle obtuse (RO), acute acute (AA), ac right angle (AR), obtuse right angle (OR), acute obtuse (AO) or obtuse obtuse (O in all the species studied except in *Alstonia scholaris*, where the predomina tertiary vein origin angle is absent. Species-wise details of angles of origin

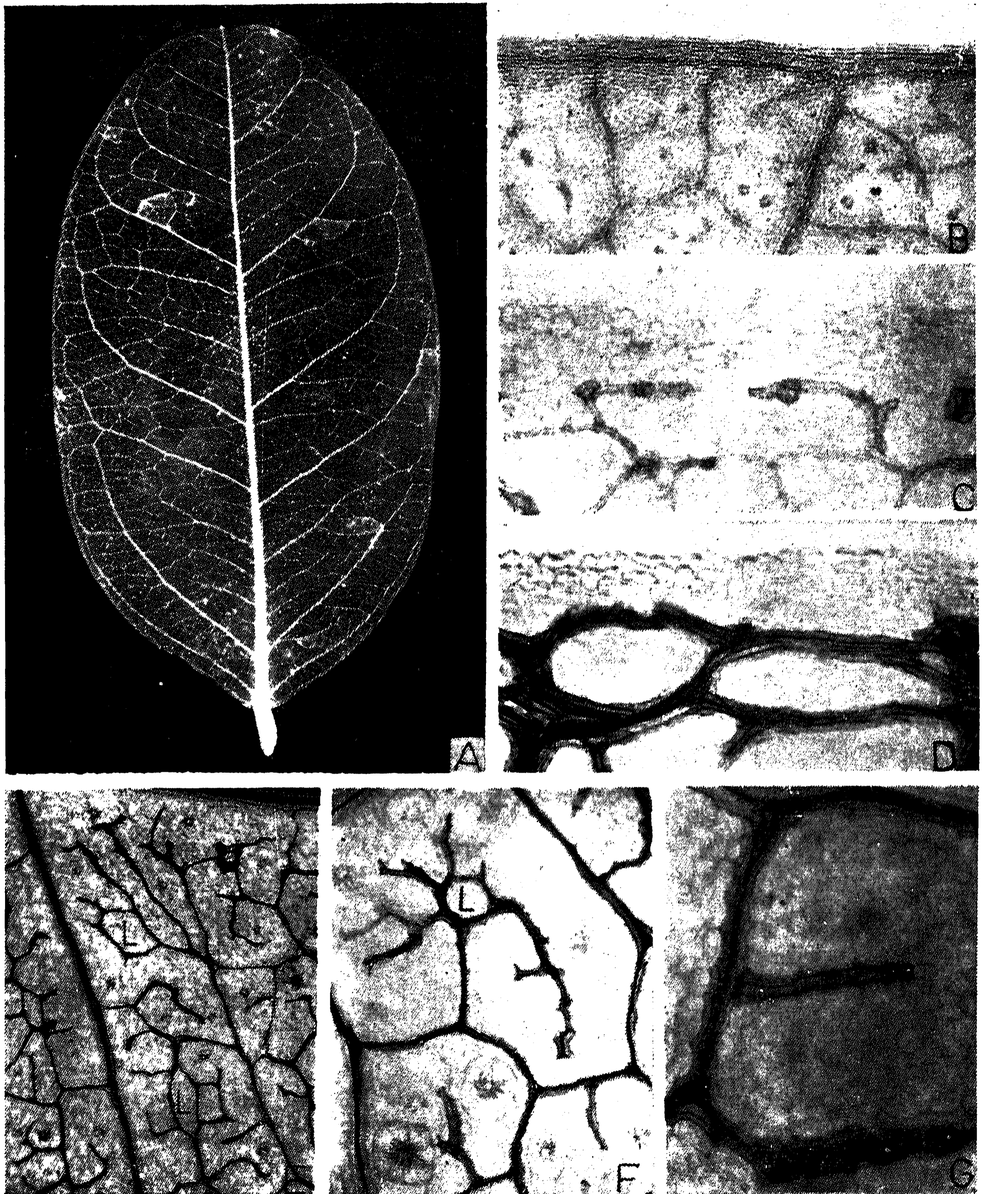


**Figure 1.** (A) direct photograph of cleared leaf showing venation pattern in *Carissa congesta* ; (B) infra-marginal vein in *Nerium indicum* ; (C) incomplete marginal ultimate venation in *Rauwolfia serpentina* ; (D) looped marginal ultimate venation in *Allamanda nerifolia* ; (E) areolation and loop formation in *Trachelospermum jasminoides* ; (F) loop formation and veinlets in *Trachelospermum jasminoides* ; (G) free vein ending and bundle sheath in *Wrightia tinctoria*

A — 2.1 × ; B — 55 × ; C — 149 × ;
D — 115 × ; E — 43 × ; F — 270 × ;
G — 335 ×

L — Loop T — tracheid

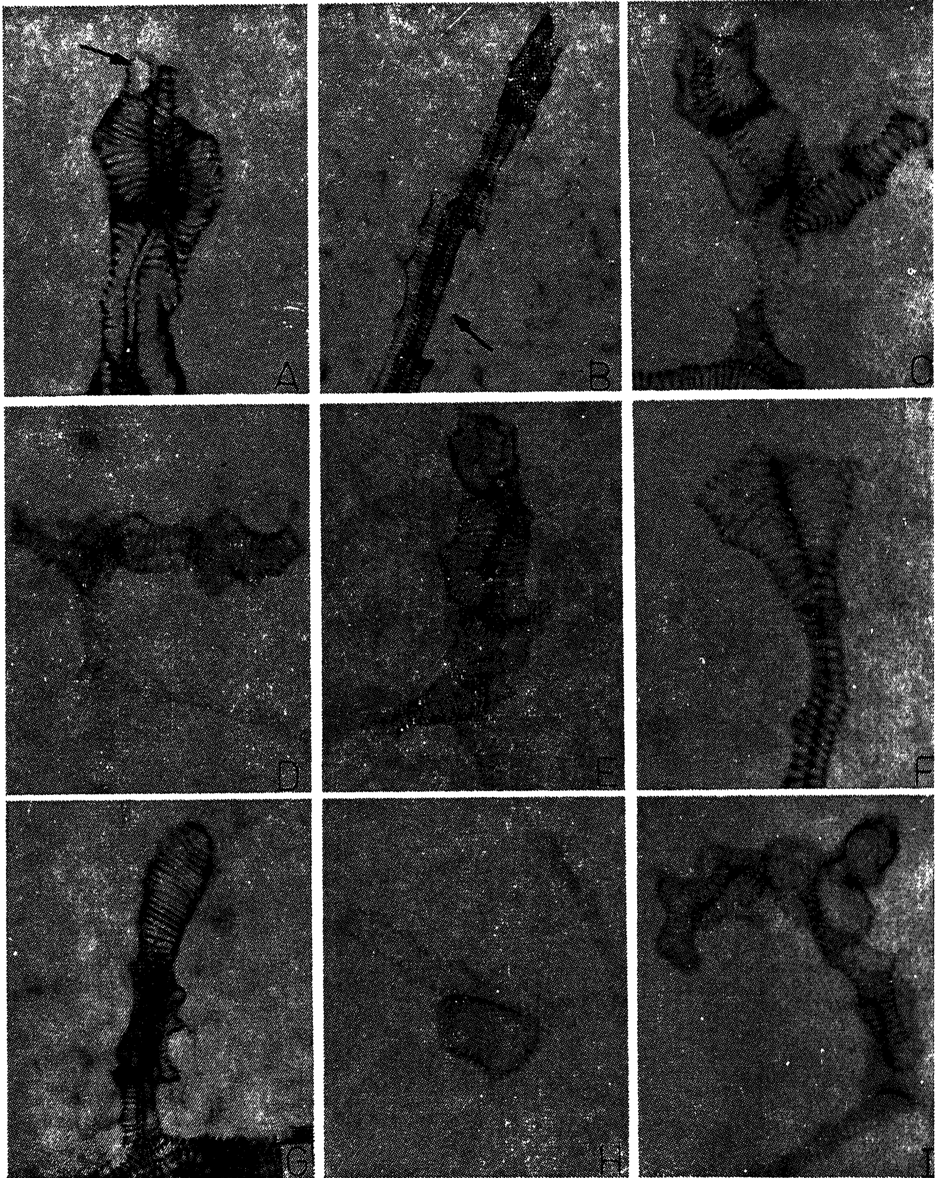


**Figure 2.** (**A**) vessel element at the terminal position of the veinlet in *Catharanthus major* ; (**B**) undifferentiated tracheary element (extension cell) in multiseriate vein in *Catharanthus major* ; (**C, D, E, F**) uniseriate or biseriate tracheids in *Rauwolfia serpentina* and *Vallaris solanacea* ; (G) uniseriate elongated tracheid in *Catharanthus major* ; (**H, I**) tracheoidal elements in *Rauwolfia serpentina* and *R. tetraphylla*.

A — 617 × ; B — 435 × ; C — 402 × ;
D — 335 × ; E — 371 × ; F — 672 × ;
G — 385 × ; H — 471 × ; I — 335 ×.

given in table 1. The pattern of the tertiary veins is either random or orthogonal reticulate (Hickey 1973) or scalariform where inter-coastal areas are bridged at regular intervals by transverse veins either at right angles or with a regular orientation and having the appearance of rungs on a ladder; transverse ramified (Hickey 1973) or dendroid - regularly or irregularly dichotomous veins occupying an areole and attached to the areolar veins at one point (Melville 1976) (figure 1 E). But in *Alstonia scholaris* admedial ramification of tertiary veins branching into higher orders without rejoining the secondary veins and oriented towards the leaf axis (Hickey 1973) or pendulous type with branching veins lying free in an intercostal area or an areolus, attached at their distal ends and appearing to be pendulous from a submarginal vein or costal vein.

### 3.3 *Minor venation pattern*

The highest order of veins is identified up to 7°, but in *Catharanthus roseus* up to 3° or 4°, in some cases up to 5° or 6°. The numerical data on the venation pattern are charted in table 1. Marginal ultimate venation is incomplete (Hickey 1973) or marginal vein simple and incomplete *i.e.* marginal vein broken, linking some of the excurrent veins but leaving others free (Melville 1976) (figure 1 C), fimbriate (Hickey 1973) or marginal vein simple and arcuate *i.e.* marginal vein formed of arching veins linking the ends of the excurrent veins (Melville 1976), looped (Hickey 1973) or marginal vein simple and irregular (Melville 1976) (figure 1 D).

The areoles are the smallest areas of the leaf tissue surrounded by the major veins which taken together form a contiguous field over most of the area of the leaf. The areoles in most of the cases are either well developed or imperfect. But in *Alstonia scholaris* the areolation is lacking. The arrangement of the areoles is either random or oriented. The shape of the areoles may be quadrangular, pentagonal, polygonal or irregular. The size of the areole is not constant, but varies in different species and even in the same species. Venation characters show variations in areole size, number of veinlets entering per areole and the organizations of terminal vein endings in different species.

### 3.4 *Veinlets*

The ultimate veins of the leaf are either simple or branched. Simple vein endings may be linear or curved (figure 1 F). The branched ones divide dichotomously once or twice symmetrically or asymmetrically (figure 1 F). The veinlets may be uniseriate (figure 1 C), biseriate or multiseriate (figure 2 B). They may be thin and long or thick and short. The veinlet number vary irrespective of areole size. The veinlets whether uni-, bi- or multiseriate without terminal tracheids are known as free vein endings (figure 1 G). Occasionally a vessel with a single scalariform perforation plate is present at the vein tip alongwith the tracheids in *Catharanthus major* (figure 2 A at arrow). In most of the cases where areoles are devoid of vein endings a loop-like structure is seen (figure 1 A, E, F) which is formed either due to the union of tracheids, veins or tracheids and veins. Rarely, in a multiseriate veinlet some of the elements fail to differentiate into tracheary elements (figure 2 B at arrow).

### 3.5 *Tracheids*

The tracheids manifest extraordinary variation in size, shape and nature and situated at the terminal position of the veinlets. Tracheids may be uniseriate (figure 2 C, G) or biseriate (figure 2 F). They may be juxtaposed (figure 2 F) or superimposed having 'V', 'T' or club-shape (figures 2 C, D, E, F; 3 F). They may be isodiametric grouped or elongated.

### 3.6 *Tracheoidal elements*

Tracheoidal elements are observed in most of the species. They lie lateral and parallel to the veins and veinlets (figures 2 H, I; 3 A). The tracheoidal elements may be isodiametric or elongated and arranged either scattered or in rows. Probably the function of tracheoidal elements is to provide mechanical support and also aid in retention of water for the leaf.

### 3.7 *Isolated tracheids*

Uniseriate or biseriate tracheids that lie free and disjunct in the mesophyll and those connected with veinlets by extension cells are called isolated tracheids (figure 3 B, C).

### 3.8 *Isolated vein endings*

Vein endings with terminal tracheids either uni- or biseriate lying free and disjunct in the areole are known as isolated vein ending (figure 3 D, E). These are common in most of the cases.

### 3.9 *Isolated free vein endings*

These are uniseriate or biseriate vein ending without terminal tracheids lying free and disjunct in the areole. These vein endings are seen in *Catharanthus roseus* (figure 3 I).

### 3.10 *Extension cells*

These are parenchymatous cells which either adjoin isolated tracheid with a vein (figure 3 G) or a vein with another vein (figure 3 H). Extension cells have failed to differentiate either into sieve or tracheary elements.

### 3.11 *Bundle sheath*

All the major and higher order veins are ensheathed by parenchymatous bundle sheath cells. The thickness of bundle sheath varies from primary to higher order veins. The shape of the bundle sheath cells may be either round, isodiametric or rectangular (figure 1 G).

## 4. Discussion

According to Hickey (1973) and Melville (1976) the Apocynaceae leaves fall under the pinnate camptodromous with festooned brochidodromous secondaries,

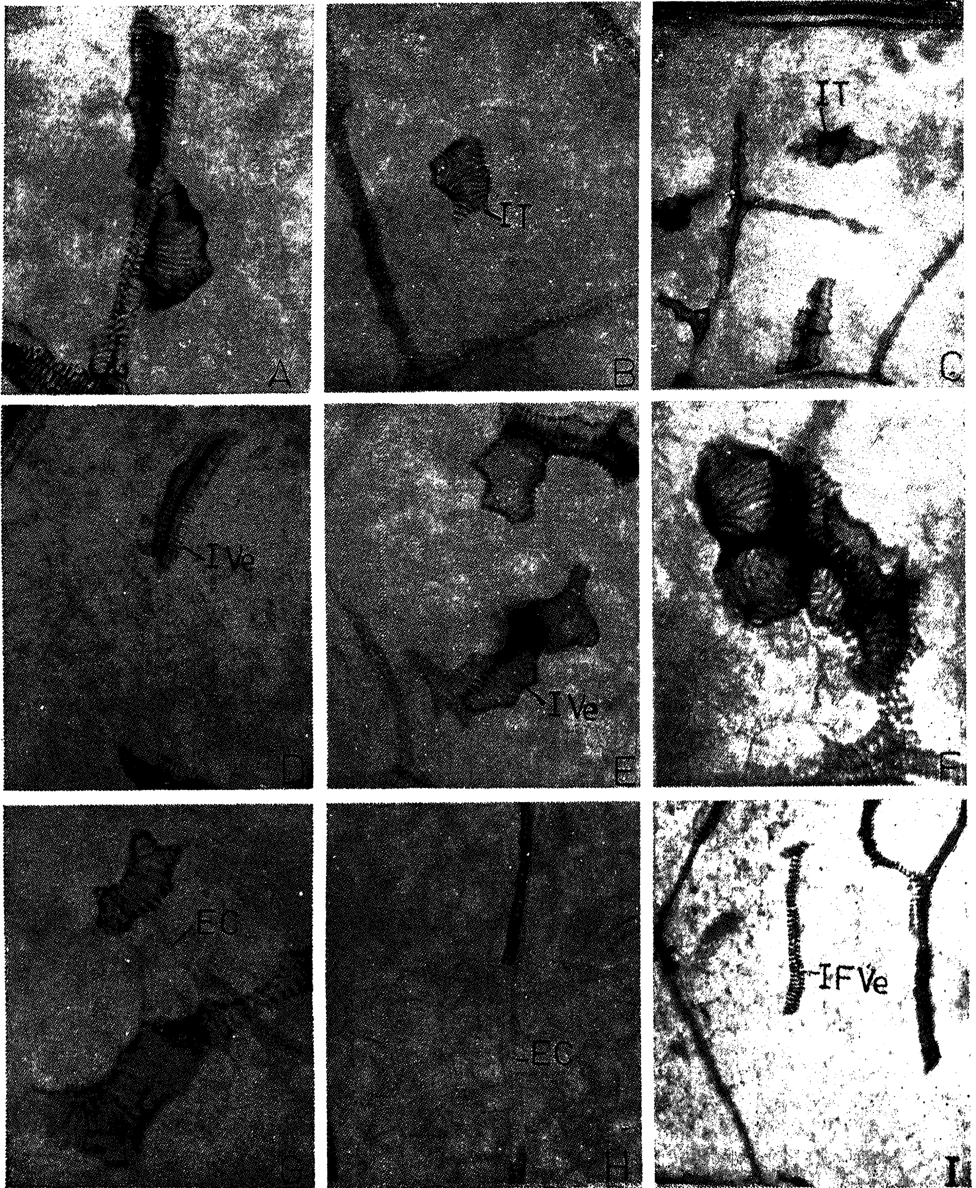


**Figure 3.** (**A**) tracheoidal element in *Catharanthus major* ; (**B**) isolated tracheid in *Rauwolfia serpentina* ; (**C**) grouped isolated tracheid in *Rauwolfia tetraphylla* ; (**D, E**) isolated vein endings in *Rauwolfia serpentina* and *Allamanda nerifolia* ; (**F**) isodiametric tracheids in *Rauwolfia tetraphylla* ; (**G**) extension cell between tracheid and vein in *Rauwolfia serpentina* ; (**H**) extension cell between vein and vein in *Catharanthus roseus* ; (**I**) isolated free vein ending in *Catharanthus roseus*.

A — 252 × ; B — 289 × ; C — 335 × ;
D — 292 × : E — 303 × ; F — 429 × ;
G — 471 × ; H — 435 × : I — 271 ×.

Ec — extension cell
IFVe — isolated free vein ending
It — isolated tracheid
IVe — isolated vein ending

rarely pinnate eucamptodromous or curvipinnate venation pattern with multi-arcuate type, rarely simple curvi-pinnate respectively. Sehgal and Paliwal (1974) classified the euphorbiaceous leaves as uni-, bi- or triveined on the basis of the number of strands entering the base of the leaf. Apocynaceae leaves fall under the univeined category.

Reports on the significant variation in the size, shape and number of vein endings entering the areole are contradictory (see Nicely 1965; Sehgal and Paliwal 1974). Sehgal and Paliwal (1974), Singh *et al* (1976), Jain (1978) and Inamdar and Murthy (1978) concluded that there is no direct relationship between size of an areole and the number of vein endings and vein termination in different species as well as in the same leaf.

Hickey (1973) classified the marginal ultimate venation into looped, fimbriate and incomplete. In *Nerium indicum* the intramarginal vein is formed by secondaries only and there are no higher order veins beyond it. Therefore, it becomes extremely difficult to classify the marginal ultimate venation according to Hickey (1973), however, it can well be classified into marginal and linear type of Melville (1976).

Tracheids or tracheoidal idioblasts (Foster 1956) also regarded as peculiar cells (Gilbort 1881; Bierhorst and Zamura 1965), storage tracheids – speichert-racheiden (Solereder and Meyer 1930), mechanical cells (Mangin 1882), water storage cells (Kny and Zimmermann 1885), water cells (Pirwitz 1931). Tucker (1974) referred to these elements as hybrid cells. According to Oltunji and Nengim (1980) the occurrence of the tracheoidal elements in many unrelated plants may be due to convergent adaptation to xerophytic habit. The occurrence of tracheoidal elements along the lateral side walls of veinlets and rarely on the 4° and 5° veins are noticed in this family. These tracheoidal elements differ from tracheids in form, arrangement and thickening.

Kasapligil (1951), Foster and Arnott (1960), Herbst (1972) reported the occurrence of isolated veins in dicotyledonous leaves. The tracheidal elements which lie free in the areole are designated as "free vein ending" by Sehgal and Paliwal (1974). The isolated tracheids, isolated vein endings and free veins are observed in most of the species. Terminologies such as vein endings, free vein endings, isolated vein endings and isolated tracheids as defined by Inamdar and Murthy (1981) are used here. Sehgal and Paliwal (1974) termed the parenchymatous sheath cells which surrounds the veins, as 'ornamented'. Bundle sheath is present in all the cases around the veins, vein ending and even tracheids.

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We thank Dr (Sr) Avita for kindly supplying some material. Mr J S S Mohan thanks the CSIR for awarding a junior research fellowship.

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# Impact of extension growth and flowering on the cambial ıctivity of *Delonix regia* Rafin.

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MS received 3 July 1981; revised 18 May 1982

**Abstract**. Shoot growth in *Delonix regia* takes place in three distinct flushes, the first commencing in mid-March, the second in late May and the third in October. The cambial reactivation starts in April after the initiation of the first flush of shoot growth, but the addition of new vascular derivatives does not take place until the second flush of shoot growth has occurred. Heavy flowering which ensues following first flush of shoot growth seems to delay cell divisions in cambial initials in April. Once the formation of cambial derivatives starts, it continues till mid-November whence the cambium enters the dormant phase. Xylogenesis begins from July and the formation of phloem in October, while the precursor phloem differentiates in early April.

**Keywords**. Tree growth; extension growth; flowering cambial activity; vascular derivatives; *Delonix regia.*

## . Introduction

Coster as early as 1927-28, considered that bud bursting and new leaf emergence .re highly significant for the initiation of cambial activity. The later works on everal tropical and temperate trees have supported Coster's contention (Chowlhury 1958, 1969; Chowdhury and Tandon 1950; Wareing *et al* 1964; Waisel and Fahn 1965; Waisel *et al* 1966; Fahn *et al* 1968; Ghouse and Hashmi 1979a). It s believed that an intricate relationship exists between bud burst, leaf emergence ınd initiation of cambial activity, the first two preceding the last. The present eport deals with the relationship between shoot growth, flowering and the cambial ıctivity in *Delonix regia*, a flowering tree which originated in West Africa and is presently grown in many parts of India. It has a deciduous habit and diffuse porous wood. The study has been undertaken at Aligarh which is located at 27° 53′ N lattitude and 78° 4′ longitude in the monsoon belt of the great gangetic plain of North India.

## 2. Materials and methods

Thirtysix trees of *Delonix regia* Rafin. planted 30 years earlier at the University Campus of Aligarh were used for the present investigation. To study the phenology

of the selected trees, 40 current year branches were tagged with aluminium label in each tree, at the rate of 10 branches facing east—west, north and south sides. Observations were recorded on leaf fall, bud burst, leaf emergence, flowering fruit setting, fruit persistence and bud burn for 3 consecutive calendar years commencing from 1974.

The daily data on atmospheric temperature, relative humidity and rainfall were collected from the local meteorological unit maintained at the University Campus. The monthly average of physical factors was calculated and represented in figure 1.

Samples of cambial strips of 2 cm sq, together with the inner bark and some sapwood were collected at fortnightly intervals for 3 consecutive calen

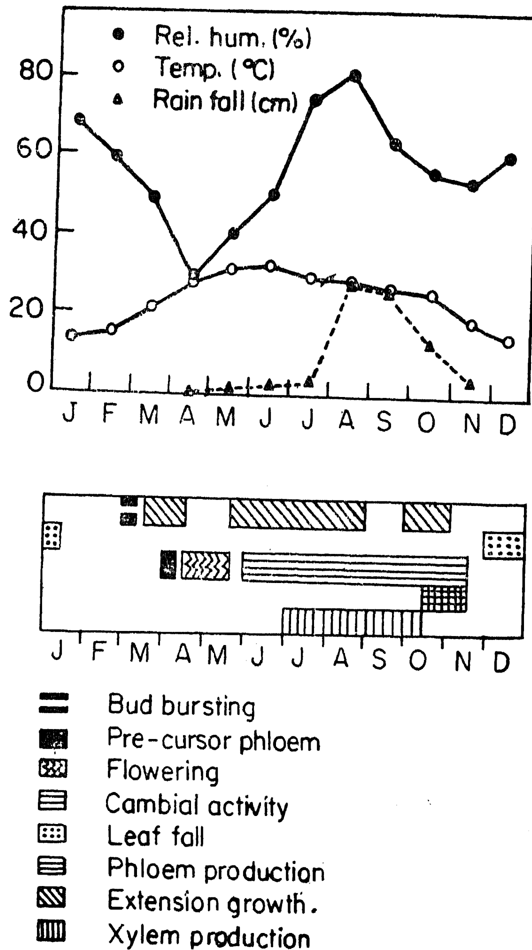


**Figure 1.** Graphs showing the relationship between the atmospheric temperature, relative humidity, rainfall, phenology and cambial activity in *Delonix regia* during a calendar year.

dar years starting from 1974. Chisel and hammer were employed to take out the blocks from the main trunks at chest height (1.5 m from ground level). Three trees were sampled on each turn and from each tree four blocks were obtained, one each from east, west—north and south side of the tree. The excised blocks were fixed on the spot in FAA (formalin-acetic acid and ethanol mixture) and aspirated after an hour. After fixation, they were softened in an alco-glycerol 1:1 mixture of 50% ethanol and 50% glycerol for 4 weeks. Sections were prepared using a sliding microtome in transverse, tangential and radial longitudinal planes at a thickness of 10 $\mu$m. The sections were stained with tannic acid and ferric chloride (Foster 1934) and with lacmoid combination (Cheadle *et al* 1953) and mounted in Canada balsam, after dehydrating in ethanol-xylol series.

## 3. Observations

### 3.1 *Extension growth*

*Delonix regia*, being a deciduous tree, starts shedding leaves from December and becomes completely bare by early January and remains so till mid-March (figure 1). In March-April, the weather becomes a little warm in day and large number of buds emerge from the naked branches of the previous year in the axils of fallen leaves. The bud grows rapidly and produces two or three bi-paripinnate leaves within a fortnight. By mid-April buds of floral axes come up in large quantity from the axils of new leaves on current year shoots. These buds develop into a large corymbose inflorescence, each having a large number of conspicuously coloured showy flowers. The trees bloom heavily in late April and early May and as a result, the green crown appears as flaming red or orange.

The first flush of extension growth which begins in late March ends by late April when reproductive growth becomes established. As a result of heavy flowering, the vegetative growth of the shoots stop completely. In late May or a little earlier, flowering declines and the fruits start setting. It may be noted that the fruits remain on the tree for about 14-15 months.

The second flush of shoot growth starts in the second half of May and continues up to August. It occurs at a rapid rate and accounts for the major part of the year's growth product.

The third flush of shoot growth occurs in October in the same year, although for a short duration because the low night temperature of November burns down the apices of the branches. Leaf fall ensues in early December and the events repeat once more (figure 1).

### 3.2 *Cambial activity*

The microscopic analysis of the cambial samples, collected at fortnightly intervals, indicates that cambial reactivation commences in the first half of April soon after bud burst and leaf emergence in first flush of extension growth. To start with

the protoplasmic contents of the cambial cells stain lighter than before. Concurrently, the nuclei also lose their chromaticity. In late May a few cells of the cambial zone enlarge in radial direction and later enter the active phase by undergoing cell division in the first week of June. The radial growth thus resuming in early June continues up to the second week of November, stretching over a period of five and a half months against the eight month extension growth occurring in three distinct flushes.

After cell division, the size of cambial zone population swells up (figures 2 C,D) touching its peak in August-September and declining later, as the derivatives continue to differentiate at a rapid rate. In October the activity slowly declines and stops by mid-November. The cambium thus enters its dormant phase in late November when the cells develop dark protoplasmic contents (figures 2 A, B). The walls of the cambial cells, especially the radial walls become thicker and develop beaded appearance on account of unthickened primary pit-fields. The cambium remains in this state till the following spring.

### 3.3 *Formation of xylem and phloem*

New xylem differentiates in trunks in July after the break of monsoon, although the cell divisions start in cambial cells one month earlier. Xylem formation takes place at a high rate throughout August and September and slows down in October and later stops by mid-October (figure 1). The phloem production, on the other hand, initiates in October, when the air temperature becomes somewhat moderate and lasts for about a month.

Earlier to the initiation of cambial activity, but after the advent of summer in early April, a few layers of cells differentiate into a narrow strip of new phloem out of the overwintered mother cells. This precursor phloem measures about 75 to 100 $\mu$m in depth in transections.

In a growth year, as viewed in transections, about 800 $\mu$m of xylem and about 500 $\mu$m of phloem are produced by *D. regia* at Aligarh conditions of weather.

## 4. Discussion

The majority of dicotyledons and gymnosperms show a sharp periodicity of shoot growth including radial growth. However, in certain exceptional cases growth may occur throughout the year without break, particularly in the tropical environment (Alvim 1964; Fahn and Sarnat 1963; Fahn *et al* 1968).

The pioneering work of Chowdhury (1958, 1968, 1969) has shown that the radial growth in certain Indian trees may extend up to 10 months in a calendar year. Other works also indicate that the tree growth in tropics takes place for a considerably longer duration than at the temperate regions (Fahn and Sarnat 1963; Lawton 1972; Rao 1972; Chau and Chiang 1973; Lu and Chiang 1975; Khan and Ghouse 1978, 1980; Ghouse and Hashmi 1979a). The present study on *D. regia* also indicates the same, as far as the extension growth is concerned. However,

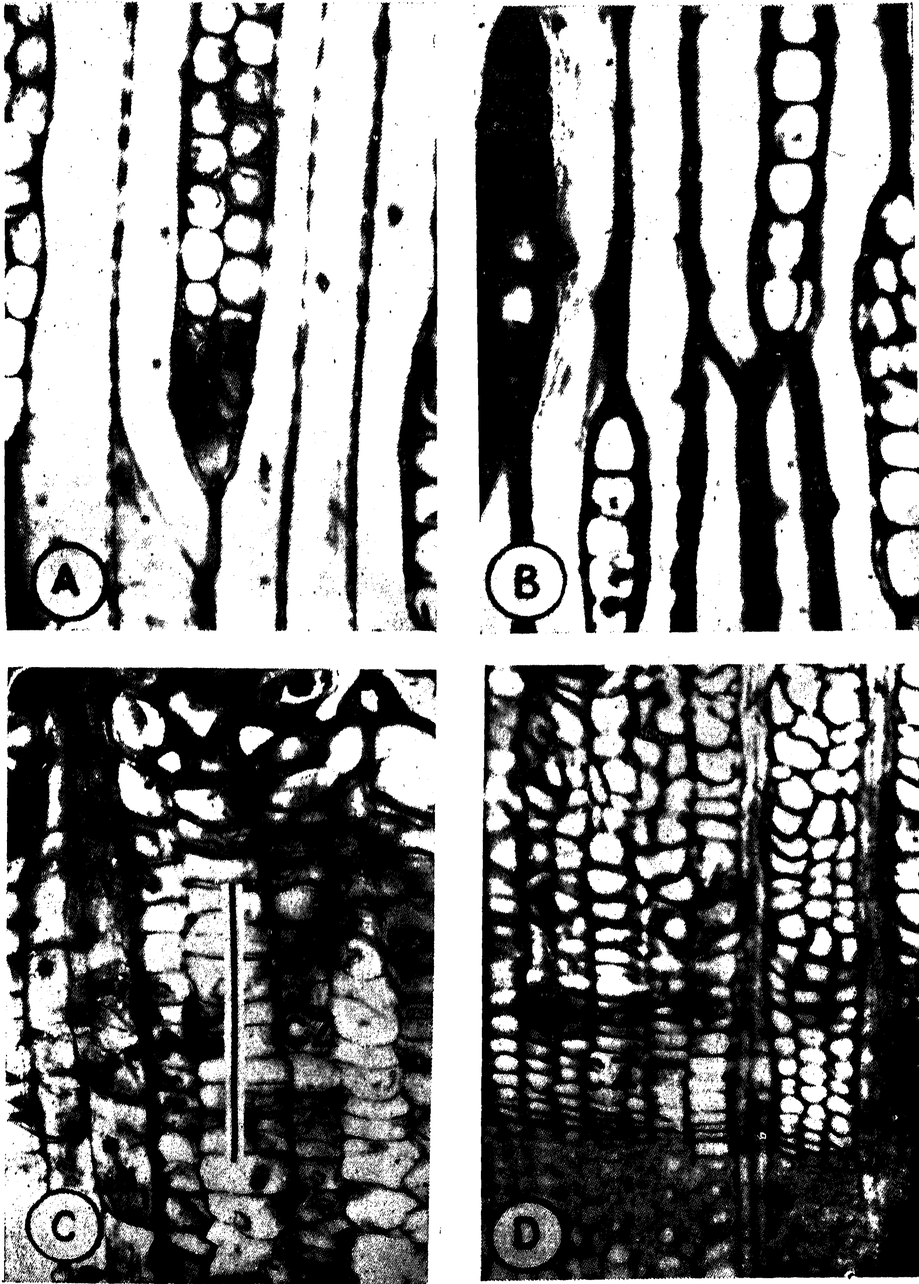


**Figure 2.** Photomicrographs showing the active (A and C) and dormant phases (B and D) of vascular cambium in tangential (A and B) and transverse (C and D) sections-A, B, C at × 372; D at × 160

radial growth in *D. regia* takes place only for a period of five and a half months in a year and it, therefore, does not resemble other trees which grow in tropics. This may be due to the flowering habit of this species in which the cell division in cambial cells is seemingly delayed by heavy flowering till June.

The direct relationship between extension growth and the cambial activity established by earlier workers like Chowdhury and Tandon (1950), Chowdhury (1958, 1969) and Ghouse and Hashmi (1979a) is further getting confirmed in the present study, since it demonstrates that the occurrence of extension growth acts as a prerequisite factor for the initiation of cambial reactivation in the investigated species. In *Polyalthia longifolia* the authors noted that the reactivation of vascular cambium is invariably preceded by the swelling phenomenon in certain cambial cells and this incidence is initiated, in turn, by the emergence of leaves (Ghouse and Hashmi 1979a). The results obtained in the present study also indicate the same for *D. regia*. In an earlier communication the authors further brought to light that high temperature and high humidity accelerate the differentiation of xylem and low humidity favours the formation of phloem in *P. longifolia* (Ghouse and Hashmi 1978). A careful scrutiny of the data obtained in the present study reveals that the requirements for differentiation of phloem and xylem are different and they follow more or less the same trend in *D. regia and P. longifolia* (Ghouse and Hashmi 1978).

The decrease in chromaticity of the protoplasmic contents, tanniferous substances and the cell wall characteristics have also been noticed in the past (Derr and Evert 1967; Tucker and Evert 1969; Ghouse and Hashmi 1979a).

Differentiation of phloem preceding that of xylem has been recorded in a number of tropical trees including those that grow in India (Lawton 1972; Ghouse and Hashmi 1978, 1979b). In the present study xylem has been noticed to differentiate first during a growth year. However, the precursor phloem differentiation as noted in *D. regia* is more commonly observed in the Indian trees (Ghouse and Hashmi 1979b, 1979c, 1980) than reported so far in the temperate trees (Evert 1960, 1963; Derr and Evert 1967; Davis and Evert 1968).

The differentiation of phloem two times in a year first in early April and next in October, as found in *D. regia* invites special attention. The phenomenon appears to be controlled more by the environmental conditions than by the internal make up of the species. A cursory look of the weather data provided in figure 1 indicates that the temperature during the periods of phloem differentiation happens to be almost the same in the present case. It appears that a slight rise in temperature above this level does not seem to favour phloem differentiation in this species. A situation of more or less similar nature has been observed in *Polyalthia longifolia* by the authors (Ghouse and Hashmi 1978).

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# Pharmacognostic studies on the flower of *Mesua ferrea* L.

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**Abstract.** Stamens of *Mesua ferrea* L. constitute the genuine 'Nagkeshar Ayurveda, a drug considered to be astringent, stomachic and expectorant. The pr communication deals with detailed pharmacognosy of the drug and includes mo logical, anatomical as well as certain phytochemical characters of the floral pa *Mesua ferrea*. Some of the distinguishing characters are : cortical fibres, num resin canals and calcium oxalate crystals in the cortex and pith of the pe anamocytic, anisocytic or paracytic stomata on sepals and petals ; and zonocolporate pollen grains with reticulate exine surface. Fluorescence an behaviour of the drug with certain chemical reagents and thin layer chromatog were also carried out.

**Keywords.** *Mesua ferrea* ; flower ; pharmacognosy ; cluseaceae.

## 1. Introduction

*Mesua ferrea* Linn. belonging to family Cluseaceae is considered the ger 'Nagkeshara' (Chunekar 1960 ; Dymock *et al* 1885 ; Mudaliar 1957 ; Va 1971 ; Waring 1868). It is known as Nagpushpa champeya in Sanskrit ; I nagkeshara in Hindi ; Nageshwara in Bengali ; Nagchampa in Madhya Prad Pilu-nagkeshara in Gujrati ; Viluha champkan in Tamil ; Iron wood tree Cobras Saffron in English.

Stamens of *M. ferrea* constitute the actual drug (Sharma 1978). It mention in all the important ancient Ayurvedic literature (Bhavmishra 19 Charaka 1949 ; Sushruta 1952) to be used as an astringent stomachic expectorant. Flowers are given in bleeding piles, in the form of a paste m with butter and sugar. These are also applied for relief in burning sensatio the feet (with old and a hundred times washed 'Ghee') (Chakradatta, See Nadk 1937; Kirtikar and Basu 1933). A syrup of the flower buds (1 in 10) is sa cure dysentery (Nadkarni 1937). The drug was also adopted by Arab Unani physicians into their Materia Medica (Anonymous 1970) accordin which it has a depressent action on uterine muscles, is digestive, diaphor antidysenteric and an emmenagogue. It is used as a constituent of abou compound Unani formulations (Anonymous 1970).

N.B.R.I. Research Publication No. 139 (New Series)

*Mesua ferrea* L. is a large, evergreen tree with a short trunk, often buttressed at the base. It is found in the Himalayas from Nepal eastwards ascending to an altitude of 1500 m. It is also cultivated in the gardens for its beautiful flowers and attractive foliage (Anonymous 1962). Detailed pharmacognostic work on the flower of *Mesua* has not been carried out so far and hence the present study.

### 1.1. *Previous Work*

Several workers have carried out market surveys of Nagkeshara. Satkopan and Thomas (1967) surveyed Gujrat markets and found only one sample from Surat as genuine 'Nagkeshara'. These authors have also tried to identify other samples being sold as Nagkeshara (Satkopan and Thomas 1967a,b ; 1968). Ali (1967) surveyed the South Indian Markets. However, he found none of the samples as *Mesua ferrea* L.

The presence of an essential oil and two bitter substances has been reported in the flowers by Boorsma in 1904 (see Chakraborty *et al* 1959). Subramanyam *et al* (1975) have isolated mesuanic acid, a new carboxylic acid, from the acetone extract of *Mesua ferrea* stamens. Subramanyam and Subba Rao (1969) had earlier isolated mammeisin from the seeds of *Mesua ferrea* L. Bala and Seshadri (1971) isolated mammeigin and mesuol as the main phenolic components from two different samples of seed oil. Dutt *et al* (1940), Chakraborty and Bose (1960) and Chakraborty and Das (1966) isolated two crystalline antibiotic principles mesuol and mesuone from the seed kernel oil. Chakraborty *et al* (1959) investigated anti-bacterial activity of mesuol and mesuone. None of the constituents, however, was found to be antifungal. Bhattacharya *et al* (1979) have synthesized an antibiotic mesuagin from *Mesua ferrea.*

## 2. Material and methods

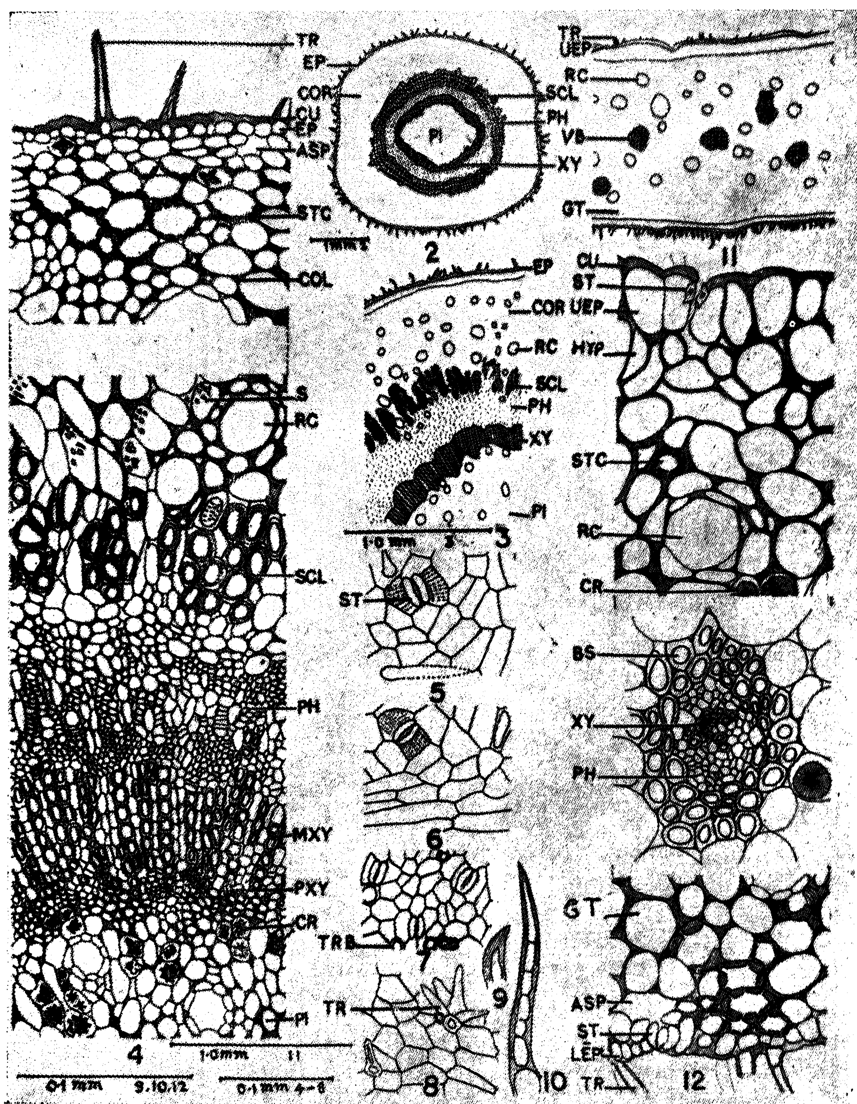
Fresh material was collected from the Forest Research Institute, Dehra Dun in the month of May and preserved in form acetic-alcohol. Hand sections stained with safranin were used for the present study. Phloroglucinol, iodine and terric chloride were used to test lignin, starch and tannin respectively. For whole mounts petals were treated with a dilute solution of nitric acid followed by clearing in chloral hydrate. Physicochemical studies were performed with the powdered, shade dried material.

## 3. External characters of the flower

The flowers are fragrant ; cream coloured ; ebracteate ; pedicellate ; pedicel short, axillary or terminal ; solitary or in pairs and 2.5-7.5 cms in diameter (figure 1). The buds are subglobose.

Sepals 4, large persistent, boat shaped, 2 outer slightly shorter than the inner ones and depressed at the base. Petals 4, large, cream coloured, spreading, cuneate, margins undulating, caducous ; stamens indefinite, golden-yellow, united

**Figure 1.** *Mesua ferrea* Linn : Two twigs with flowers × 0.425.




**Figures 2-12.** **2, 3.** T. S. pedicel (Diagrammatic) **4.** A portion of transverse section through the pedicel region showing details. **5.** Upper epidermis of a sepal showing stomata and striations. **6.** Upper epidermis of a sepal showing elongated narrow cells over the vein. **7.** Lower epidermal cells of the sepal showing trichome bases and stomata. **8.** Lower epidermal cells showing trichomes only. **9** and **10.** uni and multicellular uniseriate trichomes. **11.** T. S. sepal (Diagrammatic). **12.** A portion of the same showing details.

*Abbreviations :* ASP, airspace; BS, bundle sheath; COL, collenchyma; COR, cortex; CR, crystal; CU, cuticle; EP, epidermis; GT, ground tissue; HYP, hypodermis; LEP, lower epidermis; MXY, metaxylem; PH, phloem; Pl, pith; PXY, protoxylem; RC, resin canal; S, starch grains; SCL, sclerenchyma, ST, stomata; STC, stone cells; TR, trichome; TRB, trichome base; UEP, upper epidermis; VB, Vascular bundle; XY, Xylem.

at the base and forming a fleshy basal sheath, filaments small, anthers oblong; ovary superior, bicarpellary, syncarpous, style twice as long as stamens, stigma capitate, style and stigma persistent in young fruit but are shed away later on.

Fruit conical when young, ovoid to almost round with a prominent beak when mature, pericarp hard, warty, 2-valved after dehiscence. Seeds 1-4, angular, smooth and chestnut brown.

## 4. Histology

### 4.1 *Pedicel*

A transverse section of the pedicel is almost circular in outline. The epidermis is highly cuticularised and is formed by narrow elongated cells bearing one- to five- celled, uniseriate hairs. The cortex is 19 or 20 layered and is composed of rounded, collenchymatous cells. Cells of the outer cortical layers have characteristic semi-lunar, lignified thickenings on their walls. The inner cortical cells have fairly large starch grains as eragastic inclusions. As the pedicel matures sclerenchymatous fibres develop in the form of radially elongated patches outside the phloem. The phloem is well developed and forms a concentric cylinder. It consists of sieve tubes and phloem parenchyma. The xylem is endarch and comprises of vessels, fibres and xylem parenchyma.

The pith is well developed and collenchymatous like the cortex. Resin canals (which are lined by numerous elongated secretory cells) and Ca-oxalate crystals of rosette and prismatic types are abundant in both cortex and pith (figures 2-4).

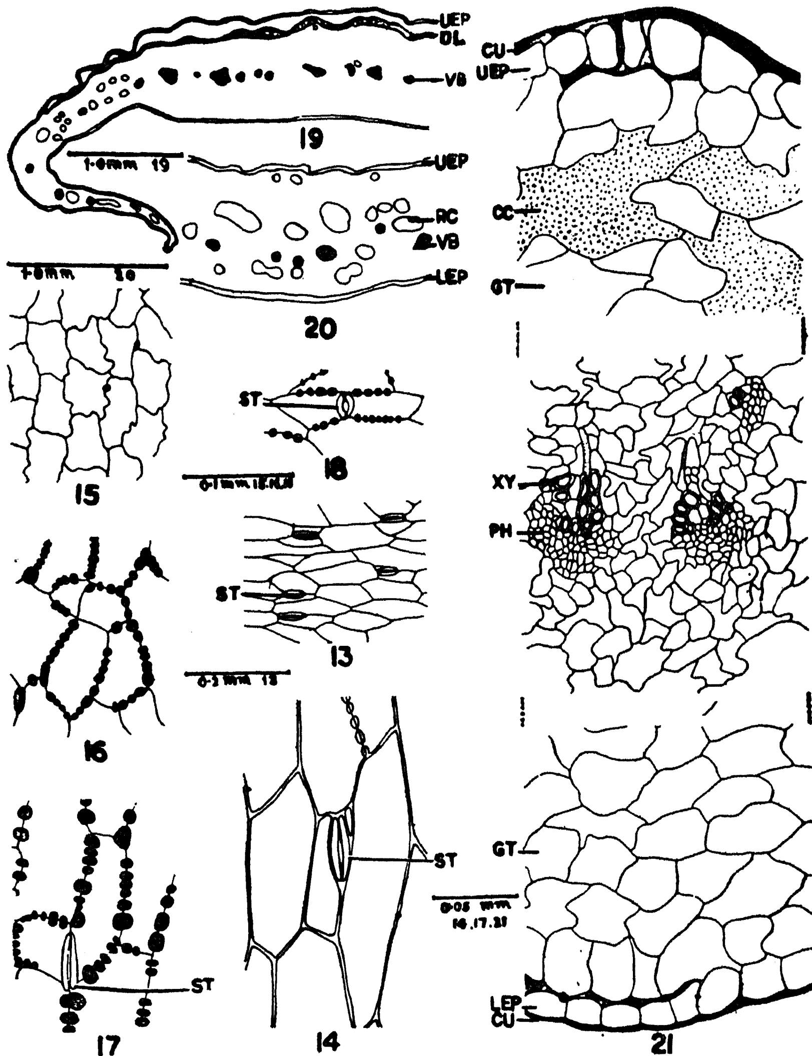
### 4.2 *Sepals*

In surface view cells of the upper epidermis are larger as compared to those of the lower one (figures 5-8). Stomata are anisocytic or paracytic (figures 5-7) and those on the upper surface are sparse and larger in size. The guard cells have parallel striations radiating out from the stomata (figures 5 and 6).

Cells over the veins are narrow and elongated (figure 6). Uni- to multicellular, uniseriate trichomes (figures 9 and 10) are present on both the surfaces but are more abundant on the lower one.

A transverse section of the sepal is semi-lunar in outline. Cells of the upper epidermis have a thick cuticle on the outside; are larger, and broader than long and have lignified walls and sunken stomata (figures 11 and 12). The epidermis is followed by a well demarcated hypodermis, again with lignified large rounded cells. In contrast the lower epidermis comprises of small, narrow, vertically elongated and unlignified cells.

The ground tissue is many layered and has rounded collenchymatous cells. Up to four layers of these have greatly thickened cells similar to those in the pedicel cortex. The vascular bundles are scattered in the middle of the ground tissue. These are usually amphicribral and are surrounded by a bundle sheath formed of two to three layered sclerenchymatous fibres. Resin canals, lined by a layer of narrow epithelial cells are present throughout the ground tissue. The




**Figures 13-21.** **13.** Upper epidermal cells of a petal. **14.** A portion from figure 13 enlarged to show the details. **15-17.** Cells of the lower epidermis of a petal from apex, middle and base respectively. **18.** Epidermal cells of a petal showing stomata arranged at right angle to the cells. **19 and 20.** T. S. of petal from lower and middle portions. (Diagrammatic). **21.** A portion from figure 19 magnified to show the details.

*Abbreviations* : CC, cell contents ; CU, cuticle ; GT, ground-tissue ; LEP, lower epidermis ; PH, phloem ; RC, resin canal ; ST, stomata ; UEP, upper epidermis ; XY, xylem.

diameter of the canals is variable, being sometimes even larger than the vascular bundles. Disc shaped Ca-oxalate crystals are also present in abundance.

### 4.3 *Petals*

The cells of the upper epidermis are uniform, laterally elongated, have anisocytic or paracytic stomata and are larger than those of the lower side (figures 13 and 14). The lower epidermal cells, on the other hand, vary considerably in shape and size from base to apex (figures 15-18). Epidermal cells in the apical region are squarish with wavy anticlinal walls (figure 15). In the lower region, however, these are narrow elongated and thick walled with comparatively smaller stomata (figures 16 and 17). The stomata are either oriented in the same plane or may be arranged at right angles to the epidermal cells (figures 17 and 18).

The transverse section of a petal near the basal region shows a thick central portion and narrow curved margins (figure 19). The upper epidermis is undulating and has a thick cuticle on the outside. Sometimes even the anticlinal inner walls may also be thickened. A little below the epidermis one or two layers of cells show conspicously brown tannin containing cells. This layer is absent at higher levels (figure 20). The lower epidermis is smooth but cuticularised and is formed of comparatively smaller cells. The ground tissue is multilayered and is formed of parenchymatous cells with irregular outlines (figure 21). Resin canals are very few at lower levels but at higher levels these are quite abundant and are larger than the vascular bundles (figure 20). The vascular bundles are arranged almost in a row, some large and some small. They are collateral and formed of a few xylem and phloem elements.

### 4.4 *Androecium*

The stamens are numerous, have short filaments and somewhat thick, elongated anther lobes. These are tetra-sporangiate (figures 22 and 23). Some fused stamens (figures 24 and 25), alongwith their respective vascular supplies, have also been observed. Occasionally the fusion may involve the anther lobes as well. In such cases, however, fusion of the anther lobes is not complete (figure 24). Another stamen in which only connectives are fused in the middle can be seen in a transverse section (figure 25).

A semi diagrammatic representation of a transverse section of dehisced anther lobe can be seen in figure 26 and details of the vascular bundle in figure 27. The cells of the connective are elongated and papillate (figure 28). They are lignified and are positive to phloroglucinol — conc. hydrochloric acid stain. The epidermis of the anther lobe is formed of large colourless highly cuticularised cells followed by a fibrous endothecium (figures 26, 29).

### 4.5 *Pollen grains*

Pollen grains are 3-(4-) zono-colporate with average size 35.89 × 48.71 $\mu$m (range 30.77 — 48.71 × 35.89 — 58.97$\mu$m) ; shape oblate to suboblate ; exine surface reticulate ; thickness 1—2 $\mu$m, muriduplibaculate, colpus measures 13 × 33$\mu$m (figures 30–32).

**Table 1.** *Mesua ferrea* Linn. : Behaviour of powder with different chemical reagents.

| Sl. No. | Treatment | Observations | |
|---|---|---|---|
| | | Flower | Stamen |
| 1. | Powder + IN NaOH in methanol | Blackish brown | Brown with yellow tinge |
| 2. | Powder + Picric acid (saturated) | Yellowish orange | Yellowish orange |
| 3. | Powder + Acetic acid | Brown | Orange yellow |
| 4. | Powder + Conc. HCl | Brown | Brown |
| 5. | Powder + Conc. $HNO_3$ | Reddish orange | Reddish orange |
| 6. | Powder + Iodine (5%) | Blackish brown | Blackish brown |
| 7. | Powder + Seliwanoff's reagent | Yellowish brown | Yellowish brown |
| 8. | Powder + Ferric chloride (5%) | Dark blackish-brown with green tinge | Dark brown with green tinge |
| 9. | Powder + 40% NaOH + a few drops of 10% lead acetate | Blackish-brown with yellowish white ppt. | Light brown with yellowish white ppt. |
| 10. | Powder + Sudan III (alcoholic) | Dark reddish orange | Reddish orange |
| 11. | Powder + Conc. $HNO_3$ + ammonia. | Orange with yellow ppt. | Orange with whitish yellow ppt |

### 4.6 *Gynaecium*

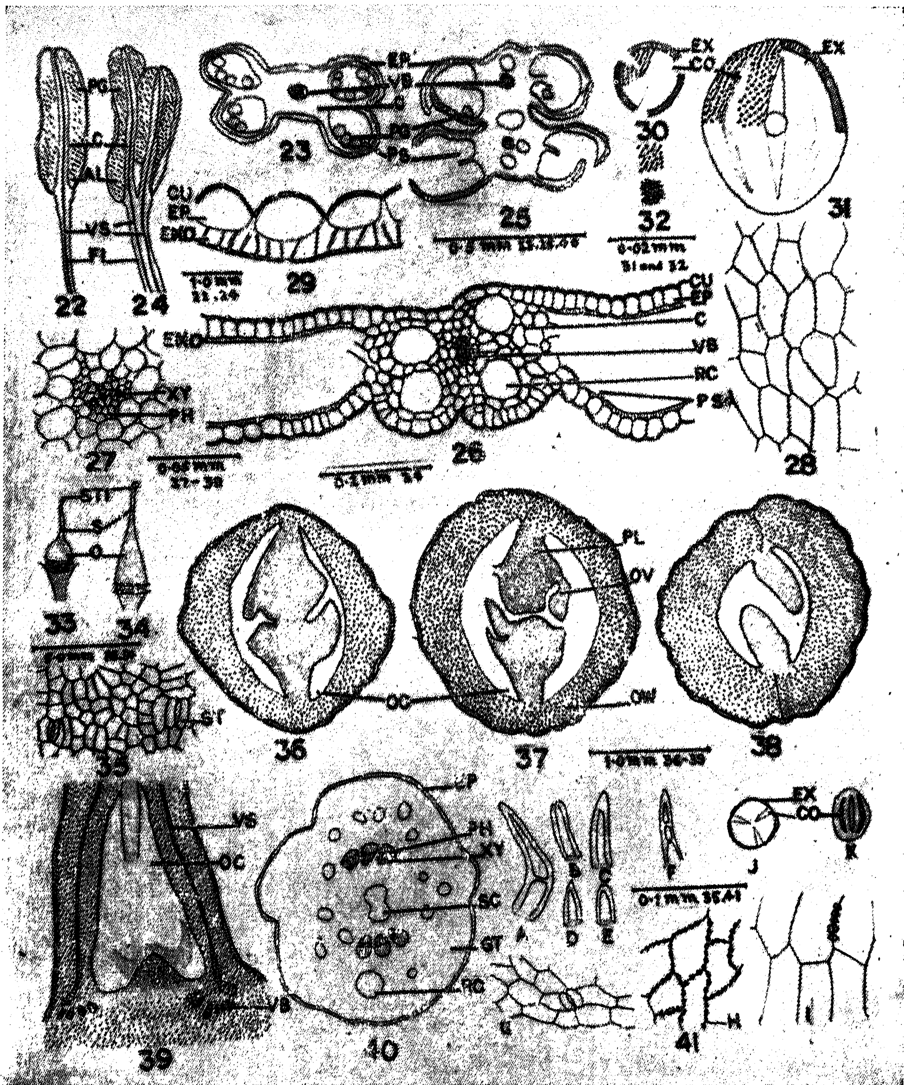
The ovary is superior, bicarpellary and syncarpous (figures 33 and 34). Epidermal cells of the ovary are small, hexagonal in surface view and have stomata measuring 21.09 × 11.59 (range 19.00 — 26.60 × 9.50 — 14.25$\mu$m (figure 35).

In transection the ovary is circular in outline (figures 36-38). The placentae are swollen and united at the base (figure 36). But at higher levels they separate (figures 36-39). The ovules arise one from each margin in each of the locules (figure 36). The placentation is thus axile tending to become parietal at higher levels. A transverse section passing through the style shows a stylar canal in the centre and three bundles, a dorsal flanked by two laterals on either side (figure 40). Resin canals are abundant even at this level.

## 5. Powder study

The powder of the whole flower is greyish brown in colour, pleasant smelling and slightly astringent in taste. The powder of stamens is golden brown in colour pleasant smelling and astringent to taste. The powder of whole flower was sieved through No. 40 mesh, cleared in chloral hydrate and mounted in glycerine. A microscopic examination revealed the following elements. (i) Pieces of trichomes (figures 41 A-F). (ii) Pieces of tissues showing stomata (figure 41 G), simple pits (figure 41 H-I). (iii) abundant pollen grains in different planes (figures 41 J and K).

The behaviour of the powdered drug with different chemical reagents was also studied and is presented in table 1.




**Figures 22-41.** **22.** A stamen showing one antherlobe. **23.** T. S. of the antherlobe showing tetra sporangiate condition (Diagrammatic). **24.** A stamen showing two anther lobes partially fused. **25.** T. S. of the fused antherlobe. (Diagrammatic). **26.** T. S. of dehisced antherlobe-(Semi-diagrammatic). **27.** Vascular bundle from figure 26 magnified to show the details. **28.** Cells from the connective (surface view). **29.** A portion of antherlobe wall magnified showing cuticularised epidermis and endothecium. **30.** A pollen grain showing wall stratification and ornamentation. **31.** A palynogram of pollen. **32.** L O pattern of pollen ornamentation. **33 and 34.** Diagrammatic representation of very young and slightly mature ovaries. **35.** Epidermal cells from ovary showing stomata in surface view. **36-38.** Serial T. S. of ovary from base to apex (Diagrammatic) **39.** L. S. of ovary (Diagrammatic). **40.** T. S. of style (Diagrammatic). **41.** A-K Different tissues from the drug powder. For details refer to the text.

*Abbreviations :* AL, antherlobe ; C, connective ; CO, Colpus ; CU, cuticle ; EP, epidermis ; EX, exine ; END, endothecium, FI, filament ; O, ovary ; OV, ovule ; OW, ovary wall ; PG, pollen grain, PH, phloem ; PL, placentum ; RC, resin canal ; SC, stylar cavity ; ST, stomata ; STI, stigma ; VB, vascular bundle ; VS, Vascular supply ; XY, Xylem.

**Table 2.** *Mesua ferrea* L. : Fluorescence analysis

| Sl. No. | Treatment | Fluorescence under UV light | |
|---|---|---|---|
| | | Stamen | Flower |
| 1. | Drug as such | Purplish brown | Blackish brown |
| 2. | Powder + Nitro-cellulose in amylacetate | Olive green | Purplish brown |
| 3. | Powder + 1N HCl | Reddish brown | Blackish brown |
| 4. | Powder + 1N HCl + Nitro-cellulose in amylacetate | Dirty green | Dark brown with purple tinge |
| 5. | Powder + aq. 1N NaOH | Dark brown | Dark brown |
| 6. | Powder + aq. 1N NaOH + Nitro-cellulose in amylacetate | Blackish brown | Blackish brown |
| 7. | Powder + 1N NaOH in Methanol | Blackish brown | Blackish brown |
| 8. | Powder + 1N NaOH in Methanol + Nitrocellulose in amylacetate | Greenish brown | Greenish brown |
| 9. | Powder + 50% Nitric acid | Reddish brown | Dark brown |
| 10. | Powder + 50% Sulphuric acid | Greenish brown with red tinge | Reddish brown |

The fluorescence analysis was carried out according to the method described by Chase and Pratt (1949) and Kokoski *et al* (1958). The observations are recorded in table 2.

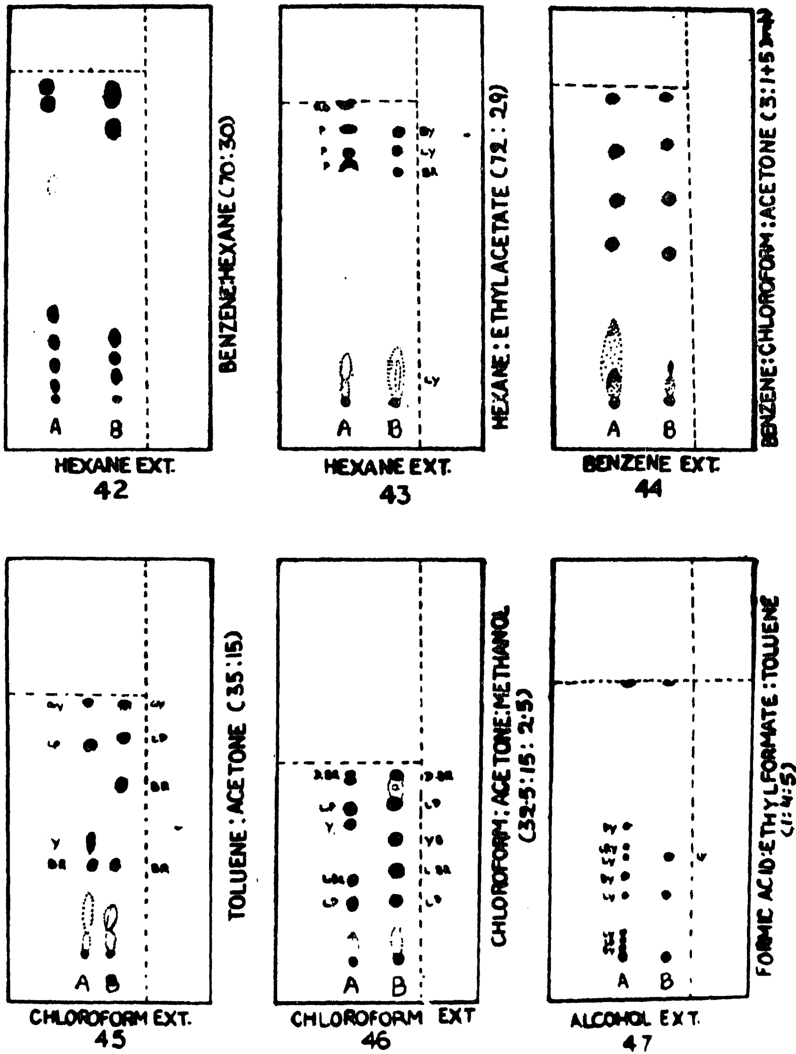
## 6. Phytochemical studies

Phytochemical characters of the drug were studied separately for the whole flower and stamens respectively. The determination of ash values, sugar, tannins, alcohol and water soluble extractives were made from air dried material. The procedures recommended by Anonymous (1966) were followed for calculating total ash, acid insoluble ash, alcohol and water soluble extractive percentages, whereas for calculating tannins, total and reducing sugars Fohlin-Denis reagent and Shaffer's Somogyi micromethods prescribed by AOAC (Anonymous 1965) were followed.

| | Flowers | Stamens |
|---|---|---|
| (1) Ash | | |
| (a) Total ash | 9.297% | 2.370% |
| (b) Acid insoluble ash | 3.730% | 0.230% |
| (2) Tannins | 4.4% | 3.76% |
| (3) Sugars | | |
| (a) Total sugars | 1.244% | 1.852% |
| (b) Reducing sugars | 0.960% | 1.160% |
| (c) Non-reducing sugars | 0.350% | 0.692% |
| (4) Volatile oil | 0.25% | 1.75% |
| (5) Alcohol soluble extractive (I.P. Method) | 21.57% | 25.31% |
| (6) Water soluble extractive (I.P. Method) | 14.86% | 15.40% |

Table 3. *Mesua ferrea* L. Preliminary phytochemical tests.

| Parameters | Hexane extractive | | Benzene extractive | | Chloroform extractive | | Ethanol extractive | | Aqueous extractive | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Flower | Stamen | Flower | Stamen | Flower | Stamen | Flower | Stamen | Flower | Stamen |
| Total percentage by weight | 30.92% | 13.52% | 2.197% | 1.5% | 0.469% | 0.784% | 0.793% | 1.4% | 9.958% | 1.53% |
| Physical appearance and consistency | Brownish yellow, oily | Bright yellow, oily | Brown, sticky | Yellowish brown, sticky | Yellowish brown, ticky | Yellow, sticky | Brown, sticky | Brown, sticky | Brown, powdery | Brown powdery |
| Alkaloids | — | — | — | — | 2s + | + | + | — | — | — |
| Flavonoids | — | — | — | — | — | — | — | — | — | — |
| Reducing sugars | — | — | — | — | — | — | 2 + | + | 3 + | 3 + |
| Resins | 4 + | 4 + | 3 + | 2 + | 2 + | 2 + | 2 + | 2 + | — | — |
| Saponins | — | — | — | — | — | — | — | — | 3 + | + |
| Steroids | — | — | — | — | — | — | — | — | — | — |
| Triterpenoids | 4 + | 4 + | 4 + | 4 + | — | — | — | — | — | — |
| Tannins | — | — | — | — | — | — | 2 + | 2 + | 3 + | 3 + |




**Figures 42-47.** A — Stamen extractive
B — Flower extractive
BR, brown ; BY, bright yellow ; D.BR, dark brown ; GY, greenish yellow ; LBR, light brown ; LP, light purple ; LRY, light reddish yellow ; LY, light yellow ; P, purple ; PY, purplish yellow; RO, reddish orange ; Y, yellow ; YB,Yellowish brown.

Beside the above 15 gms of air dried powdered flowers and stamens were extracted separately in a Soxhlet apparatus with hexane, benzene, chloroform, alcohol and water successively and percentage of each extractive calculated after evaporation of respective solvents. These were further screened for steroids, and triterpenoids (L.B. test - Peach and Tracy 1955) ; flavonoids (Shinoda's test - *Loc.Cit.*) ; alkaloids (Mayer's reagent - *Loc.Cit.*) ; and tannins (ferric chloride test-*Loc.Cit.*). The results obtained are presented in table 3.

The hexane and benzene extractives of both the parts show presence of triterpenoids and resins. Percentage of reducing sugars in alcoholic extractive, in the case of flower, was found to be quite high.

Water soluble portion of both the parts show presence of tannins and saponins. Concentration of saponins, however, was quite high in the case of flower.

Thin layer chromatography of the above extractives was also carried out (figures 42-47). The hexane soluble extractive of stamens shows a larger number of discrete spots as compared to that of the flower (figure 42). With hexane-ethyl acetate (72 : 29) as solvent system the hexane extractive separates into spots with shades of yellow and brown in the case of flower (figure 43b). Those of the stamens are, however, purple (figure 43a).

The benzene extractives (figure 44) are quite comparable. Again, chloroform extractive resolved into 4 spots each with toluene-acetone (35 : 15) as the solvent system. Of these the two upper most and the lowermost on Rf 0.96, 0.82 and 0.35 are comparable. However, an oval yellow patch at Rf 0.43 and a brown spot at Rf 0.65 in stamen and flower extracts respectively are different. The same extractive resolved into 5 spots in flower and 6 spots in stamens with solvent system chloroform : acetone : methanol, 32.5 : 15 : : 2.5 (figure 46). It is interesting to note that the alcoholic portion of the stamens gave 8 spots while the flower portion gave only 2 spots (figure 47) (Solvent system : formic acid-ethyl formate-toluene (1 : 4 : 5). The Rf values of different extractives have been recorded in a tabular form (table 4).

**Table 4.** *Mesua ferrea* Linn : TLC Rf. Values

| Extractive | Solvent system | Rf Values | |
|---|---|---|---|
| | | Flower | Stamen |
| Hexane soluble extractive | Hexane : Benzene (30 : 70) | 0.067,0.12,0.19,0.82, 0.93 | 0.04,0.10,0.17,**0.25**, **0.64**,0.89,0.95 |
| Hexane soluble extractive | Hexane : Ethyl-acetate (72 : 29) | 0.76,0.83,0.91 | 0.79,0.83,0.91,**0.99** |
| Benzene soluble extractive | Benzene : Chloroform : Acetone (3 : 1 : 0.05 cc.) | 0.46,0.63.0.80,0.95 | 0.50,0.63,0.79,0.99 |
| Chloroform soluble extractive | Toluene : Acetone (35 : 15) | 0.35,**0.65**,0.83,0.96 | 0.35,**0.43**,0.81,0.97 |
| Chloroform soluble extractive | Chloroform : Acetone : Methanol (32.5 : 15 : 2.5) | 0.28,0.43,**0.59**, 0.78,0.90 | 0.28,0.41,**0.68**,0.76, 0.90,**0.93** |
| Alcohol soluble extractive | Formic acid : Ethyl formate : Toluene (1 : 4 : 5) | **0.19,0.36,1.0** | **0.03.0.06.0.08, 0.23,0.29,0.35 0.4,0.48,0.99** |

Spraying reagent 2% $H_2SO_4$
Underlined Rfs denote spots which are distinctive.

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# Effect of $CO_2$ in ovecroming self-incompatibility barriers in *Brassica campestris* L. var. toria

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**Abstract.** $CO_2$ mediated induction of pollen adhesion, germination, tube penetration, tube growth through the stylar tissue and seed set was studied in *Brassica campestris* L. var. toria following incompatible pollination. This report stresses the effect of $CO_2$ not at a single step but at various levels which normally prevent and/or hinder successful fertilization and seed set. The possible role of $CO_2$ in overcoming these barriers is discussed.

**Keywords.** *Brassica campestris* L. var. toria; incompatibility; $CO_2$; seed set.

## 1. Introduction

The incompatibility reaction in *Brassica* is sporophytically controlled with multiple S-alleles (Bateman 1955 ; Thompson 1957). Double cross hybrids raised from self-incompatible inbred lines would give low yielding crop because of high level of cross-incompatibility with each other. In recent years, two developments have taken place to overcome such problems. One is based on the manipulation of genetic attributes (Thompson 1978) and the other involves putting the self-incompatible plants in an atmosphere of 3 to 5% $CO_2$ after selfing the flowers (Nakanishi *et al* 1969, 1975). The latter technique is now successfully employed in obtaining self-seeds.

Following incompatible pollination various check points exist at the level of pollen germination, adhesion, germination, tube penetration, tube growth through the stylar tissue and ultimately seed set. The present study describes the effect of $CO_2$ on the aforementioned levels following illegitimate pollination.

## 2. Material and methods

Seeds of *Brassica campestris* L. var. toria were obtained from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana and the plants were

raised in the Botanical Garden. Experiments were performed in full flowering season to avoid early or late season effect.

Excised pistil technique was employed to study the effect of $CO_2$ on pollen adhesion, germination, pollen tube penetration and growth through the stylar tissue. Flower buds were emasculated in the evening before opening. Next morning the flowers were removed and the pedicels with pistils were embedded in 1% agar medium in small glass vials. Self- and cross-pollinations were done with a fine brush to obtain a thin and even coating of pollen on the stigmatic surface. The vials containing self- and cross-pollinated pistils were put in a glass container which was sealed with parafilm. $CO_2$ was injected with the help of an air tight syringe to obtain 4% concentration. After incubation for 6 hr at $22 \pm 2°C$ pollen adhesion, germination and tube penetration into stigmatic papillae were studied by staining pistils with cotton blue (0.1%) in lactophenol.

Growth of pollen tubes in cross-pollinated pistils without $CO_2$ and self-pollinated pistils with 4% $CO_2$ was followed at times ranging between 6 to 30 hr after pollination. The number of pollen tubes in 0.5 mm region alongwith the length of the pistil were counted in the two situations (three replicates of six flowers each).

The emasculated flowers were pollinated with self- and cross-pollen and enclosed within known volume of polythene chamber and 4% $CO_2$ was injected with an air tight gas syringe. After exposing the flowers for 6 hr the polythene chamber was replaced by emasculating bags. The number of seeds formed per siliqua was determined 50-60 days after pollination.

## 3. Results

Due to full operation of self-incompatibility, adhesion of self pollen to the stigmas was low. Those which made contact with the papillae showed poor germination. Figure 1(a, b, c) depicts the adhesion of pollen grains in three situations. Figure 1a shows incompatible pollination with very few pollen grains adhering to the stigmatic surface and without any germination. Figure 1c is that of compatible pollination where most of the pollen grains are adhering to the papillae. Pollen grains show germination and pollen tubes grow in the stigmatic and stylar tissues. The firm attachment of pollen grains, following self-pollination, was observed when they were enclosed in an atmosphere of $CO_2$ (4%) (Figure 1b).

The data given in table 1 show that no pollen tube was recorded in the pistil following self-pollination. In majority of the styles, the longest pollen tube reached almost the same position in response to both self-pollination + $CO_2$ and cross-pollination styles. The data on percentage of pollen tubes present at different levels of the pistil after 24 hrs of pollination are set in figure 2. A gradual reduction in the number of pollen tubes from the top of the pistil down into the ovary was noticed. A decrease in cross pollen tubes as well as self pollen tubes (in the presence of $CO_2$) is evident. This decrease was more pronounced in the cross pollen tubes as compared with the self pollen tubes with $CO_2$.

Table 2 shows the number of seeds produced per siliqua after different pollination treatments. The number of seeds per siliqua following bud pollination was nearly

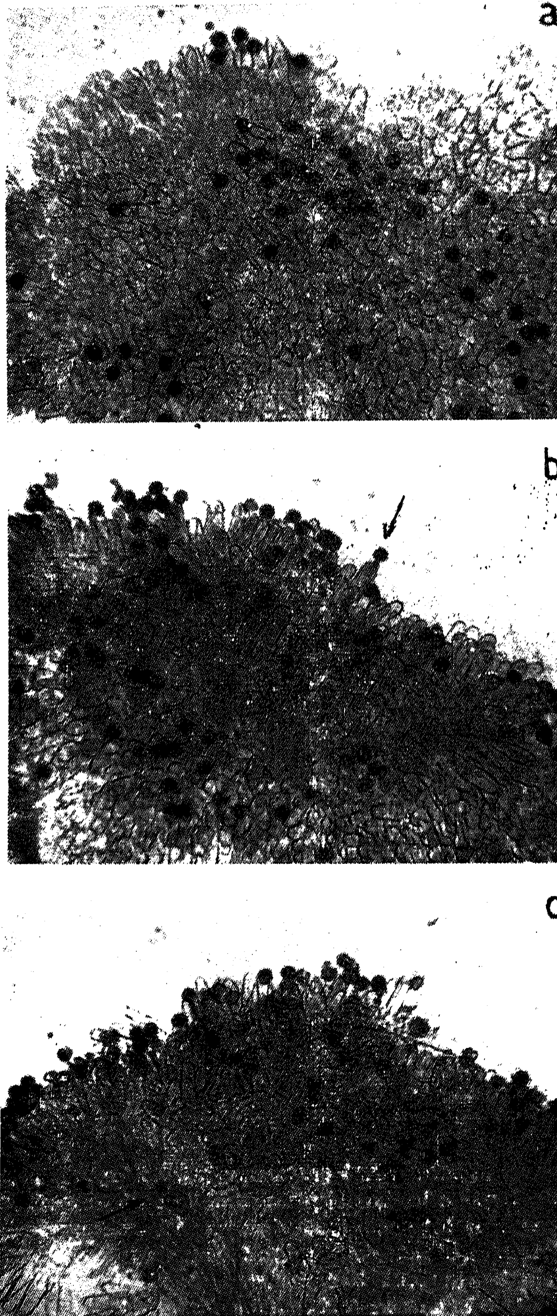


**Figure 1.** Pollen adhesion, germination and tube penetration after 6 hr pollination. The stigmas alongwith styles were stained with 0.1% cotton blue in lactophenol. **(a)** Noticed very few pollen grains adhering to the stigmatic surface following self-pollination **(b)** The number of pollen grains sticking to the stigmatic surface is more as compared with figure (a) following $CO_2$ treatment. Some pollen tubes are penetrating the stigmatic papillae. **(c)** Most of the pollen grains germinate and penetrate following cross-pollination (pollen tubes arrowed).

**Table 1.** Growth of pollen tubes in the pistil at different times after pollination in cross-, self- and self + $CO_2$ pollinations. Data are the mean of six flowers.

| Time after pollination (hr) | Pollination treatment | Maximum pollen tube length (mm) | Rate of pollen tube growth mm/hr | |
|---|---|---|---|---|
| | | | Max. rate | Mean rate |
| 6 | CP | 0.66 | 0.11 | 0.05 |
| | SP + $CO_2$ | 0.45 | 0.08 | 0.04 |
| | SP | 0.03 | 0.01 | 0.00 |
| 11 | CP | 1.40 | 0.13 | 0.07 |
| | SP + $CO_2$ | 1.35 | 0.12 | 0 05 |
| 18 | CP | 3.00 | 0.17 | 0.09 |
| | SP + $CO_2$ | 2.70 | 0.15 | 0.05 |
| 24 | CP | 3.50 | 0.15 | 0.08 |
| | SP + $CO_2$ | 3.00 | 0.13 | 0.07 |
| 30 | CP | 4.00 | 0 13 | 0.07 |
| | SP + $CO_2$ | 3.60 | 0.12 | 0.05 |

**Table 2.** Effect of $CO_2$ (4%) on seed production following self- and cross-pollination.

| Pollination treatment | Self seeds | | Cross seeds | |
|---|---|---|---|---|
| | Number of fruits | Number of seeds per fruit | Number of fruits | Number of seeds per fruit |
| Bud | 36 | 17.3 | 30 | 18.2 |
| Open flower | | | | |
| —$CO_2$ | 0 | 0.0 | 38 | 19.6 |
| +$CO_2$ | 34 | 8.2 | 30 | 18.8 |

same in the two situations i. e. self- and cross-pollinations. No seed set was recorded when the freshly opened flowers were self-pollinated. However, about 8 seeds per siliqua were formed when self-pollinated flowers were kept in 4% $CO_2$ atmosphere for about 6 hr. $CO_2$ did not affect seed set after cross-pollination.

## 4. Discussion

The strong self-incompatibility barrier exhibited by *Brassica campestris* var. toria depends on the recognition and the rejection reaction. The events grouped in these two stages constitute the various check points as mentioned earlier. The rejection response is characterized by callose deposition.

The adhesion of the pollen grain to the stigmatic surface seems to be the initial but most important event to ensure pollen germination and tube penetration. $CO_2$ plays an important role in the adhesion of the pollen grains to the self stigma surface as is evident from the presence of more pollen grains on the self stigma following $CO_2$ treatment than control (without $CO_2$) even 6 hr after pollination.

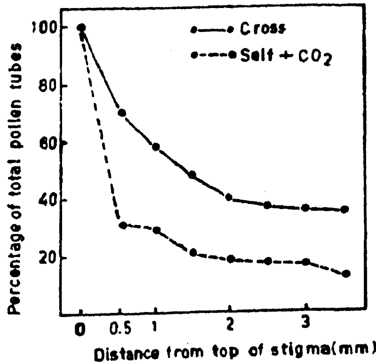


**Figure 2.** Percentage of pollen tubes at different levels from top of the stigma following 24 hr of pollination.

If pollination is compatible the tryphine is induced to 'gel' and promotes both adhesion and hydration of the pollen grain (Dickinson *et al* 1980). The number of pollen grains adhering to the stigma surface following cross-pollination is higher than self-pollination. Stead *et al* (1980) proposed that differences in the degree/extent of adhesion may result from physical or chemical alteration(s) taking place in the components that bind the pollen to the stigma surface. They suggested the involvement of stigma surface proteins in the pollen grain adhesion which have a rapid turnover rate. $CO_2$ has been shown to affect changes in the kinetic behaviour of allosteric proteins (Mitz 1979). $CO_2$ may be affecting pollen adhesion through the above mentioned processes. $CO_2$ has been reported to affect protein synthesis in maize roots (Splittstoesser 1966), *Chlorella* (Miyachi and Hogestu 1970) and germinating *Amaryllis vittata* pollen grains (Sharma *et al* 1981).

Dhaliwal and Malik (1980) and Dhaliwal *et al.* (1981) have suggested the role of $CO_2$ in pollen hydration and recognition rejection response which are essential for the activation of enzymes and acceptance of pollen grain, respectively. Non-specific esterases have been suggested to be involved in the active cutinase complex essential for the breakdown of cuticle. $CO_2$ stimulates the leaching of these esterases on to the stigma surface following illegitimate pollination (Dhaliwal and Malik 1982).

$CO_2$ increased both pollen germination and tube penetration following incompatible pollination. $CO_2$ action was correlated with the metabolic changes of pollen tubes and/or papilla cells at the time of their attachment (Nakanishi *et al* 1969). The growth rates of pollen tubes in self-pollination + $CO_2$ and cross-pollination styles indicate that the fastest self pollen tube (in presence of $CO_2$) grew nearly as fast as the fastest cross pollen tube. The gradual reduction in the number of pollen tubes from the top of the pistil down into the ovary indicate much variation in the rate of pollen tube growth. Thus, the most rapidly growing tubes registered two to three times faster rate than the average tubes. This decrease in the number is more pronounced in the cross pollen tubes as compared to self pollen tubes in the presence of $CO_2$. The variation in the growth rate may be attributed to competition between the pollen tubes for the nutrients. Clearly, single pollen

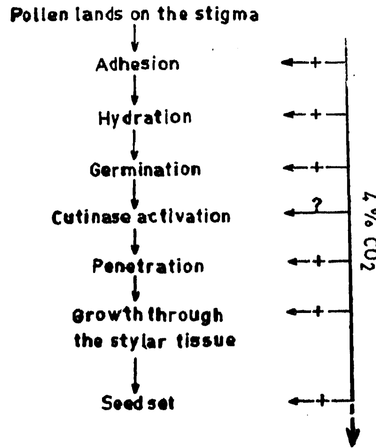


**Figure 3.** Effect of $CO_2$ (4%) on various check points that lead to self-incompatibility in *Brassica campestris* L.

tube growing through the style will have less competition with each other than several of them growing at the top of the stigma. The seed set increased when the freshly opened flowers were self-pollinated and were kept in 4% $CO_2$ atmosphere for about 6 hrs. $CO_2$ did not affect seed set after cross pollination.

Taking the present discussion in conjunction with our previous inferences on the role of $CO_2$ on various check points is indicated (figure 3). We believe that $CO_2$ act at several stages promoting successful fertilization and seed set in incompatible pollination.

## Acknowledgements

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# harmacognosy of the stems of *Portulaca quadrifida* L. and *ortulaca oleracea* L.

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**Abstract.** Pharmacognostic details of the stems of *P. quadrifida* L. and *P. oleracea* L. are reported to distinguish one from the other.

**Keywords.** Pharmacognosy; *Portulaca quadrifida* L.; comparison with *P. oleracea* L.

## Introduction

*rtulaca quadrifida* and *Portulaca oleracea,* commonly known as Chhota Luniya d Bara Luniya respectively, are succulent annual herbs and grow abundantly in ld state throughout warmer regions of India (Anon 1969). It has been suggested that m the therapeutic point of view they are quite similar and one can be used as substitute for the other by the drug dealers (Dymock *et al* 1980 ; Kirtikar and su 1975). Detailed chemical analysis of leaf and stem of *P. oleracea* was rked out by Sadana and Ahmed (1947). Recently Lal (1980) described the armacognostic features of the leaf of *P. quadrifida.* The pharmacognostic tails of the stems of both species are presented in this paper.

## Materials and methods

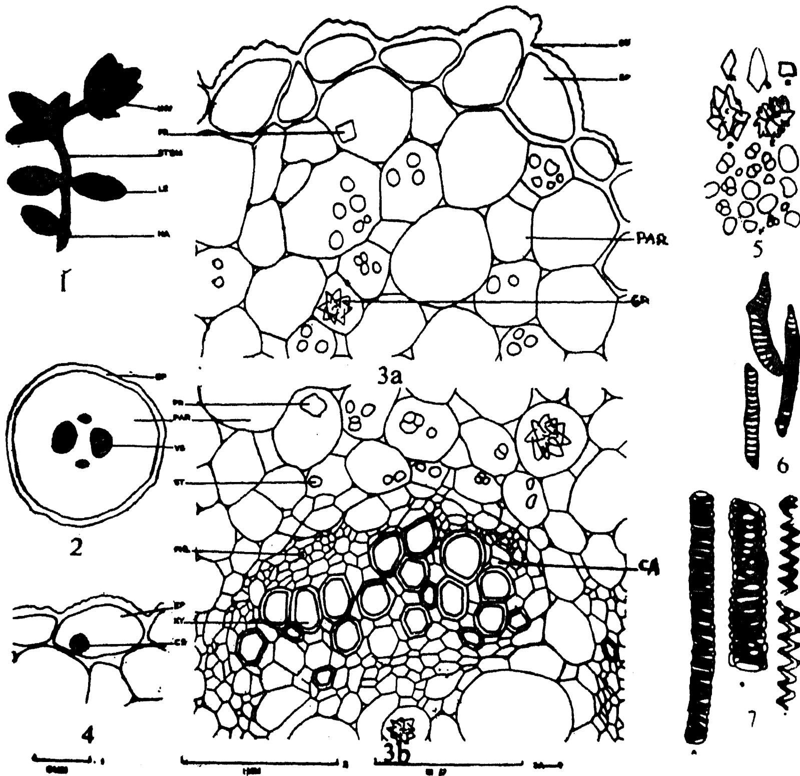
esh plants of *P. quadrifida* and *P. oleracea* collected from the Botanical Garden Aligarh Muslim University were fixed in FAA. After usual processing, free nd and microtome sections were cut and stained in safranin and fast green. uorescence analysis and extractive and ash values of the powdered mass of stems re carried out by Chase and Pratt (1949) and *Indian Pharmacopoeia* (Anon 1966) ethods respectively.

## 2.1 *Macroscopic characters*

The stem of *P. quadrifida* is succulent, diffuse, filiform, purple in colour at maturity, less than a milimeter in diameter; on crushing mucilaginous; mucilage slimy; rooting at the nodes; nodal appendages many, pilose, white; internodes 1.5 to 3 cm long; without any smell and taste acidic. The stem of *P. oleracea* (figure 8) on the other hand, is about 2 mm in diameter; internodes 1.5 to 3.5 cm long; nodal appendages less in number, minute, scarious. The other morphological characters are more or less similar to *P. quadrifida.*

## 2.2 *Microscopic characters*

The cross-section of the stems of both the species are almost circular (figures 2 and 9). The epidermal cells are polygonal in shape in both species and are surrounded externally by thick striated cuticle. The outer wall of some of the




**Figures 1-7. 1.** A twig of the plant *Portulaca quadrifida* L. (× 2.5). **2.** T.S. stem (diagrammatic) (×35). **3A, B.** Anatomical details of a portion of figure 2 (×2973). **4.** Epidermal cell in T.S. containing acicular crystals (×2973). **5A, B, C D** and **E.** Prismatic crystals and druses (×2973). **6** and **7.** Macerated xylem vessels (×2973).

epidermal cells of *P. quadrifida* slightly bulge out. Acicular crystals which appear as crystals and in cross section are present in some of the epidermal cells of *P. quadrifida* (figure 4). Epidermis is followed by 2-3 layers of collenchyma cells in the stem of *P. oleracea* (figures 9 and 10A), whereas it is parenchymatous in *P. quadrifida* (figure 3A). The parenchyma in both species consists of thin-walled, more or less isodiametric cells with large intercellular spaces. These cells are loaded with starch grains, simple as well as compound. The compound starch grains are usually 2 or 3 celled (figures 5F and 11). Druses, prismatics, acicular crystals and colourless mucilage cells are commonly present in both the species. The endodermis in both species is not well defined. Collateral vascular bundles are arranged in a ring in both but the number of bundles in *P. oleracea* is almost double or even more than those in *P. quadrifida* (figures 2 and 9). Pith consists of thin-walled isodiametric cells some of which contain calcium oxalate crystals (figure 3B). The macerated xylem consists mostly of helical and scalariform vessel elements with simple perforation (figures 6, 7A, B, C and 12) and fibres with intrusive growth.

The measurement of different tissues and cells is given in table 1.

**Table 1.** Measurement of different tissues and cells in microns.

| *P. quadrifida* | *P. oleracea* |
|---|---|
| Cuticle $M$ = 3.33 — 6.66 thickness | 3.66 — 6.89 thickness |
| Epidermis $M$ = 23.31 × 6.66 — 39.96 × 19.98 — 79.92 × 49.94 | 39.6 × 26.4 — 42.9 × 29.7 — 49.6 × 36.4 |
| Parenchyma $M$ = 6.66 — 13.32 — 93.24 diameter | 46.8 — 124.8 — 156.0 diameter |
| Collenchyma Absent | $M$ = 33.3 — 66.6 diameter |
| Vessels $M$ = 15.65 — 19.98 — 33.30 diameter | 9.99 — 26.24 — 29.97 diameter |
| $T$ = 79.92 × 23.31 — 123.21 × 63.27 — 404.0 × 31.10 | 90.0 × 28.54 — 223.31 × 68.27 — 532.98 × 35.30 |
| Fibres $M$ = 15.55 — 23.32 — 31.10 diameter | |
| $T$ = 155.50 × 23.32 — 233.25 × 31.10 | |
| Pith $M$ = 16.65 — 23.31 — 66.60 diameter | 46.8 — 124.8 — 153.50 diameter |
| Druse $M$ = 33.30 — 39.96 — 49.96 diameter | 46.8 — 78.0 — 124.8 diameter |
| Starch grains $M$ = 3.33 — 9.99 — 13.32 diameter | 1.66 — 3.33 — 16.50 diameter |

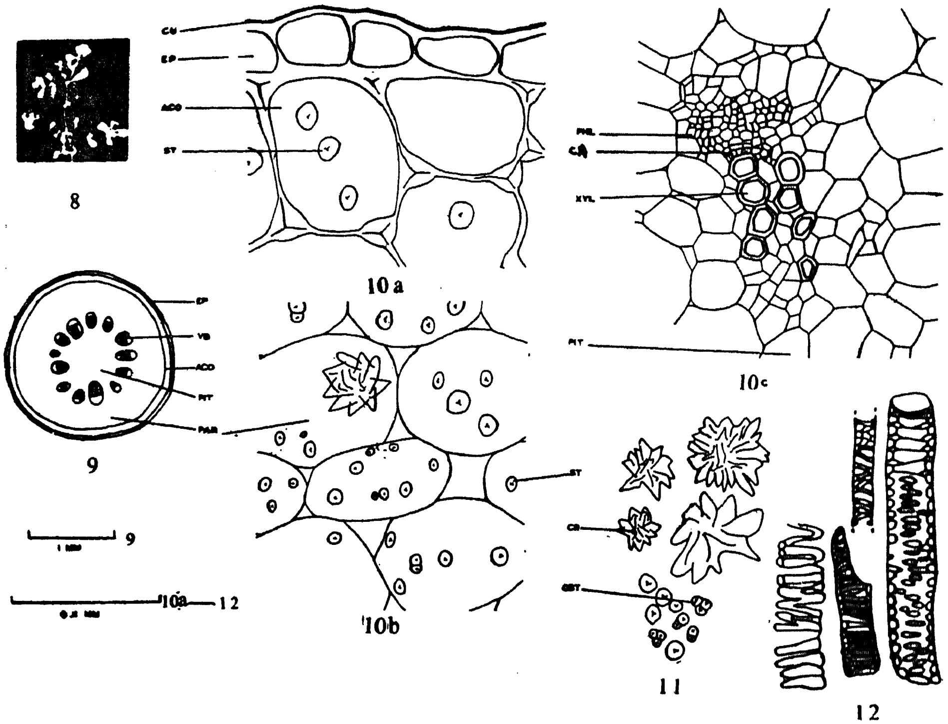
$M$ = measurement in cross-section; $T$ = measurement of macerate

### 2.3 *Macerate*

Macerate consists of cuticle, parenchyma cells, xylem vessel elements, fibres, starch grains and druses (figures 5, 6, 7, 11 and 12).

### 2.4 *Extractive and ash values*

Extractive and ash values were determined according to Anon (1966) and the results are given in table 2.




**Figures 8-12. 8.** A twig of the plant *Portulaca oleracea* L. (×1). **9.** T.S. stem (diagrammatic) (×20). **10A, B and C.** Anatomical details of a portion of figure 9 (×2973). **11.** Starch grains and druses (×2973). **12.** Maccrates (×2973).

**Table 2.** Extractive and ash values of the stems of *P. quadrifida* and *P. oleracea.*

| Extractive and ash values | *P. quadrifida* | *P. oleracea* |
|---|---|---|
| Water soluble extractive (chloroform water) | 19.73% | 25.00% |
| Alcohol soluble extractive | 10.32% | 18.50% |
| Total ash | 9.09% | 25.18% |
| Acid insoluble ash | 0.63% | 3.18% |

## 2.5 *Fluorescence analysis of the powdered drugs*

The stem powders prepared by drying fresh specimens at 60°C were chemically treated and exposed to ultraviolet light. The fluorescence observed is recorded in table 3.

**Table 3.** Fluorescence analysis of the stem powders of *P. quadrifida* and *P. oleracea*.

| Chemical treatments | Fluorescence | |
|---|---|---|
| | *P. quadrifida* | *P. oleracea* |
| Powder as such | Light green | Dark green |
| Powder mounted in 1N NaOH in methanol | Yellowish green | Green |
| Powder mounted in nitrocellulose | Orange | Brownish green |
| Powder treated with 1N NaOH in methanol and mounted in nitrocellulose | Brown | Dark brown |

### 2.6 *Chromatographic studies*

Alcohol extracts of the stems of *P. quadrifida* and *P. oleracea* were subjected to thin layer chromatography with the solvent system methanol : chloroform (3:7). The plates were developed by iodine vapours. They showed the presence of four spots (figure 13) with Rf values 0.05, 0.65, 0.73, 0.90 and 0.05, 0.65, 0.76, 0.88 respectively. This indicates that the two species have more or less the same chemical constituents.

## 3. Conclusion

The two species differ considerably in their measurements of cells and tissues (table 1), extractive and ash values (table 2) and fluorescence analysis of the powdered drugs under UV light (table 3). Little or no differences were obtained in TLC studies as shown in the chromatogram (figure 13). Undoubtedly, the

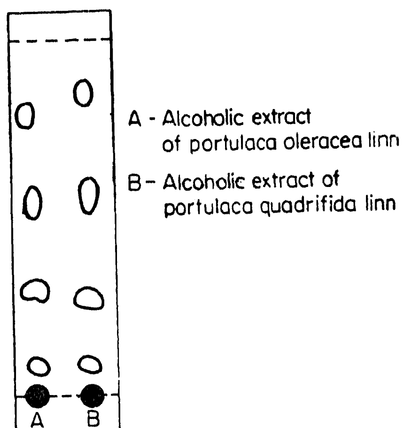



**Figure 13.** Chromatogram.

stems of *P. quadrifida* and *P. oleracea* differ morphologically and anatomically. They also differ in extractive and ash values as well as in fluorescence analysis under UV light; yet in view of the similarities in therapeutic properties the stem of *P. quadrifida* can be used as a substitute for that of *P. oleracea*.

**Acknowledgements**

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**Abbreviations :** CA : Cambium; ACO : Angular collenchyma; CR : Druses; CS : Crystal sand; CST : Compound starch grains; CU : Cuticle; EP : Epidermis; INV : Involucre; LF : Leaf; NA : Nodal appendages; PAR : Parenchyma; PHI : Phloem; PIT : Pith; PR : Prismatic crystal; ST : Starch grains; VB : Vascular bundle; XY : Xylem.

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# Structure and function of a sub-tropical humid forest of Meghalaya I. Vegetation, biomass and its nutrients

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**Abstract.** The peripheral disturbed zone of a 50-year old stand of the forest at Lailad was dominated by *Dendrocalamus hamiltonii*, an early successional bamboo characteristic of a secondary successional fallow of not more than 20-25 years while the central undisturbed zone had *Schima wallichii*, *Castanopsis indica* and *Shorea robusta* as important components. The biomass of the forest was computed as 137 × $10^{3}$ kg/ha of which 64.7% was in the central zone. The contribution by different species both in the central and peripheral zone of the forest was worked out. Linear relationship between *dbh*/$d^2h$ and biomass was worked out for different species. The standing crop had : N, 953 ; P, 284; K, 600; Ca, 2281 ; and Mg, 450 kg/ha, of which 60% was in the central zone of the forest.

**Keywords.** Biomass ; nutrients; sub-tropical forest.

## 1. Introduction

Understanding of the structural and functional attributes of a given forest ecosystem is important for proper management of the environment and utilization of the resource potential (Grantham and Ellis 1974). The present study on a 50-year old humid tropical forest ecosystem of north-eastern India is important as it represents a stage in secondary succession after slash and burn agriculture (locally known as jhum) which is a wide spread land use practice in this region (Ramakrishnan *et al* 1981). The present series of three papers deal with forest ecosystem from the point of view of its organization, biomass and nutrient flow through litter and through water.

The structure of a vegetational unit depends upon the species composition and their relative number (Gleason 1926). Biomass data form an important component of the structure of any ecosystem (New Bould 1967). Though much information on this and inventory of nutrients is available on different forest types from the parts of the world, little is known on Indian forest types except for a few studies (Dadhichi 1979; Agrawal 1980; Vyas *et al* 1980). This paper is related

to phytosociology and biomass analysis alongwith nutrient inventory of the forest type mentioned above.

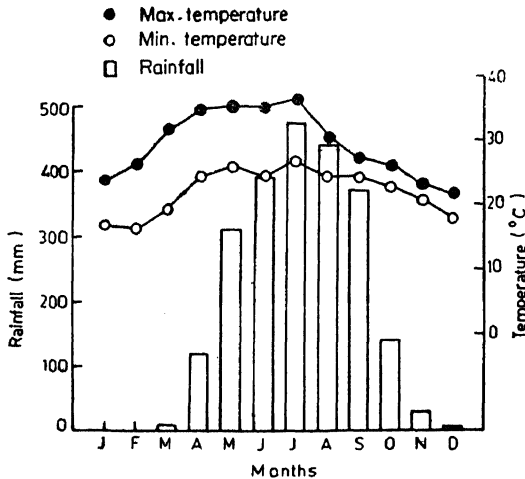


**Figure 1.** Climate of Lailad based on the average of two years - 1977 and 1978 (data obtained from Department of Silviculture, Government of Meghalaya).

## 2. Study area, geology and climate

The study area located at Lailad (25°45″– 26°0″ N Latitude and 91°45″ – 92°0E Longitude) at an altitude of 296 m, is tropozoidal shaped and is a part of a reserve forest known as Nonghyllem reserve under the Meghalaya Forest Department since 1910. The peripheral zone is subjected to biotic disturbances, such as cutting of trees for firewood and removal of bamboo for fencing and house building by the local tribal population. Therefore, only the central zone of the forest is representative of a 50-year old stand. The soil is red, sandy loam and is of laterite origin. The pH ranged from 5.8 to 6.3. The climate is typically monsoonic with most of the rainfall (84%) during May to September. The monsoon season is followed by a mild winter (mid November to mid February). March and early April represent a brief dry summer period (figure 1).

### 2.1 *Methods of study*

Phytosociological studies were done in July 1976, at the peak of the growing season both in the outer peripheral zone of about three hectare. The density, frequency, basal area and importance value index were based on 20 quadrats of 10 × 10m for trees, 5 × 5m quadrats for shrubs and 1 × 1m quadrats for herbs along a transect running from the periphery to the centre of the forest

(Misra 1968; Kershaw 1973). Species diversity was calculated using the formula given by Shannon and Weaver (1949)

$$\text{as } H = -\left(\frac{ni}{N}\right) \quad \log\left(\frac{ni}{N}\right)$$

where $H$ = Shannon index of general diversity, $ni$ = importance value index of species $i$, $N$ = Importance value index in the community.

The index of dominance of the community was calculated according to Simpson (1949).

$$\text{as } C = \left(\frac{ni}{N}\right)$$

where $C$ = concentration of dominance ; $ni$ · importance value of species $i$, $N$ = total importance value for the community.

Biomass estimation of tree species was done in a specified area during August at the time when the leaves were fully mature. Three different girth classes with 3 replicates of 11 major tree species, namely, *Shorea robusta, Schima wallichii, Castonopsis indica, Artocarpus chaplasha, Gmeline arborea, Garcinia cowa, Miliusa roxburghiana, Sterculia villosa, Dillenia indica, Vitex peduncularis and Dendrocalamus hamiltonii* were harvested. Various parameters like diameter of the bole at base, middle and top, total height of the tree, diameter and length of the branches and total number of leaves were recorded after harvest. The fresh weight of all the branches and leaves were determined in the field and sub-samples of branches, small twigs and leaves were brought to the laboratory in polythene bags. Small discs (2-3 cm thick) from the base and top of the bole were taken for compulation of dry weight. All the sub-samples were oven dried at 80° – 85° C to constant weight. The regression equations were obtained relating the biomass parameters to combinations of diameter at breast height (*dbh*) and diameter$^2$ × height ($d^2h$). Regression equations were used to estimate the biomass of the standing crop.

Plant samples were ground and passed through a 20-mesh screen and chemically analyzed for nitrogen, phosphorus, potassium, calcium and magnesium using standard methods as described by Peach and Tracy (1956) and Jackson (1958). Thus, nitrogen was determined by micro-kjaldahl method and phosphorus was estimated colorimetrically by molybdenum blue method. After dry ashing the samples, calcium and magnesium were analyzed by EDTA-filtration method and potassium by flame emission method.

## 3. Results and discussion

### 3.1 *Vegetation structure*

Some of the most important component of the peripheral disturbed zone were *Dendrocalamus hamiltonii, Mesua ferrea, Millusa roxburghiana, Vitex peduncularis, Schima wallichii, Castonopsis indica* and *Shorea robusta*. It may be noted that *D. hamiltonii* which is more dominant in the peripheral zone is an early successional

**Table 1.** Density, frequency, basal area and importance value index of tree, shrub and herbaceous species in the peripheral and central zones of the sub-tropical forest at Lailad.

| Species | Density/ha | | Frequency | | Basal area ($m^2$/ha) | | IVI | |
|---|---|---|---|---|---|---|---|---|
| | Peripheral zone | Central zone | Peripheral zone | Central zone | Peripheral zone | Central zone | Peripheral zone | Central zone |
| **Trees** | | | | | | | | |
| *Artocarpus chaplasha* | 24 | 16 | 4 | 14 | 13.77 | 9.18 | 14.88 | 15.37 |
| *Castanopsis indica* | 92 | 122 | 4 | 64 | 13.23 | 17.55 | 35.09 | 43.42 |
| *Dillenia indica* | 18 | 14 | 21 | 17 | 1.98 | 1.55 | 6.39 | 4.96 |
| *Dendrocalumus hamiltonii* | 770 | 350 | 54 | 21 | 5.37 | 2.44 | 83.40 | 41.56 |
| *Garcinia peniculata* | — | 2 | — | 4 | — | 0.08 | — | 3.00 |
| *Gmelina arborea* | 12 | 16 | 11 | 43 | 2.33 | 3.10 | 4.51 | 6.59 |
| *Miliusa roxburghiana* | 90 | 32 | 75 | 36 | 0.72 | 0 25 | 18.60 | 7.59 |
| *Mesua ferrea* | 8 | — | 11 | — | 0.63 | — | 3.87 | — |
| *Sterculia villosa* | 20 | 8 | 29 | 11 | 0.52 | 0 21 | 5.29 | 3.72 |
| *Schima wallichii* | 144 | 204 | 68 | 89 | 1.45 | 20.60 | 39.51 | 60.60 |
| *Shorea robusta* | 62 | 90 | 60 | 50 | 9.20 | 13.35 | 27.87 | 30.87 |
| *Vitex peduncularis* | 44 | 32 | 21 | 49 | 1.94 | 1.25 | 16.59 | 14.52 |
| **Shrubs** | | | | | | | | |
| *Annona wallichii* | 72 | 48 | 50 | 40 | 160.02 | 100.24 | 25.74 | 17.96 |
| *Croton oblongifolium* | 240 | 120 | 60 | 53 | 200.62 | 100.42 | 42.41 | 28.41 |
| *Crombretum decandrum* | 160 | 80 | 40 | 30 | 17.22 | 8.38 | 12.69 | 13.96 |
| *Litsaea khasyana* | 160 | 120 | 73 | 40 | 261.60 | 201.02 | 26.63 | 21.00 |
| *Leea sambucina* | 104 | 138 | 27 | 30 | 121.34 | 140.62 | 29.44 | 26.71 |
| *Micromelum pubescence* | 144 | 96 | 47 | 37 | 12.32 | 8.70 | 16.37 | 24.05 |
| *Randia densiflora* | 160 | 200 | 60 | 87 | 40.62 | 40.42 | 16.80 | 24.64 |
| **Herbs** | | | | | | | | |
| *Cyperus elegance* | 2.02 | 2.06 | 55 | 67 | 1.02 | 0.80 | 19.9 | 18.33 |
| *Fimbristylis dichotoma* | 1.74 | 1.60 | 73 | 63 | 0.90 | 1.00 | 14.10 | 14.12 |
| *Hedychium gracile* | 1.40 | 0.80 | 67 | 50 | 0.48 | 0.40 | 16.28 | 9.35 |
| *Hedychium cornonarium* | 0.80 | 0.86 | 50 | 63 | 0.26 | 0.08 | 9.01 | 7.36 |
| *Oplismenus compositus* | 1.00 | 0.60 | 73 | 47 | 0.60 | 0.40 | 11.65 | 7.87 |
| *Panicum khasianum* | 17.54 | 4.00 | 83 | 67 | 1.80 | 8.00 | 40.23 | 15.53 |
| *Passiflora nepalensis* | 0.20 | 0.46 | 20 | 30 | 1.32 | 0.80 | 10.21 | 11.14 |

species and reaches its peak in a 20-year old community after which it declines (Ramakrishnan and Toky 1978). On the other hand species like *Schima wallichii*, *Castonopsis indica* and *Shorea robusta* were more dominant towards the central zone of the forest with higher IVI values. In forest community as a whole, dominant tree species were *Dendrocalamus hamiltonii*, *Schima wallichii*, *Castonopsis indica*, *Shorea robusta*, *Milusa roxburghiana* and *Artocarpus chaplasha* (table 1). *M. roxburghiana* had high density and frequency but low IVI values due to low basal area whereas *Artocarpus chaplasha* and *Vitex peduncularis* had high IVI values in spite of low density and frequency because of their greater basal area.

*Croton oblongifolium*, *Litsaea khasyana*, *Leea sambucina*, *Annona wallichii*, *Randia demiflora* and *Micromelum pubescence* were the important of the shrub layer of the forest as a whole. It may be noted that species like *Croton oblongifolium* had high IVI values towards the peripheral zone of the forest along with species like *Annona wallichi*, *Combretum decundrum*, *Litsaea khasyana*, etc. In the forest as a whole, *Panicum khasianum*, *Cyperus elegans*, *Hedychium gracile*, etc. are predominant amongst herbaceous species (table 1). However, a number of these species possess greater IVI in the peripheral zone in comparison to the central zone of the forest. This may be due to lesser number of tree species with reduced canopy cover which permitted greater light penetration and less competition in the peripheral zone.

### 3.2 *Biomass*

The pattern of biomass distribution in the forest for the important species may be related to the species diversity. Of the total biomass 64.7% was along the central zone due to greater species diversity whereas peripheral zone contributed about 35.3%. *Schima wallichii*, *Castonopsis indica* and *Shorea robusta* contributed maximum in the peripheral zone as well as central zone. However, these three species contributed more than 2-fold in the central zone of the forest compared to the peripheral zone (table 2).

*Leea sambucina*, *Annona wallichii*, *Sterculia coccinia*, *Litsaea khasyana* and *Croton oblongifolium* account for the largest biomass contributed by the shrubs in the forest. Of the total biomass contributed by shrubs, 54.3% was along the peripheral zone and the remaining 45.7% was in the central zone of the forest. Of the total plant biomass in the forest as a whole 64.5% was in the central zone and only 35.5% along the peripheral zone. Along the periphery, shrubs and herbs contributed larger share of the total biomass whereas trees contributed mostly towards the central zone of the forest (table 3).

The average biomass on an unit area basis in the forest was $137 \times 10^3$ kg/ha, the value being lower than those reported by other workers for the tropical forests (Jordan and Kline 1972). Whittaker and Likens (1973 a, b) reported a mean value of $35\text{-}45 \times 10^4$ kg/ha biomass for some tropical and seasonal forests. However, the present value lies between 60-350 m/ha reported by Whittaker (1975) for some temperate evergreen and tropical seasonal forests and much higher than that for temperate coniferous forest (Akai *et al* 1968; Smith *et al* 1971).

**Table 2.** Biomass contribution by major tree, and shrub species in the peripheral and central zones of the forest at Lailad (values in parentheses represents the percentage of the total amount).

| Species | Biomass (kg/ha) | |
|---|---|---|
| | Peripheral zone | Central zone |
| Trees | | |
| *Artocarpus chaplasha* | 3796.2 (1.4) | 9124.2 (3.3) |
| *Castonopsis indica* | 22484.1 (8.2) | 45199.9 (16.4) |
| *Dendrocalamus hamiltonii* | 6914.8 (2.5) | 3638.2 (1.3) |
| *Dillenia indica* | 4594.6 (1.7) | 2938.4 (1.7) |
| *Gmelina arborea* | 2033.8 (0.7) | 4589.2 (1.7) |
| *Garcinia cowa* | 1967.3 (0.7) | 5118 7 (1.9) |
| *Miliusa roxburghiana* | 6185 7 (2.2) | 4744.5 (1.7) |
| *Schima wallichii* | 35113.9 (12.8) | 74900.4 (27.2 |
| *Shorea robusta* | 8416.3 (3.1) | 21395.9 (7.8) |
| *Sterculia villosa* | 4674.8 (1.7) | 2614.4 (0.9) |
| *Vitex peduncularis* | 1052.1 (0.4) | 1698.7 (0.6) |
| Shrubs | | |
| *Anona wallichii* | 196.2 (6.2) | 65.4 (2.1) |
| *Actinodaphne angustifolia* | 141.2 (4.5) | 94.4 (3 0) |
| *Croton oblongifolium* | 184.0 (5.8) | 147.2 (4.6) |
| *Combretum decandrum* | 127.4 (4.0) | 72.8 (2.3) |
| *Litsaea khasyana* | 186.0 (5.9) | 248.0 (7.8) |
| *Leea sambucina* | 265.0 (8.3) | 371.0 (11.7) |
| *Morinda umbellata* | 151.2 (4.8) | 108.0 (3 4) |
| *Phlogacanthus thyrsiflorus* | 208 0 (6.5) | 124.8 (3.9) |
| *Randia densiflora* | 73.6 (2.3) | 92.0 (2.9) |
| Others | 193.0 (6.1) | 131.2 (4.1) |

**Table 3.** Biomass contribution by major tree species, shrub and herbaceous layers in the peripheral and central zones of the forest at Lailad (values in parentheses represents the percentage of the total amount).

| Different layers | Biomass (t/ha) | |
|---|---|---|
| | Peripheral zone | Central zone |
| Tree | 97.234 (35.3) | 177.984 (64.6) |
| Shrub | 0.172 (0.01) | 0.146 (0.05) |
| Herbaceous | 0.008 (0.03) | 0.004 (0.01) |

Relationship between morphological growth parameters *viz.*, diameter (*dbh*), and diameter$^2$ $\times$ height ($d^2h$) on the one hand and biomass of individual tree species as well as fractional plant parts on the other was found to be highly significant for all the tree species except *D. hamiltonii* where significant correlations were found with *dbh* (tables 4,5).

Table 4. Linear regression equations for *dbh* with above ground, bole, branch and leaf biomass for

| Species | Above ground biomass | Bole biomass | Branch biomass | Leaf biomass |
|---|---|---|---|---|
| *Artocarpus chaplasha* | $Y = -233.191+18.276X$<br>$r = 0.988$ | $Y = -144.347+12.0255X$<br>$r = 0.986$ | $Y = -74.0+5.433X$<br>$r = 0.979$ | $Y = -5.424+0.596X$<br>$r = 0.992$ |
| *Castanopsis indica* | $Y = -63.06+10.560X$<br>$r = 0.991$ | $Y = -67.328+8.553X$<br>$r = 0.925$ | $Y = -74.0+5.433X$<br>$r = 0.979$ | $r = 0.979$ |
| *Dendrocalamus hamiltonii* | $Y = -16.987+4.20X$<br>$r = 0.849$ | $Y = -9.544+2.62X$<br>$r = 0.817$ | $Y = -0.736+1.870X$<br>$r = 0.971$ | $Y = -3.819+0.791X$<br>$r = 0.849$ |
| *Dillenia indica* | $Y = -122.297+13.065X$<br>$r = 0.991$ | $Y = -69.562+8.790X$<br>$r = 0.957$ | $Y = -3.324+0.753X$<br>$r = 0.672$ | $Y = -7.741+0.641X$<br>$r = 0.993$ |
| *Gmelina arborea* | $Y = -229.852+70.451X$<br>$r = 0.984$ | $Y = -163.85+0.660X$<br>$r = 0.993$ | $Y = -55.38+4.00X$<br>$r = 0.985$ | $Y = -4.464+0.391X$<br>$r = 0.989$ |
| *Garcinia cowa* | $Y = -91.137+10.887X$<br>$r = 0.997$ | $Y = -60.879+7.797X$<br>$r = 0.963$ | $Y = 61.377+4.403X$<br>$r = 0.975$ | $Y = -0.312+0.225X$<br>$r = 0.985$ |
| *Miliusa roxburghiana* | $Y = 4.435+5.219X$<br>$r = 0.997$ | $Y = -11.323+3.35X$<br>$r = 0.993$ | $Y = -29.946+2.$[illegible]$68X$<br>$r = 0.991$ | $Y = -1.518+0.362X$<br>$r = 0.991$ |
| *Schima wallichii* | $Y = -144.678+144.678X$<br>$r = 0.987$ | $Y = -98.449+10.251X$<br>$r = 0.979$ | $Y = -5.455+1.519X$<br>$r = 0.995$ | $Y = -2.357+0.328X$<br>$r = 0.963$ |
| *Shorea robusta* | $Y = -163.332+15.09X$<br>$r = 0.993$ | $Y = -95.49+9.975X$<br>$r = 0.981$ | $Y = -43.50+$[illegible]$.735X$<br>$r = 0.991$ | $Y = -6.543+0.671X$<br>$r = 0.932$ |
| *Sterculia villosa* | $Y = -80.00+6.50X$<br>$r = 0.942$ | $Y = -70.576+5.512X$<br>$r = 0.971$ | $Y = -57.64+4.325X$<br>$r = 0.992$ | $Y = -3.04+0.230X$<br>$r = 0.932$ |
| *Vitex peduncularis* | $Y = -115.659+9.998X$<br>$r = 0.956$ | $Y = -84.71+7.29X$<br>$r = 0.959$ | $Y = -23.280+1.112X$<br>$r = 0.991$ | $Y = -2.943+0.328X$<br>$r = 0.952$ |

$Y$ = Above ground biomass of components parts.

$X$ = *dbh* of different tree species

**Table 5.** Linear regression equations for $d^2 h$ ($dbh^2 \times$ height) with above ground, bole branch and leaf biomass for different tree species.

| | | | | |
|---|---|---|---|---|
| *Artocarpus chaplasha* | $Y = 99.318+8.28\times10^{-5}X$<br>$r = 0.991$ | $Y = 71.127+5.53\times10^{-5}X$<br>$r = 0.963$ | $Y = 23.714+2.50\times10^{-5}X$<br>$r = 0.983$ | $Y = 5.51+2.67\times10^{-6}X$<br>$r = 0.962$ |
| *Castanopsis indica* | $Y = 174.700+3.56\times10^{-5}X$<br>$r = 0.972$ | $Y = 125.55+2.88\times10^{-5}X$<br>$r = 0.970$ | $Y = 40.90+6.41\times10^{-5}X$<br>$r = 0.971$ | $Y = 6.743+1.01\times10^{-6}X$<br>$r = 0.971$ |
| *Dendrocalamus hamiltonii* | — | — | — | — |
| *Dillenia indica* | $Y = 173.751+4.87\times10^{-5}X$<br>$r = 0.961$ | $Y = 128.654+1.41\times10^{-5}X$<br>$r = 0.973$ | $Y = 36.785+1.41\times10^{-5}X$<br>$r = 0.992$ | $Y = 6.863+2.37\times10^{-6}X$<br>$r = 0\ 953$ |
| *Gmelina arborea* | $Y = 40.435+5.81\times10^{-5}X$<br>$r = 0.973$ | $Y = 5.242+4.74\times10^{-5}X$<br>$r = 0.970$ | $Y = 3.47+1.86\times10^{-5}X$<br>$r = 0.983$ | $Y = 1.00+1.41\times10^{-6}X$<br>$r = 0.943$ |
| *Garcinia cowa* | $Y = 65.793+9.18\times10^{-5}X$<br>$r = 0.981$ | $Y = 51.863+6.54\times10^{-5}$<br>$r = 0.981$ | $Y = 11.200+2.44\times10^{-5}X$<br>$r = 0.983$ | $Y = 2.881+1.89\times10^{-6}X$<br>$r = 0.971$ |
| *Miliusa roxburghiana* | $Y = 68.591+3.64\times10^{-5}X$<br>$r = 0.963$ | $Y = 52.126+2.32\times10^{-5}X$<br>$r = 0.962$ | $Y = 12.985+1.057\times10^{-5}X$<br>$r = 0.981$ | $Y = 2.590+2\ 58\times10^{-6}X$<br>$r = 0.990$ |
| *Schima wallichii* | $Y = 151.530+5.91\times10^{-5}X$<br>$r = 0.913$ | $Y = 114.371+4.21\times10^{-5}X$<br>$r = 0.900$ | $Y = 33.352+1.56\times10^{-5}X$<br>$r = 0.921$ | $Y = 3.991+1.49\times10^{-6}X$<br>$r = 0.982$ |
| *Shorea robusta* | $Y = 67.852+1.02\times10^{-5}X$<br>$r = 0.972$ | $Y = 57.533+6.73\times10^{-5}X$<br>$r = 0.961$ | $Y = 8.225+2.69\times10^{-5}X$<br>$r = 0\ 982$ | $Y = 3.813+4.48\times10^{-6}X$<br>$r = 0.900$ |
| *Sterculia villosa* | $Y = 70.00+2.4\times10^{-5}X$<br>$r = 0.946$ | $Y = 25.00+2.88\times10^{-5}X$<br>$r = 0.952$ | $Y = 5.00+5.31\times10^{-5}X$<br>$r = 0.962$ | $Y = 1.51+1.00\times10^{-6}X$<br>$r = 0\ 913$ |
| *Vitex peduncularis* | $Y = 25.453+8.16\times10^{-5}X$<br>$r = 0.964$ | $Y = 18.147+5.94\times10^{-5}X$<br>$r = 0.962$ | $Y = 6.044+1.93\times10^{-5}X$<br>$r = 0.980$ | $Y = 1\ 863+2.54\times10^{-6}X$<br>$r = 0.921$ |

$Y$ = Above ground biomass and biomass of component part ; $X = d^2 h$

.3 *Nutrient content in biomass*

ın analysis of the concentration of N, P, K, Ca and Mg in different plant omponents (bole, branches and leaves) showed that the leaf had higher percenage of N, P, K, and Mg while bole alongwith bark contained higher percentage f Ca in all the species except *Dendrocalamus hamiltonii* where the concentration f Ca was more in leaves. Next to the leaves, the branches had higher levels f N, P, K and Mg and the bole had the least concentration. On the other hand, Ca oncentration was the least in branches with intermediate values from leaves :able 6).

**Table 6.** Concentration of different nutrients in different compartments of different tree species at Lailad.

| Species | Compart-ment | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|---|
| | Bole | 0.41 | 0.12 | 0.31 | 3.00 | 0.14 |
| *Artocarpus chaplasha* | Branches | 0.61 | 0.23 | 0.32 | 0.82 | 0.49 |
| | Leaves | 2.10 | 0.64 | 0.59 | 2.96 | 0.77 |
| | Bole | 0.64 | 0.14 | 0.58 | 0.84 | 0.47 |
| *Dendrocalamus hamiltonii* | Branches | 0.70 | 0.28 | 0.52 | 0.69 | 0.70 |
| | Leaves | 1.76 | 0.59 | 0.83 | 1.99 | 0.88 |
| | Bole | 0.85 | 0.14 | 0.26 | 2.90 | 0.18 |
| *Castonopsis indica* | Branches | 0.90 | 0.28 | 0.40 | 1.00 | 0.34 |
| | Leaves | 1.90 | 0.49 | 0.66 | 2.40 | 0.79 |
| | Bole | 0.58 | 0.15 | 0.26 | 2.80 | 0.29 |
| *Miliusa roxburghiana* | Branches | 0.75 | 0.28 | 0.65 | 1.00 | 0.83 |
| | Leaves | 1.65 | 0.47 | 0.85 | 3.20 | 1.09 |
| | Bole | 0.59 | 0.16 | 0.48 | 3.36 | 0.26 |
| *Schima wallichii* | Branches | 0.62 | 0 31 | 0 63 | 0.70 | 0.45 |
| | Leaves | 1.86 | 0 66 | 1.19 | 2.60 | 0.67 |
| | Bole | 0.42 | 0.11 | 0.34 | 2.40 | 0.30 |
| *Shorea robusta* | Branches | 0.82 | 0.26 | 0.44 | 0.88 | 0.68 |
| | Leaves | 1.90 | 0.76 | 1.20 | 1.78 | 1.08 |

On hectare basis, the total amount of different elements contributed by different pecies (table 7) showed that *Schima wallichii*, *Castonopsis indica* and *Shorea obusta* contributed maximum with respect to N, P, K, Ca and Mg. This could e related to the large biomass in the standing crop of these tree species 1 the same order as given above. Among other species a great variation 'as observed with regard to percentage contribution of different elements 'hich may be partly due to the concentration of nutrients. Thus, *Dendroalamus hamiltonii* stands next to *Artocarpus chaplasha* and *Miliusa roxburhiana* in biomass but contributed more in terms of P, K and Mg due to igher concentration of these three elements in the plant tissue. Because of low 'a concentration in the tissue coupled with low biomass the contribution of Ca y *Dendrocalamus hamiltonii* was far less than that of *Artocarpus chaplasha* and *Iiliusa roxburghiana.*

The distribution of nutrients in different tree compartments showed that bole contained maximum amount of all nutrients followed by branches and leaves. This is in spite of the higher concentration of N, P, K and Mg in the leaf tissue This compartmentalization of nutrient is highly exaggerated for Ca due to the fact that this nutrient also had the highest concentration in the bole (figure 2).

**Table 7.** Amount of different elements contributed by different species alongwith percentage contribution of the total for the forest given in parentheses.

| Species | Nutrient (kg/ha) | | | | |
|---|---|---|---|---|---|
| | N | P | K | Ca | Mg |
| *Artocarpus chaplasha* | 34.7<br>(3.8) | 11.3<br>(4.0) | 21.2<br>(3.6) | 135.8<br>(4.8) | 17.2<br>(3.9) |
| *Dendrocalamus hamiltonii* | 32.4<br>(3.[illegible]) | 22.0<br>(7.9) | 31.7<br>(5.4) | 36.1<br>(1.4) | 29.2<br>([illegible]) |
| *Dillenia indica* | 23.7<br>(2.6) | 8.1<br>(2.9) | 21.1<br>(3.6) | 74.6<br>(2.7) | 10.3<br>(2.3) |
| *Castanopsis indica* | 291.7<br>(32.0) | 61.4<br>(22.0) | 102.5<br>(17.4) | 439.9<br>(25.7) | 79.3<br>(17.9) |
| *Gmelina arborea* | 18.8<br>(2.1) | 5.2<br>(1.9) | 16.6<br>(2.8) | 63.8<br>(2.0) | 12.7<br>(2.9) |
| *Garcinia cowa* | 18.1<br>(2.0) | 8.5<br>(3.0) | 10.5<br>(1.8) | 61.2<br>(2.2) | 12.8<br>(2.9) |
| *Miliusa roxburghiana* | 27.7<br>(3.0) | 11.0<br>(3.9) | 11.7<br>(2.0) | 113.1<br>(4.7) | 25.3<br>(5.7) |
| *Schima wallichii* | 346.7<br>(38.1) | 115.0<br>(41.2) | 294.0<br>(49.9) | 1086.6<br>(45.5) | 174.0<br>(39.3) |
| *Shorea robusta* | 83.7<br>(9.2) | 25.2<br>(9.0) | 58.1<br>(9.9) | 181.7<br>(8.9) | 61.0<br>(13.8) |
| *Sterculia villosa* | 23.1<br>(2.5) | 9.1<br>(3.2) | 14.6<br>(2.5) | 54.0<br>(1.9) | 16.8<br>(3.6) |
| *Vitex peduncularis* | 10.1<br>(1.1) | 2.5<br>(0.1) | 7.0<br>(1.2) | 22.4<br>(0.9) | 3.8<br>(0.7) |

Figure 3 indicates the percentage contribution (per hectare) by the different elements in the different compartments of the standing crop. The quantities of the different nutrients in the three compartments of the tree in a decreasing order are as follows :

$$Ca > N > K > Mg > P.$$

The pattern of distribution of nutrients by trees, shrubs and herbs along the periphery and the centre of the forest showed that about 60% of the total nutrient pool was in the undisturbed central zone and the rest along the disturbed peripheral zone (table 8). However, along the peripheral zone the contribution by shrub and herb species was more (0.9 and 0 3% respectively) in comparison with the central zone (0.7 and 0.2% respectively). Tree species contributed more towards the nutrient pool of the living biomass (59.9%) in the central zone than along the peripheral zone (37.9) of the forest.

**Table 8.** Distribution of different elements in the peripheral and in the central zones of the forest by trees, shrubs and herbs (kg/ha).

| Elements | Peripheral zone | | | Central zone | | | Mean value |
|---|---|---|---|---|---|---|---|
| | Trees | Shrubs | Herbs | Trees | Shrubs | Herbs | |
| **Nitrogen** | 609.34 | 34.40 | 13.86 | 1212.12 | 29.20 | 6.82 | 952.77 |
| **Phosphorus** | 194.68 | 4.74 | 1.88 | 363.58 | 4.02 | 0.94 | 284.92 |
| **Potassium** | 417.90 | 8.94 | 3.54 | 760.16 | 76.60 | 1.20 | 599.67 |
| **Calcium** | 1920.88 | 25.80 | 10.24 | 2577.58 | 21.90 | 5.00 | 2280.70 |
| **Magnesium** | 326.28 | 6.02 | 2.38 | 558.36 | 5.12 | 1.20 | 449 68 |

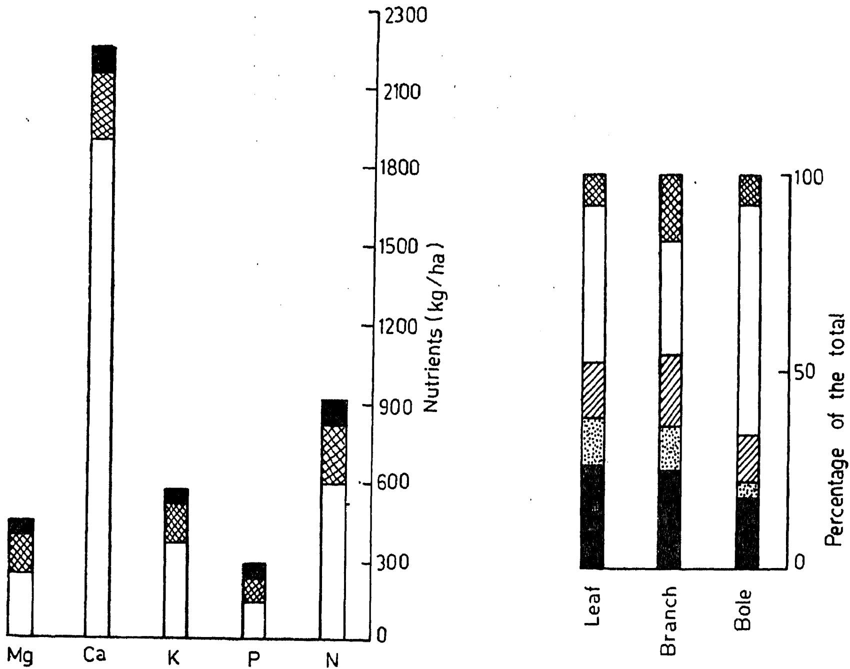


**Figure 2.** Pattern of compartmentalization of nutrients in trees at Lailad forest. Open column bole; hatched column branches; closed column leaves.

**Figure 3.** Proportion of different nutrients in different components in living biomass of trees (percentage of the total amount in kg/ha). Closed column N; strippled column P; hatched column K; open column Ca; cross hatched column Mg.

The total standing crop of nutrients as worked out in the present studies was higher when compared with broad leaved temperate forests (Ovington 1958; Grier *et al* 1974); Divigneud *et al* (1968) but the values are lower when compared with tropical forests of Ghana (Greenland and Kowa 1960) and some Indian forest type (Deshbandhu 1970 ; Faruqi 1972)

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# Structure and function of a sub-tropical humid forest of Meghalaya II. Litter dynamics and nutrient cycling

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**Abstract.** The litter production in a 50 year old humid sub-tropical forest at Lailad was found to be 5.5 t/ha/yr, 77% of which was through leaf and the remainder through wood. Litter production was more in the peripheral disturbed zone compared to the undisturbed central zone which is related to the successional status of the community. Species differences in the rate of decomposition of litter was noted. Besides litter production and decomposition pattern were related to seasonal differences in temperature and humidity. The present results have been discussed in the light of the data from other studies done elsewhere.

**Keywords.** Litter dynamics ; nutrient cycling.

## 1. Introduction

Studies on litter production and its decomposition are important for understanding of energy flow, nutrient cycling and primary production in the ecosystem. While much is known on these aspects for temperate forests (Olson 1963 ; Bray and Gorham 1964 ; Rodin and Bazilevich 1967 ; Gosz *et al* 1972) fewer studies are available from the tropics (Jenny *et al* 1949 ; Loudelot and Meyer 1954 ; Cornforth 1970 ; Singh and Gupta 1977 ; Edwards 1977). Little is known on the litter dynamics of forests in India (Seth *et al* 1963 ; Singh 1969 ; Singh and Gupta 1977). Further, many of these studies pertain to leaf litter alone and that derived from wood is often ignored.

In an earlier paper in this series (Part I), the phytosociology, biomass and nutrient inventory of a 50 year old forest developed after slash and burn agriculture (Ramakrishnan *et al* 1981) at Lailad in Meghalaya was considered. The present study deals with the estimation of leaf and wood litter, its decomposition and nutrient cycling in this forest stand.

## 2. Methods of study

For collection of litter, 20 permanent quadrats (1 × 1 m) made of wood (10 cms high) were randomly laid out in each site. Litter was collected at monthly

intervals, classified into leaves and wood and further sub-divided intc different important tree species and a miscellaneous category. The litter wa: oven-dried, weighed and passed through a 20-mesh screen for chemical analysis The peripheral part of the forest was considered separated from the centra zone (of Part I).

Decomposition of leaf litter was studied by litter bag technique (Shanks anc Olson 1961 ; Singh and Gupta 1977). Freshly fallen leaves in April wer collected in bulk from the forest and sorted out into five important tre species, namely, *Shorea robusta, Schima wallichii, Castonopsis indica Dendrocalamus hamiltonii* and *Artocarpus chaplasha* and the remainder wer treated as miscellaneous. The litter was brought to the laboratory and air dried at constant temperature. Litter bags of nylon (10 × 14 cm) with 1 mn mesh size containing 30 g samples were used. 50 bags of each species wer prepared and decomposition of litter samples were evaluated by placing th bags on the surface of the soil. Three replicate samples of each categor were recovered from the fields at monthly intervals. The material from th litter bags was washed with water using a 100$\mu$ mesh screen to remove all soi particles. It was dried at 80°C, weighed and preserved for chemical analysis.

The rate of decomposition of wood litter was estimated using the metho given by Yoneda (1975). Freshly fallen wood pieces of moderate diamete (3-4 cm) of the above-mentioned species were collected in April, air-dried a constant temperature, cut into 12 cm long pieces and initial weight of eac piece was determined. 50 pieces for each species were randomly placed on th surface of the soil and three replicate of each species were picked at monthl interval. These were processed in the laboratory using similar procedures a used for leaf litter bags.

Chemical analysis of the litter was done following standard procedure (Allen 1974). Thus, nitrogen was determined by the Kjeldahl method, anc phosphorus was estimated colorimetrically by the molybdenum-blue method calcium and magnesium by EDTA method while potassium by flame emissio method after dry ashing the samples in a muffle furnace at 450°C.

Soil respiration was measured by the alkali absorption method (Colema 1973 ; Gupta and Singh 1977 ; Gupta and Singh 1981) using 16.6 cm diamete metallic cylinder with 50 ml of 1 N KOH solution. The alkali solution wa kept in the cylinders for 24 hours. Soil respiration was measured in two type of situations. (i) On the mineral soil after removing the litter layer, and (ii) O the soil without removing the litter layer. A third set served as the blank for whic the beaker was placed on a wooden platform lined by a layer of polythene sheet Three replicates were done for each treatment.

## 3. Results and Discussion

### 3.1 *Litter production*

Total litter production was estimated to be 5 5 t/ha/yr of which 77% wa through leaves and the remaining through wood. Litter production was mor

along the periphery than the central zone of the forest. *Dendrocalamus hamiltonii* (bamboo) a comparatively early successional species was an important component in the periphery due to frequent disturbances like felling of dicot trees for timber and fuel wood and occurrence of accidental fire ; this species alone contributed 19% of the total litter. This species has been shown to have a high rate of turnover of biomass through leaves in the younger fallows up to 20 years (Ramakrishnan and Toky 1981). In the central zone of the forest contribution of litter was chiefly through *Shorea robusta*, *Schima wallichii* and *Castonopsis indica* with 13, 10 and 8% respectively of the total (table 1).

The average litterfall of the forest as a whole (5.5 t/ha/yr) was slightly lower than the values obtained for other tropical forests. At one end of the range are the values recorded by Mitchell (cited by Bray and Gorham 1964) for Malaya (5.5-7.2), Klinge and Rodrigues (1968) for Amazonia (7.3) and Edwards (1977) for lower Montane Rainforest in New Guinea (7.5). The values reported on higher side are by Bernhard (1970) for Ivory Coast (8.3-13.4) and Ewel (1976) for Guatemala (9.0). Toky and Ramakrishnan (1980) reported litterfall of 9.7 t/ha/yr in a successional forest of 20-year dominated by bamboo (*D. hamiltonii*) in the same area where this study was done. This may be due to the fast developing vegetation during the early successional stages and the consequent rapid turnover of biomass. Since an early successional community undergoes fast changes in species composition, often entire individual may contribute to litter production resulting in an over shoot of litter production in early successional phase (Toky and Ramakrishnan 1980 ; Ewel 1976).

**Table 1.** Distribution of litter along the peripheral and central zones of the sub-tropical forest at Lailad (kg/ha/yr). (Figures in parentheses represent the percentage of the total litter in each zone).

| Species | Peripheral zone | Central zone |
|---|---|---|
| *Leaf litter :–* | | |
| *Artocarpus chaplasha* | 102.2 (2.3) | 204.5 (3.1) |
| *Castanopsis indica* | 245.8 (5.4) | 491.8 (7.5) |
| *Dendrocalamus hamiltonii* | 846.6 (18.7) | 282.2 (4.3) |
| *Dillenia indica* | 25.2 (0.6) | 33.1 (0.5) |
| *Garcinia cowa* | 48.8 (1.1) | 145.0 (2.2) |
| *Machillus khasiana* | 4.0 (0.1) | 5.0 (0.1) |
| *Mesua ferrea* | 40.2 (0.9) | 14.4 (0.2) |
| *Miliusa roxburghiana* | 311.3 (6.9) | 140.7 (2.2) |
| *Shorea robusta* | 261.9 (5.8) | 849.5 (13.0) |
| *Schima wallichi* | 297.8 (6.6) | 643.6 (9.9) |
| *Sterculia villosa* | 64.7 (1.4) | 26.3 (0.4) |
| *Vitex peduncularis* | 36.6 (0.8) | 43.4 (0.7) |
| Other species (leaf) (miscellaneous) | 1530.5 (33.9) | 1796.0 (27.6) |
| Total | 3815.6 | 4675.5 |
| *Wood litter :–* | | |
| Branches, twigs, barks. (all species) | 701.0 (15.5) | 1833.8 (28.2) |
| Total (leaf + wood) litter | 4516.6 | 6509.3 |
| Mean of peripheral and central zone | | 5512.9 |

Wood litter (1.3 t/ha/yr) formed 23% of the total litterfall and the value is comparable to 1.2 t/ha/yr (16% of the total) as reported by Edwards (1977) in Lower Montane Rain forest in New Guinea. For temperate forests a range of 22-78% of the total contribution through wood litter has been estimated (Carlisle *et al* 1966; Anderson 1970).

The distribution of litterfall was markedly seasonal with a maximum leaf litterfall (57%) during the dry months of February, March and April. Wood litterfall showed a peak during April to July (figure 1). A similar seasonal trend in litterfall was also found in other tropical rain forests (Nye 1961), Klinge and Rodrigues (1968). Data of Laudelot and Meyer (1954) for young secondary forests showed two periods of litterfall that come at the end of the drier season. This may be related to the formation of abscission layer in leaves due to the severity of drought stress during dry periods. During the present study, the peak of the wood litterfall extended into the rainy season. This may be related to storms prevailing during the rainy season, as also reported elsewhere (Edwards 1977).

### 3.2 *Litter decomposition*

Most of the workers who have considered the rate of decomposition of litter on the forest floor (Jenny *et al* 1949; Olson 1963; Edwards 1977) have assumed that there is an exponential loss in weight as a result of decomposition, *i.e.*

$$X/x_o = \exp(-kt)$$

where $x_o$ is the initial weight, $x$ is the weight at time $t$, and $k$ is the annual decomposition constant. This model expresses the loss as a negative exponential function of the fraction and calculation of $k$ remains the most convenient means of comparing forests.

During the present study the rate of decomposition was rapid in *D. hamiltonii*, *Schima wallichii* and miscellaneous litter (high $k$ values) than other types of litter (table 2). The overall rate of decomposition in this forest for wood and leaf litter was found to be lower than the values reported by Laudelot and Meyer (1954) for Zaire and Singh (1968) for deciduous forest

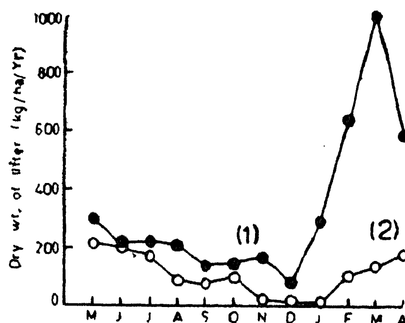



**Figure 1.** Monthly litter production in a humid sub-tropical forest at Lailad (1977-78).

at Varanasi (India) and was comparable to the values reported by Edwards (1977) for New Guinea. Nitrogen content and the texture of the litter play a great role in decomposition (Ewel 1976; Singh and Gupta 1980), however, Singh (1968) correlated various chemical constituents and the rate of decomposition in tropical tree species and found that not only nitrogen but numerous chemicals interact to affect the rate of decomposition. The nitrogen content of leaf litter (table 3) in *S. robusta*, *C. indica and A. chaplasha* was quite high but still a low rate of decomposition was observed in these species. This may be due to decay resistant petiole and mid-rib of leaves due to high content of lignin (Singh 1968). The lower decay rate may also partly be due to the smaller mesh size of litter bags which do not allow larger fauna to enter (Edwards and Heath 1963).

After a period of one year, the highest loss of litter was observed for *D. hamiltonii* (78%) and least for *S. robusta* (56%) (figure 2) 40-45% of litter was lost during May to August due to higher temperature and humidity. Subsequently the rate of decomposition slowed down (figure 3) due to low temperature and moisture levels. This pattern of decomposition was also evident from the evaluation of $CO_2$ from litter layer on the mineral soil (figure 4).

**Table 2.** Decay constants and time required for the loss of one-half and 95% of the original leaf and wood dry weight in different species.

| Species | Time parameter (years) | | | |
|---|---|---|---|---|
| | Half-time $\frac{(0.693)}{K}$ | | 95% $\frac{(3)}{K}$ | |
| | Leaf | Wood | Leaf | Wood |
| *Artocarpus chaplasha* | 1.99 | 0.79 | 1.50 | 3.78 |
| *Castanopsis indica* | 1.87 | 1.10 | 1.61 | 2.78 |
| *Dendrocalamus hamiltonii* | 2.17 | 3.63 | 1.38 | 0.83 |
| *Shorea robusta* | 1.87 | 0.76 | 1.61 | 3.97 |
| *Schima wallichii* | 2.18 | 1.19 | 1.38 | 2.52 |
| Other species (miscellaneous) | 2.17 | | 1.38 | |

**Table 3.** Chemical composition of leaf litter collected in April 1977 from Lailad forest.

| Species | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|
| *Artocarpus chaplasha* | 0.67 | 0.35 | 0.35 | 2.50 | 0.66 |
| *Castanopsis indica* | 0.82 | 0.46 | 0.36 | 1.88 | 0.59 |
| *Dendrocalamus hamiltonii* | 0.68 | 0.32 | 0.22 | 1.15 | 0.31 |
| *Shorea robusta* | 0.85 | 0.63 | 0.63 | 1.12 | 0.51 |
| *Schima wallichii* | 0.76 | 0.54 | 0.44 | 1.93 | 0.65 |
| Other species (miscellaneous) | 0.79 | 0.56 | 0.46 | 2.08 | 0.67 |

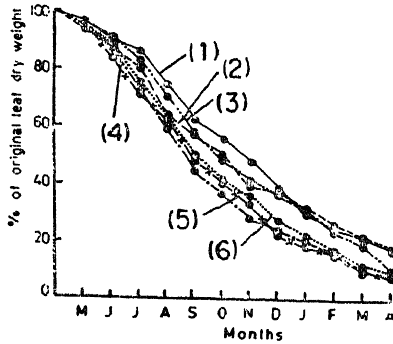



**Figure 2.** Rate of decomposition of leaf litter expressed as percentage of the original dry weight of leaves remaining after various periods of decomposition.

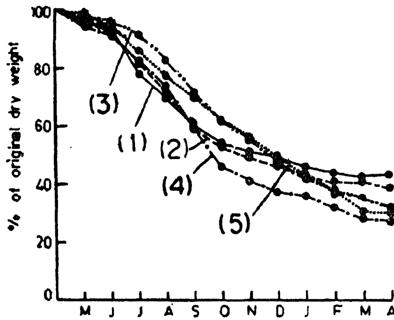



**Figure 3.** Rate of decomposition of wood litter expressed as percentage of the original dry weight remaining after various periods of decomposition.

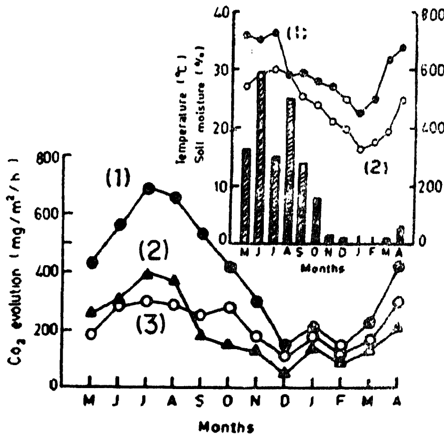



**Figure 4.** Monthly pattern of $CO_2$ evolution from the forest floor at Lailad.

### 3.3 *Nutrient content in litter*

The seasonal variations in concentration of nitrogen, phosphorus and potassium was well marked in both leaf and wood litter (figures 5, 6). This may be explained

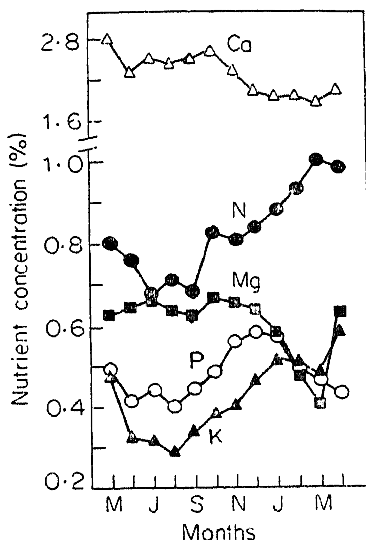



**Figure 5.** Monthly variation in concentration of different nutrients in leaf litter at Lailad forest.

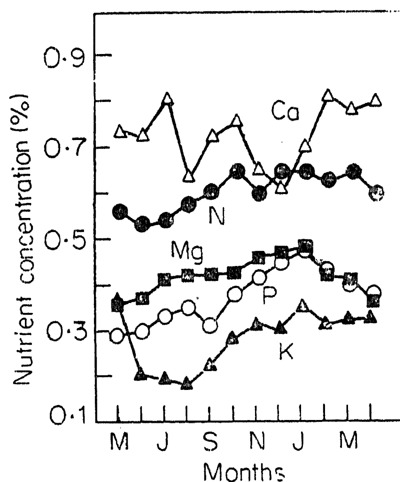



**Figure 6.** Monthly variation in concentration of different nutrients in wood litter at Lailad forest.

as due to a number of factors like translocation of nutrients from leaves before senescence, leaching of nutrients particularly potassium through rainfall and the extent of decomposition of leaves and twigs before they fall (Nye 1961; Tukey 1970; Gosz *et al* 1972). The concentration of N, P and K was higher in dry periods than during the rainy season; this may be mainly due to leaching of nutrients through rainfall.

Average concentration for the 12-months period, for various species showed great variations. Nitrogen and calcium content was highest in *Mesua ferrea*, *Miliusa roxburghiana* while phosphorus and magnesium concentration was found

**Table 4.** Nutrients content in leaf litter of different species and wood litter (Composite sample for all species).

| Species | N (%) | P (%) | K (%) | Ca (%) | Mg (%) |
|---|---|---|---|---|---|
| *Leaf litter :–* | | | | | |
| *Artocarpus chaplasha* | 0.68 | 0.35 | 0.35 | 2.92 | 0.66 |
| *Castanopsis indica* | 0.85 | 0.46 | 0.36 | 2.28 | 0.59 |
| *Dendrocalamus hamiltonii* | 0.74 | 0.32 | 0.22 | 1.39 | 0.36 |
| *Dillenia indica* | 0.64 | 0.24 | 0.54 | 1.86 | 0.28 |
| *Garcinia cowa* | 0.52 | 0.31 | 0.31 | 1.65 | 0.35 |
| *Machillus khasiana* | 0.85 | 0.40 | 0.30 | 1.98 | 0.65 |
| *Mesua ferrea* | 0.99 | 0.27 | 0.47 | 3.23 | 0.58 |
| *Miliusa roxburghiana* | 0.87 | 0.22 | 0.32 | 3.16 | 0.65 |
| *Shorea robusta* | 0.86 | 0.63 | 0.63 | 1.35 | 0.51 |
| *Schima wallichii* | 0.77 | 0.54 | 0.44 | 2.31 | 0.66 |
| *Sterculia villosa* | 0.66 | 0.30 | 0.40 | 1.33 | 0.42 |
| *Vitex peduncularis* | 0.57 | 0.33 | 0.23 | 1.81 | 0.35 |
| Other species (miscellaneous) | 0.79 | 0.56 | 0.46 | 2.51 | 0.67 |
| *Wood litter :–* | | | | | |
| Branches, twigs and barks (all species) | 0.60 | 0.38 | | 0.73 | 0.45 |

to be more in *Shorea robusta, Artocarpus chaplasha* and *Schima wallichii.* For other tree species a great variety in concentration of different elements was observed. In general calcium content was more followed by nitrogen in leaf and wood litter (table 4). Ewel (1976) observed a reverse trend where nitrogen was the predominant element followed by calcium.

### 3.4 *Nutrient return through litterfall*

The percentage contribution by different species not only depends upon litter biomass contributed by them but also on the nutrient concentration in their litter. Thus, even though *Dendrocalamus hamiltonii* had greatest litter biomass in the forest as a whole, yet there were other species like *S. robusta* which contributed higher amount of P through litter. In contrast a species like *M. ferrea* which had high nutrient level in the leaf tissue for N and Ca, had very low total contribution of these elements through litter because of lesser litter production. *Shorea robusta, S. wallichii* and *C. indica* in that order contributed high percentage of the nutrients both because of high litter production and high nutrient concentration in their litter. The total contribution of nutrients through litter was higher in the central zone of the forest than the periphery, though the total production of litter was higher in the periphery than the central zone (table 5). This may be related to the difference in the species composition in the zones due to difference in maturity of the forest community.

A comparison of nutrient returned through litterfall in the present study has been compared with other tropical and temperate forest ecosystems (table 6). The values for all the elements were lower (except phosphorus) than the values reported by Laudelot and Meyer (1954) for Zaire, and Nye (1961) for Ghana,

**Table 5.** Contribution of nutrient elements by different tree species in the peripheral and central zones of the forest at Lailad (kg/ha/yr).

| Species | Peripheral zone | | | | | Central zone | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | N | P | K | Ca | Mg |
| *Leaf litter :–* | | | | | | | | | | |
| *Artocarpus chaplasha* | 0.70 | 0.36 | 0.36 | 3.00 | 0.67 | 1.40 | 0.72 | 0.72 | 5.97 | 1.34 |
| *Castanopsis indica* | 2.08 | 1.13 | 0.89 | 5.61 | 1.45 | 4.16 | 2.27 | 1.77 | 10.22 | 2.91 |
| *Dendrocalamus hamiltonii* | 6.25 | 2.74 | 1.90 | 11.76 | 1.04 | 2.08 | 0.91 | 0.63 | 3.93 | 1.01 |
| *Dillenia indica* | 0.16 | 0.06 | 0.14 | 0.47 | 0.07 | 0.21 | 0.08 | 0.18 | 0.61 | 0.09 |
| *Gracinia cowa* | 0.26 | 0.15 | 0.15 | 0.81 | 0.16 | 0.76 | 0.45 | 0.45 | 2.39 | 0.51 |
| *Machillus khasiana* | 0.03 | 0.02 | 0.01 | 0.08 | 0.03 | 0.04 | 0.22 | 0.12 | 0.10 | 0.03 |
| *Miliusa roxburghiana* | 2.72 | 0.69 | 1.00 | 12.01 | 2.02 | 0.02 | 0.31 | 0.45 | 5.43 | 0.91 |
| *Mesua ferrea* | 0.40 | 0.11 | 0.19 | 1.50 | 0.23 | 0.14 | 0.04 | 0.07 | 0.54 | 0.08 |
| *Shorea robusta* | 2.86 | 1.64 | 1.87 | 4.02 | 1.42 | 6.18 | 4.25 | 4.03 | 8.69 | 3.30 |
| *Schima wallichii* | 2.01 | 1.60 | 1.15 | 6.05 | 1.60 | 6.52 | 4.38 | 3.68 | 19.87 | 5.52 |
| *Sterculia villosa* | 0.43 | 0.19 | 0.25 | 0.86 | 0.27 | 0.17 | 0.08 | 0.11 | 0.35 | 0.11 |
| *Vitex peduncularis* | 0.21 | 0.12 | 0.08 | 0.66 | 0.13 | 0.25 | 0.14 | 0.10 | 0 79 | 0.15 |
| Other species (miscellaneous) | 12.08 | 8.57 | 6.32 | 38.40 | 10.30 | 14.18 | 9.73 | 7.99 | 45.12 | 11.70 |
| *Wood litter :–* | | | | | | | | | | |
| Branches, twigs, barks (all species). | 4.21 | 2.69 | 1.99 | 5.12 | 3.16 | 11.04 | 7.05 | 5.21 | 13.38 | 8.27 |
| Total (leaf + wood) | 34.40 | 20.07 | 17.01 | 90.32 | 24.78 | 48.35 | 30.43 | 25.41 | 48.13 | 35.94 |

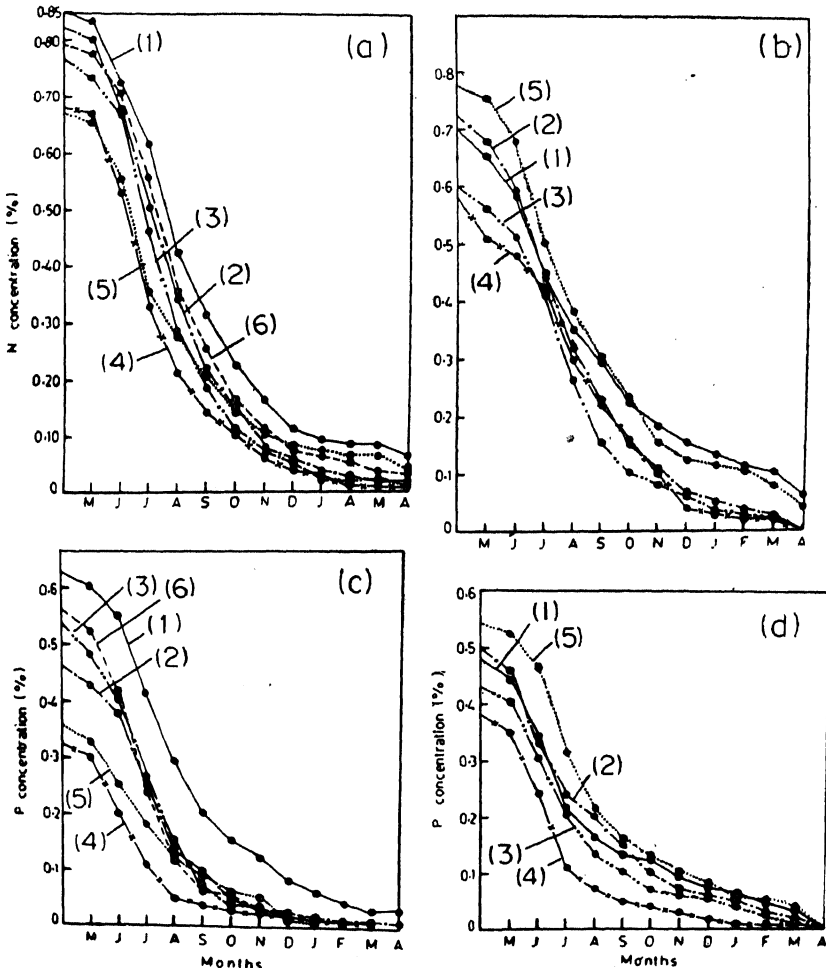
**Table 6.** Amount of mineral nutrients returned through litter fall in some tropical, sub-tropical and temperate forests of the world.

| Vegetation | Location | Nutrients (kg/ha/yr) | | | | | Source |
|---|---|---|---|---|---|---|---|
| | | Nitrogen | Phosphorous | Potassium | Calcium | Magnesium | |
| Mixed tropical | Zaire | 154-224 | 7-9 | 48-87 | 84-105 | 44-53 | Laudelot and Mayer (1954) |
| Mixed tropical | Kade, Ghana | 199.4 | 7.2 | 68.3 | 206.1 | 44-8 | Nye (1961) |
| Amazonian tropical rainforest | Brazil | 106 | 2.1 | 13 | 18 | 13 | Klinge and Rodrigues (1968) |
| Tropical deciduous. | Guatemala | 169 | 5.8 | 20 | 88 | 64 | Ewel (1976) |
| Birch forest | U.S.S.R. | 66 | 5 | 13 | 54 | 19 | Russian authors cited by Ovington (1959) |
| Scot Pine forest | England | 125 | 10 | 57 | 49 | 9 | Ovington (1959) |
| Temperate forest *Pinus caribae* | Nigeria | 7.3 | 0.4 | 6-9 | 11.4 | 2.7 | Egunjobi and Fasehum (1972) |
| Sessile Oak forest | England | 21-27 | 1-9 | 7-4 | 14-15 | 2-2.8 | Carlisle *et al* (1966) |
| Mixed tropical deciduous forest | Varanasi (India) | 18-54 | 1-28 | 6-31 | 15-184 | 44-30 | Present study |
| Mixed tropical forest | Meghalaya (India) | 41.36 | 25.26 | 21.21 | 104-22 | 30-36 | |

but higher than that for (except nitrogen) Amazonian tropical Rain-forest Klinge and Rodrigues (1968) and mixed deciduous forests (except calcium which is comparable) at Varanasi (Singh 1969).

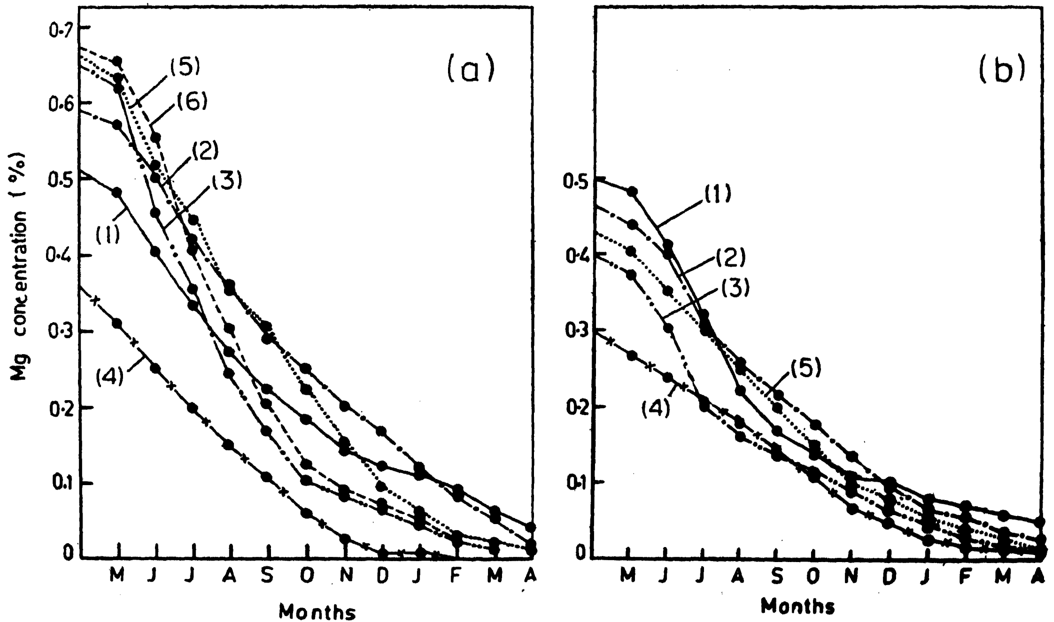
### 3.5 *Nutrient release through decomposition*

During the first 4 months after the placement of litter bags in the field, there was a rapid fall in the concentration of all the elements ; potassium and nitrogen were lost more rapidly. Loss of calcium and magnesium was comparatively slower. A similar trend of decline in concentration of nutrients, was found during wood decomposition too (figures 7, 8, 9). These results are similar to the trends shown by Gupta and Singh (1977) and Ewel (1976).




**Figures 7 a-d. a.** Loss of nitrogen from leaf litter through decomposition. **b.** Loss of nitrogen from wood litter through decomposition. **c.** Loss of phosphorus from leaf litter through decomposition. **d.** Loss of phosphorus from wood litter through decomposition.

**Figures 8 a-d.** **a.** Loss of potassium from leaf litter through decomposition. **b.** Loss of potassium from wood litter through decomposition. **c.** Loss of calcium from leaf litter through decomposition. **d.** Loss of calcium from wood litter through decomposition.




**Figures 9 a-b.** **a.** Loss of magnesium from leaf litter through decomposition. **b.** Loss of magnesium from wood litter through decomposition.

It may be concluded that (i) the turnover of litter is more in the peripheral sturbed zone of the forest than in the central zone due to comparatively ırly successional stage of the community, (ii) litter production and decomposi- on was markedly seasonal related to the temperature and rainfall pattern. The sults are compared with data on litter production and decomposition of her forest types.

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# Structure and function of a sub-tropical humid forest of Meghalaya III. Nutrient flow through water

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**Abstract.** This paper deals with water and nutrient flow through incident rainfall, throughfall and stemflow through a 50-year old stand of forest at Lailad. A larger proportion of water was accounted as throughfall and stemflow in March-April and was related to canopy density and this was inversely related to interception loss which was maximum in December. Many of the nutrient concentrations like that of N, K, Ca, etc. increased with the maturation of the leaves during October-November. Though concentration of nutrients was higher in stemflow, the total quantity was more *via* throughfall because of larger quantity of water passing through this compartment. More of Ca and K was lost through run-off and percolation due to greater release of these nutrients through litter. The significance of these results have been discussed in the context of secondary succession after slash and burn agriculture (jhum) of which this forms a later stage of community development.

## 1. Introduction

Atmosphere is a source of chemical inputs to terrestrial ecosystems which come through direct fall, throughfall and stemflow (Ovington 1959; Carlisle *et al* 1965) Generalization of these aspects are chiefly based on temperate forests and studies on tropical forests are meagre (Jackson 1971). In India the few studies done on nutrient flow through water are on plantations (Debral and Subba Rao 1968; George 1978) and no work has been done on natural forests.

The earlier two papers in this series (Part I and II) pertain to a 50 year old forest fallow at lailad in Meghalaya and deal with phytosociology, biomass and nutrient inventory and cycling through leaf and wood litter. The present study deals with the pattern of water and nutrient circulation through stemflow and throughfall and quantity interception, run-off and percolation losses in this forested ecosystem. For the present study, only the central zone of the forest representing the undisturbed 50-year stand has been considered.

## 2. Methods of study

Stemflow was sampled using a spiral polythene gutter of 6 cm diameter fitted in each stem and sealed with paraffin wax. The gutter was fixed at a height of 1.5 m above the soil surface on the tree trunk. A plastic funnel was attached to the two cut ends of the gutter and connected to a polythene container of 5 litre capacity. A nylon filter 1 mm mesh size was placed in the mouth of the funnel to prevent entry of extraneous matter. Three replicates each for two girth classes namely 30 and 90 cm were selected for each of the following important species : (1) *Shorea robusta* (ii) *Schima Wallichii,* (iii) *Castanopsis indica,* (iv) *Gmelina arborea* and (v) *Artocarpus chaplasha.*

Water of throughfall and incident rainfall was collected in polythene containers, the mouth of each being fitted with 20 cm diameter funnel which was provided with 1 mm mesh nylon filter to prevent entry of foreign matter. Three containers were kept outside the forest in open places to collect the water from incident rain. Twelve containers of the same size were kept under the forest canopy to measure the throughfall. In order to measure the atmospheric precipitation two standard rain gauges were kept in the open. All the containers were kept 50 cm above the surface on a wooden platform to avoid splashing of soil particles into the funnels. 2 ml toluene was added to the container to prevent microbial activity. Sampling was done at intervals ranging from 2 to 7 days depending upon the intensity and the frequency of rainfall during the monsoon. At the time of sampling, 500 ml of well homogenised water from stemflow/throughfall/incident rain was brought to the laboratory and the samples were filtered through a Whatman no. 44 filter paper and chemically analysed for N, P, K, Ca and Mg.

For studies pertaining to run-off water and sedimentation, the loss from a confined area of 1×10 m along the slope was collected in drums of 200 litre capacity and periodically removed for analysis. Percolation studies were done using a simple lysimeter of the Russian type (Buckman and Brady 1980). The soil was cut out vertically to expose the profile. A small tunnel was excavated at a depth of 40 cm (this is the depth at which root density is high) and a collector of 30 × 30 × 15 cm was placed inside the tunnel. The water percolating through soil was tapped out from time to time and chemically analysed for N, P, K, Ca and Mg using standard methods as described by Jackson (1958) and Allen (1974). Thus, nitrogen was determined by micro-kjeldahl method and phosphorus estimated colorimetrically by molybdenum-method. Calcium and magnesium were analysed by EDTA titration method and potassium by flame emission method.

## 3. Results and discussion

### 3.1 *Throughfall*

A summary of the seasonal distribution (table 1) shows that the percentage of rainwater coming as throughfall is maximum in the month of March-April (over 70% of the total) while in other months it was much less with minimum

percentage in December. The high proportion of throughfall in March-April was due to reduced crown density at this time when 41% leaf fall occurred. The proportion of throughfall showed a decreasing trend with increase in canopy density in subsequent months. Similar relationship between crown density and throughfall was also reported by Szabo (1975). The average value of 52.4% throughfall during the course of this study was higher than the values obtained for other broad leaved forests (Debral and Subba Rao 1968 ; Aldridge and Jackson 1973) but lower than a few others (Ovington 1959; Leonard 1961; Szabo 1975). The quantity of throughfall is directly proportional to the gross rainfall and the relationship is indicated in figure 1a ($P < \cdot 05$).

### 3.2 *Stemflow*

The average stemflow during the period of study was measured at 8% of the precipitation (table 1). A low proportion of stemflow was measured in the month of November due to lesser intensity and frequency of rain. On the other hand,

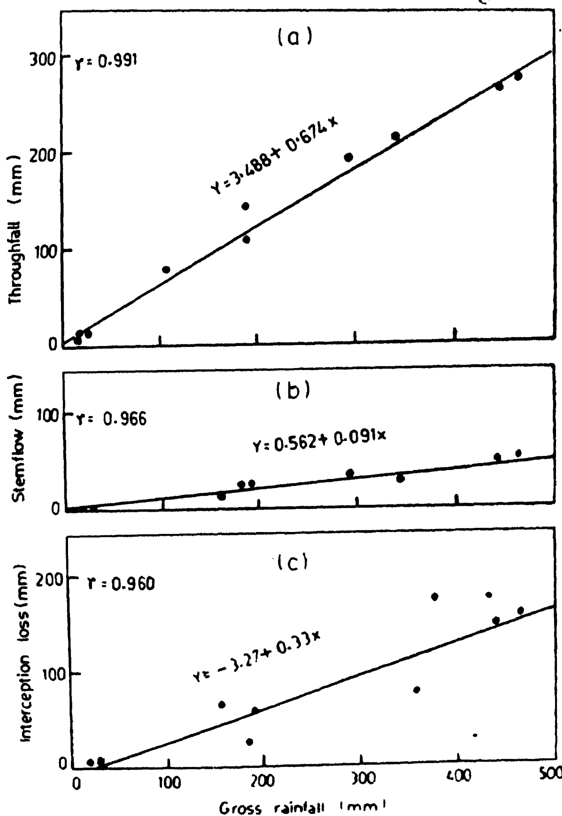



**Figure 1.** Relationship between gross rainfall and (a) throughfall, (b) stemflow and (c) interception.

reduced canopy density in the month of March and April allowed more water flow through stem. The gradual decline in percentage stem flow from May-October could be related to the high canopy density which in turn resulted in greater proportion of interception losses. The present value of stemflow (8%) was found to be higher than the values reported by Ovington (1959) and Szabo (1975) which ranged from 0.12—3.10% but lower than the values reported by a few others (Eidmam 1959 ; Aldridge and Jackson 1973). The relationship between gross rainfall and stemflow was found to be highly significant which is expressed by a linear regression line (figure 1b).

### 3.3 *Interception loss*

The proportion of the rainfall intercepted by the canopy was inversely related to the proportion of throughfall and stemflow with maximum percentage values recorded in December and minimum in March. This high percentage of water transmission from the canopy during October-December may be attributed to the fact that rainfall in these months was not regular in comparison to the monsoon period (April-September) and the vegetation often remained dry. Thus the greater quantity of water necessary to wet the vegetation accounted for the high interception loss. The low interception values during March and April were due to reduced crown density due to maximum leaf fall during this period. Similar observation with low interception losses during heavier leaf fall was made by Szabo (1975) in their study on Hungarian Oak forest ecosystem. The relationship between gross rainfall and interception loss which follow the same pattern as stemflow and throughfall is shown in figure 1c.

### 3.4 *Water balance of the ecosystem*

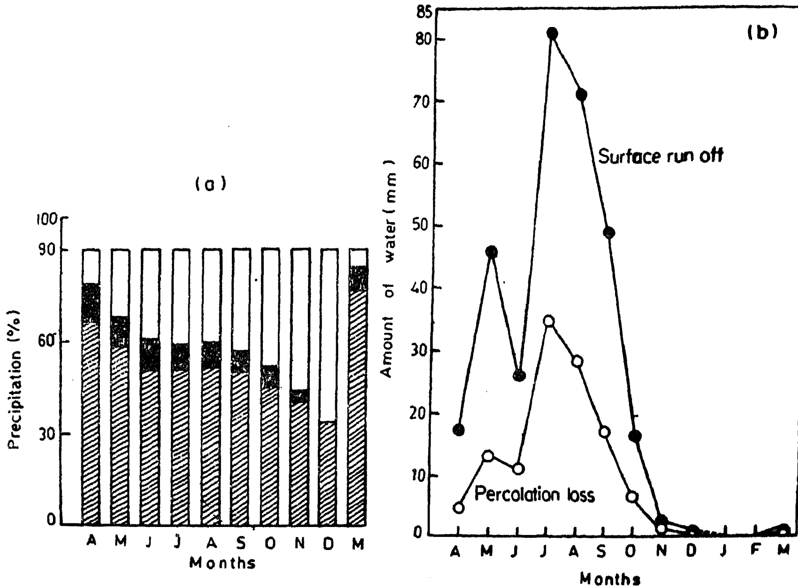
The total amount of water and percentage values given in table 1 are estimates based considering forest canopy as entirely closed. But in order to explain the total quantity of water in one hectare forest surface, the forest gaps are also to be considered. The total canopy coverage in the present forest was 90% and the rest 10% was accounted for gaps where the precipitation could reach the forest floor almost directly without any interruption. After making allowances (Szabo 1975) for gaps in the forest canopy, the percentage distribution of the incident rainfall in a forest of one hectare is shown in figure 2a. However, it may be mentioned that the stemflow values would be on the higher side as only selected important tree species of same girth classess were considered and assumed contribute to the total cover of the forest.

### 3.5 *Loss through run-off and percolation*

Studies on surface run-off and percolation losses of rainwater at Lailad forest showed that they represented 19.6% and 6.78% respectively of the total annual

**Table 1.** Throughfall, stemflow and interception loss at Lailad forest for the year 1977-78 with standard errors.

| Month | Gross rainfall (mm) | Throughfall (mm) | % | Stemflow (mm) | % | Interception loss (mm) | % |
|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| April | 188.5 | 139.5 ± 2.8 | 74.0 | 25.0 ± 1.3 | 13.3 | 24.0 ± 1.8 | 12.7 |
| May | 292.5 | 190.0 ± 2.0 | 64.9 | 30.0 ± 1.1 | 10.3 | 72.5 ± 1.9 | 24.8 |
| June | 187.5 | 105.5 ± 1.8 | 56 3 | 21.5 ± 0.8 | 11.5 | 60.5 ± 1.7 | 32.3 |
| July | 463.0 | 264.5 ± 1.7 | 57.1 | 45.0 ± 0.7 | 9.7 | 153.5 ± 1.7 | 33.1 |
| August | 440.0 | 253.5 ± 0.7 | 57.6 | 40.0 ± 0.5 | 9.1 | 146.5 ± 0.9 | 33.3 |
| September | 375.0 | 209.5 ± 1.8 | 55.9 | 27.0 ± 0.7 | 77.2 | 138.5 ± 1.7 | 36.9 |
| October | 160.0 | 80.0 ± 1.6 | 50.0 | 12.0 ± 1.0 | 7.5 | 68.0 ± 1.5 | 42.5 |
| November | 26.5 | 12.0 ± 1.6 | 45.3 | 1.0 ± 1.2 | 3.4 | 13.5 ± 1.6 | 50.9 |
| December | 14.5 | 5.5 ± 2.5 | 37.0 | — | — | 9.0 ± 2.3 | 69.1 |
| March | 18.5 | 15.7 ± 2.4 | 84.9 | 1.5 ± 0.7 | 8.1 | 1.3 ± 2.1 | 7 0 |




**Figures 2a-b. a.** Distribution of precipitation in the forest (per hectare). Hatched columns, throughfall ; closed columns, stemflow ; open column, intercepted loss. **b.** Monthly pattern of surface run-off and percolation loss of Lailad forest.

rainfall, during the year. The monthly pattern of losses of water could be related to the rainfall pattern with maximum run-off and percolation losses occurring during May-September (with 87% and 37% of the total run-off and percolation losses respectively), with peak values in the months of July (figure 2b). Both, the high frequency and intensity of rainfall during monsoon contribute to heavy losses during this period.

### 3.6 *Nutrient return by throughfall, stemflow and incident rainfall*

The mean monthly concentration (mg/l) of the various elements throughout the study period in throughfall, stemflow and incident rainfalls are shown in figures 3a, b, c, d, e, The concentration of total nitrogen in throughfall and stemflow was low during March-May, followed by a steady rise reaching a maximum towards October-November which could be related to the subsequent maturity of the leaves. Similar increase in N concentration of throughfall with increasing leaf maturity was shown by Tukey *et al* (1958) with subsequent decrease after the formation of abscission layer. The concentration of nitrogen in stemflow was more than that of throughfall which may be due to (i) release

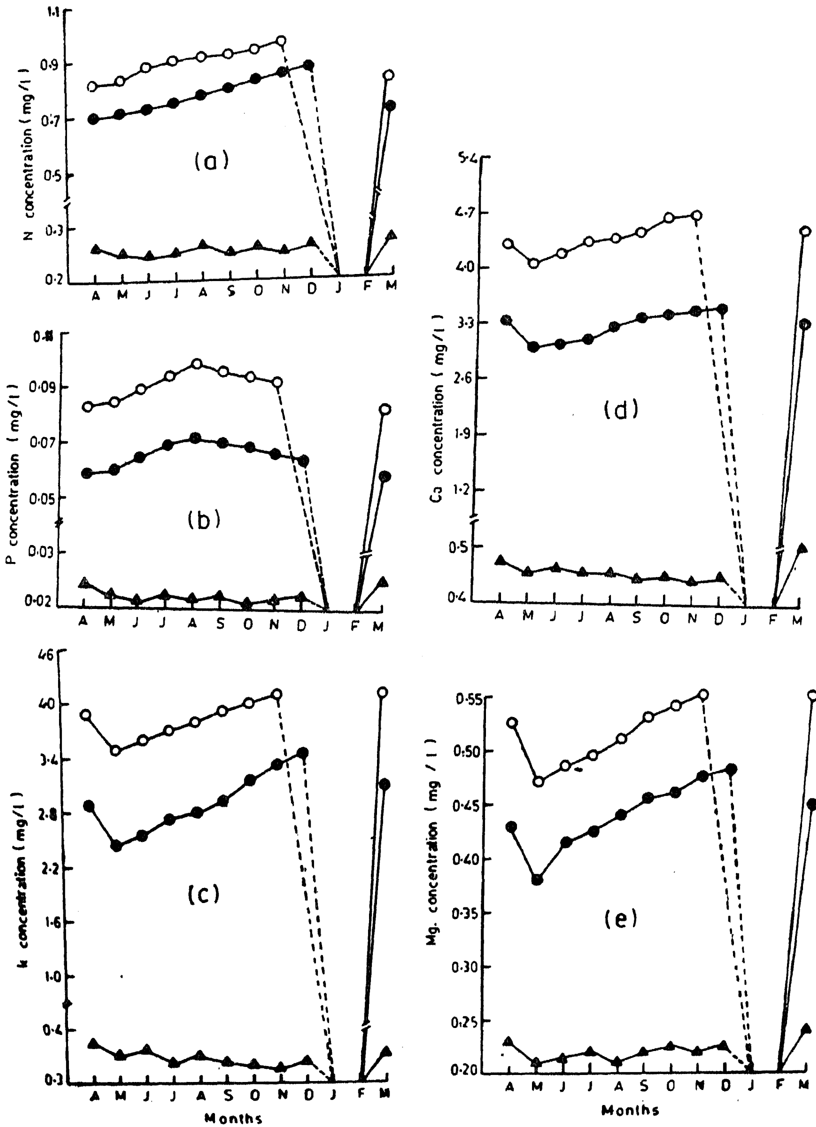



**Figure 3.** Monthly variation in concentration of different elements, **a**, nitrogen ; **b**, phosphorus ; **c**, potassium ; **d**, calcium and **e**, magnesium in different water samples.

of nutrient during bark decay during the rainy season (ii) the wash out of nutrients from leaves and (iii) low quantity of water in stemflow and consequent high concentration of nutrients. Monthly variation in the concentration of N in incident rainwater could also be observed though the values were low compared to that of the throughfall and stemflow.

The concentration of phosphorus in incident rain was very low during the rainy season. A slightly higher level was noted during March-April which could be due to (i) high level of dust particles in the atmosphere during the preceding dry period, (ii) presence of partly burnt particles of organic matter in the air due to burning of slash in the neighbourhood during this period due to shifting agriculture and (iii) low rainfall during this period with lesser dilution of this element. In throughfall, the level of P gradually increased reaching a maximum in August followed by a slow decrease in subsequent months. The gradual increase in concentration up to August may be attributed to the presence of high pollen grains of the tree species and also the production of new leaves which may have higher concentration of phosphorus as was reputed by Carlisle *et al* (1967) working on a sessile oak forest in England. A similar pattern of monthly concentration changes was also observed for stemflow except that the concentration of P on the average was two times higher than that for throughfall due to release of this nutrient from the decayed bark.

The monthly pattern for potassium, calcium and magnesium concentration in throughfall, stemflow and incident rainfall was similar (figures 3c, d, e). The gradual increase in concentration from the month of May to December is probably related to gradual maturation of new leaves produced in April and the consequent increase in release of some of these nutrients from more mature leaves, an observation also made by Tukey *et al* (1958) and Deneyear-DeSmet (1966). Amongst the three elements, the concentration of Ca was more in stemflow due to its high level in the bark.

The total amount of nutrients (kg/ha/yr) contributed through stemflow, throughfall and incident rain is shown in table 2. Throughfall contributed 98% of all the nutrients. The low addition through stemflow is in spite of the high concentration of nutrients in the stemflow water, as the total quantity of stemflow is far less than that due to throughfall. Amongst the cations, calcium and potassium were highly leachable with heavy washout through stemflow and throughfall.

**Table 2.** Nutrient return through stemflow, throughfall and rainwater at Lailad forests.

| | | Nutrients (kg/ha/yr) | | | | |
|---|---|---|---|---|---|---|
| | | N | P | K | Ca | Mg |
| Stemflow | | 0.17 | 0.02 | 0.71 | 0.84 | 0.10 |
| Throughfall | | 8.39 | 0.89 | 31.28 | 35.19 | 5.03 |
| | Total | 9.56 | 0.91 | 31.99 | 35.93 | 5.13 |
| Rainwater | | 4.33 | 0.43 | 7.80 | 9.96 | 4.77 |
| | Difference | 5.23 | 0.48 | 24.19 | 24.97 | 0.63 |

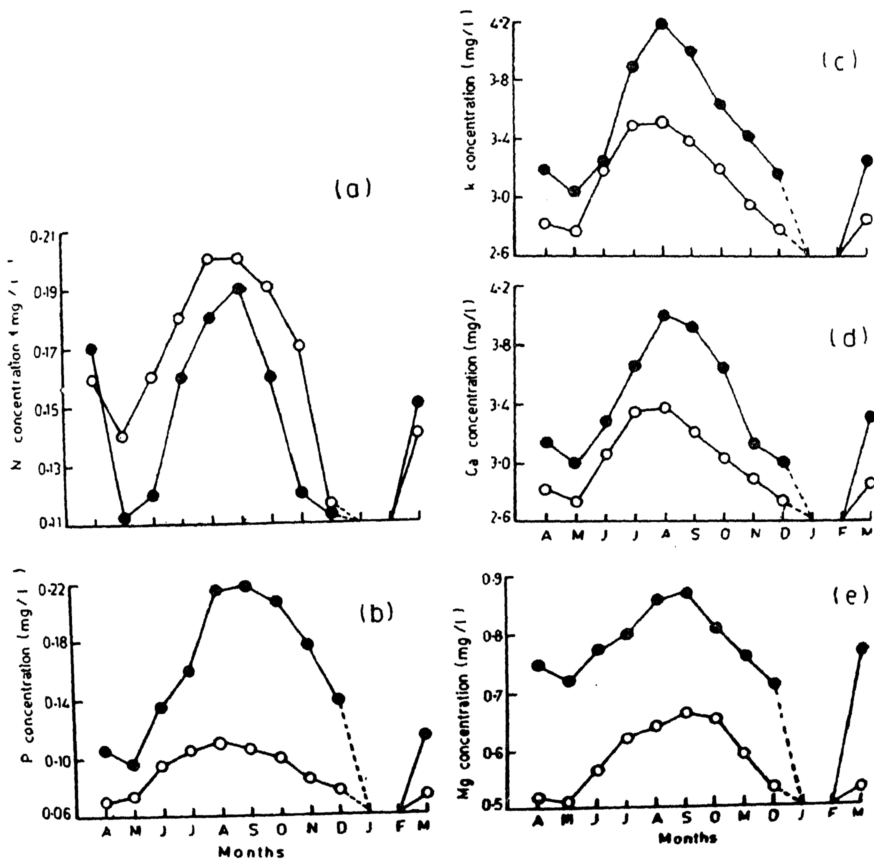



**Figure 4.** Monthly variation in concentration of different elements, **a**, nitrogen; **b**, phosphorus; **c**, potassium ; **d**, calcium and **e**, magnesium in different water samples.

Between nitrogen and phosphorus, leaching of the former was far greater. The total quantities of different nutrients reaching the soil by throughfall, stemflow and incident rain was in the order Ca> K> N> Mg> P.

## 3.7 *Nutrient lossess through run-off and percolation of water*

The concentration of different elements namely N, P, K, Ca and Mg which were lost through run-off showed much fluctuation in different months. The high concentration of N in run-off water in March-April (figures 4a, b, c, d, e) may be due to lesser rainfall in these months and the consequent low dilution effect. The peak concentration was reached during August-September and this may be related to greater release of nutrients from freshly decomposing litter on the forest floor, whereas concentration of most of the nutrients was more in the run-off water, N concentration was generally higher in percolation water.

**Table 3.** Annual loss of water (mm) and nutrients (kg/ha) from different successional ages of fallows (partly based on Toky and Ramakrishnan, unpublished)

| Age of the fallows (year) | Surface run-off (mm) | Percolation loss (mm) | N | | P | | K | | Ca | | Mg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Surface run-off | Percolation loss | Surface run-off | Percolation loss | Surface run-off | Percolation loss | Surface run-off | Percolation loss | Surface run-off | Percolation loss |
| 0 (after burning and cropped) | 557.9 | 357.1 | 8.1 | 14.05 | 1.1 | 0.1 | 77.7 | 20.8 | 210.3 | 6.9 | 15.2 | 3.5 |
| 50 | 312.6 | 124.0 | 0.5 | 0.2 | 0.5 | 0.1 | 11.0 | 3.8 | 10.7 | 3.7 | 2.4 | 0.7 |

The concentration of nutrients in percolation water also followed a similar pattern as that in the run-off water, in that peak values were higher during July-September with a minor peak during March-April (figures 4a, b, c, d, e).

Since the present forest represented a secondary successional fallow after jhum (Toky and Ramakrishnan unpublished) a comparison of the pattern of losses with a 0-year freshly burnt site would be interesting (table 3). The loss of Ca and K from a 50-year old fallow was higher compared to other nutrients may be due to greater release of these nutrients from the vegetation through litter decomposition (Timmons *et al* 1977). In fact even though the quantity of water lost from a deforested side through run-off and percolation was not more than 2-3 fold compared to the Lailad forest, nutrient losses increased as much as 6–7 times compared to the forested site. This may be due to sudden release of nutrients through burnt and lack of vegetation cover to hold the same (Ramakrishnan *et al* 1980).

## 4. Conclusion

The three series of papers on the ecosystem structure and function of a 50-year old secondary successional fallow is significant as it represents a comparatively more stable forested ecosystem in the successional gradient. An early successional weed stage which dominates up to about 5 years of fallow development after jhum is soon replaced by a bamboo dominated stand up to about 20 years beyond which dicot tree species gain importance (Ramakrishnan *et al* 1981). The peripheral zone of the Lailad forest (Part I in this series) represents such a stage due to frequent disturbances in this zone. The central zone, however, represents the 50-year old stand of a mixed broad leaved forest. Apart from the high species diversity and biomass and nutrient stored in the living compartment alongwith efficient cycling through litter (Ramakrishnan *et al* 1980), the loss of nutrients from the system is also minimal compared to younger fallows (Toky and Ramakrishnan 1981). In fact there is negligible loss from the system through both run-off and percolation as seen from comparison presented in Part III in this series. This presented in a dramatic way the damage done due to deforestation during shifting agriculture. With the climate and steep topographic conditions prevailing in the north-eastern hill region, the present study highlights the significance of maintaining a forested ecosystem for environmental stability.

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# Anatomy of the seedling of the Leguminosae — I

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**Abstract.** Anatomy of the juvenile nodes in the seedling of fourteen genera of Leguminosae is described. The cotyledonary node exhibits a two-trace, unilacunar condition. The two traces show various degrees of approximation of the two traces leading to one-trace, unilacunar condition in some genera. At the second node level, only two genera show the one-trace, unilacunar pattern ; 11 of the remaining genera have a three-trace trilacunar supply and one genus exhibits an intermediate type. At the third node level all the genera show a three-trace, trilacunar pattern. The present data suggest that the three-trace, trilacunar condition is derived by the addition of a lateral trace on either side of the median trace and that the one-trace, unilacunar condition appears to be a result of the approximation of the two traces at the cotyledonary node.

**Keywords.** Nodal anatomy ; seedlings ; Leguminosae.

## 1. Introduction

Anatomy of the seedling in the Leguminosae has been studied by Compton (1912), Winter (1932), McMurray and Fisk (1936), Weaver (1960), Pillai and Sukumaran (1969), Pillai *et al* (1970, 1974), Bairathi and Nathawat (1974) and Narang (1978). Most of these studies are, however, restricted to the cotyledonary node and root-stem transition region. The evolutionary sequence followed by the different types of nodal structures has always been of interest to plant anatomists. The present investigation was, therefore, undertaken to find out if the anatomical studies in the seedling of some dicots would be of help in this regard. For this purpose the anatomy of the juvenile nodes of the seedling of 14 species belonging to 14 genera of the Leguminosae were studied.

## 2. Materials and methods

Seedlings of the plants studied were raised in the garden of the Institute of Science, Bombay, from seeds collected locally or purchased in the market. Portions of the

axis obtained by cutting a little below and above the first (cotyledonary), second and the third nodes were fixed in FAA. The usual paraffin method was followed. Sections of the paraffin infiltrated material were cut on a microtome at a thickness of 15–20 microns. The sewere stained with crystal/gentian violet using erythrosin/ orange G as counter strain

## 3. Observations

*Crotalaria juncea* Linn. The hypocotyledonary axis has a ring of about a dozen bundles (figure 1). Two traces depart from each of the two sides (figure 2). The two traces come closer to each other in their upward course but retain their separate entity at the cotyledonary base (figure 3). Axillary buds are present in the axil of the cotyledons (figure 3).

The epicotyledonary axis has a ring of about 15–20 bundles (figure 4). Three traces, one median and a lateral on either side of it, emerge from the ring (figure 5). The laterals in their upward course shift closer to the median and can hardly be differentiated from each other in the petiole (figure 6).

The third node has, likewise, a three trace, trilacunar supply to the leaf (figure 7). The laterals after giving off a branch to the stipule shift closer to the median and almost merge with it so as to become indistinguishable (figures 8–9). As a variation, some series cut off at the second and third nodes exhibit a four-trace, four lacunar condition *i.e.* a median with two laterals on one side and one on the other (figures 10-11).

The nodal structure at the first three nodes in *Medicago sativa* Linn., *Tephorosia purpurea* (Linn.) Pers., *Cassia tora* Linn. and *Mimosa pudica* Linn. is similar to *Crotalaria juncea* except for minor differences regarding the degree of distinctiveness of the two traces to the cotyledons as they extend upwards and that of the lateral traces of the leaves of the second and third nodes from the median trace, as these extend upwards into the petiole. In *Mimosa pudica* the cotyledons separate out from the axis in the form of a ring which organizes into two cotyledons, and two non-vascularized stipular structures (figure 12).

In *Arachis hypogaea* Linn., the cotyledonary node is two traced unilacunar with the two traces remaining distinct in the cotyledons (figures 13–14). The leaves at the second and the third nodes are three–traced and trilacunar (figure 15) with the laterals as well as the median bundles dividing in their upward course (figure 16). Some of the branches of the laterals extend into the stipules while the remaining ones alongwith the branches of the median and the median itself, extend into the petiole (figure 17).

The cotyledonary node in *Pisum sativum* Linn. is two–traced, unilacunar, traces remaining distinct from one another in their upward course (figures 18–19). The leaves of the second and the third node, are three–traced and trilacunar. This plant is characterized by the precocious emergence of the lateral traces as compared to the median trace of the same leaf. Thus, the lateral traces of the leaf of the second node emerge a little above the cotyledonary node and those of the leaves of the third node emerge almost alongwith the median bundle of the second node (figure 20). These precociously emerged lateral traces, extend outwards from the

vascular ring (figures 21 and 22). The median bundle and the lateral traces divide in their upward course (figures 21–23). While some of the branches of the laterals extend into the stipules, the remaining ones alongwith the branches of the median and the median itself, extend into the petiole (figures 22,24).

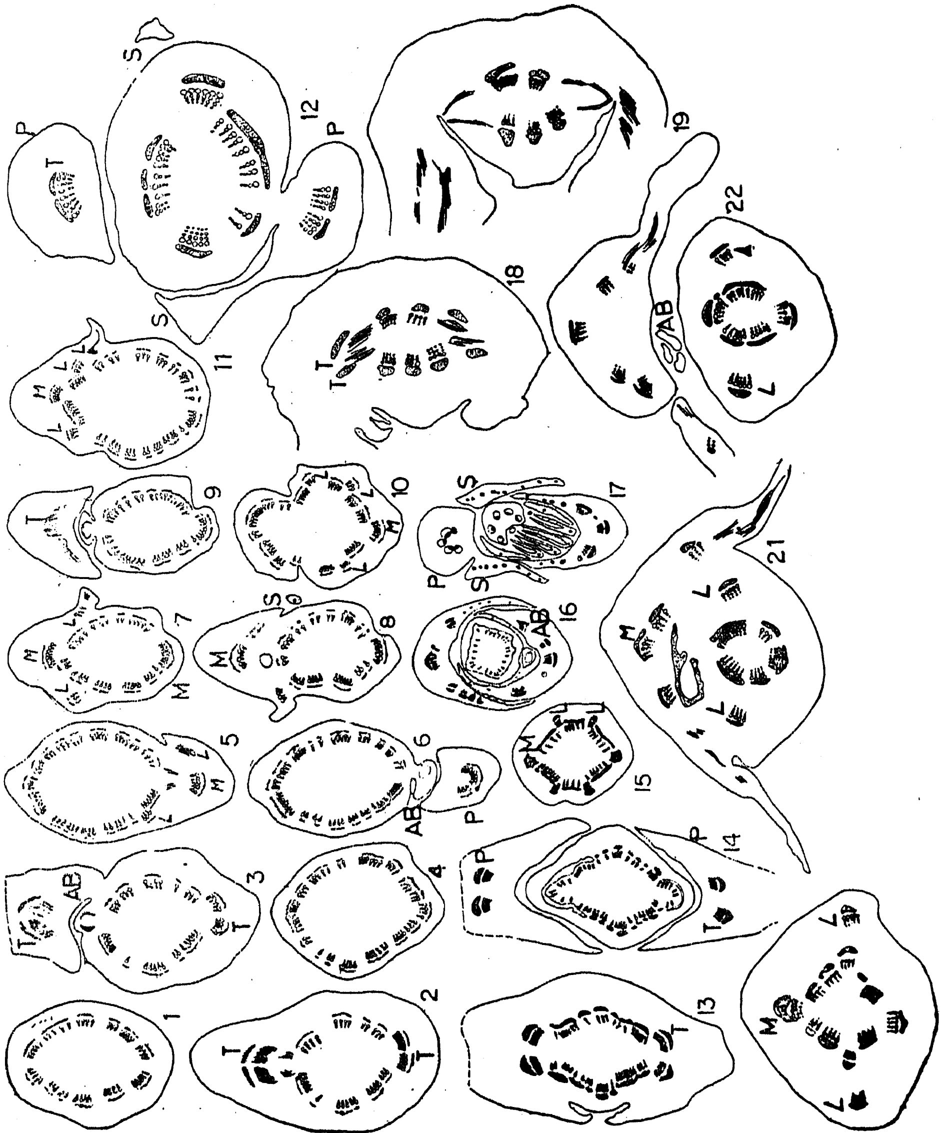
The cotyledonary node in *Clitoria ternatea* Linn. is two–traced, unilacunar. The two traces come together in their upward course. They ultimately lose their distinctiveness. The leaves of the second node are opposite in phyllotaxy. Each leaf receives a single median trace and a common lateral only on one side of the axis (figure 25). The single common lateral splits into two (figure 26). The branches of the split lateral, after giving off branches to the stipule, shift closer to the median in their upward course and ultimately merge with it forming a concentric bundle (figures 26–27). The stipules on the other side of the leaves receive their vascular supply from the median bundle itself (figure 26). The phyllotaxy at the third node is alternate. The leaves at this node receive a three–trace vascular supply. The lateral traces, as usual, in their upward course, at first, give off a branch to the stipule and later come closer to the median and merge with it.

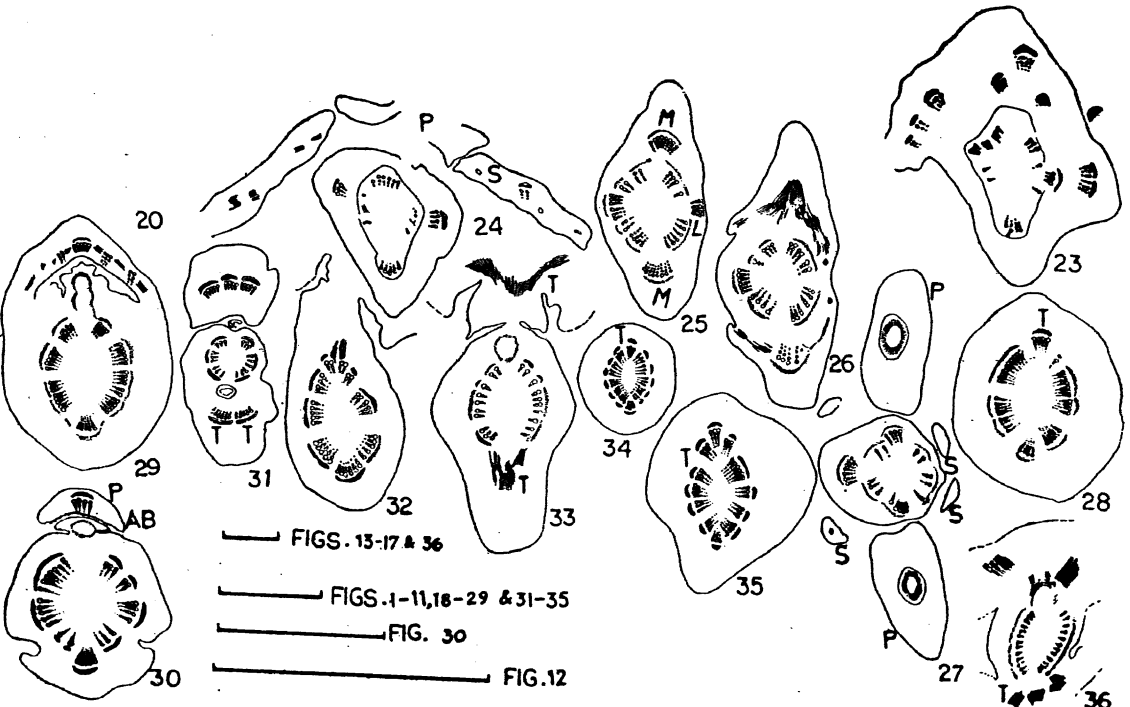
The cotyledonary node in *Abrus precatorius* Linn. and *Cicer arietinum* Linn. is two–traced, unilacunar. The two traces come closer to each other in their upward course. The leaves of the second node are one-traced, unilacunar (figures 28,30). The single trace splits into many small strands, upwards (figure 29). The leaf of the third node, is as usual three–traced, trilacunar.

The cotyledons in *Leucaena glauca* Linn. and *Albizia lebbeck* (Linn.) Benth receive two traces, which in their upward course merge into one and divide into three at the point of entry into the cotyledonary base (figures 31,32,33). The cotyledons in the *Tamarindus indica* Linn. and *Pithecellobium dulce*, (Roxb.) Benth are characterized by one–trace, unilacunar supply (figure 34). This single trace divides into three right at the point of emergence (figures 35–36). The vascular supply to the leaves of the second and the third nodes in the above mentioned plants is of the three trace, trilacunar type with the lateral traces in their upward course, at first giving off branches to the stipules and then shifting closer to median.

## 4. Discussion

Anatomists have treated different types of nodal structures in different ways, phylogenetically. Thus, Sinnott and Bailey (1914) suggest that the three–trace, trilacunar type is primitive as compared to unilacunar and multilacunar types. Ozenda (1949) on the other hand treats the multilacunar type as more primitive. Marsden and Bailey (1955) describe the so-called " fourth type " *i.e.*, a two trace, unilacunar type as the primitive type of node. They, further, consider that the unilacunar node with one trace arose by the fusion of two traces. Bailey (1956) reexamined the earlier viewpoint which he shared with Sinnott (Sinnott and Bailey 1914) and reached a conclusion that the three–trace, trilacunar and multitrace, multilacunar types in angiosperms were derived at some stage in their evolution from the one-trace, unilacunar type. Canright (1955), Eames (1961), Esau (1960,1965) and Carlquist (1961) have more strongly emphasized the primitiveness of the unilacunar node with two traces and consider it as basic in the evolution of angiosperm nodal structure.






STUDIES IN THE ANATOMY OF THE SEEDLING IN THE LEGUMINOSAE-I HEGDE & TILAK

Serial T.S. of the axis of the seedling **Figures. 1-36. 1-11.** *Crotalaria juncea*, 1-3. hypocotyledonary region and cotyledonary node, two-trace, unilacunar. **4-6.** second node, 7-9. third node, 10, 11. 2nd and 3rd nodes, 4 trace, 4 lacunar; **12.** *Mimosa pudica* cotyledonary node; **13-17.** *Arachis hypogaea*; **13, 14.** cotyledonary node, **15-17.** 2nd and 3rd nodes; **18-24,** *Pisum sativum*, **18, 19.** cotyledonary node, **20-22.** 2nd node. **23, 24.** 3rd node, **25-27,** *Clitoria ternatea* 2nd node, **28, 29.** *Cicer arietinum* 2nd node; **30.** *Abrus precatorius* 2nd node; **31.** *Leucaena glauca*, cotyledonary node; **32, 33.** *Albizia lebbek* cotyledonary node;. **34, 35.** *Pithecellobium dulce* cotyledonary node; **36.** *Tamarindus indica*, cotyledonary node.

*Abbreviations*: AB, axillary bud, L, lateral trace; $L_2$, lateral trace of 2nd node; $L_3$, lateral trace of 3rd node; M, median trace; P, petiole; S, stipule, T, traces.

Pant and Mehra (1964) and Benzing (1967) do not treat the two–trace, unilacunar node as primitive. Instead, Benzing (1967) proposes that the three–traced condition is likely to be more primitive in the angiosperms than the two–trace, unilacunar type. Takhtajan (1969) conceives of a hypothetical " fifth type " of node with a central gap having two traces. This type is supposed to have given rise to all other known types.

All the plants studied show a basically two traced, unilacunar nodal pattern at the cotyledonary node. Some of these have a two–traced, unilacunar structure with varying degrees of independence or approximation of the two traces from the point of emergence through their extention into the cotyledons. Other plants have a one-traced, unilacunar type. Thus, there is a trend towards the approximation of the two traces leading to a one trace, unilacunar type of node.

At the second node level *Abrus precatorius* and *Cicer arietinum* have a one–trace unilacunar condition. *Clitoria ternatea* presents an intermediate condition. It has a median trace, each, for the two leaves of the opposite phyllotaxy and a common lateral on one side of the axis. All the remaining plants have a three–trace, trilacunar supply to the leaves of the second node. Further, all the plants studied have a three–trace, trilacunar structure at the third node. Thus, it is only in *Abrus precatorius* and *Cicer arietinum* that the three trace, trilacunar pattern is attained at the third node. Rest of the plants achieve this condition at the second node itself.

The present data, therefore, shows that the three–trace, trilacunar condition is a characteristic feature of the more mature second and third nodes while the two–trace, unilacunar and one trace, unilacunar patterns are seen at the cotyledonary nodes. Similar observations have been made during the course of a study of twentytwo genera of the Malvales (Rao 1980). Further, in no plant either of the Leguminosae or of the Malvales could a condition be recorded wherein a three–trace, trilacunar condition of the cotyledonary or second node is succeeded by a one–trace, unilacunar type at the second and third nodes. Bailey (1956) records similar observations. These observations may be taken as an evidence in support of the primitiveness of the one trace, unilacunar type as compared to the three–trace, trilacunar type.

In *Abrus precatorius* the single trace at the second node divides into many small strands. In *Clitoria ternatea* the branches of the split lateral give off a branch to the stipule and shift closer to the median and merge with it. The stipule on the other side receives its vascular supply from the median itself. In *Arachis hypogaea* and *Pisum sativum* the median as well as the laterals divide into smaller strands. While some of the branches of the laterals enter into the stipule, the remaining ones alongwith the branches of the median and the median itself extend into the petiole. In rest of the plants both at the second as well as the third nodes the laterals at first give off a branch to the stipule and their remaining portions shift closer to the median. These portions of the laterals may remain distinct from the median in plants such as *Medicago sativa* and *Tephrosia purpurea* or merge with the median as in *Crotalaria juncea and Cassia tora.* Thus, the laterals, give vascular supply to the stipules, however, when absent, the stipules receive branches from the median itself.

Vascular bundles outside the main vascular ring have been recorded in the inter node of *Pisum sativum* by Kupicha (1975). He designates these as cortical bundles but they appear to be precociously emerged lateral traces.

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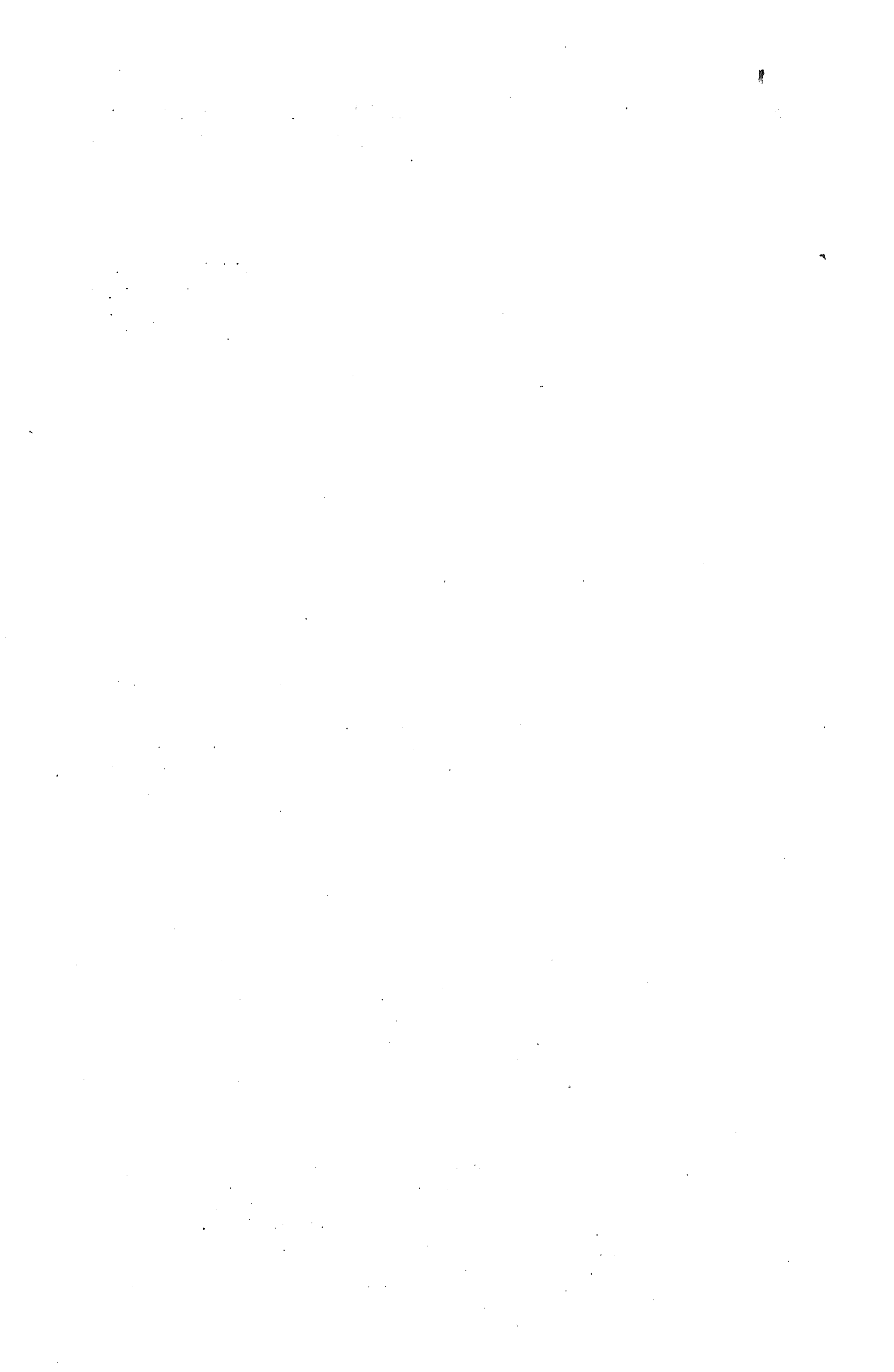

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# Cork-warts in *Eucalyptus* species

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**Abstract.** Four different types of cork-warts, the ordinary cork–warts, the d-scars, wound cork and trichome scars have been described from *E. citriodora* and *E. torellina*. The differences between them are discussed.

**Keywords.** *Eucalyptus* ; cork-warts ; d-scars ; wound cork ; trichome scars ; d-stomata.

## 1. Introduction

The occurrence of cork–warts or holes in the epidermis surrounded by radially arranged concentric rows of suberised cells has been mentioned among others by Solereder (1908), Metcalfe and Chalk (1950) and Stace (1965). Their presence has been explained either as a diagnostic character or as a result of mechanical injuries. During a general survey of epidermal characters of a number of *Eucalyptus* species, cork–warts were noticed in some of them. As no detailed accounts of their structure and development is available it was thought worthwhile to investigate them in detail.

## 2. Material and methods

Young and mature leaves of *E. citriodora* Hook. and *E. torellina* F.VM. were obtained from Tamil Nadu. Peels of the epidermis were obtained by scrapping and those of the cuticle by maceration in a 1 : 1 mixture of nitric acid and chromic acid (both 10%). The peels were mounted in Safranin glycerine jelly. Transparencies of the leaves were also prepared by the method of Arnott (1959).

## 3. Observations

In both species, numerous black or brown spots are scattered over the upper and lower epidermis (figures 1,12,13). A closer examination of these spots and study of their early developmental stages show four distinct types of structures.

### 3. 1. *d-stomata and d-scars*

In the young epidermis, alongwith developmental stages of stomata, hairs etc., a few large fully formed stomata are also present. They persist in the mature leaf and occur isolated between groups of normal sized stomata (figure 2). Some of these large stomata become darker in colour and a dark zone is demarcated around them in the ordinary epidermal cells (figures 3,9). Others persist as large stomata, without further development. The stomata with a dark zone develop further. The surrounding cells divide concentrically around the stoma (figure 4). The pore of the stoma enlarges and becomes thin walled (figure 4). The guard cells appear to lose their contents. Finally the guard cells are ruptured and lost and the surrounding cells become thick-walled and darker. Similar stomata have been described in *Ilex* species by Korn and Fredrick (1973) and have been named d-stomata (or developmentally important stomata) and the replacement periderm tissue is termed the d-scar.

### 3.2. *Ordinary cork-warts*

Intermixed between the d-stomata and d-scars are other structures with similar concentric rings of periderm like tissue which enclose either a single ordinary sized stoma, or several ordinary stomata (figures 5,10) or even ordinary epidermal cells without stomata. After the thickening of the walls of the periderm like tissue the enclosed cells break down and disappear (figure 8).

### 3.3. *Wound cork*

Occassionally the epidermis also shows rounded or slit like gaps in it which appear like punctures by insects or accidental wounds respectively. Later a periderm like activity is seen around them (figure 6), the cells finally becoming thick walled and cork-like with the hole in the centre.

### 3.4. *Trichome scars*

Young leaves of both these species show multicellular, multiseriate hairs or emergences with a broad multicellular base. In *E. torellina*, some of these trichomes persist even on mature leaves especially over the margins and veins. When the hair falls its base is surrounded by the thick-walled, somewhat papillate cells (figure 7). A periderm like tissue develops around the hair base. These form the trichome scars (figure 11).

## 4. Discussion

Cork-warts have been mentioned by Solereder (1908), Haberlandt (1914), Metcalfe and Chalk (1950) and others. Stace (1965) was perhaps the first to suspect their composite nature and described them under "cork-warts and similar structures". The function of the cork-warts has invariably been attributed as

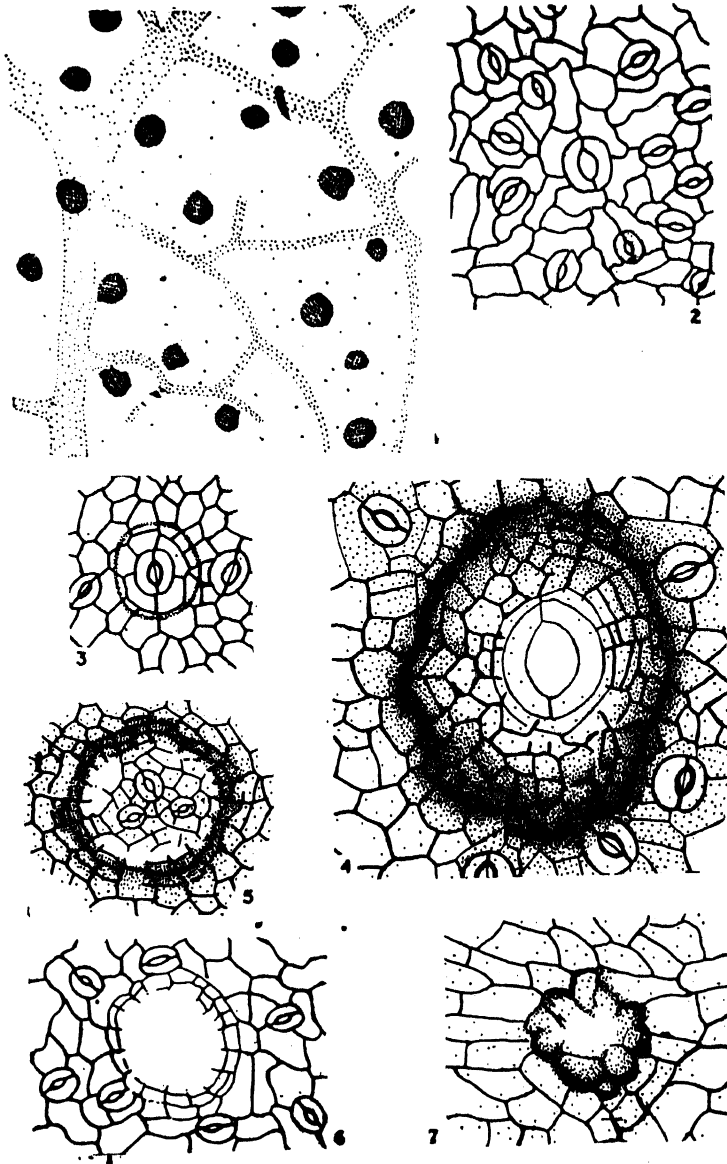



**Figure 1–7. 1.** *E. torellina* : Low power view of the leaf surface showing distribution of cork warts (× 20). **2.** *E. torellina* : Large d-stoma surrounded by normal sized stomata (× 340). **3.** *E. citriodora*: d-stoma showing a zone of darker area around it (× 340). **4.** *E. citriodora* : d-stoma and d-scar (× 340). **5.** *E. torellina*: Cork-wart showing three stomata within it (×175). **6.** *E. torellina*: hole, presumably an insect puncture showing beginning of formation of wound—cork around it (× 340). **7.** *E. torellina* : Hair base with papillate cells and beginning of periderm in lower right hand corner (× 340).

mechanical and to heal and repair holes caused by insect punctures or mechanical injuries. Stace (1965) has observed that cork–warts are not abundunt in nature and that they are present in certain species irrespective of wounds or insect punctures and, therefore, are of diagnostic value. He considers them as distinct from those caused by mechanical or insect injury.

d-stomata and d-scars have been reported only from the leaves of some species of *Ilex* (Korn and Fredrick 1973). They are formed around large stomata which can be distinguished from the early developmental stages of the leaf. In some mangrove genera, Stace (1965) had earlier mentioned cork formation around some large stomata, which he suspected to be water stomata. As far as I am aware, they have not been reported from any other plant. Hence, their presence in species of *Eucalyptus* is interesting.

The large d-stomata in the young epidermis, and the few that persist in the mature condition without the formation of a periderm like tissue, appear very similar to the giant stomata reported in a number of plants (Stace 1965 ; Sitholey and Pandey 1971 ; Farooqui 1979 and others). It is possible that such giant stomata at a later stage form a d-scar around them. It may be worthwhile to reinvestigate plants with giant stomata from such a stand point.

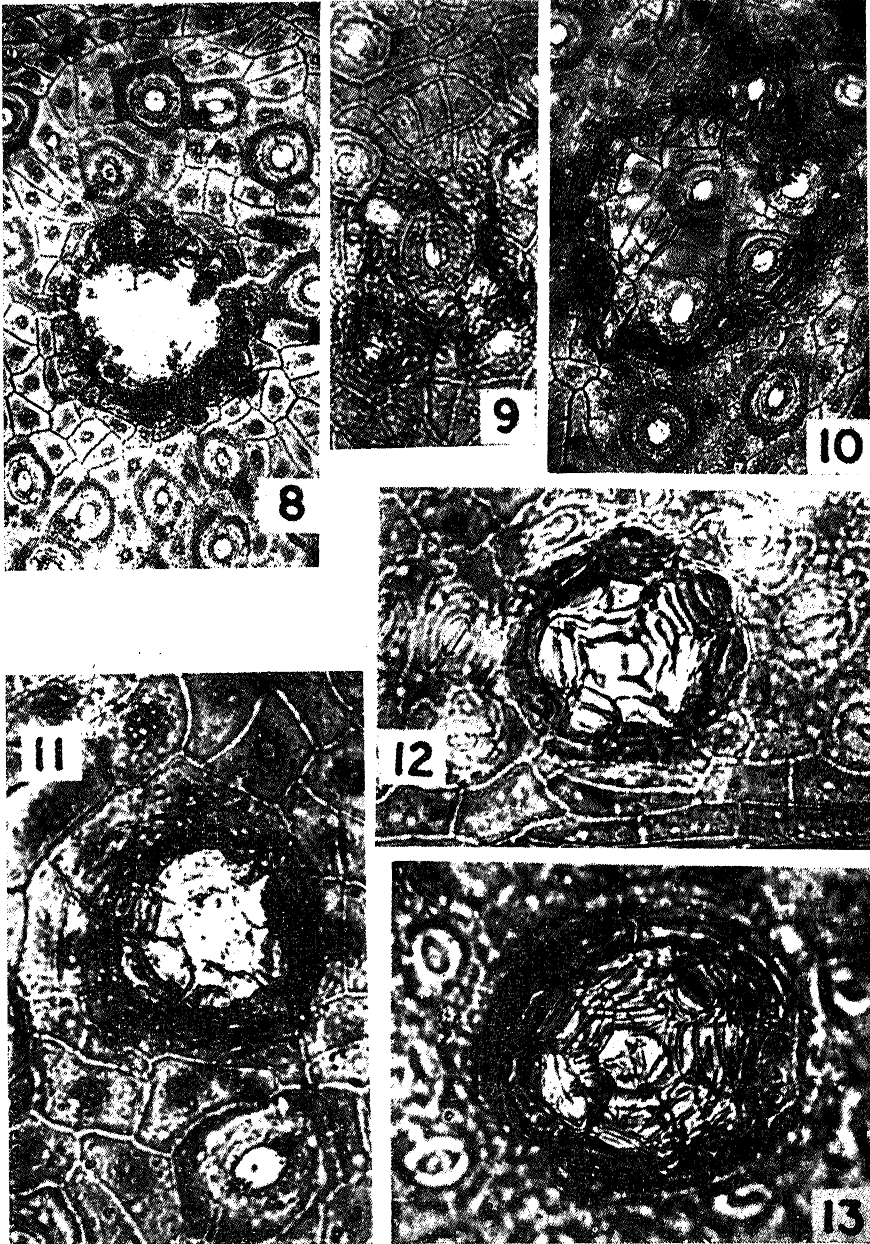
According to Korn and Fredrick (1973), the d-stomata produce a zone of inhibition that prevents additional d-stomata from forming during subsequent growth. Their scattered distribution and absence of any contiguous d-stomata either in *Ilex* or *Eucalyptus* supports such an interpretation.

Wound cork formed as a result of injury may also be distinguished into two types. As pointed out by Stace (1965), purely mechanical accidental wounds may be variously shaped but are usually long and scar like. On the other hand insect punctures are usually more or less rounded. The size of the insect punctures may sometime indicate the identity of the insect visitor as it will correspond to the size of the stylet of the insect.

The formation of a trichome scar after the falling out of the trichome is also interesting. Usually hair fall off without leaving any mark on the epidermis or their bases are left as such or they may become slightly thick walled. Here, periderm like tissue is formed around the trichome base.

In the mature condition, when the inner plug of tissue is completely formed or has fallen off, the four types of structures described here, appear very similar to each other (figures 12, 13). However, developmentally they are different and may be distinguished on the following basis :

| | d-stoma and d-scar | Cork–wart | Wound cork | trichome scar |
|---|---|---|---|---|
| (a) | d-stoma is formed early in development and later forms the d-scar. The position of the d-scar is therefore, predetermined. | Formed towards the beginning of leaf maturation. Position random. | Formed when injury occurs. Position determined by site of injury. | Formed towards leaf maturation. Position determined by site of the falling trichome. |




**Figures 8–13.** *E. citriodora* : Cork-wart with the central plug fallen (× 400). **9.** *E. citriodora*: d-stoma showing formation of a dark zone around it (× 400). **10.** *E. citriodora* : cork-wart showing four stomata within it ( × 500). **11.** *E. torellina* : hair scar(× 1000). **12, 13.** *E. torellina*: cork-warts in different stages of development (×600).

| ) Only one large stoma is involved. | One, several or no stomata are involved. It may include only epidermal cells. | Tissue involved is determined by the size of the wound. | Only one trichome base is involved. |
|---|---|---|---|
| ) Cork forms around stoma first, stoma breaks up later. | Cork formed first and tissue internal to it breaks up later. | Break up or injury occurs first and cork formed later. | Trichome falls off first. Cork is formed later. However, some basal cells of the trichome are already thick-walled. |
| .) May be of diagnostic value as they have been reported in rare cases only. | According to Stace (1965) of diagnostic value. | Of no diagnostic value as they are purely accidental. | Falling hairs leaving scars in the epidermis may be of diagnostic value. |

The present study has clearly shown four distinct types of cork–warts. It is .erefore, important that in all future studies where cork–warts are found, their :velopmental stages should also be studied in order to assign them to the correct 'pe.

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# Pericarpial sclereids in some Mimosaceae

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**Abstract.** Structure, ontogeny and distribution of sclereids in the pericarp of seven species of Mimosaceae are studied. Their occurrence is recorded in the epidermis hypodermis, mesocarp and endocarp. They may be macrosclereids or brachysclereids. Their structure and ontogeny are described and their taxonomic utility is pointed out.

**Keywords.** Pericarp ; sclereids ; Mimosaceae.

## 1. Introduction

Extensive ltierature is available on foliar sclereids (Metcalfe and Chalk 1979 ; Rao 1951, 1980 ; Rao and Bhupal 1973 ; Rao and Bhattacharya 1978 ; Rao and Das 1979), but it is very meagre on the occurrence and ontogeny of pericarpial sclereids (Gupta and Lamba 1981), especially in Leguminoseae (Halliburton *et al* 1975). In this paper, therefore, an account of the distribution, structure and ontogeny of sclereids in the pericarp of seven species belonging to five genera of Mimosaceae is given and their use to delimit the investigated taxa is also indicated.

## 2. Materials and methods

Fresh fruits of *Acacia auriculiformis* Car., *A. pennata* Willd., *A. torta* Craib, *Albizia lebbeck* Bth., *Calliandra* sp., *Pithecellobium dulce* Bth. and *Samanea saman* Merr. were collected at different stages of their development from the University Botanical Garden. The materials were processed through the usual procedures after fixing in FAA. Voucher specimens are deposited in the Herbarium of Sardar Patel University. Materials were macerated in Jane's fluid (Jane 1956). Histochemical localization of lignin was done by Phloroglucinol–HCL method (Johansen 1940).

## 3. Observations

Sclereids occur at various places in the mature pericarp and are found scattered among parenchyma. They are recognized by lignified cell walls, orderly pitting and size. Their distribution is characteristic in the different species under study.

There are two categories of sclereids in the investigated taxa : (a) non-idioblastic tissue forming sclereid layers and (b) idioblastic sclereids. In all the investigated taxa non-idioblastic tissue is present. In *Acacia pennata* and *A. torta* it constitutes 2–3 hypodermal layers (figures 3,4, and 6) whereas in *Calliandra* almost an equal number of layers occur below a hypodermal layer (figure 7). One to three layers of sclereids are present in the endocarp of *Albizia*, *Pithecellobium* and *Samanea* (figures 9–11). Idioblastic sclereids are present among the parenchyma of mesocarp in small or large groups in *A. auriculiformis* (figures 1,2) or isolated and scattered in *A. torta* (figure 6 at arrow). Besides isolated sclereids they occur in groups of 2–3 in the outer epidermis in the latter species (figure 5 at arrow). In *Calliandra* they are also present in small patches at the junction of sutural region and the valve.

Sclereids vary in shape. Brachysclereids, which are more or less isodiametric (figures 12, 16 at arrow), are more frequent than the other types of sclereids. Other types of sclereids observed are the macrosclereids, which are rod-shaped (figures 12 at dart, 13 at arrow, 14), fusiform, which are broader at the centre and gradually taper at both ends (figure 16 at dart), spindle-shaped, which are also broader at the centre but abruptly taper at the ends (figure 15 at arrow) and kidney-shaped (figure 15 at dart).

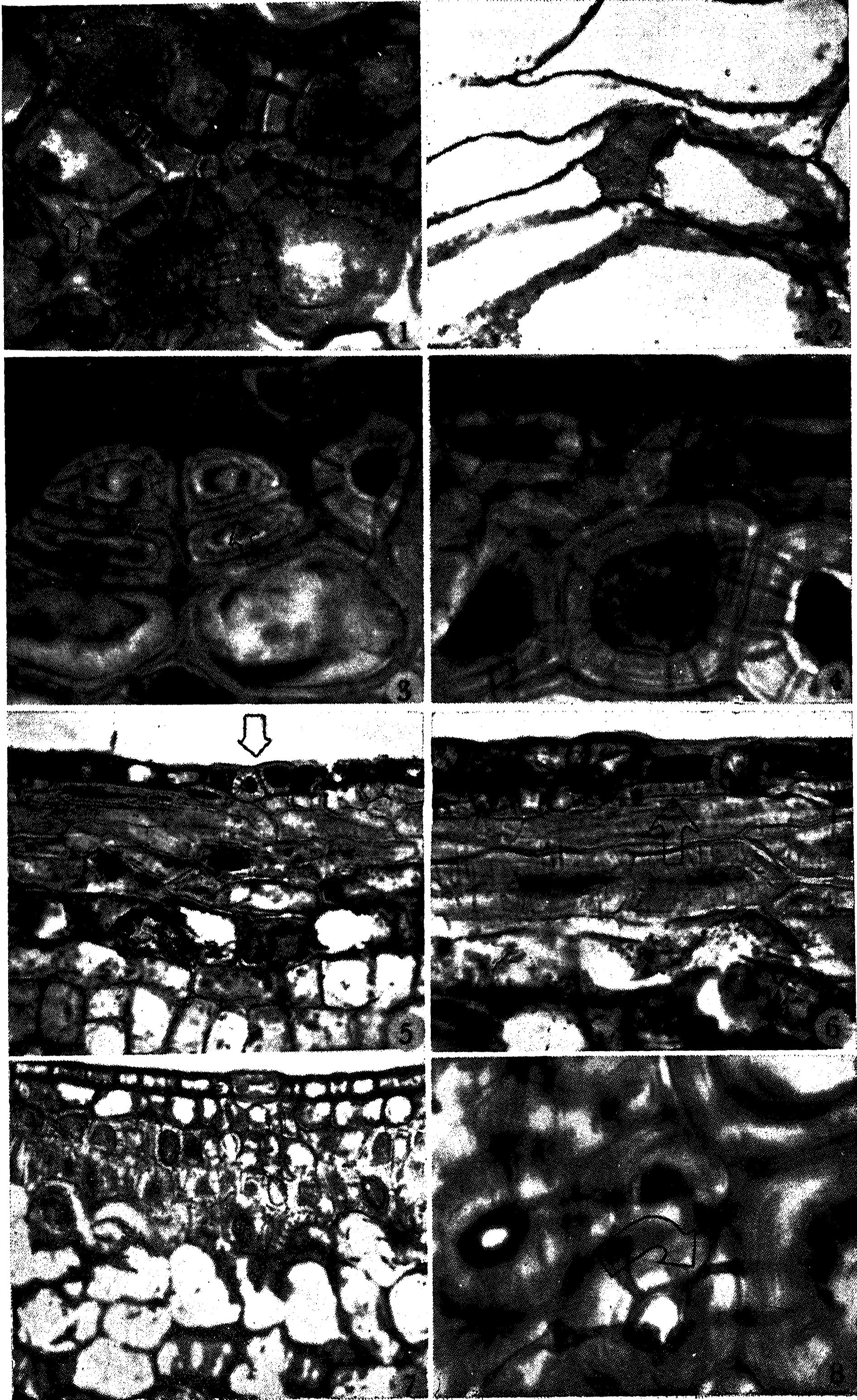
In transection they may be spherical (figures 1,4,5,8), oval (figure 1), angular (figure 7) or columnar and palisade like (figures 9–11). Sclereids have thick lignified and stratified walls with either broad or narrow lumen. In the endocarpic sclereids, however, the lumen is very much reduced and slit-like (figures 9–11). Sometimes it is obliterated (figure 2). Usually mature sclereids are occluded with tanniferous contents, as evidenced by ferric chloride staining. However, tanniferous contents are absent or very rare in the endocarpic sclereids (figures 9–11). Pit canals are visible in the epidermal, hypodermal and mesocarpial sclereids (figures 1,3–8) but not in the endocarpial ones (figures 9–11). They may be simple (figures 1,3,4) or branched (figure 8 at arrow).

Differentiation of sclereids is evident at the mature stage of fruit development. In early stages a sclereid initial is distinguished by its size larger than the adjoining cells, dense cytoplasm and prominent nucleus with a single nucleolus (figure 1 at arrow). In the course of differentiation the cell wall stratification increases with the deposition of lignin. Consequently, the lumen size is reduced. Finally the nucleus and the cytoplasm are autolyzed. In all the species, except endocarpic sclereids in *Albizia*, *Pithecellobium* and *Samanea*, pit canals become prominent and the lumen is filled up with tanniferous contents. Further mature sclereids are devoid of cytoplasm and nucleus.

## 4. Discussion

As far as the authors are aware this is the first account of the presence of sclereids in the pericarp of Mimosaceae (Metcalfe and Chalk 1979) though endocarpic

**Figures 1–8.** All transections of fruits showing sclereids. **1, 2.** *Acacia auriculiformis*. **3, 4.** *A. pennata*. **5, 6.** *A. torta*. **7, 8.** *Calliandra* sp. (1, 3, 4, 8 × 1080 ; 2, 5, 7 × 270 ; 6 × 430).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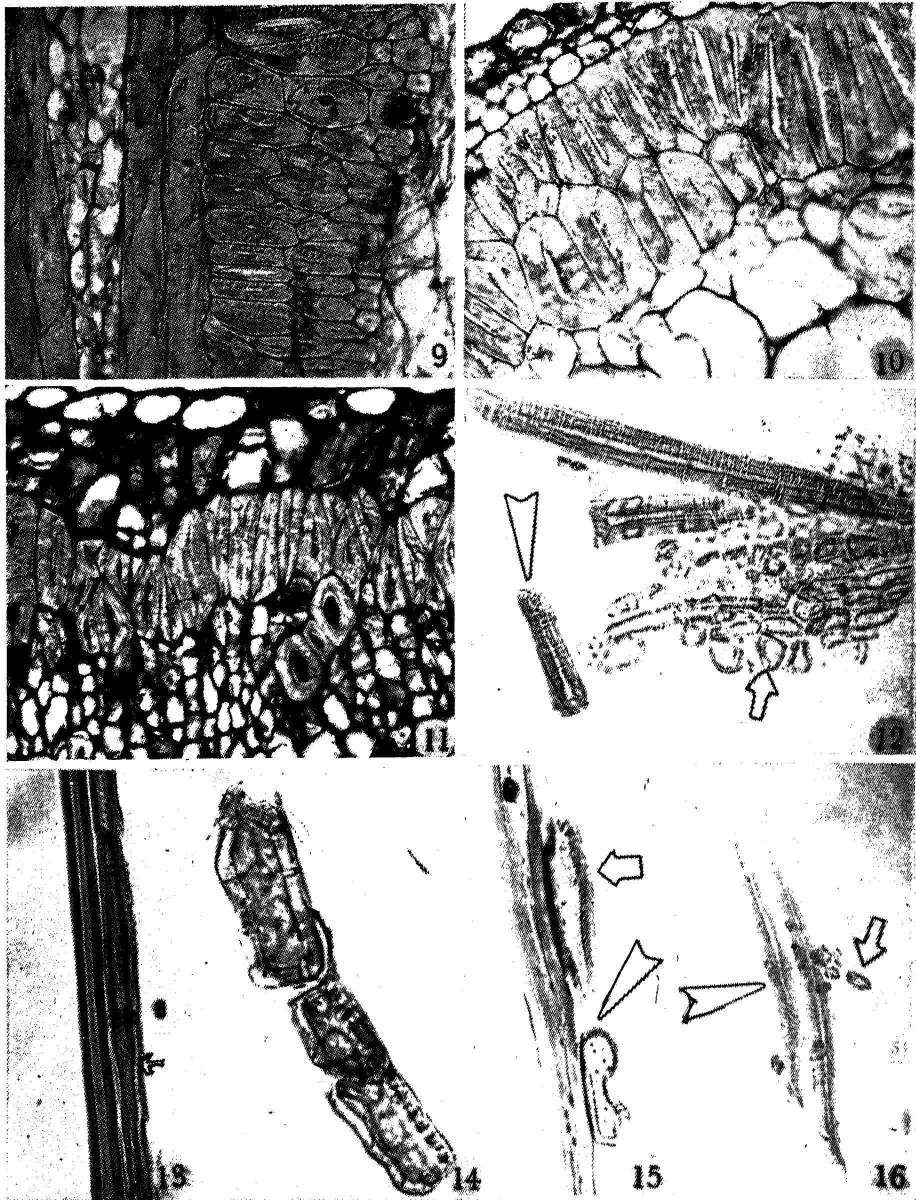


**Figures 9–11.** Transections of fruits. **12–16.** Macerated sclereids of fruits. **9.** *Albizia lebbeck*. **10.** *Pithecellobium dulce*. **11.** *Samanea saman* **12, 16.** *Acacia auriculiformis*. **13–15.** *Calliandra* sp. (9–11 × 270 ; 12, 13, 15 × 230 ; 14 × 340 ; 16 × 75).

sclereids of three types-vermisosclereid (worm-like), calceusosclereids (boot-like) and cruxosclereids (cross or T-like), are reported in *Arachis* of the Papilionaceae (Halliburton *et al* 1975). The only other detailed report of pericarpial sclereids is that of Gupta and Lamba (1981). They have accounted for the structure of sclereids in the endocarp of *Rauvolfia serpentina.* In the species investigated by us we have observed them in the epidermis of *Acacia torta*, hypodermal layers of *A. pennata* and *A. torta*, and in the mesocarp of *A. auriculiformis*. In *Calliandra* sp. sclereids occur at the junction of the sutural region and the valve in addition to subhypodermal layers. Endocarpic sclereids occur in *Albizia, Pithecellobium* and *Samanea.*

The position and type of sclereids can also be useful to delineate the taxa studied as follows :

Sclereids only non-idioblastic :

Only brachysclereids present :

- Sclereids endocarpial ............................ *Albizia, Samanea*
- Sclereids hypodermal ............................ *Acacia pennata*
- Sclereids fusiform ............................ *Pithecellobium*

Sclereids idioblastic in addition to non-idioblastic ones :

- Sclereids mesocarpial ............................ *Acacia auriculiformis*
- Sclereids solitary or in groups of 2–3 in the epidermis .................. *Acacia torta*
- Sclereids below a hypodermal layer ............................ *Calliandra*


## Acknowledgement

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# Viability and infectivity of zoospores of *Sclerospora graminicola* (Sacc.) Schroet in the soil

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**Abstract.** In the present study an attempt has been made to establish the fate of sporangia of *Sclerospora graminicola* (Sacc.) Schroet deposited in the soil. A technique has been standardised to demonstrate the germination of sporangia and the viability and infectivity of zoospores in the soil under laboratory conditions. For how long the zoospores remain motile in the soil is one of the many unanswered questions in the zoospore biology. From the present study it is seen that, the sporangia can germinate in the soil and liberate zoospores. The zoospores can move against gravity, remain viable and infective for 5 hrs in the soil. Survival of zoospores in the soil indicated that, they may serve as a potential secondary source of inoculum through soil under field conditions.

**Keywords.** *Sclerospora graminicola* ; pearl millet ; zcospoe ; soil ; green ear disease.

## 1. Introduction

Data have accumulated in evidence of zoospore serving as inoculum through water currents in soil-borne plant pathogenic phycomycetes like *Phytophthora* (Bewley and Buddin 1921 ; Klotz *et al* 1949 ; Mehrotra 1961 ; Nolla 1928 ; McIntosh 1964 ; Zentmyer and Richards 1952). No studies have been made on these lines in the host parasite relationship of the "green ear disease" of pearl millet caused by *Sclerospora graminicola.* Turner (1960) found that zoospores of *Phytophthora palmivora* remained viable for six months in the soil at 50% water holding capacity. Royle (1963) established that, zoospores of *Pythium aphanidermatum, P. cryptogea, P. fragariae* and *Aphanomyces euteiches* retained motility 1–3 hrs in non-sterile soil. Following water column technique, Mehrotra (1970) demonstrated that zoospores could move for a limited distance through soil towards plant roots, such movement being largely dependent on movement of water through the soil. Although infectivity of sporangia and zoospores (Thakur and Kanwar 1977)have been established no attempt has been made to study fate of sporangia and zoospores in the soil. In the present study an attempt has been made to study the germination, viability and infectivity of the sporangia of *S. graminicola* in the soil,

## 2. Materials and methods

### 2.1. *Zoospore release and viability*

Three corning glass funnels of 10 cm diameter were used in the experiment. A cheese cloth with a 1 mm pore was inserted in the funnel and 3/4 of it was filled with sterilised red loamy soil. A rubber tubing carrying a pinch-cock was introduced at the narrower end of the two funnels, which were used for porangial release. The third funnel was used for sowing surface sterilised seeds of susceptible bajra cultivar HB-3. Distilled water was added to the funnels and allowed to become saturated from the bottom until the soil surface was covered with 2–4 mm of water. Inoculum was prepared by following the procedures given by Safeeulla (1976). Sporangial suspension was obtained by incubating leaf bits collected from systemically infected bajra plants in petri dishes lined with moist filter paper and scraping sporangia, thus obtained in distilled water. In order to observe the release of zoospores from the sporangia in the soil, freshly collected sporangial suspension was added to the soil in the two funnels and the water film on the surface of the soil was periodically observed for zoospore release. After the liberation of zoospores, water was periodically drained from one of the funnels at regular interval of 1 hr for ten hrs to test the viability of zoospores.

### 2.2. *Infectivity and upward movement of zoospores*

For testing the infectivity and upward movement of zoospores in the soil, the following method was used. Three day old susceptible bajra seedlings were kept in contact with the periodically drained water to test the infectivity of zoospores. The seedlings thus inoculated were incubated for 12 hrs and transferred to pots containing sterilised soil. To test the upward movement of zoospores, the second funnel containing the zoospores was connected with the help of a rubber tubing to the narrower end of the funnel containing 4-day old bajra seedlings and the soil was saturated with water so that a continuous column of water was maintained in the rubber tubing connecting the two funnels (figure 1).

## 3. Observations

### 3.1. *Release and viability of zoospores*

Zoospores were released from the sporangia 30 min after sowing in the funnels. Observation of zoospores at hourly intervals revealed that they were motile for a period of 5 hrs from the time of release.

### 3.2. *Infectivity and upward movement of zoospores*

Pearl millet seedlings kept in contact with drained off water, developed downy mildew symptoms indicating the infectivity of zoospores up to 5 hrs after their release in the soil. However the percentage of infection decreased with the increase in the duration of retention following their release. Retention for 1–2 hrs resulted in 60% infection. At 3rd, 4th and 5th hrs it decreased to 36%, 11% and 9% respectively. Beyond 5 hrs no infection was noticed. Seedlings

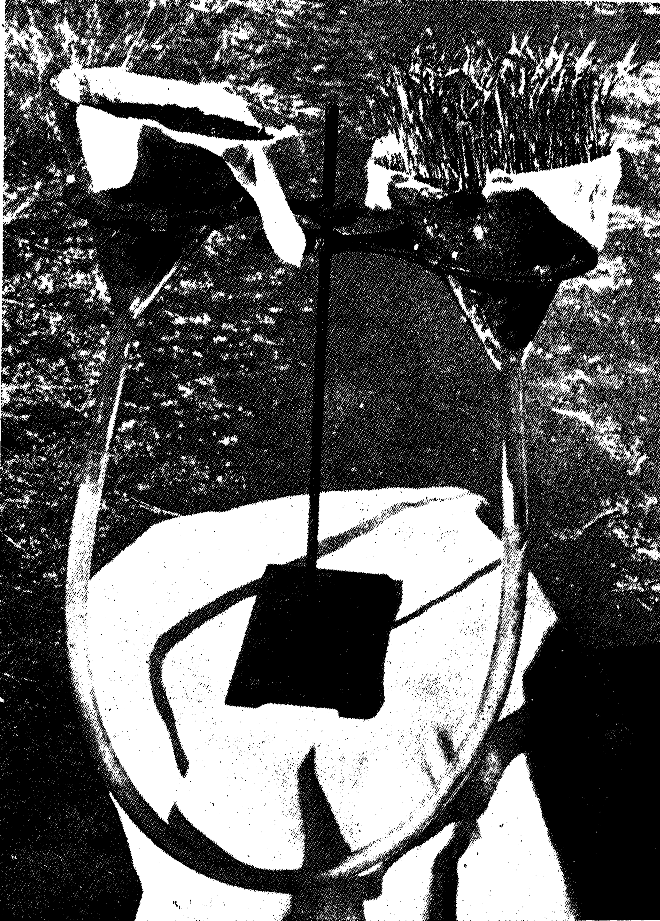

**Figure 1.** Viability and upward movement of zoospores.

raised in the funnel connected to the zoospores source showed downy mildew symptoms 6 days after inoculation indicating the upward movement of zoospores.

## Discussion

*Sclerospora graminicola* produces a large number of asexual sporangia during night under field conditions. Out of the large number of sporangia produced and liberated, some get into the air, a few get deposited on the same or on the neighbouring plants and a few fall to the ground. The significance of the sporangia falling on the soil was not realised so far in *Sclerospora graminicola.* Sporangia are produced during the humid night hours, which is congenial for the release of zoospores from the sporangia. The present study has indicated that sporangia can germinate in the soil liberating zoospores, which can thrive for 5 hrs without loosing their viability. The earlier reports (Haensler 1925 ; Lockwood and Ballard 1959 ; Esmarch 1927 ; Kuhlman 1964 ; Chupp 1917) have failed to establish the distance travelled by zoospores in the soil unaided by water movement. From the present study it is clear that the zoospores have moved upwards along the rubber tube, stem of the funnel and the soil profile to infect the seedlings in the funnel. Since zoospores can thus remain viable and infective in the soil, they may serve as a potential source of secondary inoculum for causing infection in the field. The texture and moisture content of the soil might also influence the viability and infectivity of zoospores. The decline in the percentage of infection of seedlings with the increase in the retention period might be due to the reduction in the number of motile zoospores coming in contact with the roots. This also indicates that, zoospore loose their motility in the soil when they are retained for longer periods.

## Acknowledgements

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# Initiation, development and structure of root nodules in some members of the tribe Trifolieae (Papilionaceae)

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**Abstract.** Initiation and development of root nodules are studied in 7 species and the structure in 4 species, belonging to 3 genera of the tribe Trifolieae. The shape of the mature nodules may be spherical, cylindrical, fan-like or coral-like. The bacterial threads enter the root through the intact epidermis and cause proliferation in cortex by liberating the bacteria. The origin of nodules in the investigated taxa is exogenous and they belong to the 'apical' type in Kodama's classification. A mature nodule comprises of meristematic zone, cortex with vascular bundles and the bacteroid zone. The bacteroid zone is heterogeneous and is composed of infected and uninfected cells.

**Keywords.** Trifolieae ; root nodule ; exogenous origin ; bacterial thread ; proliferation of cortex ; bacteroid zone.

## 1. Introduction

The root nodules have been a subject of investigation because of nitrogen fixing function. Though extensive studies have been made on physiology, cytology and histology of root nodules in the leguminosae in general, only a few investigators have paid attention to the nodule anatomy in the tribe Trifolieae (Peirce 1902; Thornton 1930; Nutman 1948; Dart and Mercer 1963, 1964; Jordan *et al* 1963 ; Mosse 1964 ; Munns 1968 ; Tu 1977). The present investigation is a supplement to the existing data based on the study of initiation and development of nodules in *Medicago orbicularis* All., *M. scutellata* Mill., *M. truncatula* Gaertn., *Melilotus officinalis* Pallas, *M. wolgica* Poir., *Trigonella corniculata* L. and *T. foenumgraecum* L. and structure of nodules in *Medicago sativa* L., *Melilotus alba* Med. *M. indica* All. and *Trigonella foenum-graecum* L.

## 2. Materials and methods

Seeds of *Trigonella corniculata* were obtained locally from a seedsman whereas fully developed nodules of *Melilotus indica* were collected from the plants growing wild in the University Campus. The rest of the species were raised from the seeds obtained from Berlin in the University Botanical Garden. Low viability

of the seeds precluded the investigation of nodular structure of some species. The roots and rootlets of seedlings and root nodules of different stages of development were fixed in FAA (Johansen 1940) and stored in 70% alcohol after 48 hrs. Longitudinal and transverse sections of roots and nodules (5–8 μm) were stained with safranin O and fast-green FCF (Berlyn and Miksche 1976) and made permanent in a customary way.

## 3. Observations

### 3·1. *Morphological description*

The developing nodules are spherical, but the fully developed ones are commonly cylindrical and variously lobed often becoming fan-like or coral-like and rarely spherical. They occur on primary, secondary and tertiary roots. There is no variation in size except that the smallest nodules are in *Melilotus alba* and the largest in *Trigonella foenum-graecum* (figures 1–4).

### 3·2. *Infection, initiation and development*

The entry of bacterial threads (BT) into the root cortex through the intact epidermis (E) is observed only in *Melilotus officinalis*, *Medicago truncatula* and *Trigonella foenum-graecum* (figure 5). The cortical cells through which the threads pass are relatively larger than the remaining cells (figure 6, at arrows). The threads are often found in close proximity or in contact with the host cell nucleus (N) while passing through the cells (figures 5, 7). Further, they develop bulbous or funnel-shaped swellings (S) adjacent to the cell wall in *Medicago truncatula*, *Melilotus officinalis* and *M. wolgica* (figures 7, 8). The threads rupture and liberate the bacteria into the middle of the cortex. The bacterial infected cells contain dense cytoplasm and distinct nucleus, referred here as "proliferation cortical initials" (PCI) (figure 9). By repeated divisions these initials produce a mass of cells, each with dense cytoplasm and a nucleus (figure 10). Gradually, the divisions become restricted to the distal end as a result of which the mass of cells attains spherical shape (figure 11). The developing spherical nodule is now distinguishable into 2–3 layers of peripheral tangentially elongated cells (PL) around the inner mass of cells (MC) (figure 12). It is at this stage the inner mass

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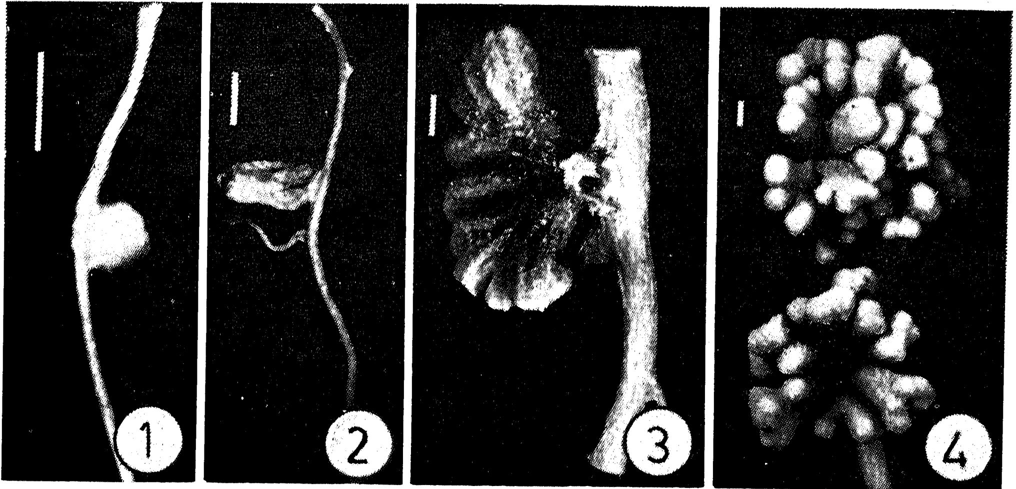
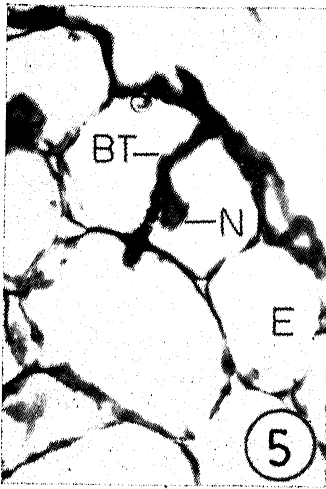
**Figures 1–10.** **1–4.** Mature root nodules. **1.** *Melilotus alba*. **2.** *Medicago sativa*. **3** and **4.** *Trigonella foenum-graecum*. **5–10.** T.S. of roots. **5.** *Melilotus officinalis* showing the entry of bacterial thread into the root. **6.** *Medicago truncatula* showing large cortical cells through which bacterial threads pass at arrows. **7.** *Melilotus wolgica* showing the contact of bacterial thread with host cell nucleus. **8.** *Melilotus officinalis* showing the swelling of bacterial thread near cell wall, note the breaking of thread at arrow. **9.** *Melilotus wolgica* showing proliferation cortical initials. **10.** *Melilotus wolgica* showing mass of proliferated cells. 1–4 line indicates 1 mm; 5 × 260; 6 × 160; 7 × 650; 8 × 380; 9 × 430; 10 × 160.

*Abbreviations*: BT, bacterial thread; N, host cell nucleus; E, epidermis; S, swelling; PCI, proliferation cortical initials.

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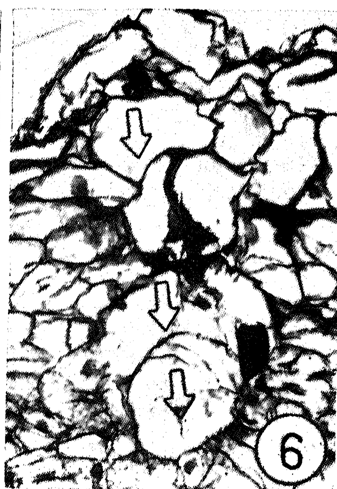

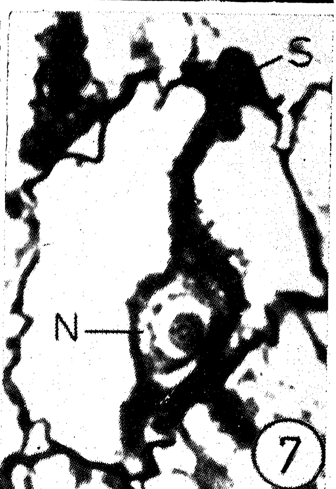
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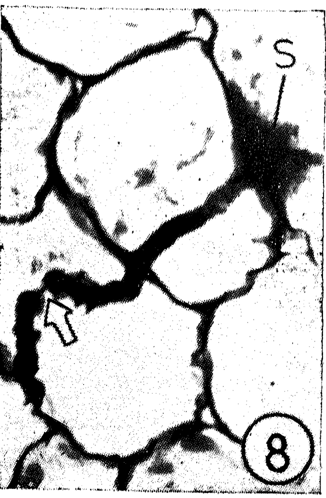
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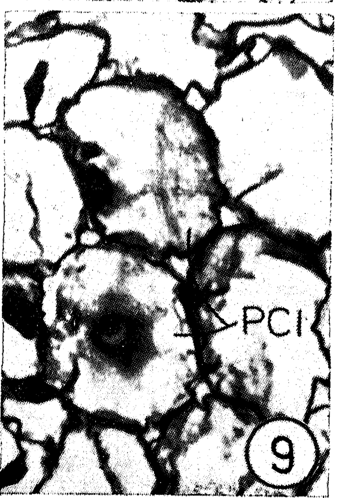
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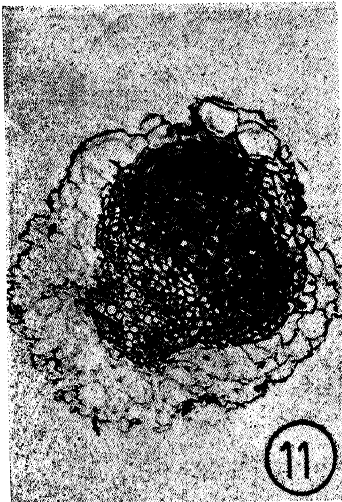
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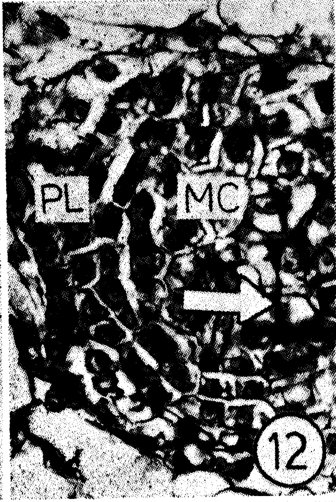
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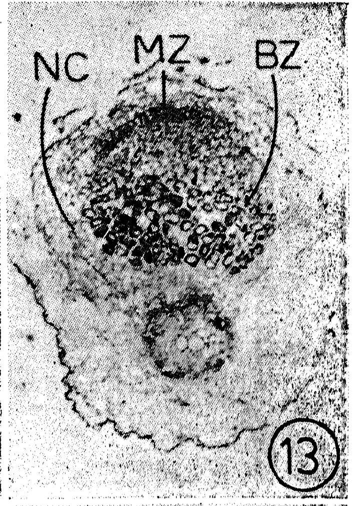
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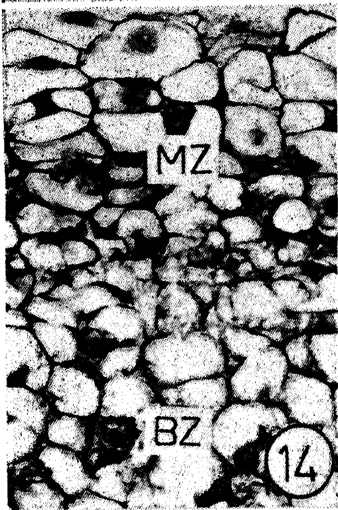
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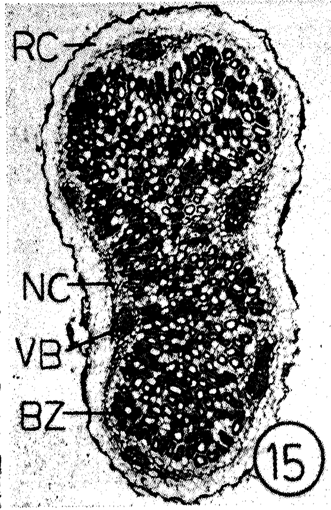
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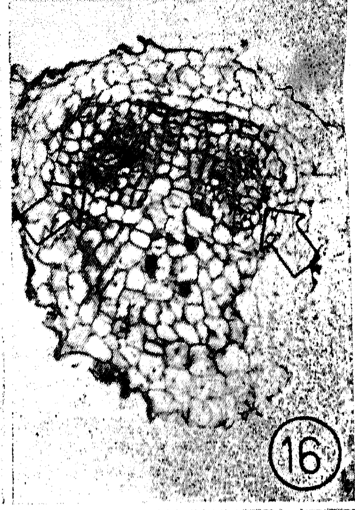
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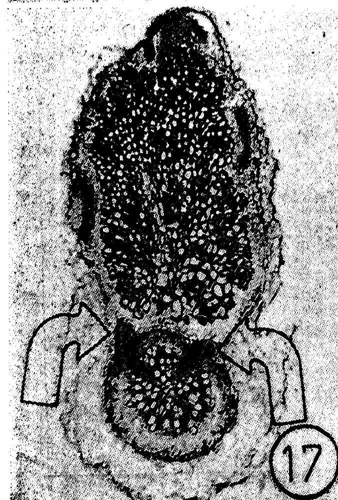
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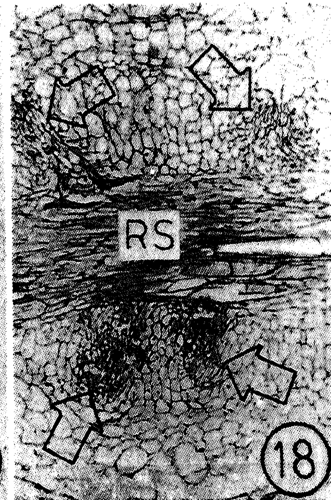
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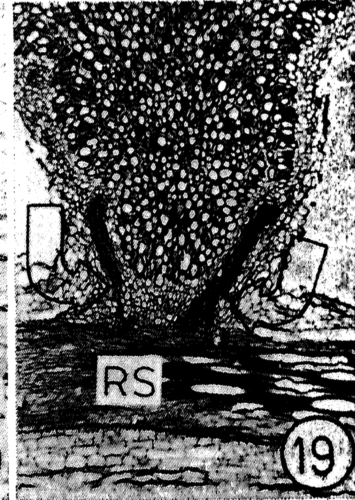
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of cells transforms into the bacteroid zone (BZ) and the peripheral layers into nodule cortex (NC) with distinct apical meristematic zone (MZ) (figure 13). The ruptured threads even after liberating the bacteria remain in the developing nodule (figure 12 at arrow). The developing nodule now protrudes from the root with a protective covering of a few layers of the root cortical cells (figure 13).

### 3.3. *Structure*

A mature nodule consists of meristematic zone (MZ), nodule cortex (NC) with vascular bundles and the central bacteroid zone (BZ).

The meristematic zone is situated at the apex of the nodule, composed of multilayered, thin walled, tangentially elongated cells with dense cytoplasm and prominent nuclei, arranged compactly in regular rows (figure 14).

The nodule cortex is homogeneous comprising of 3 to 6 layers of compact parenchymatous cells with vascular bundles (figures 15, 20). In *Medicago sativa*, *Melilotus alba* and *M. indica* 2 vascular bundles enter the base of the nodule (figures 16, 17 at arrows), but in *Trigonella foenum-graecum* there are 4 vascular traces two of which supply to each side of the nodule (figures 18, 19 at arrows). The vascular strands arise opposite to the protoxylem of the root stele (figure 17). The vascular strands during their upward course, branch repeatedly within the nodule cortex, but do not come in contact with the bacteroid zone (figure 20). The vascular bundles are "inversely collateral" and conjoint, surrounded by an endodermis (EN) (figure 21).

The bacteroid zone is heterogeneous composed of approximately 75% infected cells (IC) and 25% uninfected cells (UC) (figure 22). The uninfected cells are packed with spherical starch grains and interspersed within the tissue of infected cells (figure 23 at arrow). The young infected cells are with distinct nucleus and contain bacteria (figure 24). In the maturing infected cells small vacuoles (V) appear (figure 25) and subsequently their fusion tend to form a large vacuole, pushing the contents and the nucleus towards periphery of the cell (figures 26, 27) and at this stage the mature infected cells are about four times larger than the uninfected ones (figure 22). At a later stage the disappearance of the nucleus

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**Figures 11–19. 11–13.** T.S. of roots. **11.** *Medicago orbicularis* showing developing spherical nodule. **12.** *Trigonella corniculata* showing differentiation of peripheral layers and inner mass of cells in the spherical nodule. **13.** *Trigonella foenum-graecum* showing protrusion of nodule from the root. **14.** L.S. of nodule of *Trigonella foenum-graecum* showing meristematic zone. **15.** T.S. of nodule of *Medicago sativa*. **16.** T.S. of the basalmost region of nodule of *Medicago sativa* showing vascular bundles, at arrow. **17.** T.S. of root with nodule of *Melilotus indica* showing vascular connections with root stele, at arrows. **18.** T.S. of the basalmost region of nodule of *Trigonella foenum-graecum* showing vascular traces, at arrows. **19.** L.S. of the root with nodule of *Trigonella foenum-graecum* showing vascular connections with root stele, at arrows.

11 × 532 ; 12 × 380 ; 13 × 43 ; 14 × 450; 15 × 32; 16 × 100; 17 × 48; 18 × 80; 19 × 38.

*Abbreviations* : PL, peripheral layers ; MC, mass of cells ; NC, nodule cortex; BZ, bacteroid zone ; MZ, meristematic zone ; VB, vascular bundle ; RC, root cortex; RS, root stele.

and clumping of the bacteroids of the infected cells lead to the senescence of the nodule (figures 28, 29 at arrow).

## 4. Discussion

The mode of invasion of bacterial thread into the roots is considerably interesting. In most of the legumes the infection takes place through root hairs (Thornton 1930 ; Bond 1948 ; Harris *et al* 1949 ; Arora 1956b, c ; Nutman 1959 ; Dart and Mercer 1963, 1964 ; Narayana 1963 ; Narayana and Gothwal 1964 ; Kapil and Kapil 1971), but in a few cases it is reported to enter through the intact root epidermal cells (McCoy 1929 ; Schaede 1940), broken epidermal cells (Bieberdorf 1938) and wounded and ruptured cortical cells during the emergence of lateral roots (Allen and Allen 1940 ; Arora 1954). Narayana and Gothwal (1964) reported the infection thread to enter through root hair in *Trigonella foenum-graecum*, but we have noted its entry through the intact root epidermis in *Trigonella foenum-graecum* and also in *Medicago sativa* and *Melilotus officinalis.*

From the data presented regarding the depth of penetration of infection thread into the root cortex (Bieberdorf 1938 ; Bond 1948 ; Arora 1956c ; Narayana 1963 ; Kapil and Kapil 1971) it appears that there is no correlation between the depth of penetration of the thread and the structure (thickness) of the root cortex. In the plants of present investigation also the penetration of the infection thread is up to middle region of the cortex in primary, secondary and tertiary roots.

The dissemination of the bacteria can be either by the invasion of the infection threads of the newly produced cells (Bond 1948 ; Harris *et al* 1949 ; Narayana 1963 ; Narayana and Gothwal 1964 ; Kapil and Kapil 1971) or by the division of the infected cells (McCoy 1929 ; Allen and Allen 1940 ; Arora 1954). In our plants it is by the second method.

Several view points have been put forth to explain the formation of the funnel shaped swellings in the infection threads (McCoy 1929 ; Thornton 1930 ; Harris *et al* 1949 ; Arora 1956c ; Narayana 1963 ; Narayana and Gothwal 1964 ; Dixon 1964). Such swellings are observed in the present investigation and may be due to emaciation of the bacterial mass caused by the stretching of the thread during the enlargement of the host cell harbouring it (Arora 1956c). The breaking of

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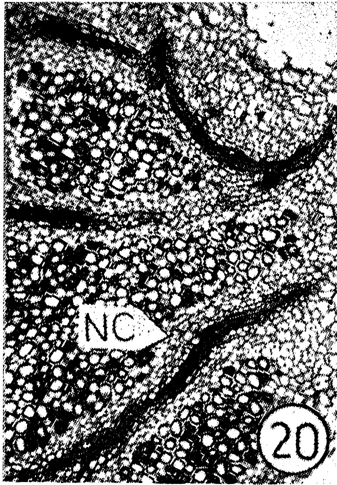
**Figures 20–29.** L.S./T.S. of nodules. **20.** L.S. of nodule of *Trigonella foenum-graecum* showing branching of vascular strands. **21.** *Melilotus indica* showing vascular bundle. **22.** Bacteroid zone of *Medicago sativa* showing infected and uninfected cells. **23.** *Melilotus indica* showing starch grains in the uninfected cells, at arrow. **24–26.** *Melilotus alba*. **24.** Young infected cells. **25.** Maturing infected cell showing vacuoles. **26.** Fusion of vacuoles in the maturing infected cell. **27.** Mature infected cell of *Melilotus indica* showing large central vacuole and peripheral contents. **28–29.** *Trigonella foenum-graecum* showing infected cells at the senescence. **28.** Early stage of bacterial clumping, at arrows. **29.** Late stage of bacterial clumping, at arrow.

20 × 38 ; 21 × 768 ; 22 × 200 ; 23 × 380 ; 24 × 1040 ; 25 × 960 ; 26 × 640 ; 27 × 350 ; 28 × 1600 ; 29 × 560.

*Abbreviations* : NC, nodule cortex ; EN, endodermis ; XY, xylem ; PH, phloem ; UC, uninfected cell ; IC, infected cell ; N, nucleus ; V, vacuole.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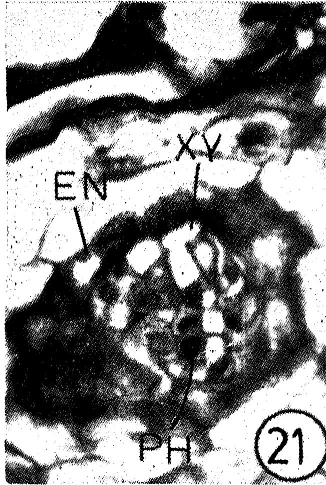
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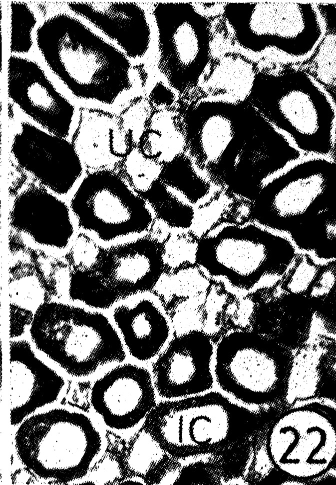
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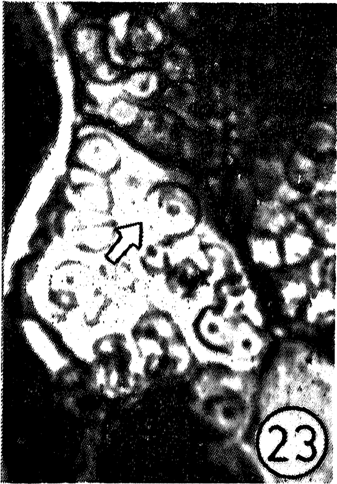
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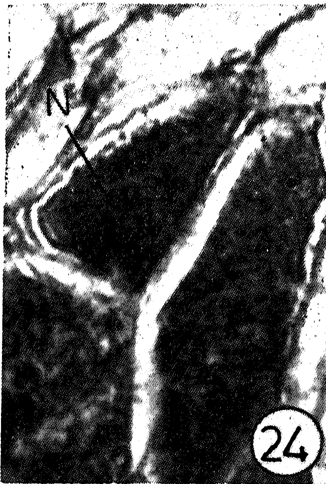
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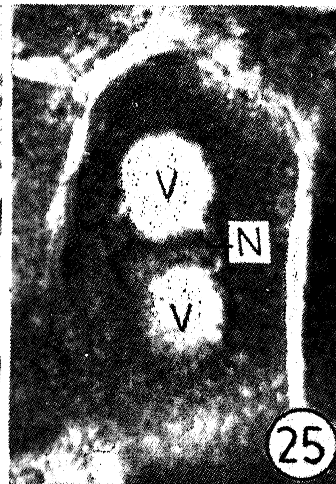
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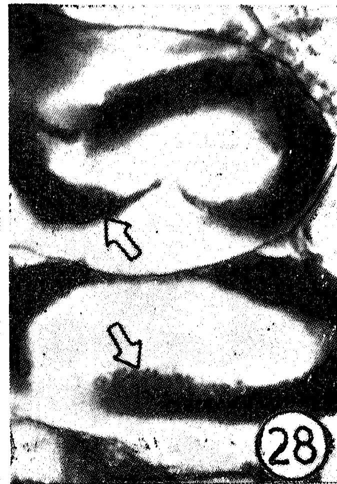
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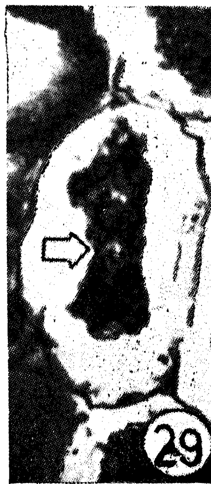
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the thread in the middle (Narayana 1963) further supports our conclusion (figure 8 at arrow).

The meristematic zone may be situated at the apex or at several places surrounding the nodule and accordingly they may be "apical" or "spherical" respectively (Kodama 1967). In the species of the present investigation the meristematic zone occurs at the apex and thereby the nodules confirm to "apical" type of Kodama (1967).

The structure of the nodule cortex is reported to be heterogeneous in some legumes (McCoy 1929 ; Harris *et al* 1949 ; Arora 1954 ; Allen *et al* 1955 ; Narayana and Gothwal 1964 ; Kapil and Kapil 1971), but it is homogeneous, comprising of 3–6 layers of compact parenchyma cells surrounded by 3–6 layers of root cortex in the species studied by us.

There is a great variation in the number and orientation of vascular strands connecting the root stele in different leguminous nodules (Bieberdorf 1938 ; Bond 1948 ; Harris *et al* 1949 ; Arora 1954, 1956a–c ; Narayana 1963 ; Kapil and Kapil 1971). We have observed four vascular connections in *Trigonella foenum-graecum* (see also Narayana and Gothwal 1964) and two in *Medicago sativa, Melilotus alba* and *M. indica.*

The arrangement of phloem and xylem in the vascular bundles in different leguminous nodules is variable (Allen and Allen 1940 ; Allen *et al* 1955 ; Kapil and Kapil 1971). It is inversely collateral in the nodules of the investigated species (see Kapil and Kapil 1971).

All the cells in the bacteroid zone contain bacteria (Allen and Allen 1940 ; Arora 1954, 1956a) or some of them may be infected by bacteria and others may not (Harris *et al* 1949 ; Arora 1956b, c ; Narayana 1963 ; Kapil and Kapil 1971). The latter situation is observed in our plants and the percentage of infected and uninfected cells is 75% and 25% respectively.

We are in agreement with the observations of Allen and Allen (1958) who report that the earliest indication of senescence is the change in colour of the bacteroid zone from red to green and a change in the nodule surface from smooth to wrinkled. Clumping of bacteria is quite common during the nodule senescence.

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# *Turnera ulmifolia* var. *elegans* × *T. ulmifolia* var. *angustifolia* crosses and its bearing on the taxonomy of the species

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**Abstract.** The heterostylous taxon *T. ulmifolia* var. *elegans* Urb. ($2n = 20$) and the homostylous taxon *T. ulmifolia* var. *angustifolia* Willd. ($2n = 30$) were selfed and intercrossed. Both pin and thrum forms of var. *elegans* are self incompatible while the variety *angustifolia* is self compatible. Among the intervarietal combinations only *angustifolia* × *elegans* (thrum) crosses were successful though the hybrid seeds were inviable.

From an analysis of the results of *in vivo* pollen germination studies in the incompatible crosses, it is concluded that *angustifolia* pollen are of the thrum type and its pistil is of the pin type. It is suggested that var. *angustifolia* has evolved by a rare crossing over within the super gene complex for heterostyly in the parent taxon, which might be the hexaploid ($2n = 30$) heterostylous *T. ulmifolia* Linn. The failure of the apparently 'legitimate' cross *elegans* (pin) × *angustifolia* is suggested to be due to the ploidy difference between the two varieties. On morphological cytological and biochemical grounds the separation of the *elegans* element from, *T. ulmifolia* complex and assigning of species status to it is suggested.

**Keywords.** *Turnera* ; heterostyly ; intervarietal crosses ; incompatibility.

## 1. Introduction

*Turnera ulmifolia* L. is a polymorphic weedy species of the New World Tropics with a fairly high caffeine content in the seeds (Raffauf 1970; Tarar and Patil 1974). *T. ulmifolia* var. *elegans* Urb. and *T. ulmifolia* var. *angustifolia* Willd. occur as introduced weeds in South India (Gamble 1915). The former variety is heterostylous and is self incompatible while the latter is homostylous and self and cross compatible (Barrett 1978). Earlier cytological studies have shown that *T. ulmifolia* var. *elegans* has $2n = 20$ chromosomes (Raman and Kesavan 1963; Barrett 1978) and *T. ulmifolia* var. *angustifolia* has $2n = 30$ chromosomes (Barrett 1978; Tarar and Dnyanasagar 1976, 1979). Results of intervarietal crosses between these two taxa are reported here.

## 2. Materials and methods

Plants of natural populations of the two varieties occurring in the Kariavattom Campus of the University of Kerala were used in this study. The flowers of

*T. ulmifolia* var. *angustifolia* open by about 6 am while those of *T. ulmifolia* var. *elegans* open by 7·30 am. Flowers of the former taxon were emasculated on the evening previous to blooming. Emasculation on the day previous to blooming caused serious damage to the buds of *T. ulmifolia* var. *elegans* and hence these were emasculated only about one hour before the flowers open. Flowers of both the varieties were pollinated at 7·30 am.

White's (1954) culture medium solidified with 4% agar and supplemented with 100 ppm indole acetic acid was used to culture the hybrid seeds. *In vivo* germination of pollen grains was studied by staining the styles and stigmas after treatment in lactophenol for 17 hrs at 70°C. Pollen tubes taken from 9 styles were studied. Measurements of leaves, bracteoles, stamens, styles and pollen are given from 25 observations in each case.

## 3. Results

### 3·1. *Intra- and inter- varietal crosses*

*Turnera ulmifolia* var. *elegans* is a profusely branched, bushy, distylous herbaceous perennial growing up to a height of 40 cm (figure 1). The leaves are 2·5 cm to 4·25 cm long, roundish with crenate margin. The bracteoles are linear, up to 2 cm long. The flowers are cream coloured with dark violet spots at the base of the petals (figures 2,3). The pin styles are on an average 10·56 mm long while the thrum styles measure only 5·66 mm. The average length of pin and thrum stamens were 6·00 mm and 10·74 mm respectively. The pollen also showed dimorphism with an average measurement of $P \times E = 64{\cdot}15\mu \times 58{\cdot}93\mu m$ in pin types and $P \times E = 70{\cdot}12\mu m \times 63{\cdot}35\mu m$ in the thrums. Both the types showed an average of 20 seeds per capsule.

Plants of *T. ulmifolia* var. *angustifolia* are sparcely branching erect shrubs, reaching more than 1 m in height (figure 4). Leaves are elliptic-lanceolate, 10–12·5 cm long with irregularly serrate margin. Bracteoles are foliaceous up to 3·1 cm long and 0·75 cm broad. Flowers are long-homostylous (figure 5) with bright yellow petals. The styles are 19·2 mm long and the stamens 20·44 mm. The fruits are larger than that of the former variety and contain about 70 seeds in each.

Self and cross pollinations in *T. ulmifolia* var. *angustifolia* and both pin and thrum forms of *T. ulmifolia* var. *elegans* and the intervarietal crosses were made (table 1). *T. ulmifolia* var. *angustifolia* was found to be self and cross compatible. In *T. ulmifolia* var. *elegans* only crosses between the thrum and pin forms were compatible. Among the intervarietal crosses only the cross *T. ulmifolia* var. *angustifolia* × *T. ulmifolia* var. *elegans* (thrum) was found to be successful.

The hybrid seeds obtained failed to germinate when sown on wet cotton in petri dishes and also in soil. The hybrid seeds cultured in White's medium with 100 ppm indole acetic acid also failed to germinate.

### 3·2. *In-vivo pollen germination*

*In vivo* germination of pollen grains in the three unsuccessful intervarietal crosses were studied with a view to elucidate the causes of their failure. In the successful

**Figures 1–5. 1.** var. *elegans*, plant habit. **2.** Dissected thrum flower of var. *elegans*, arrow points to stigma × 1. **3.** Dissected pin flower of var. *elegans*, arrow points to stigma × 1. **4.** Var. *angustifolia*, plant habit. **5.** Dissected flower of var. *angustifolia*, arrow points to stigma in a black background × 1.

**Figures 6–11.** **6–9 and 11.** × 80. **10** × 2. Arrow point to ends of pollen tubes. **6.** *Elegans* (pin) stigma showing inhibition of *elegans* (pin) pollen tubes. **7.** *elegans* (thrum) stigma showing inhibited *angustifolia* pollen tubes. **8, 9.** *elegans* (pin) style bases showing *angustifolia* pollen tubes. **10.** *elegans* (pin) gynoecium after (a) pollination with *angustifolia* pollen and (b) selfing. **11.** *Angustifolia* style showing inhibited *elegans* (pin) pollen tubes.

**Table 1.** Details of inter and intravarietal pollinations in *T. ulmifolia* var. *elegans* and *T ulmifolia* var. *angustifolia*

| Type of pollination | No. of flowers pollinated | No. of capsules formed | Total no. of seeds | Average no. of seeds per capsule |
|---|---|---|---|---|
| *Intravarietal* | | | | |
| 1. *elegans* (pin) × *elegans* (pin) (cross) | 17 | .. | .. | .. |
| 2. *elegans* (pin) × *elegans* (pin) (self) | 15 | .. | .. | .. |
| 3. *elegans* (thrum) × *elegans* (thrum) (cross) | 17 | .. | .. | .. |
| 4. *elegans* (thrum) × *elegans* (thrum) (self) | 15 | .. | .. | .. |
| 5. *elegans* (pin) × *elegans* (thrum) | 11 | 3 | 67 | 22 |
| 6. *elegans* (thrum) × *elegans* (pin) | 11 | 1 | 13 | 13 |
| 7. *angustifolia* × *angustifolia* (self) | 10 | 7 | 518 | 73 |
| 8. *angustifolia* × *angustifolia* (cross) | 10 | 9 | 603 | 67 |
| *Intervarietal* | | | | |
| 9. *elegans* (pin) × *angustifolia* | 21 | .. | .. | .. |
| 10. *elegans* (thrum) × *angustifolia* | 14 | .. | .. | .. |
| 11. *angustifolia* × *elegans* (pin) | 39 | .. | .. | .. |
| 12. *angustifolia* × *elegans* (thrum) | 22 | 9 | 117 | 13 |

cross *T. ulmifolia* var. *angustifolia* × *T. ulmifolia* var. *elegans* (thrum type), the *elegans* pollen tubes were found to reach the stylar base in 4 to 5 hrs after pollination. Therefore, the styles in the unsuccessful intra- and intervarietal crosses were examined 7 hrs after pollination. In both pin × pin and thrum × thrum crosses in *elegans* the pollen grains germinated, but failed to enter the style (figure 6). The ends of pollen tubes got enlarged and burst at about the basal region of the stigma. In the three unsuccessful intervarietal crosses also the pollen germinated *in vivo*. In *elegans* (thrum) × *angustifolia*, the *angustifolia* pollen tubes were inhibited at the stigma style joint. The ends of most of the tubes got enlarged and burst at this region (figure 7). In *elegans* (pin) × *angustifolia* crosses the *angustifolia* pollen tubes were found to reach the stylar base in about 7 hrs after pollination (figures 8,9). Pollinated flowers were invariably found to fall off, but only after 5–7 days during which period the ovary showed some enlargement (figure 10a). Long styled flowers of *elegans* emasculated and bagged for a day to prevent pollination took 5–6 days to fall off, but the ovaries in these were not enlarged (figure 10b).

In *angustifolia* × *elegans* (pin) crosses the *elegans* pollen tubes entered the style and grew down ; but most of the pollen tubes stopped growth just below the stigma, where their ends ballooned and burst opened (figure 11). The maximum length attained by pollen tubes in 9 cross pollinated styles are given in table 2. It is seen that the maximum length of pollen tubes observed was 95% of the length of the style.

**Table 2.** *In vivo* pollen tube growth in *T. ulmifolia* var. *angustifolia* × *T. ulmifolia* var. *elegans* cross

| No. of styles | Length of style (mm) | Maximum growth of pollen tubes (mm) | Length of pollen tube as percentage of stylar length |
|---|---|---|---|
| 1 | 15·0 | 10·1 | 67·33 |
| 2 | 15·5 | 11·7 | 75·48 |
| 3 | 15·0 | 11·5 | 76·67 |
| 4 | 16·0 | 12·7 | 79·38 |
| 5 | 15·5 | 12·7 | 81·94 |
| 6 | 15·5 | 13·0 | 83·87 |
| 7 | 16·0 | 13·9 | 86·88 |
| 8 | 15·5 | 13·9 | 89·68 |
| 9 | 16·0 | 15·2 | 95·00 |

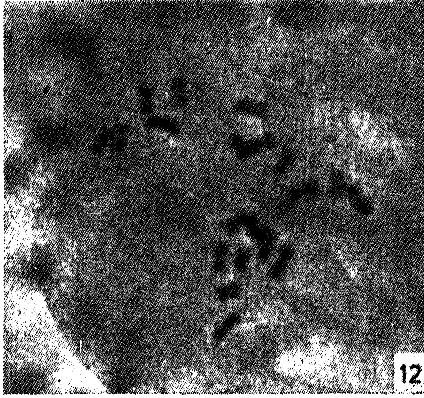
## 4. Discussion

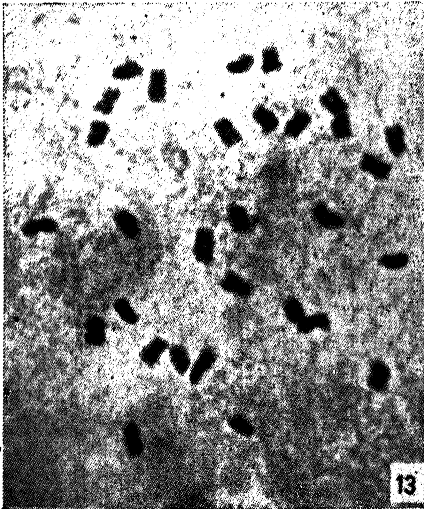
### 4·1. *Causes of intervarietal incompatibility*

Intervarietal crosses between *angustifolia* (female) and *elegans* thrum (male) were successful with 40·9% fruit set. There was no fruit set in the reciprocal cross : though the *angustifolia* pollen grains germinated *in vivo*, the tube growth was inhibited in the stigma itself. The nature of inhibition of pollen tubes in this cross is similar to that in the incompatible intravarietal crosses of the heterostylous *elegans*. Moreover, in *elegans* pin (female) × *angustifolia* (male) crosses, *angustifolia* pollen grew to the base of the styles in pin plants of *elegans*. These facts may suggest that with regard to its incompatibility reaction to the pin and thrum forms of *elegans*, *angustifolia* pollen are of the thrum type.

In the intervarietal cross using pin forms of *elegans* as males, the pin pollen readily grew into the styles of *angustifolia*. But none of the pollen tubes was found to grow beyond 95% length of the style and the vast majority of the pollen tubes stopped growth much earlier. Their ends became bulged and were burst. It is clear from this that this cross is incompatible. This may further indicate that the long styled *angustifolia* is, with regard to the incompatibility reaction of the pistil, a pin type. This inference is supported by the observed compatibility of the intervarietal cross when thrum plants of *elegans* were used as pollen parents.

Since *angustifolia* is a homostyle with 'thrum type' behaviour of pollen grains, the intervarietal cross *elegans* pin (female) × *angustifolia* (male) is an apparently 'legitimate' cross. In this cross the *angustifolia* pollen tubes could be traced to the very base of pin styles of *elegans*. Though this together with the enlargement and the slightly delayed abscission of the cross pollinated ovaries might




**Figures 12–13.** Photomicrographs of somatic chromosomes × 1500. **12.** var. *elegans* ; **13.** var. *angustifolia*.

suggest the possibility of fertilization in the cross, there was no fruit set. Normal growth of pollen tube without fruit set have been reported in such 'legitimate' crosses between heterostylous (pin) *Linum perenne* and homostylous *L. lewisii* also (Baker 1961). It is not clearly understood whether the failure of fruit set observed in the present cross is related to the interaction between the heterostylous and homostylous systems. However, *L. perenne* and *L. lewisii* are reported to have the same chromosome number (Fedorov 1969) whereas *elegans* used in this study is a tetraploid with $2n = 20$ (figure 12) and *angustifolia,* a hexaploid with $2n = 30$ (figure 13). It is known that plants differing in chromosome numbers may show differences between reciprocal crosses and that in such cases greater success may be met with when the taxon with larger chromosome number is used as the seed parent (Thompson 1930). It is likely that the above cross is unsuccessful due to the lower level of ploidy in *elegans* (pin), used as the seed parent, than in *angustifolia.*

### 4·2. *The probable origin of var. angustifolia*

The pistil of *angustifolia* exhibited incompatibility reactions characteristic of long styled plants and its anthers exhibited incompatibility reactions characteristic of short styled plants. This shows that this variety is a long homostyle. Lewis (1954, 1979) and Baker (1961) have suggested that such long homostyles may be formed as segregants in the progeny of polyploids of heterostylous species, as a result of rare cross-overs between $I_1$ and $I_2$ genes in the super gene complex $GSI_1I_2AP$, controlling the heterostyly system. Instances of homostylous segregants have been reported among the offsprings of polyploids of the heterostylous species *Fagopyrum esculentum* (Esser 1953) and *Primula obconica* (Dowrick 1957). It is highly probable that the long homostylous *angustifolia* is derived from a heterostylous progenitor. The variety *angustifolia* is a hexaploid ($2n = 30$). The only other hexaploid taxon so far known in this species complex is *T. ulmifolia* (Hamel 1965). Apart from the fact that *T. ulmifolia* is heterostylous and *T. ulmifolia* var. *angustifolia* is a long homostyle, these taxa resemble each other very closely in other morphological characters (Mudaliyar and Rao 1951). Therefore it is likely that var. *angustifolia* is a segregant of *T. ulmifolia.*

### 4·3. *Taxonomic considerations*

Crosses between *T. ulmifolia* var. *angustifolia* and short styled plants of *T. ulmifolia* var. *elegans* yielded some seeds. But seed set per capsule in the cross was found to be very low compared to seed set by selfing in the seed parent (table 1). Moreover, the hybrid seeds were inviable. These facts reveal the existence of a total reproductive barrier between the two taxa. *Turnera ulmifolia* var. *angustifolia* with $2n = 30$ chromosomes (Tarar and Dnyanasagar 1976; Barrett 1978) is cytologically distinct from *T. ulmifolia* var. *elegans* with $2n = 20$ (Raman and Kesavan 1963; Barrett 1978). *T. ulmifolia* var. *elegans* is endowed with a stable distyly system having self incompatibility. The plants of this variety are of a spreading type, with roundish leaves, 2·5 to 4·25 cm long having crenate margin with prominent scarlet spots in the basal parts of petals. Plants of the variety *angustifolia*

on the other hand are erect and shruby, plants lack the corolla spots, have orange yellow petals and elliptic-lanceolate leaves, 10 to 12·5 cm long and with irregularly serrate margin. Besides, with respect to their foliar phenolic constituents these two varieties showed isolation values as high as 66·66% for *elegans* and 68·18% for *angustifolia*, which denote significant difference between them (Rajeev, Kuriachan and Ninan, unpublished). The foliar phenolic components of *T. ulmifolia* (*s.s*) are not known. Though it is heterostylous (Martin 1965) as *T. ulmifolia* var. *elegans*, in other morphological characters it resembles *T. ulmifolia* var. *angustifolia* more closely than *T. ulmifolia* var. *elegans* (Mudaliar and Rao 1951). Cytologically also *T. ulmifolia* with $2n = 30$ (Hamel 1965) is nearer to *T. ulmifolia* var. *angustifolia* than *T. ulmifolia* var. *elegans*. Thus evidence from crossability, cytology, morphology and foliar phenolic constituents support the separation of the '*elegans*' element from *Turnera ulmifolia* complex and assignment of species status to it.

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# Airborne pollen grains of Visakhapatnam : A combined field and air sampling study

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**Abstract.** Field assessments at regular intervals from April 1975 to March 1979 recorded 61 plant species comprising 13 grasses, 20 weeds and 28 trees and shrubs as emitting appreciable amounts of pollen into the atmosphere of Visakhapatnam. The data also showed relative prevalence of these taxa in different zones of the city and their flowering periods. Pollen output in terms of number per anther and per flower was determined for 29 taxa. Air sampling with rod traps of 0·53 cm diameter enabled the identification of 23 different pollen types in the atmosphere with Poaceae accounting for Ca. 37% of the total pollen load. *Casuarina* contributed to 13% followed by Cyperaceae 6%, *Eucalyptus* 5·8%, *Dodonaea* 3·8%, Amaranth–Chenopod and *Phoenix* each 3·4%, *Borassus* 2·4% and *Peltophorum* 2%. Of the total identified pollen, ca 85% belonged to anemophilous taxa. There was no pollen-free day. The total pollen and individual types displayed seasonality quite closely corresponding with the blooming seasons of the source plants. Three pollen peaks, two in the wet period (June-November) and one in the dry period (December—May) were evident. Year to year variations in pollen abundance occurred and urban growth affected pollen frequency pointing to the need for routine monitoring of the atmosphere.

**Keywords.** Pollination calendar ; pollen productivity ; airborne pollen ; atmospheric biopollutants ; Poaceae ; *Casuarina*.

## 1. Introduction

Allergic responses to airborne biogenic particles impose major adverse effects on the physical and economic health of mankind (Davis 1972). Airborne pollen grains belong to this group of particles and have long been recognised as the incitants of rhinitis and asthma. In order to identify the offending pollen agent(s) it is necessary to monitor the pollen particles in free air, their prevalence and emission patterns through systematic air sampling. Field assessments recording the relative abundance and blooming phenology of source plants complement and greatly increase the value of such information. In areas of industrial atmospheric pollution there is every likelihood that the airborne pollen and chemical pollutants interact with each other and result in the aggravation of human discomfort (Newmark 1970 ; Nilsson and Nybom 1978).

Visakhapatnam, situated in Andhra Pradesh on the east-coast of India is an industrial area but which also has a rich vegetation (Venkateswarlu *et al* 1972). It is thus likely that the air over the city is being charged with pollen from vegetation and gaseous emanations from industries that may cause suffering to the inhabitants. The experience of clinicians in the King George Hospital and of private practitioners support this supposition. The situation thus calls for serious efforts at routine atmospheric monitoring so that necessary measures can be taken to alleviate the human affliction. Efforts at trapping the airborne pollen were begun a decade ago (Subba Reddi 1970), but the information at hand is still far from complete. In this paper we present data describing the composition of the airborne pollen, relative prevalence of the constituents and their seasonal emission patterns over a period of four years from April 1975 to March 1979 together with the distribution, blooming phenology of source plants and their pollen productivity per anther and per flower. We suggest these data serve as a basis for local clinical strategy and as a comparison with pollen spectra established elsewhere in India and abroad.

## 2. Materials and methods

### 2·1. *Physiography and climate of the study area*

The physiography of Visakhapatnam (latitude 17° 42′ N and longitude 82° 18′ E) and its surroundings is shown in figure 1. On two sides the city is bounded by hill ranges ; on the north-eastern side the famous pilgrimage centre Simhachalam or Kailasa hill range with an average height of 300 m and about 16 km long and on the southern side Yarada hill range running for about 8 km with an average height of 300 m and projecting prominently into the Bay, and popularly known as Dolphin's nose. The shore of Bay of Bengal forms the eastern boundary and the western side is bounded by the tidal basin called locally Vupputeru.

The most important feature of the climate is the alternation of monsoon seasons classified by Indian Meteorologists as : (i) The northeast monsoon season (December-February), (ii) The hot weather season (March–May), (iii) The southwest monsoon season (June–September) and (iv) The retreating southwest monsoon (October–November).

Figure 2 shows the mean distribution of temperature and rainfall for the period when spore trapping was done. The mean monthly temperature was 27·4° C and variation of the mean from month to month was approximately 2°C. The difference between mean monthly minima was no more than 9°C. The hottest weather occurred in May, while the coolest in January. Most precipitation fell during June–November. The total precipitation during the 4–year period declined in each successive year.

### 2·2. *Field assessments*

Visakhapatnam was divided into seven zones (figure 1). Zone–I constitutes the thickly built up area ; zone–II a sparsely built up area with vacant land patches ;

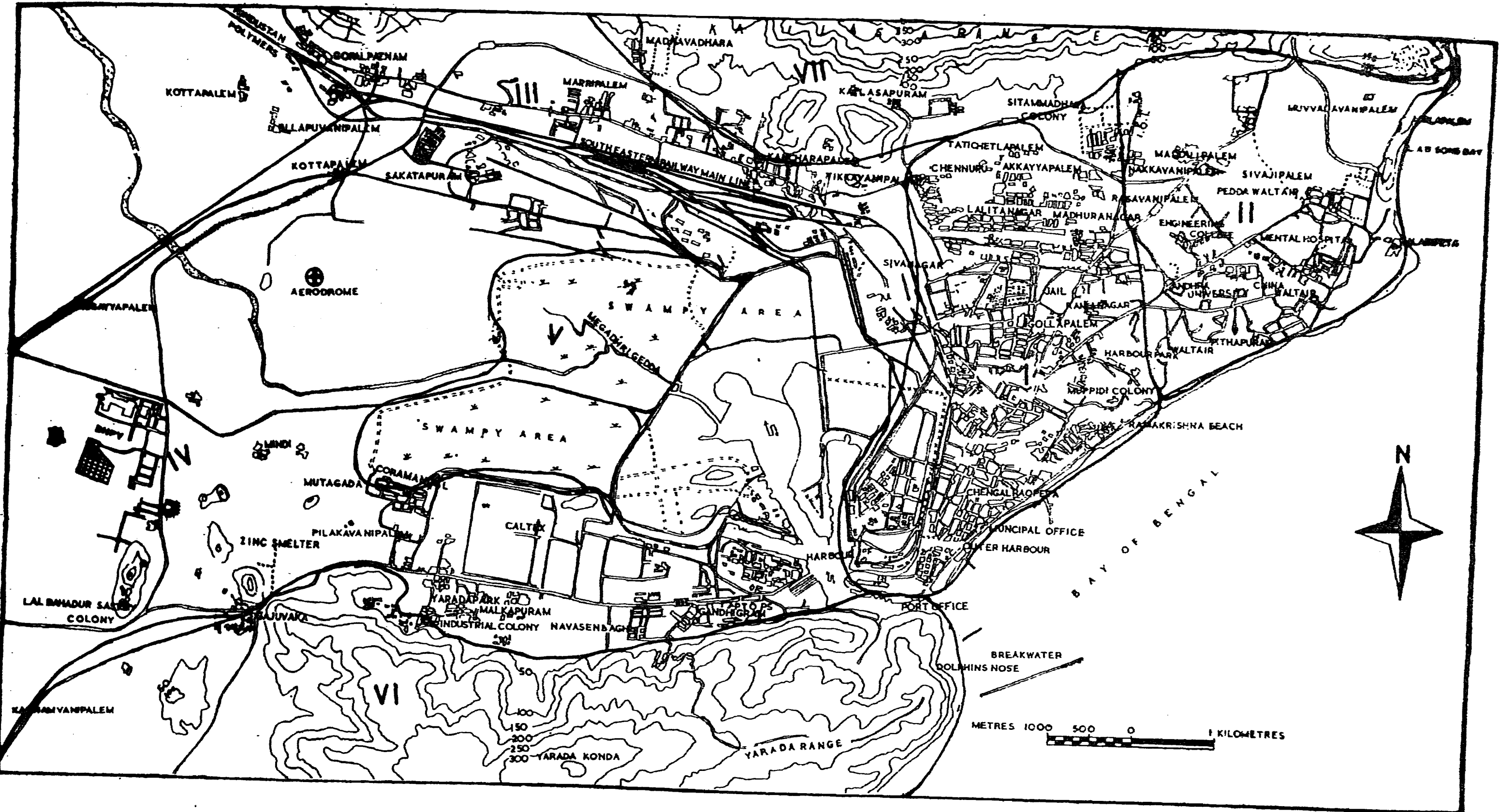



**Figure 1.** Physiography of Visakhapatnam and its surroundings showing the seven field study zones.

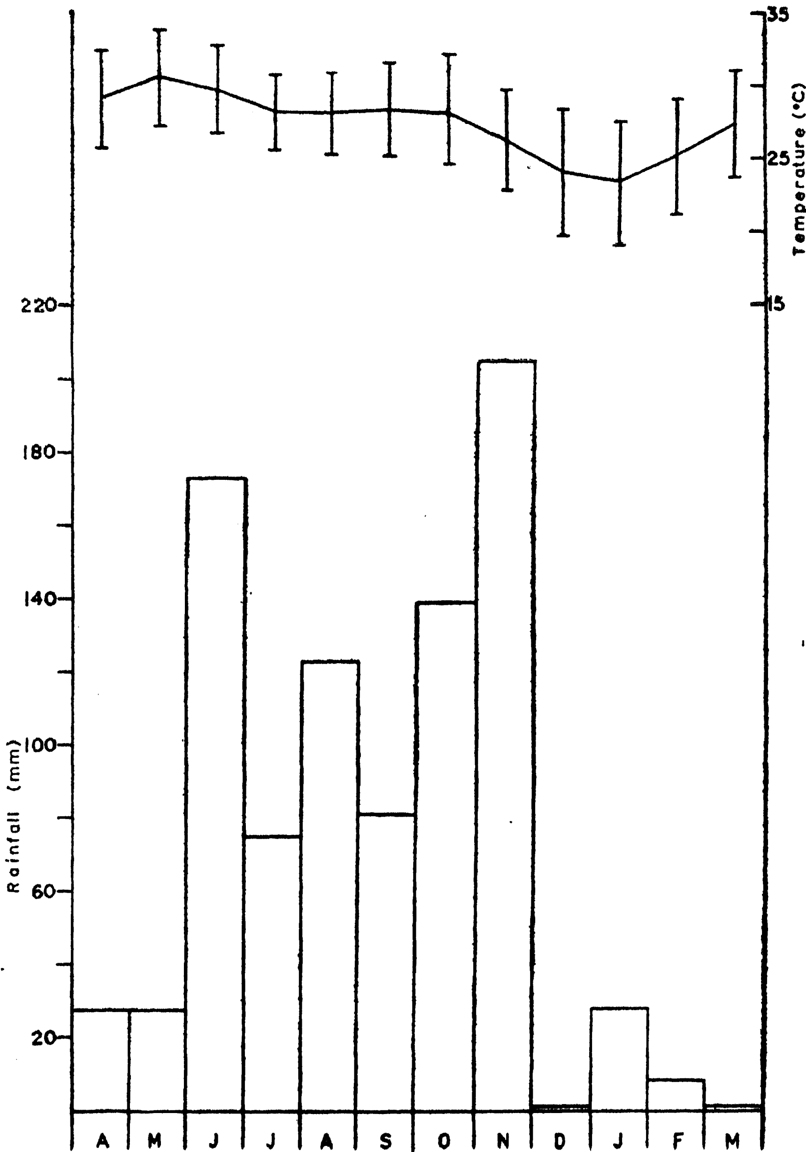



**Figure 2.** Mean monthly, mean monthly maximum and mean monthly minimum temperature and rainfall at Visakhapatnam for the period of the study, April 1975 to March 1979.

zone–III the areas adjacent to the Railway line ; zone–IV the industrial belt ; zone–V a swampy area ; zone–VI the Yarada hill range and zone–VII the Kailasa hill range. The last three zones were not included in the survey as the vegetation of the two hill ranges is sparse scrub with *Dodonaea* occurring in considerable frequency and the swampy area has mainly *Avicennia* plants of stunted growth. Trips were made to the other four zones at approximately weekly intervals during 1975–1979 to record the prevalence of anemophilous and such entomophilous

plants capable of liberating sizeable amounts of pollen into the ambient air. Arbitrary units like sparse, common, more common and abundant were used to indicate the prevalence. Concurrently observations were made on the onset, intensity, duration and termination of flowering of these plants.

Taxonomic identification of the plants was done using the Flora of Visakhapatnam by Venkateswarlu *et al* (1972) as well as comparison with the authenticated herbarium specimens available in the Botany Department, Andhra University.

### 2·3. *Pollen productivity*

The output of pollen per anther was assessed by the method described by Subba Reddi (1976). Mature and undehisced anthers were taken into clean, dry, stoppered tubes with the aid of forceps and allowed to dry. Care was taken not to injure the anthers while transferring them into tubes. After the completion of dehiscence, a measured volume of 50% alcohol containing methyl green stain was added to each tube with a small quantity of the wetting agent 'Tween 20' and the contents shaken thoroughly to get a uniform suspension of pollen grains. From the suspension thus obtained, one ml was pipetted out into a counting chamber and the pollen counted. Three counts were made for each sample and from them was calculated the number liberated by the anthers in that sample. Afterwards the emptied anthers were mounted on a microscope slide and examined for remaining pollen to make any necessary correction.

Knowing the number of pollen grains produced by the number of anthers included in a particular sample, the pollen productivity was then estimated per anther as well as flower.

### 2·4. *Air sampling*

This was done using glass rods of 0·53 cm diameter and 23 cm long wrapped with 18 mm square sticky cellophane strips. Each rod was supported vertically and exposed for 24-hour periods (1700–1600 hr) in shelters of the type used by Hyde and Williams (1945) so as to protect the cylinder from rain other than that experienced during violent storm. Airborne pollen grains are impacted by the wind onto the traps.

The details of preparing, exposing cylinders, mounting of the trace and scanning were the same as described by Ramalingam (1968) and Subba Reddi (1970). The pollen counts are expressed as no/cm$^2$ of the trap surface.

## 3. Results and discussion

### 3·1. *Plant species, their prevalence and flowering periods*

A variety of plant taxa was reported to occur in the study area (Venkateswarlu *et al* 1972). However, field observations on the mode of pollination indicated that only a relatively small number would emit pollen into the ambient air in sizeable amounts. Table 1 lists such plants with data on their relative prevalence in diffe-

**Table 1.** Prevalence and flowering periods of anemophilous and such entomophilous plant taxa capable of liberating sizable amounts of pollen into the air of Visakhapatnam.

| Plant species | Prevalence | | | | Flowering period |
|---|---|---|---|---|---|
| | Zone I | II | III | IV | |
| **Gramineae or poaceae** | | | | | |
| *Andropogon pumilus*, Roxb. | +++ | – | ++ | + | September–December |
| *Bothriochloa pertusa*, (Linn.) A. Camus | +++ | + | +++ | + | September–December |
| *Pennisetum polystachyon*, Schult | ++ | – | – | – | September–December |
| *Brachiaria distachya* (Linn.) Stapf | ++++ | + | +++ | ++ | July–September |
| *Cenchrus ciliaris*, Linn. | +++ | + | ++ | + | August–Decem'er |
| *Cynodon dactylon*, (Linn.) Pers. | ++++ | + | ++ | ++ | Major part of year |
| *Dactyloctenium aegyptium*, (Linn.) Beauv. | ++ | – | ++ | + | July–November |
| *Dichanthim annulatum*, (Forssk.) Stapf | ++++ | + | +++ | ++ | August–October |
| *Panicum repens*, Linn. | ++ | + | ++ | + | July–November |
| *Chloris barbata*, Sw. | +++ | + | ++ | + | Major part of year |
| *Trachys muricata*, Pers. ex Trim | ++ | – | – | – | July–September |
| *Setaria verticillata*, (Linn.) P. Beauv. | ++ | – | – | – | September–December |
| *Digitaria adscendens* (HBK) Henr. | | | | | |
| **Weeds** | | | | | |
| Capparidaceae | | | | | |
| *Cleome viscosa*, Linn. | +++ | + | +++ | ++ | July–December |
| Papavaraceae | | | | | |
| *Argemone mexicana*, Linn. | ++ | + | ++ | + | January–June |
| Caryophyllaceae | | | | | |
| *Polycarpaea corymbosa*, (Linn.) Lamk. | ++ | – | – | – | August–November |
| Asteraceae | | | | | |
| * *Artemisia vulgare*, Linn. | + | + | + | – | September–November |
| *Echinops echinatus*, Roxb. | + | – | – | + | September–May |
| *Tridax procumbens*, Linn. | ++++ | + | ++ | ++ | Throughout year |
| * *Xanthium strumarium*, Linn. | ++ | – | + | + | September–March |

| | | | | | |
|---|---|---|---|---|---|
| Amaranthaceae | | | | | |
| * *Allmania nodiflora*, R. Br. | ++ | – | + | + | Throughout year |
| * *Amaranthus polygamous*, Linn. | ++ | + | ++ | ++ | August–November |
| * *Amaranthus gracilis*, Linn. | +++ | ++ | ++ | ++ | Throughout year |
| * *Amaranthus spinosus*, Linn. | +++ | ++ | ++++ | +++ | Throughout year |
| Euphorbiaceae | | | | | |
| * *Acalypha indica*, Linn. | +++ | + | +++ | + | June–December |
| *Croton bonplandianum*, Baill. | ++++ | ++ | ++++ | ++ | Throughout year |
| Typhaceae | | | | | |
| *Typha angustata*, Bory and Chaub. | + | – | – | + | September–March |
| Cyperaceae | | | | | |
| * *Cyperus compressus*, Linn. | ++ | + | ++ | + | June–October |
| * *Cyperus rotundus*, Linn. | ++++ | ++ | ++++ | ++ | June–November |
| * *Bulbostylis barbata*, (Rottb.) Cl. | ++ | – | ++ | + | July–October |
| * *Eleocharis atropurpuris*, (Retz.) Kunth. | + | – | + | + | August–November |
| * *Fimbristylis monostachya*, (Linn.) Hassk. | +++ | + | +++ | ++ | July–October |
| * *Scirpus articulatus*, Linn. | +++ | ++ | +++ | + | July–October |
| Trees (and Shrubs) | | | | | |
| Elaeocarpaceae | | | | | |
| *Muntingia calabura*, Linn. | + | – | – | – | Major part of year |
| Simaroubaceae | | | | | |
| * *Ailanthus excelsa*, Roxb. | + | – | – | – | January–March |
| Meliaceae | | | | | |
| *Azadirachta indica*, A. Juss. | +++ | ++ | ++ | ++ | February–June |
| Sapindaceae | | | | | |
| * *Dodonaea viscosa*, (Linn.) Jacq. | +++ | – | – | ++ | October–February |
| Anacardaceae | | | | | |
| *Lannea coromandalica* (Houtt.) Merr. | + | – | + | + | February–April |
| Moringaceae | | | | | |
| *Moringa olifera*, Lamk. | ++ | +++ | + | + | Major part of year |
| Leguminosae | | | | | |
| *Acacia arabica*, (Linn.) willd. | + | + | + | + | June–November |
| *Albizzia lebbeck*, (Linn.) Benth. | + | + | + | + | March–June |
| *Peltophorum pterocarpum* (DC.) Baker ex Heyne. | ++ | ++ | + | – | March–October |
| *Pithecolobium dulce*, (Roxb.) Benth. | + | + | + | ++ | January–May |
| *Pongamia pinnata*, (Linn.) Pierre | ++ | ++ | ++ | ++ | March–June |
| *Prosopis juliflora*, (Sw.) DC. | +++ | + | ++ | ++ | Major part of year |
| *Pterocarpus santalinus*, Linn. | + | – | – | – | March–May |
| *Tamarindus indica*, Linn. | +++ | + | ++ | ++++ | February–July |

Table 1—*Contd.*

| Plant species | Prevalence | | | | Flowering period |
|---|---|---|---|---|---|
| | Zone I | II | III | IV | |
| Myrtaceae | | | | | |
| *Eucalyptus globulus*, Labill. | ++ | + | + | + | Major part of year |
| *Syzygium jambolanum*, DC. | ++ | + | + | + | April–August |
| Sapotaceae | | | | | |
| * *Madhuca indica*, Gmelin | + | – | – | – | February–May |
| * *Mimusops elengi*, Linn. | + | + | + | + | Mjaor part of year |
| Euphorbiaceae | | | | | |
| * *Cicca disticha*, Linn. | ++ | +++ | ++ | + | Throughout year |
| * *Emblica officinalis* Gaertn. | + | + | + | + | February–May |
| * *Ricinus communis*, Linn. | ++ | ++ | ++ | + | Throughout yea r |
| .lmaceae | | | | | |
| * *Holoptelea integrifolia*, (Roxb.) Planch. | + | + | – | – | November–Aprl |
| Moraceae | | | | | |
| * *Morus alba*, Linn. | + | + | + | + | January–June |
| Casuarinaceae | | | | | |
| * *Casuarina equisetifolia*, Linn. | +++ | ++ | ++ | ++ | Throughout year |
| Arecaceae | | | | | |
| * *Borassus flabellifer*, Linn. | ++++ | + | +++ | +++ | February–May |
| * *Cocos nucifera*, Linn. | +++ | ++ | +++ | ++ | Throughout year |
| * *Phoenix sylvestris*, (Linn.) Roxb. | +++ | + | ++ | +++ | December–March |
| Pandanaceae | | | | | |
| * *Pandanus fascicularis*, Lamk. | + | – | – | – | June–November |

+ = Sparse ; ++ = Moderately common ; +++ = Common ; ++++ = Abundant ; – = Absent ; * = Anemophilous.

ent zones of Visakhapatnam and their periods of flowering. The pollination alendar of some of the more important taxa is graphically presented in figure 3 which shows clearly that, unlike temperate regions of the world, one or the other f the diverse plant taxa was in flower at all times of the year with consequent beration of pollen into the ambient atmosphere. During two periods a maximum umber of wind-pollinated plants were in bloom. Most Poaceae and other weeds vere in bloom during late June–early December, while most of the trees bloom uring January–April. Some of the weeds and grasses flowered in both periods

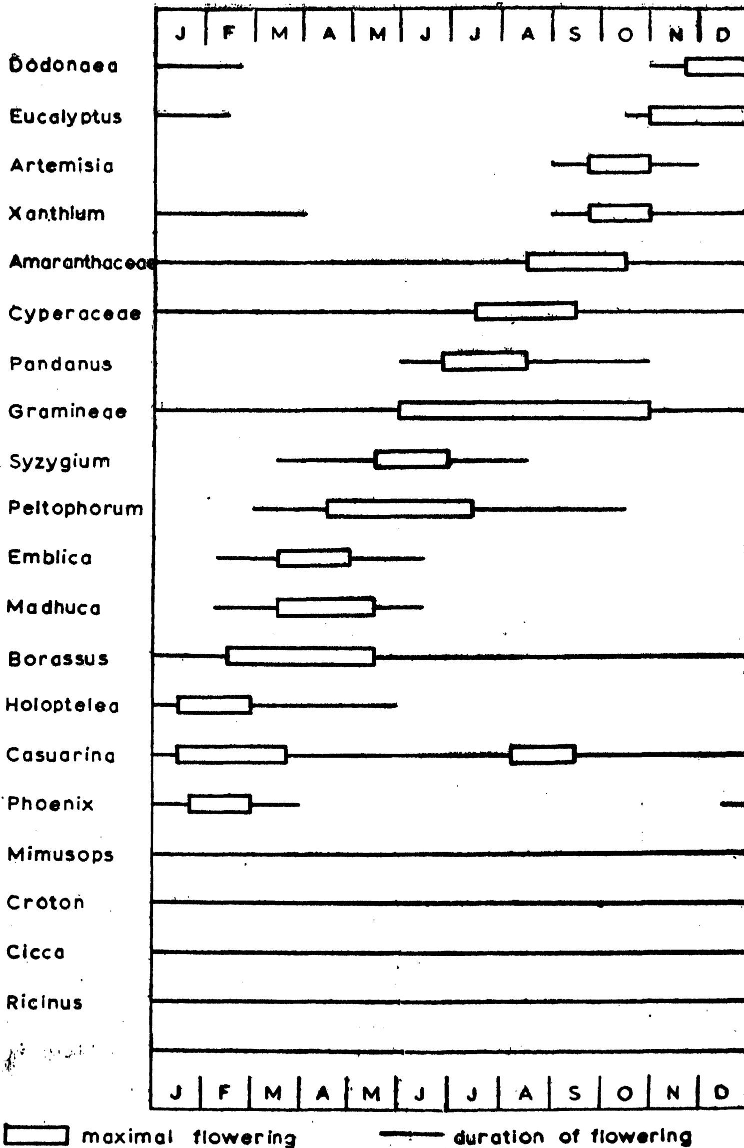



**Figure 3.** Pollination calendar of some plant species at Visakhapatnam.

but they were more numerous during late June–early December. These observations are in general agreement with the earlier records of the blooming periods of the plant species of this area (Subba Reddi 1974).

### 3·2. *Pollen productivity*

Table 2 gives the estimated pollen productivity per anther as well as flower determined for 29 of the 61 plant taxa listed in table 1. The sample size (the number of anthers included in a sample, percent of pollen that failed to get liberated into the medium together with the size of individual pollen types are also included in the table.

Among these 29 plants, the highest pollen output per anther was for *Phoenix*, whereas maximum number per flower was for *Ricinus*. The lowest production both per anther and per flower was for *Cyperus compressus*. It might be expected that pollen productivity in different plants would be influenced by the size of grains as well as anthers, but this is not borne out by the results (table 2).

The pollen productivity data given in table 2 indicate the relative capability of the different taxa to pollute the ambient air with their pollen. However, in some cases all the pollen produced might not be emitted into the air because of the hindrance offered by the plant habit. This is so in the case of *Phoenix* which was first for pollen productivity but the thick crown of leaves obstructs the free dissemination of pollen.

Distinguishing plant species by light microscope examination of pollen is not always possible, *e.g.*, the pollen of stenopalynous groups like Poaceae and Amaranthaceae–Chenopodiaceae are not readily separated. In such cases the relative importance of the taxa contributing to the airspora might be determined by a knowledge of their pollen productivity coupled with their distributional data. For instance, the two species of *Amaranthus* greatly vary in the quantity of pollen produced (table 2) and by knowing their relative abundance in a region, one can easily assess which species is important in the area. Assessed in this way, at Visakhapatnam *A. spinosus* is more important than *A. gracilis*.

### 3·3. *Components of airborne pollen flora and their relative contributions*

Over the 4-year period a total of 34976 pollen/cm² of trap area were counted. Of these, 30851 pollen were identified and assigned to 23 different pollen types which are listed in table 3 alongwith the percent contribution of each to the total pollen flora. Numerically Poaceae ranked first with a mean contribution of 37·32% followed by *Casuarina* (13·11%), Cyperaceae (6·15%), *Eucalyptus* (5·83%), *Dodonaea* (3·8%), Amaranth–chenopod and *Phoenix* each 3·44%, *Borassus* (2·45%) and *Peltophorum* (2·21%). It may be noted here that the Cardiff aerobiologists are of the opinion that the numbers alone are misleading as guides to the relative importance of aeroallergens (Hyde and Adams 1960; Hyde and Williams 1961). They stressed how the relationship between number and pollen volume might be important in allergy, because some pollen although numerically minor, might assume dominance in terms of 'bulk concentration'. However, they did not stress enough that allergens that are superficial or migrate through

| Sl. No. | Plant name | No. of anthers per flower | No. of anthers in the sample | Pollen grains No. per anther | Pollen grains No. per flower | Pollen size ($\mu$m) | % of pollen that could not be liberated |
|---|---|---|---|---|---|---|---|
| 1. | *Phoenix sylvestris* | 5 | 30 | 89262±11910 | 446310 | 16–20 | 5·0 |
| 2. | *Mimusops elengi* | 8 | 32 | 19933±2148 | 159464 | 32–40 | 1·6 |
| 3. | *Dodonaea viscosa* | 8 | 41 | 18574±8183 | 167166 | 24–33 | 0·0 |
| | | (average of 30 flowers) | | | | | |
| 4. | *Madhuca indica* | 28 | 38 | 14495±246 | 406000 | 40–50 | 0·0 |
| 5. | *Cacos nucifera* | 6 | 42 | 10620±1593 | 63720 | 40–45 | 2·8 |
| 6. | *Argemone mexicana* | 58 | 35 | 8008±1010 | 464464 | 40–50 | 3·4 |
| | | (average of 10 flowers) | | | | | |
| 7. | *Pennisetum typhoides* | 3 | 30 | 5706±925 | 17118 | 29–52 | 0·0 |
| 8. | *Peltophorum* | 10 | 25 | 5332±388 | 53320 | 40–60 | 4·0 |
| 9. | *Amaranthus spinosus* | 5 | 30 | 5076±310 | 25380 | 20–40 | 1·8 |
| 10. | *Scirpus articulatus* | 3 | 30 | 4755±770 | 14265 | 24–46×20–33 | 4·2 |
| 11. | *Cyperus rotundus* | 3 | 12 | 3628±213 | 10884 | 33–44×20–32 | 0·0 |
| 12. | *Emblica officinalis* | 3 | 30 | 3461±205 | 10383 | 20–23 | 0·0 |
| 13. | *Lannea coromandelica* | 8 | 57 | 2569±629 | 20552 | 24–34 | 3·3 |
| 14. | *Holoptelea integrifolia* | 10 | 30 | 2437±316 | 24370 | 20–25 | 0·0 |
| | | (average of 18 flowers) | | | | | |
| 15. | *Borassus flabellifer* | 6 | 30 | 2002±80 | 12012 | 45–56 | 2·6 |
| 16. | *Amaranthus gracilis* | 3 | 36 | 1959±131 | 5877 | 20–35 | 0·0 |
| 17. | *Pennisetum polystechyon* | 3 | 50 | 1414±124 | 4242 | 28–52 | 0·0 |
| 18. | *Xanthium strumarium* | 5 | 50 | 1314±190 | 6570 | 24–28 | 4·0 |
| 19. | *Trachys muricata* | 3 | 30 | 1077±80 | 3231 | 26–52 | 0·0 |
| 20. | *Bulbostylis barbata* | 3 | 24 | 878±139 | 2634 | 26–38×20–28 | 0·0 |
| 21. | *Setaria verticillata* | 3 | 50 | 781±25 | 2343 | 32–52 | 0·0 |
| 22. | *Tridax procumbens* | 5 | 50 | 740±66 | 3700 | 24–34 | 0·0 |
| 23. | *Artemisia nilagirica* | 5 | 60 | 689±171 | 3445 | 18–32 | 0·0 |
| 24. | *Casuarina eguisetifolia* | 1 | 50 | 680±80 | 680 | 24–41 | 0·0 |
| 25. | *Cynodon dactylon* | 3 | 40 | 582±84 | 1746 | 18–38 | 0·0 |
| 26. | *Digitaria adscendens* | 3 | 50 | 548±54 | 1644 | 27–41 | 0·0 |
| 27. | *Ricinus Communis* | 1133 | 116 | 451±54 | 510983 | 28–36 | 12·0 |
| | | (average of 3 flowers) | | | | | |
| 28. | *Chloris barbata* | 3 | 50 | 279±13 | 837 | 24–36 | 0·0 |
| 29. | *Cyperus compressus* | 3 | 40 | 183±13 | 549 | 28–35×18–25 | 0·0 |

**Table 3.** Components of the airborne pollen flora and their relative contribution.

| Pollen type | 4 year average seasonal total | Contribution |
|---|---|---|
| Gramineae | 3263 | 37.15 |
| *Casuarina* | 1146 | 13.05 |
| Cyperaceae | 536 | 6.10 |
| *Eucalyptus* | 510 | 5.81 |
| *Dodonaea* | 338 | 3.85 |
| *Phoenix* | 301 | 3.43 |
| Amaranth-chenopod | 300 | 3.42 |
| *Borassus* | 214 | 2.44 |
| *Peltophorum* | 193 | 2.20 |
| *Syzygium* | 178 | 2.03 |
| **Muntingia* | 142 | 1.62 |
| *Ricinus* | 130 | 1.48 |
| *Embliea* | 99 | 1.13 |
| Asteraceae | 98 | 1.12 |
| *Cocos* | 73 | 0.83 |
| Mimosoideae | 56 | 0.64 |
| *Holoptelea* | 53 | 0.60 |
| *Tamarindus* | 29 | 0.33 |
| *Croton* | 25 | 0.28 |
| *Artemisia* | 25 | 0.28 |
| *Morus* | 19 | 0.22 |
| *Cicca* | 15 | 0.17 |
| *Xanthium* | 9 | 0.10 |
| Damaged and unidentified pollen | 1031 | 11.74 |

pollen surface might be more potent in unit weight of small rather than large pollen (Hirst 1973). In this connection it is worth mentioning that the variety of size was one of the features of the complete airspora that impressed Gregory in 1951 (Gregory 1973) and led him to stress how size would influence dispersal.

### 3.4. *Pollen groups counted*

In allergy literature plant species are generally grouped into grasses, weeds and trees (Duchaine 1959). Following this tradition, the pollen recognised, were differentiated into these three groups (table 4). Further, a division of these pollen according to the mode of pollination of the source plants was made. The average contribution of grass, weed and tree (including shrubs) pollen to the total

Table 4. Pollen group counts

| Name of group | % contribution to the total identified pollen | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | |
| Gramineae | 50·69 | | 39·08 | | 38·82 | | 41·35 | |
| Weeds | 9·11 | | 17·26 | | 10·89 | | 13·33 | |
| Anemophilous | | 8·04 | | 25·24 | | 9·96 | | 10·98 |
| Entomophilous | | 1·06 | | 2·02 | | 0·93 | | 2·35 |
| Trees and shrubs | 40·22 | | 43·66 | | 50·28 | | 45·33 | |
| Anemophilous | | 30·57 | | 27·18 | | 32·52 | | 34·60 |
| Entomophilous | | 9·63 | | 16·48 | | 17·77 | | 10·73 |
| Total anemophilous | 81·31 | | 81·50 | | 81·30 | | 86·93 | |
| Total entomophilous | 10·70 | | 18·50 | | 18·70 | | 13·07 | |

I = 1975–76 II = 1976–77 III = 1977–78 IV = 1978–79.

identified pollen were 42, 13, 45% respectively. The tree pollen were distributed over 16 types and the weed pollen over 6 types. Of the four seasons, the 1st year witnessed the highest incidence of grass pollen (50·69%) ; it even excelled the tree pollen count (40·22%) in this year. The 2nd year registered maximal incidence of weed pollen (17·2%), and in the 3rd year, tree pollen (50·28%). As expected, pollen from anemophilous taxa were predominant in the catches. On average, 84·7% of the total identified pollen were of wind-pollinated taxa.

### 3·5. *Seasonal periodicity in airborne pollen*

Table 5 and figure 4 give the trends in the seasonal emission of the different pollen types recognised and of total pollen. While arriving at the data of table 5 each month was considered to consist of a uniform 30 days and the monthly totals were got by applying a correction factor. In figure 4 the counts of the same month over the four-year period were averaged and plotted as a function of time. Additional curves were also drawn through the higher and lower points. Such a graphical representation of the data gives an indication of the most likely density of pollen to be expected and the probable range about the mean (Ogden *et al* 1974 ; Solomon 1976).

As was thought of from a consideration of the pollination calendar, no period altogether free from airborne pollen occurred. The total pollen frequency was significant all through the season with three periods of pronounced occurrence. These were July–September, November–December and February–March. Corresponding to these periods of higher pollen incidence three peaks : two in the wet period and one in the dry period, were evident. Of the two peaks in the wet period, one was confined to the southwest monsoon. Of the three peaks, the one in August is the highest and, the February and November peaks follow in that order,

**Table 5.** Month-wise distribution and seasonal totals of each of the components of the airborne pollen flora enounctered in different years of the study period, April 1975 to March 1979.

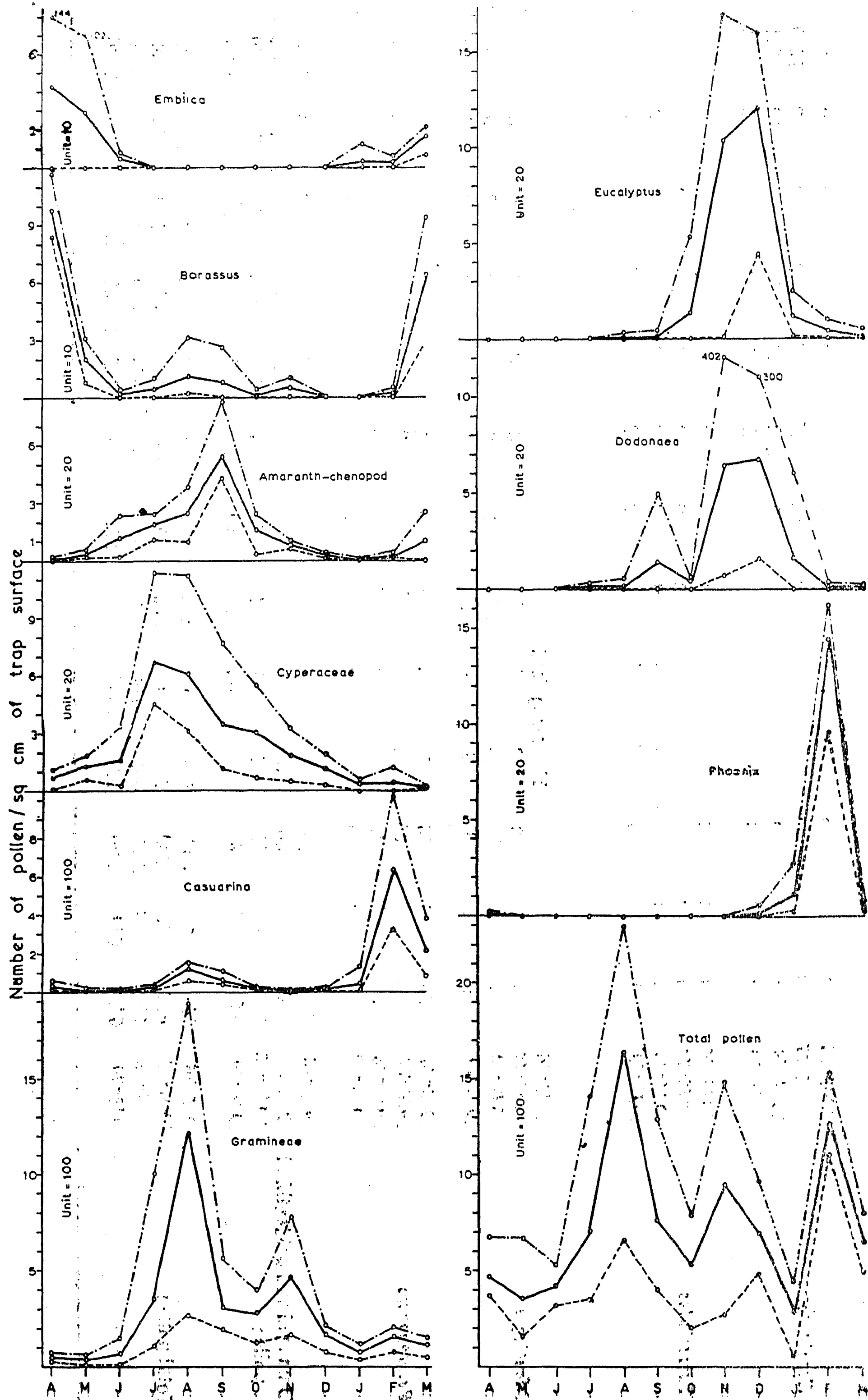
| | Year of Study | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | March | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gramineae | 1975–76 | 22 | 2 | 10 | 161 | 1894 | 196 | 125 | 777 | 171 | 68 | 201 | 137 | 3764 |
| | 1976–77 | 41 | 31 | 51 | 132 | 1228 | 565 | 314 | 717 | 211 | 86 | 75 | 111 | 3562 |
| | 1977–78 | 45 | 54 | 144 | 1002 | 265 | 222 | 397 | 206 | 196 | 117 | 169 | 144 | 2961 |
| | 1978–79 | 78 | 61 | 58 | 109 | 1442 | 240 | 281 | 162 | 72 | 33 | 184 | 44 | 2764 |
| Weeds | | | | | | | | | | | | | | |
| Cyperaceae | 1975–76 | 9 | 37 | 6 | 128 | 106 | 23 | 13 | 41 | 12 | 0 | 24 | 2 | 401 |
| | 1976–77 | 21 | 23 | 32 | 227 | 224 | 154 | 110 | 66 | 38 | 12 | 11 | 2 | 920 |
| | 1977–78 | 2 | 12 | 68 | 92 | 63 | 36 | 61 | 22 | 35 | 10 | 0 | 4 | 405 |
| | 1978–79 | 22 | 31 | 28 | 92 | 96 | 66 | 60 | 10 | 6 | 4 | 0 | 4 | 419 |
| Amaranth-chenopod | 1975–76 | 2 | 0 | 4 | 21 | 22 | 85 | 6 | 14 | 6 | 2 | 8 | 7 | 177 |
| | 1976–77 | 2 | 4 | 22 | 41 | 76 | 165 | 30 | 12 | 4 | 0 | 0 | 21 | 377 |
| | 1977–78 | 0 | 10 | 47 | 36 | 19 | 94 | 48 | 20 | 8 | 0 | 11 | 50 | 343 |
| | 1978–79 | 4 | 12 | 18 | 48 | 77 | 86 | 41 | 12 | 2 | 2 | 2 | 0 | 304 |
| *Artemisia* | 1975–76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 10 | 12 |
| | 1976–77 | 0 | 0 | 0 | 0 | 0 | 24 | 40 | 0 | 4 | 0 | 2 | 0 | 70 |
| | 1977–78 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 4 | 0 | 0 | 0 | 0 | 12 |
| | 1978–79 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Xanthium* | 1975–76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 3 | 0 | 7 |
| | 1976–77 | 0 | 2 | 0 | 0 | 0 | 4 | 14 | 0 | 0 | 2 | 0 | 0 | 22 |
| | 1977–78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1978–79 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 2 | 0 | 0 | 0 | 0 | 7 |
| Asteraceae | 1975–76 | 2 | 0 | 2 | 2 | 0 | 0 | 2 | 10 | 0 | 10 | 33 | 5 | 66 |
| | 1976–77 | 4 | 2 | 6 | 6 | 4 | 11 | 42 | 2 | 35 | 10 | 2 | 13 | 137 |
| | 1977–78 | 2 | 0 | 4 | 4 | 0 | 8 | 13 | 6 | 4 | 8 | 0 | 4 | 53 |
| | 1978–79 | 18 | 50 | 16 | 6 | 8 | 11 | 5 | 4 | 4 | 4 | 9 | 2 | 137 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Croton* | 1975–76 | 2 | 0 | 0 | 0 | 5 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 13 |
| | 1976–77 | 0 | 4 | 0 | 0 | 8 | 2 | 2 | 16 | 15 | 0 | 0 | 0 | 47 |
| | 1977–78 | 2 | 0 | 0 | 0 | 2 | 10 | 2 | 0 | 2 | 0 | 0 | 0 | 18 |
| | 1978–79 | 2 | 2 | 0 | 2 | 4 | 0 | 2 | 4 | 4 | 0 | 0 | 0 | 20 |
| Trees and Shrubs | | | | | | | | | | | | | | |
| *Casuarina* | 1975–76 | 29 | 0 | 4 | 6 | 151 | 40 | 10 | 8 | 4 | 4 | 324 | 300 | 880 |
| | 1976–77 | 10 | 0 | 0 | 12 | 122 | 103 | 14 | 4 | 0 | 134 | 645 | 86 | 1130 |
| | 1977–78 | 0 | 2 | 2 | 34 | 136 | 72 | 10 | 6 | 19 | 33 | 550 | 380 | 1244 |
| | 1978–79 | 54 | 10 | 0 | 19 | 60 | 45 | 21 | 8 | 0 | 2 | 1017 | 94 | 1330 |
| *Borassus* | 1975–76 | 117 | 16 | 4 | 4 | 5 | 0 | 0 | 2 | 0 | 0 | 5 | 94 | 247 |
| | 1976–77 | 107 | 23 | 0 | 0 | 2 | 4 | 0 | 8 | 0 | 0 | 2 | 60 | 206 |
| | 1977–78 | 89 | 8 | 0 | 10 | 31 | 26 | 4 | 10 | 0 | 0 | 0 | 30 | 208 |
| | 1978–79 | 84 | 31 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 194 |
| *Cocos* | 1975–76 | 22 | 0 | 19 | 8 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 5 | 60 |
| | 1976–77 | 10 | 13 | 0 | 2 | 8 | 0 | 10 | 23 | 4 | 4 | 33 | 8 | 115 |
| | 1977–78 | 21 | 2 | 4 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 2 | 49 |
| | 1978–79 | 2 | 17 | 2 | 0 | 4 | 0 | 16 | 4 | 0 | 0 | 4 | 17 | 66 |
| *Phoenix* | 1975–76 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 190 | 10 | 258 |
| | 1976–77 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 260 | 4 | 274 |
| | 1977–78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 21 | 383 | 14 | 428 |
| | 1978–79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 234 | 4 | 242 |
| *Dodonaea* | 1975–76 | 0 | 0 | 0 | 0 | 0 | 17 | 13 | 402 | 108 | 0 | 3 | 5 | 548 |
| | 1976–77 | 0 | 0 | 0 | 0 | 0 | 98 | 12 | 80 | 31 | 4 | 0 | 0 | 225 |
| | 1977–78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 300 | 121 | 6 | 0 | 443 |
| | 1978–79 | 0 | 0 | 0 | 0 | 0 | 12 | 5 | 14 | 99 | 4 | 0 | 0 | 134 |
| *Ricinus* | 1975–76 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 8 | 2 | 16 |
| | 1976–77 | 0 | 33 | 3 | 27 | 10 | 0 | 0 | 0 | 35 | 67 | 136 | 115 | 426 |
| | 1977–78 | 6 | 8 | 4 | 6 | 10 | 6 | 0 | 0 | 0 | 6 | 0 | 2 | 48 |
| | 1978–79 | 0 | 0 | 0 | 2 | 0 | 4 | 0 | 0 | 6 | 0 | 19 | 0 | 31 |

Table 5. *Contd.*

| | Year of Study | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | March | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Morus* | 1975–76 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 8 | 0 | 19 |
| | 1976–77 | 0 | 0 | 3 | 2 | 0 | 11 | 4 | 0 | 0 | 6 | 11 | 0 | 37 |
| | 1977–78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| | 1978–79 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 6 | 6 | 16 |
| *Holoptelea* | 1975–76 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 152 | 10 | 170 |
| | 1976–77 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 0 | 0 | 8 |
| | 1977–78 | 2 | 4 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 14 |
| | 1978–79 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 13 | 19 |
| *Emblica* | 1975–76 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 5 | 17 | 40 |
| | 1976–77 | 19 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 6 | 44 |
| | 1977–78 | 8 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 18 | 40 |
| | 1978–79 | 144 | 102 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 273 |
| *Cicca* | 1975–76 | 11 | 0 | 14 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 |
| | 1976–77 | 0 | 0 | 0 | 4 | 2 | 0 | 4 | 0 | 6 | 0 | 0 | 0 | 16 |
| | 1977–78 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 1978–79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 2 | 2 | 8 |
| *Syzygium* | 1975–76 | 0 | 2 | 352 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 368 |
| | 1976–77 | 155 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 161 |
| | 1977–78 | 0 | 8 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 49 |
| | 1978–79 | 2 | 42 | 88 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 132 |
| Mimosoideae | 1975–76 | 0 | 0 | 0 | 0 | 2 | 20 | 8 | 19 | 0 | 0 | 3 | 0 | 52 |
| | 1976–77 | 4 | 2 | 0 | 0 | 4 | 4 | 8 | 12 | 4 | 0 | 0 | 0 | 38 |
| | 1977–78 | 0 | 0 | 2 | 4 | 4 | 16 | 4 | 18 | 6 | 0 | 0 | 0 | 54 |
| | 1978–79 | 0 | 0 | 0 | 0 | 4 | 28 | 16 | 24 | 8 | 0 | 0 | 0 | 80 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Eucalyptus* | 1975–76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 88 | 6 | 0 | 0 | 117 |
| | 1976–77 | 0 | 0 | 0 | 2 | 6 | 0 | 106 | 507 | 369 | 50 | 0 | 0 | 1040 |
| | 1977–78 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 292 | 267 | 33 | 11 | 10 | 621 |
| | 1978–79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 237 | 2 | 19 | 2 | 262 |
| *Tamarindus* | 1975–76 | 0 | 0 | 2 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| | 1976–77 | 0 | 0 | 13 | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| | 1977–78 | 0 | 0 | 14 | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 20 |
| | 1978–79 | 0 | 13 | 14 | 10 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 |
| *Muntingia* | 1975–76 | Not recognised | | | | | | | | | | | | |
| | 1976–77 | 0 | 0 | 0 | 0 | 4 | 39 | 4 | 0 | 0 | 0 | 0 | 0 | 47 |
| | 1977–78 | 0 | 6 | 43 | 20 | 33 | 226 | 2 | 0 | 0 | 0 | 0 | 0 | 330 |
| | 1978–79 | 2 | 0 | 0 | 0 | 36 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 49 |
| *Peltophorum* | 1975–76 | 29 | 0 | 16 | 54 | 36 | 9 | 4 | 2 | 0 | 0 | 0 | 7 | 157 |
| | 1976–77 | 10 | 77 | 63 | 23 | 18 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 195 |
| | 1977–78 | 31 | 13 | 72 | 118 | 31 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 281 |
| | 1978–79 | 86 | 19 | 6 | 4 | 0 | 21 | 2 | 0 | 0 | 0 | 0 | 2 | 140 |
| Damaged and unidentified pollen | 1975–76 | 111 | 101 | 58 | 95 | 62 | 20 | 21 | 84 | 121 | 62 | 138 | 194 | 1067 |
| | 1976–77 | 72 | 187 | 140 | 47 | 78 | 102 | 76 | 29 | 65 | 42 | 42 | 158 | 1038 |
| | 1977–78 | 170 | 61 | 72 | 72 | 65 | 32 | 73 | 38 | 111 | 100 | 81 | 74 | 949 |
| | 1978–79 | 168 | 277 | 82 | 60 | 42 | 51 | 69 | 26 | 43 | 6 | 30 | 217 | 1071 |
| Total pollen | 1975–76 | 369 | 160 | 497 | 518 | 2290 | 404 | 204 | 1398 | 516 | 218 | 1113 | 805 | 8492 |
| | 1976–77 | 457 | 418 | 339 | 531 | 1796 | 1288 | 790 | 1476 | 821 | 433 | 1219 | 584 | 10152 |
| | 1977–78 | 378 | 188 | 527 | 1402 | 659 | 782 | 632 | 640 | 968 | 449 | 1217 | 734 | 8576 |
| | 1978–79 | 672 | 669 | 320 | 352 | 1804 | 565 | 525 | 274 | 485 | 61 | 1528 | 501 | 7756 |


Embilica
Unit=10
Borassus
Unit=10
Amaranth-chenopod
Unit=20
Cyperaceae
Unit=20
Casuarina
Unit=100
Gramineae
Unit=100
Number of pollen / sq cm of trap surface
Eucalyptus
Unit=20
Dodonaea
Unit=20
402
300
Phoenix
Unit=20
Total pollen
Unit=100
A M J J A S O N D J F M

The chief donors to the pollen peak in August were Poaceae, *Casuarina*, Cyperaceae in that order. In February the chief donors were *Casuarina*, *Phoenix* and Poaceae and to that in November, Poaceae, *Dodonaea*, *Eucalyptus* and Cyperaceae contributed more.

The pollen of Poaceae, Cyperaceae, Amaranth-chenopod and *Casuarina* were encountered all through the year. Poaceae pollen exhibited two distinct seasons : one major and the other minor. The former practically started in early July with a great uprush in the counts from late July to mid September followed by a gradual decrease till the 2nd week of October. On the average, 50% of the annual catch was recovered in this season. Peak incidence was recorded in the 2nd week of August. The minor season can be said to have started by the 2nd week of October and continued up to the 3rd week of December; maximal numbers usually occurred in November. The main Cyperaceae season occurred in July–October ; on average, 60% of the annual catch was recovered here. Relatively high numbers of Amaranth-chenopod type were noticed during June–November period with maximal incidence in September. With *Casuarina*, two periods of higher incidence were apparent ; the main period extending from January to April and the subsidiary from July to October. Peak incidence occurred in February during the main and in August during the subsidiary period ; on average, 80% and 19% of the annual catch were recovered in the respective seasons.

Pollen types like Asteraceae, *Cocos* and *Ricinus* were caught rather irregularly all round the year. Types other than these were more or less confined to a particular period of the year. Thus the *Syzigium* pollen season was from late April to early July with maximal incidence in June. *Emblica* pollen were caught from early March to early June with higher numbers during April–May. *Peltophorum* pollen were registered from March to November (though not regularly) with higher frequency from April to September. *Tamarindus* pollen were mainly caught from May to August ; greater frequency was from June to July. *Muntingia* pollen season was from April to October ; the numbers abounded in August–September. Mimosoideae pollen though encountered occasionally in several months, were rather more common from August to December. *Eucalyptus* pollen were noticed from July to March with maximal incidence during November–December. The *Dodonaea* pollen season extended from September until March but was at its maximum during November–January. *Phoenix* pollen began to appear in air from late December, reached a peak in February, decreased through March and disappeared by April. *Borassus* pollen were trapped in all months (though not every year) except in December–January. They abounded during March–May with a peak in April.

The pollen of *Holoptelea*, *Artemisia*, *Xanthium*, *Croton*, *Morus* and *Cicca* were of spasmodic occurrence over a major part of the year.

### 3.6. *Between-year variation in pollen abundance*

The different pollen types recognised as well as total pollen counted, varied considerably in their abundance from year to year (tables 5 and 6). The catches of

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**Figure 4.** Seasonal curves of maximum, minimum and average incidence of major pollen types as well as total pollen at Visakhapatnam.

Table 6. The four-year average annual figures and the range of variation for the airborne pollen

| Pollen type | 4-year average (No./sq cm) | Range of variation (%) | Difference |
|---|---|---|---|
| *Borassus* | 214 | 90·65–115·42 | 24·77 |
| Gramineae | 3263 | 84·71–115·35 | 30·64 |
| *Casuarina* | 1146 | 76·79–116·06 | 39·27 |
| *Phoenix* | 301 | 80·40–142·19 | 61·79 |
| Amaranth–chenopod | 301 | 58·80–125·25 | 66·45 |
| *Peltophorum* | 193 | 72·54–145·60 | 73·06 |
| Mimosoideae | 56 | 67·86–142·86 | 75·00 |
| Asteraceae | 98 | 54·08–139·80 | 85·72 |
| *Cocos* | 73 | 67·12–157·53 | 90·41 |
| Cyperaceae | 536 | 74·81–171·64 | 96·83 |
| *Tamarindus* | 29 | 68·97–186·21 | 117·24 |
| *Dodonaea* | 338 | 39·64–162·13 | 122·49 |
| *Croton* | 25 | 52·00–188·00 | 136·00 |
| *Syzygium* | 178 | 27·53–206·74 | 179·21 |
| *Eucalyptus* | 510 | 22·94–203·92 | 180·98 |
| * Muntingia | 142 | 33·10–232·39 | 199·29 |
| *Emblica* | 99 | 40·40–275·76 | 235·36 |
| *Artemisia* | 25 | 16·00–280·00 | 264·00 |
| *Holoptelea* | 53 | 15·09–320·75 | 305·66 |
| *Ricinus* | 130 | 12·31–327·70 | 315·38 |
| Total pollen grains | 8744 | 88·70–116·10 | 27·40 |

* average of 3 years

Poaceae were higher in 1975–76 than in the other years monitored, and decreased progressively in the subsequent years. These varied between 85% of 4-year average in 1978–79 and 115% in 1975–76. The total weed pollen registered in 1976–77 were greater than in other years. They ranged from 68% to 158% of average. The pollen of anemophilous weeds preponderated almost equally in 1976–77, and 1977–78. They ranged from 69% to 160% of average. The pollen of entomophilous weeds of 1976–77 outnumbered others. They varied from 58% to 150% of average. The pollen of trees preponderated in almost equal magnitude in 1976–77 and 1977–78. They ranged from 86–115% of average. The total anemophilous as well as total entomophilous tree pollen were more in 1976–77 ; the former ranged from 95% to 104%, and the latter from 67% to 140% of respective averages. The total pollen preponderated in the 2nd year of this study and ranged from 89% to 116% of average. From the range of variations it becomes evident that group-wise, the pollen of trees and of grasses remained relatively steady. Much more steady were the pollen of anemophilous trees. This observation regarding tree pollen incidence is at variance with Dua

and Shivpuri (1962), Subba Reddi (1970), Mittre and Khandelwal (1973) from India and Hyde (1972) from UK, Solomon and Buell (1969) from USA who reported a high degree of annual variation.

Meteorological differences from year to year may account at least in part for the recorded variation in annual pollen emission rates. This is especially so with Poaceae and other weeds which grow and subsequently flower most abundantly in response to the amount and distribution of precipitation. Vertical cylinder traps are somewhat sensitive to wind speed and this may also account for some of the observed variations, but the constancy of tree pollen counts suggest this was not important.

### 3·7. *Effect of urbanisation on airborne pollen*

A comparison of the results of an earlier survey conducted during April 1966–March 1968 (Subba Reddi 1970) and those of the present study revealed a considerable decline in pollen abundance between the two study times (figure 5) indicating that increasing urbanisation has had an effect on the airspora. Due to increased industrialisation of the city the demand for residential houses increased with the result the neighbouring suburban and rural areas have been intensively used for extensive building construction. This has drastically curtailed the sites available for plants especially of herbaceous flora.

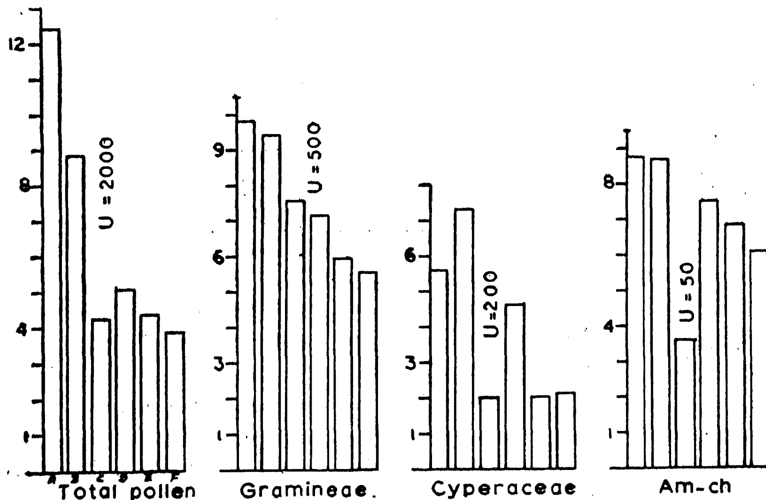


Figure 5. The frequency of some pollen types at Visakhapatnam in six years from 1966 to 1979 (A, 1966–67 ; B, 1967–68 ; C, 1975–76 ; D, 1976–77 ; E, 1977–78; F, 1978–79).

## 4. Conclusions

As is true of other tropical regions, no season was altogether free of pollen at Visakhapatnam. Nor were any clear-cut tree, grass and weed pollen seasons characteristic of spring, summer and autumn of temperate zones distinguished. The different pollen types encountered, are to be considered as pernicious and important pollutants of biological origin in the air of Visakhapatnam adding to

the overall dangers of atmospheric pollution of this growing industrial city. This warrants an urgent inquiry into the clinical significance of these biopollutants as well as the interaction between these and the chemical pollutants, and the possible resulting synergistic adverse effects on human health.

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# The floral anatomy of *Kniphofia uvaria* Hook. (Liliaceae: Kniphofieae)

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**Abstract.** The floral anatomy of *Kniphofia uvaria* Hook. is described. The tepals are anatomically similar and one-traced. The stamens are one-traced. The outer whorl consists of shorter stamens. The placentation is parietal, nectary ovarian and septal. The extension of the carpellary ventrals into the style is an important anatomical feature. The trend towards development of an inferior ovary is noted. Evidence from floral morphology and other disciplines is discussed and it is inferred that the alleged affinity of *Kniphofia* and the Kniphofieae with the Aloineae and the Hemerocallideae is rather remote.

**Keywords.** Floral anatomy ; *Kniphofia uvaria.*

## 1. Introduction

In earlier contributions, the floral anatomy of the Aloineae and Hemerocallideae was presented (Vaikos, Markandeya and Pai 1978, 1981). The tribe Kniphofieae is thought to chiefly comprise of the genus *Kniphofia* (cf. Stebbins 1971) although two more genera *Blandfordia* and *Notosceptrum* are included in it by Hutchinson (1959). The present paper deals with the vascular anatomy of the flower of *Kniphofia uvaria* Hook.

## 2. Materials and methods

The flowering material was obtained from the Curator, Government Botanical Gardens, Ootacamund. The flower buds were fixed in FAA. The usual paraffin method was followed. The microtome sections cut at 9–12$\mu$ were stained with crystal violet using erythrosin as counter stain.

## 3. Observations

The pedicel contains a ring of three large bundles (figure 1). These are laterally connected upwards to develop an anastomosis (figure 2). The six tepal strands

emerge first (figure 2). The six staminal strands emerge quickly upwards (figure 3). The remainder of the vascular tissue resolves into three carpellary dorsals and six carpellary ventrals (figure 3).

The hypanthium is adnate to the ovary for a short length (figures 4, 5). The tepals and stamens separate out simultaneously (figure 5). The tepals are united into a tube for a considerable length (figures 5–11). Each of the tepals receives a single vascular bundle (figures 5–11).

The stamens have cylindric filaments (figures 5–8). Those of the outer whorl are antheriferous first and are also shorter (figures 9–10). The inner three stamens are antheriferous at a much higher level (figures 10–11). Each of them receives a single vascular bundle which continues upwards into the connective without a division and ends beneath the tip of the anther (figures 5–10). The connective splits and ends together with the anther lobes (figure 10). The anthers are introrse and two–celled.

The ovary is trilocular at the base with the ovules arranged in two rows in each loculus (figures 4–6). The carpellary ventrals of adjacent carpels are united to form the composite placental bundles which are lodged on septal radii (figures 4–6). These split into the constituent ventrals at the beginning of the ovuliferous zone and bear traces to the ovules of adjacent carpels (figure 6). Upwards, the placentae separate in the centre rendering the ovary unilocular (figure 7). The ventrals of adjacent carpels extend at the inner end of the septa, continue to bear traces to the ovules of adjacent carpels (figure 7) and enter the base of the style (figure 8). The carpellary ventrals end in the basal half of the style.

The septal nectaries are developed from the base of the ovary (figure 4). These open at the base of the style (figure 8). The ovarian loculus is continued upwards into the style as a triradiate canal (figure 8). The stylar canal is closed in the centre in some flowers to result in three cavities (figure 9). It is lined with transmitting tissue. The carpellary dorsals extend into the style (figures 8–9) and end much beneath its tip (figure 10). The style has three grooves along which it splits into three non-vascular stigmatic lobes (figure 11).

### 3·1. *Abnormal flower*

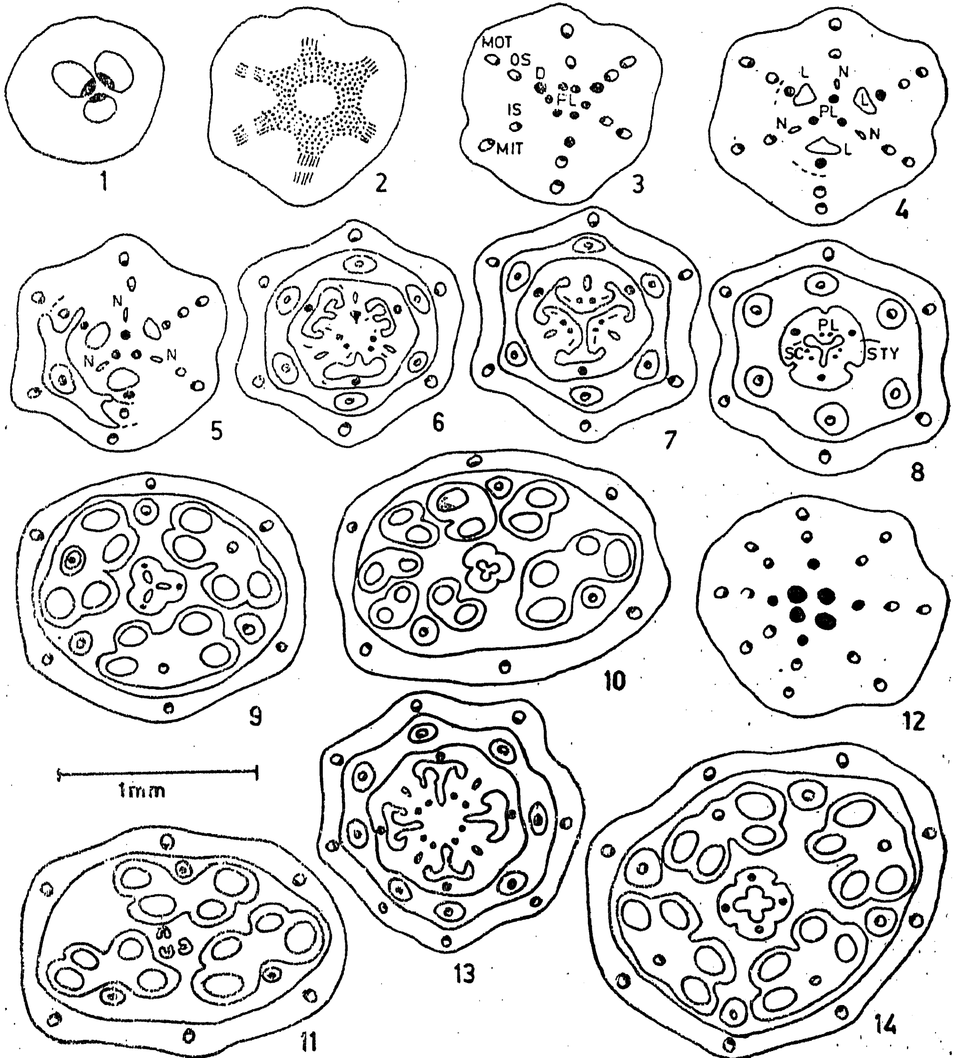
This flower has eight tepals, eight stamens and four carpels. The vascular supply is derived in conformity with the tetramerous structure (figure 12). The septal glands are four (figure 13). The style receives four carpellary dorsals and it is four-lobed (figure 14).

## 4. Discussion

The six tepals are arranged in two whorls and are united to develop a prominent tube. Each of them receives a single bundle and, anatomically, both the whorls are similar. This is a condition observed in many liliaceous genera, *e.g.*, *Ophiopogon*, *Convallaria*, *Polygonatum*, *Maianthemum*, *Eucomis*, *Asphodelus*, *Urginea*, etc. In the allegedly allied Aloineae and Hemerocallideae (Vaikos, Markandeya and Pai 1978, 1981), the tepals are three–traced ; the three traces may arise separately

from the stele or the laterals of the outer and inner tepals may be derived through a bifurcation of the common or commissural bundles.

The stamens are also one-traced ; this is characteristic of most lilies studied. The similarity in the vascular supply to the tepals and the stamens does not seem to indicate a staminal origin for the perianth as is sometimes inferred (cf. Leinfellner 1963).




**Figures 1–14.** *Kniphofia uvaria* : **1–11.** Transections of the flower from the base upwards. **12–14.** Transections of the abnormal flower, showing tetramerous condition.

*Abbreviations* D, carpellary dorsal ; IS, inner staminal strand ; L, locule ; MIT, median bundle of an inner tepal ; MOT, median bundle of an outer tepal ; N, nectary ; OS, outer staminal strand ; PL, placental bundle ; SC, stylar canal ; STY, style.

The outer stamens are shorter than the inner ones. This condition is characteristic of species of Aloineae, Allieae and some other genera (Markandeya 1978 ; Vaikos 1974 ; Vaikos *et al* 1978, 1981). It represents a trend towards differentiation of the two androecial whorls and ultimate reduction of one or more of the shorter stamens or a whorl of stamens as occurs in Allieae (Markandeya 1978).

The outer floral whorls are adnate to the base of the ovary indicating a trend towards the development of an inferior ovary. This is a trend which sporadically occurs throughout the many genera and tribes of the family (Markandeya 1978 ; Vaikos 1974 ; Vaikos *et al* 1978, 1981).

The gynoecium is tricarpellary and trilocular at the base and unilocular upwards. The carpellary ventrals are lodged on the alternate septal radii and bear traces to the ovules of adjacent carpels. The placentation is anatomically and morphologically parietal (cf. Puri 1952).

The nectaries are ovarian and are typical septal glands. They develop at the base of the ovary and open at the base of the style. The placental bundles which lie close to the glands have to be associated with them in their function (Agthe 1951 ; Frei 1955 ; Pai and Tilak 1965).

The style receives the carpellary dorsals and the carpellary ventrals, although the latter end early. The extension of the carpellary ventrals into the style is a less advanced feature.

Tetramerous structure is noted in a few flowers. The vascular supply is derived in conformity with tetramery. Tetramerous flowers occur normally in *Aspidistra.*

The genus *Kniphofia* forms a component of the Aloineae in the Englerian scheme (cf. Melchior 1964), whereas Hutchinson (1959) erects a tribe in its name. In the old Bentham and Hooker's (1883) system it is placed under the tribe Hemerocalleae.

This paper demonstrates that the tepals in *Kniphofia* are one-traced while in Aloineae and Hemerocallideae they are three-traced (Vaikos *et al* 1978, 1981). In the studied plants of the tribes Aloineae and Hemerocallideae, the placentation is axile, while it is parietal in *Kniphofia.*

In the taxa of the Aloineae studied, all or few stamens are adnate to the base of the ovary and, as a variation, the stamens and the style may also be fused up to the top to develop a prominent column—the gynostemium (Vaikos and Markandeya 1976 ; Vaikos *et al* 1978). In *Hemerocallis,* the stamens are adnate to the perianth (Vaikos *et al* 1981). However, in *Kniphofia* the stamens are neither adnate to the ovary nor to the tepals.

Embryological evidence shows that *Kniphofia* is best treated distinct and not as a component of the Hemerocallideae (cf. Di Fulvio and Cave 1964). The study of vessel structure shows the vessels in *Kniphofia* are less specialised than those in the Hemerocallideae, as also the Aloineae (Cheadle and Kosakai 1971).

Anthraquinones are detected in *Aloe,* whereas they are absent in *Kniphofia* (Van Oudtshoorn and Van Rheede 1964).

The karyotype of *Kniphofia* is symmetric with $n = 6$ (Moffett 1932 ; Stebbins 1971). The Aloineae have the characteristic bimodal $4L + 3S$ karyotype (Brandham 1971 ; Darlington 1963 ; Stebbins 1971), whereas *Hemerocallis* of the Hemerocallideae has $n = 11$ and the karyotype is not very asymmetric and shows rather a close similarity with *Amaryllis* (Sato 1942).

Hutchinson (1959) considers the further development of the Kniphofieae to the Aloineae. Cheadle and Kosakai (1971) consider this as a plausible suggestion for they find the vessels in the Kniphofieae less specialised than those in the Aloineae. Furthermore, " no member of the Aloineae has vessels less specialised than those most specialised in the *Kniphofieae* ".

Stebbins (1971) infers a karyological affinity between the Kniphofieae and Aloineae and suggests that, " increasing asymmetry and heterogeneity of the relatively symmetrical karyotype of the Kniphofieae, consisting chiefly of the genus *Kniphofia*, together with the addition of a chromosome to the complement through fixation of a centric fragment, would lead to the asymmetrical karyotype of the Aloineae with $n = 7$ ". This appears to be too speculative a statement at the present stage of our knowledge and further karyological studies of more species of the genus should be in order. It may be noted that the bimodal karyotype of Aloineae is clearly marked with chromosomal markers for it. The present study would demonstrate that *Kniphofia* is best placed distinct from the Aloineae as also the Hemerocallideae. Further studies on more species of the genus are obviously in order for a categorical conclusion.

Hutchinson's treatment of the tribe may also need a review. Di Fulvio and Cave (1964) doubt the inclusion of *Blandfordia* in the tribe. Both *Blandfordia* and *Notosceptrum* merit a floral anatomical study.

## Acknowledgements

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# Transmission of seed-borne inoculum of *Macrophomina phaseolina* from seed to plant

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**Abstract.** *Macrophomina phaseolina* is a serious pathogen which is externally as well as internally seed-borne. It causes failure of seed germination and browning and rotting of seedlings. The presence of pathogen in infected and healthy looking seedlings was tested by clearing, sectioning and incubation techniques. After 8 weeks almost every surviving plant developed pale yellow to brown circular or oval concentric spots on leaves, stem and capsules. Mycelium and microsclerotia were observed in the peripheral region of the lesions. Splitted root, stem and capsule also showed the presence of microsclerotia. Cleared wholemounts of leaf and stem and T.S. and L.S. of stem showed inter- and intracellular mycelium in cortex, xylem and pith cells. Microsclerotia were also observed. In capsule, infection was recorded on its inner wall, septum, placenta and seeds spreading from base to apex.

**Keywords.** *Macrophomina phaseolina*; seed-borne transmission; *Sesamum indicum*.

## 1. Introduction

Root, stem or charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is a serious seed-borne disease of sesame in India (Pearl 1923; Mc Rae 1930; Sundararaman 1931, 1932; Mehta 1951; Jain and Kulkarni 1965; Parasar and Suryanarayana 1971; Mishra *et al* 1973; Gemawat and Verma 1974; Verma and Daftari 1974). It has been reported from many parts of the world and Meiri and Solel (1963) regarded it as the most destructive disease of sesame in Israel. It is soil as well as seed-borne (Noble *et al* 1958; Meiri and Solel 1963; Richardson 1979). Singh and Singh (1979) have shown that in sesame *M. phaseolina* is surface as well as internally seed-borne. The effects of seed-borne inoculum of *M. phaseolina* on germination and the mode of disease transmission from seed to plant have not been precisely investigated so far and therefore this study was taken up.

## 2. Material and methods

Six samples ac. nos. 7, 9 (Ajmer), 15, 17, 18 (Chittorgarh) and 11 (Udaipur investigated for deep location of *M. phaseolina* were selected for the study. Of

these ac. no. 11 was free from the infection (figure 1) and used as control, whereas, the remaining samples carrying various degrees of infection were categorized into three groups (i) seeds without microsclerotia, (ii) seeds with moderate number of microsclerotia, and (iii) seeds with abundant microsclerotia (figure 2). The following three methods were used to investigate the transference of disease from seed to plant. Seeds harvested in experimental plants were tested by standard blotter method.

## 2·1. *Petriplate method*

One hundred seeds of each sample were treated with 2% available chlorine aqueous solution of sodium hypochlorite and tested by standard blotter method. Observations were made on percentage of *M. phaseolina* infection, radicle emergence, germination, rotting and deformed seedlings, seedlings with pycnidia and ungerminated seeds at 24 hr intervals.

## 2·2. *Seedling symptom test*

Ten ml of 2% agar water was poured into each test tube and sterilized. One hundred seeds of each sample after pretreatment with 1% available chlorine were transferred aseptically to test tubes (one seed per test tube) and incubated for 8 days at 22° C under 12 hr of alternating cycles of day light and darkness.

## 2·3. *Growth test*

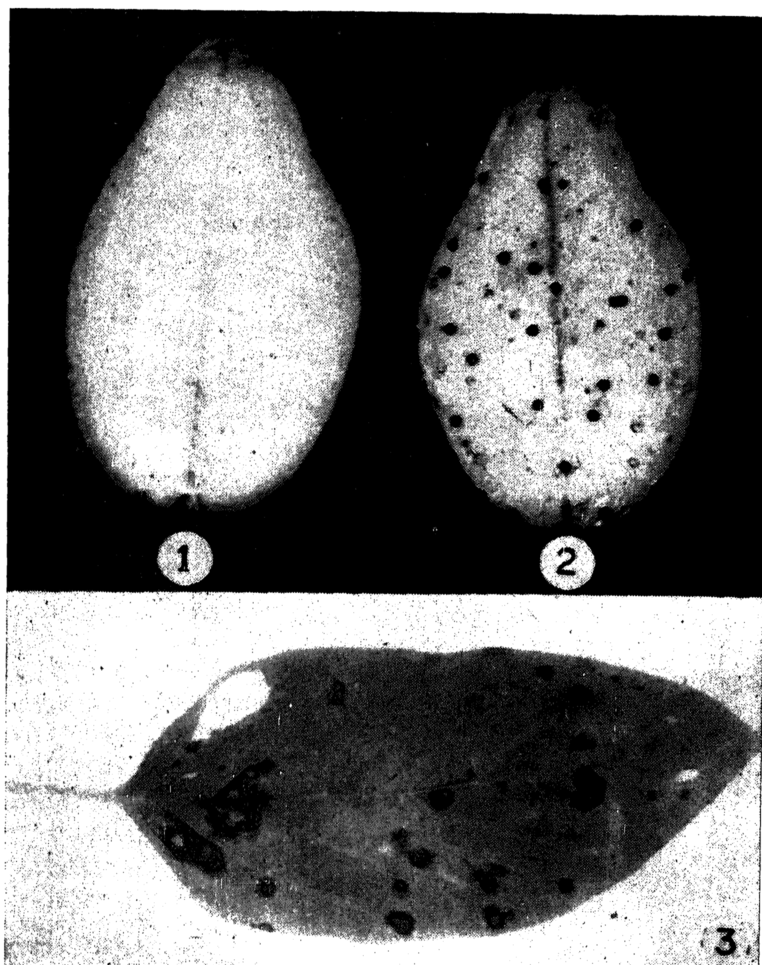
One hundred seeds of each sample untreated and pretreated with 2% and 5% available chlorine for 5 min were sown in pots containing sterilized soil. Mortality, seedling survival, symptoms and transmission of disease were recorded at weekly intervals. Isolations were made from wilting and healthy looking seedlings and plants at regular intervals. For isolation, seedlings were carefully uprooted, washed in running water, segmented and after surface washing with 1% available chlorine were spaced on blotters and incubated for 7 days. For this study different parts of seedlings (radicle, plumule, hypocotyl, cotyledon) and plants (roots, stems, leaves, flower buds, flowers, green and dry capsules and seeds) were incubated.

After 14 weeks dried plants were uprooted and the capsules were harvested for further observations. The root system was separated, washed thoroughly and splitted longitudinally. External and internal surfaces were observed by naked eye and under stereobinocular microscope.

Stem portions were also split longitudinally and divided into three parts basal, middle and apical.

To detect the presence of *M. phaseolina* on the surface and inside the dried capsules, they were kept in 70% ethanol overnight. Capsule surface and its parts showing microsclerotia were photographed while under 70% ethanol.

Clearing of plant parts carrying infection was made by using lactic acid, potassium hydroxide and ethanol. Hand sections were also cut and stained with safranin and fast-green combination.




**Figures 1–3. 1.** Uninfected seed of sesame, **2.** Seeds infected with *Macrophomina phaseolina* ; note pin head like microsclerotia on seed surface, **3.** Leaves infected with *M. phaseolina* ; note dark brown leaf spots with pale centre. Shot holes also seen.

## 3. Results

### 3·1. *Effect on germination and survival*

In blotter test germination was only slightly affected (table 1). The radicle emerged out in 19–29% seeds in five samples within 24 hr and in 55–75% seeds by 48 hr. The germination ranged from 73–92%. Seedling abnormalities such as seedlings without radicle and seedling with short radicle were observed and the abnormalities ranged from 1–8%. Germination was found to be better in seeds with superficial infection (ac. nos. 15,18) in comparison to other samples having a higher percentage of embryonal infection (ac. nos. 7, 9 and 17). Pycnidia formation was frequent on rotted seedlings and in ungerminated seeds.

The growth test using categorised seeds, seeds without microsclerotia and with microsclerotia in five samples, showed that seedling emergence was considerably affected (table 2) and ranged from 75–82% in seeds without microsclerotia (untreated) and 56–78% in seeds with microsclerotia. The germination was only 56–66% in samples with deep infection and was 77 and 78% for remaining samples with superficial infection. Survival of seedlings in samples with superficial infection was also high. Seedlings obtained from seeds without microsclerotia were more vigorous to start with than those from microsclerotial seeds.

Chlorine pretreatment in growth test promoted emergence. Pretreatment with 2% Cl accelerated germination in both the categories and was 70–97% in seeds without microsclerotia and 60–93% in seed with microsclerotia. 5% Cl-pretreatment was phytotoxic and germination showed significant decline (61–88%) in seeds without microsclerotia and was only 27–75% in seeds with microsclerotia. Seedling survival was also higher in 2% Cl pretreatment (table 2). The Cl-pretreatment (2%) brought about a slight improvement in germination even in the control sample but the phytotoxic effects of higher concentration of Cl pretreatment caused 36 and 37% decrease in germination and survival respectively as compared to those of 2% Cl treatment. 5% Cl treatment probably inhibited root growth as in petriplate experiment in 12–15% seeds. The embryo was observed to emerge after rupturing the seed coat at the chalazal end. The cotyledons in such cases turned green later on. The radicle remained lodged in the seed coat and its growth was negligible.

### 3·2. *Seedling symptom test*

Three categories of seeds *viz.*, (i) seeds without microsclerotia (ii) seeds with moderate number of microsclerotia and (iii) seeds with abundant microsclerotia were used in the water agar seedling test (table 3). Seedling from first category showed an initial vigorous growth but subsequently 20–25% of them became diseased. Seeds of third category failed to germinate in sample ac. nos. 7,9,17 and gave only 9 and 7 percent germination in seeds of ac. nos. 15 and 18 respectively. Ungerminated seeds were covered with heavy mycelial growth of *M. phaseolina*. In moderately infected seeds of second category the percentage of ungerminated seeds was 5–12. The seedlings were usually stunted as compared with those from seeds without microsclerotia. The incidence of seedling infection of *M. phaseolina* was high in ac. nos. 7,9,17 ranging from 51–70% and 37 and 19% in ac. nos. 15 and 18 respectively. The percentage of deformed seedlings was also

**Table 1.** Sequential studies on percentage germination, seedling abnormalities and disease symptoms of *Macrophomina phaseolina* in blotter test, 100 seeds per sample and 10 seeds per plate.

| Sample Number | 7 | | | | | | | 9 | | | | | | | 15 | | | | | | | 17 | | | | | | | 18 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days / Factors studied | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| *Macrophomina phaseolina* (%) | 0 | 0 | 4 | 9 | 15 | 22 | 22 | 0 | 0 | 4 | 10 | 18 | 23 | 24 | 0 | 0 | 0 | 3 | 9 | 15 | 15 | 0 | 0 | 2 | 15 | 24 | 26 | 29 | 0 | 0 | 0 | 3 | 12 | 14 | 17 |
| Radicle emergence | 25 | 74 | 81 | 81 | 81 | 81 | 21 | 68 | 89 | 91 | 91 | 92 | 92 | 19 | 65 | 89 | 89 | 89 | 91 | 91 | 19 | 55 | 72 | 72 | 80 | 88 | 88 | 29 | 75 | 79 | 81 | 87 | 92 | 92 | 92 |
| Germination | 0 | 0 | 31 | 69 | 77 | 77 | 81 | 0 | 0 | 25 | 78 | 78 | 78 | 78 | 0 | 0 | 29 | 70 | 89 | 90 | 90 | 0 | 0 | 0 | 19 | 44 | 69 | 73 | 0 | 0 | 50 | 71 | 87 | 92 | 92 |
| Seedling without radicle | 0 | 0 | 0 | 2 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 1 | 3 | 3 | 0 | 0 | 0 | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| Seedling with or short radicle | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 5 | 5 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Seedling rot | 0 | 0 | 0 | 3 | 7 | 7 | 9 | 0 | 0 | 0 | 2 | 7 | 11 | 12 | 0 | 0 | 0 | 4 | 6 | 6 | 7 | 0 | 0 | 1 | 9 | 13 | 13 | 16 | 0 | 0 | 0 | 7 | 10 | 13 | 13 |
| Seedling with pycnidia | 0 | 0 | 4 | 9 | 13 | 19 | 19 | 0 | 0 | 4 | 10 | 16 | 19 | 20 | 0 | 0 | 0 | 3 | 9 | 13 | 13 | 0 | 0 | 2 | 13 | 22 | 22 | 24 | 0 | 0 | 0 | 3 | 9 | 11 | 14 |
| Ungerminated seeds with pycnidia | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 4 | 5 | 0 | 0 | 0 | 0 | 3 | 3 | 3 |

**Table 2.** Data on seedling emergence and seedling survival beyond 10 weeks in six samples (1 control and 5 infected) in pot experiment. 100 seeds used per treatment

| Sample number | Seeds without microsclerotia | | | | | | Seeds with microsclerotia | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Emergence (%) | | | Survival (%) | | | Emergence (%) | | | Survival (%) | | |
| | Un-treated | Pretreated 2% | 5% | Un-treated | Pretreated 2% | 5% | Un-treated | Pretreated 2% | 5% | Un-treated | Pretreated 2% | 5% |
| 11 (Control) | 93 | 98 | 62 | 89 | 95 | 58 | ... | ... | ... | ... | ... | ... |
| 7 | 75 | 79 | 65 | 46 | 48 | 45 | 65 | 70 | 55 | 34 | 30 | 25 |
| 9 | 79 | 84 | 67 | 66 | 69 | 55 | 56 | 60 | 27 | 36 | 27 | 19 |
| 15 | 81 | 97 | 88 | 67 | 75 | 59 | 77 | 93 | 75 | 59 | 60 | 50 |
| 17 | 76 | 70 | 61 | 40 | 66 | 34 | 66 | 74 | 53 | 36 | 44 | 26 |
| 18 | 82 | 90 | 74 | 76 | 78 | 70 | 78 | 86 | 70 | 68 | 76 | 54 |

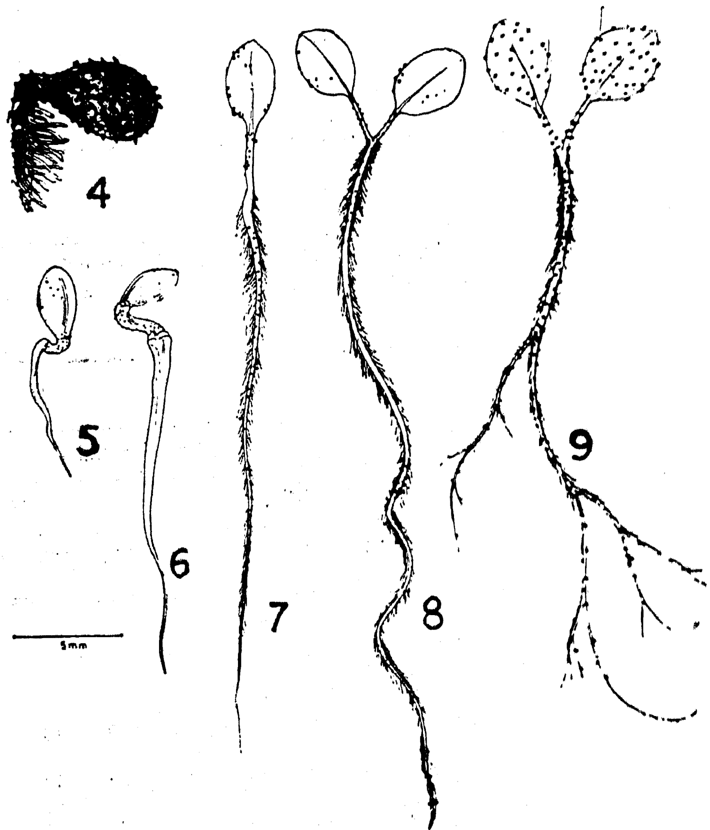
**Table 3.** Percentage of ungerminated seeds (US) and healthy (HS) deformed (DS) and infected seedlings (IS), infected with *Macrophomina phaseolina*, from non-microsclerotial and microsclerotial seeds having mild and heavy infection (100 seeds/sample).

| Sample No. | Seeds | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-microsclerotial | | | | Microsclerotial | | | | | | | |
| | | | | | Mild | | | | Heavy | | | |
| | US | HS | DS | IS | US | HS | DS | IS | US | HS | DS | IS |
| 11 (Control) | ... | 100 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 7 | 11 | 60 | 9 | 20 | 12 | 26 | 11 | 51 | 100 | 00 | 00 | 00 |
| 9 | 9 | 56 | 13 | 22 | 10 | 10 | 10 | 70 | 100 | 00 | 00 | 00 |
| 15 | 7 | 62 | 9 | 22 | 8 | 53 | 6 | 37 | 91 | 3 | 00 | 6 |
| 17 | 10 | 50 | 15 | 25 | 10 | 10 | 10 | 70 | 100 | 00 | 00 | 00 |
| 18 | 00 | 69 | 8 | 23 | 5 | 67 | 9 | 19 | 93 | 00 | 7 | 00 |

higher in the former as compared with those of the latter. It may be mentioned here that the fungus grew readily on agar medium and therefore, seeds with microsclerotia, moderate or heavy infection, caused seed failure of a high incidence of seedling infection in comparison to the seedling infection recorded in the blotter test.

## 3·3. *Symptoms and effect on host*

*M. phaseolina* caused seed rot, browning and rotting of seedlings in blotter (figure 4) and water agar tests. The initial infection usually resulted at the root shoot transition zone (figures 5,6) and progressed towards the root and shoot tips (figure 7). Browning and damping of root took place more readily than that of the shoot. Initially it manifested in the form of pale to brown streaks but later on the whole root turned brown and pulpy. In some cases when the seed coat having microsclerotia is carried alongwith the cotyledons during germination and remain attached with for a longer period, the infection spreads downwards from cotyledons to other pasts of the seedlings. Formation of microsclerotia was quite common on shoot and root (taproot and lateral roots) systems (figures 8, 9).




**Figures 4–9.** Diagrams to show symtoms of *M. phaseolina* on sesame seedling in blotter test. **4.** Rotting of seed with pycnidia and microsclerotia. **5, 6, 7** Seedlings showing streaks and microsclerotia. **8, 9.** Seedlings showing distribution of microsclerotia; note infection of primary as well as secondary roots in (9

In growth tests first disease symptoms were observed on the cotyledons after a fortnight. In a few days (by 20th day after germination) these became well marked as pale yellow to brown or blackish circular or oval and concentric spots. Each spot had a whitish area in the centre. The spots enlarged, became irregular and necrosis caused shot-holes. Similar spots were recorded on leaves after six weeks of germination (figure 3). Infected plants were weak and had leaves smaller than those on healthy plants. After 8 weeks almost every surviving plant from untreated as well as Cl-pretreated seeds developed symptoms of *M. phaseolina*. Infection on stem appeared as yellowish brown discoloured patches. Their periphery turned dark brown to black subsequently (figure 12). Pedicel and green capsules also revealed the occurrence of yellowish patches.

Under stereobinocular microscope mycelial growth was observed on leaf, stem, pedicel and capsule. Although no microsclerotia were seen on leaf surface, they occurred in abundance in black peripheral region of the patches on stem. Scattered microsclerotia were also seen on other parts of stem.

### 3·4. *Isolation and observation of fungus*

Isolations were made from seedlings and plants raised from untreated and pretreated seeds at regular intervals. They were classified into two categories *viz.*, rotted and healthy looking seedlings. The two types of seedlings, were treated separately for incubation. For the latter, 25 seedlings were used but for the rotted all the seedlings available on the day of setting the experiment were used which were usually more than 10.

Ten-day old rotting seedlings yielded *M. phaseolina* from all the parts including cotyledons. The incidence was more than 80% and usually higher in transition zone than the other components. Rotted seedlings from pretreated seeds yielded the pathogen in slightly low incidence in comparison to those of untreated seeds. In healthy looking seedlings, *M. phaseolina* was recorded in not more than 41% cases. Interestingly in such cases the incidence of pathogen recovery was higher in sample nos. 7 and 17 as compared to that of sample nos. 9, 15, 18.

Similar data were also collected on 50-day old plants which yielded *M. phaseolina* in very high percentage. No significant difference in percentages was recorded in plants from untreated and pretreated seeds in all the five samples. The infection percentage on different plant parts were also comparable.

Mature but not dead dry plants (75-day old) yielded the fungus in 100% cases from roots (figure 10) and transition zone in sample nos. 7,9,17 and 18. It was found to be 80 and 83 (roots) and 87 and 86 (transition zone) in plants from untreated and pretreated seeds of sample no. 15. The recovery of fungus was usually high from stem and leaves than the other plant parts *i.e.*, flower buds, flower (figure 11) and capsules.

*M. phaseolina* was also recovered in very high percentages from root, stem, whole capsule and its parts (Placenta, seed and septum) from dry plants in blotter test. Visibility of microsclerotia was slightly difficult on the hairy surface of capsule, whereas the placenta and septum turned black.

Dry plant parts were also examined for the presence of pathogen. Dry plants of all the five samples were harvested and examined with unaided eye or 10 × handlens. Microsclerotia were observed on and inside the tap root and stem from

base to tip (table 4). Lateral roots could not be split. The incidence of microsclerotia on roots and basal part of stem surface ranged from 78–100% and 70–100% respectively. In the interior part of root and basal stem it ranged from 9–14% and 24–53% respectively. Maximum infection (86%) was recorded on the entire surface of the root and stem (figure 12) (base to apex). However, a gradual decrease in its incidence was recorded inside the stem from its basal to the apical part (figure 13), incidence being 53% (base), 33% (middle) and 22% (apical part). In the remaining sample apical region yielded low percentage of infection on the outside as well as inside.

The surface and the interior of the capsules showed abundant microsclerotia on different parts including seeds (figures 14,15). A higher incidence of microsclerotia was observed in the basal and distal region of capsule surface (outer and inner), septa, placenta and seeds (table 5). Most of the seeds showed abundant microsclerotia and a thick net of black mycelium on the micropylar region (figure 15). The chalazal end was either free or showed mild infection. In septum the incidence of fungus was rather low (19–67%).

### 3·5. *Clearing and section cutting*

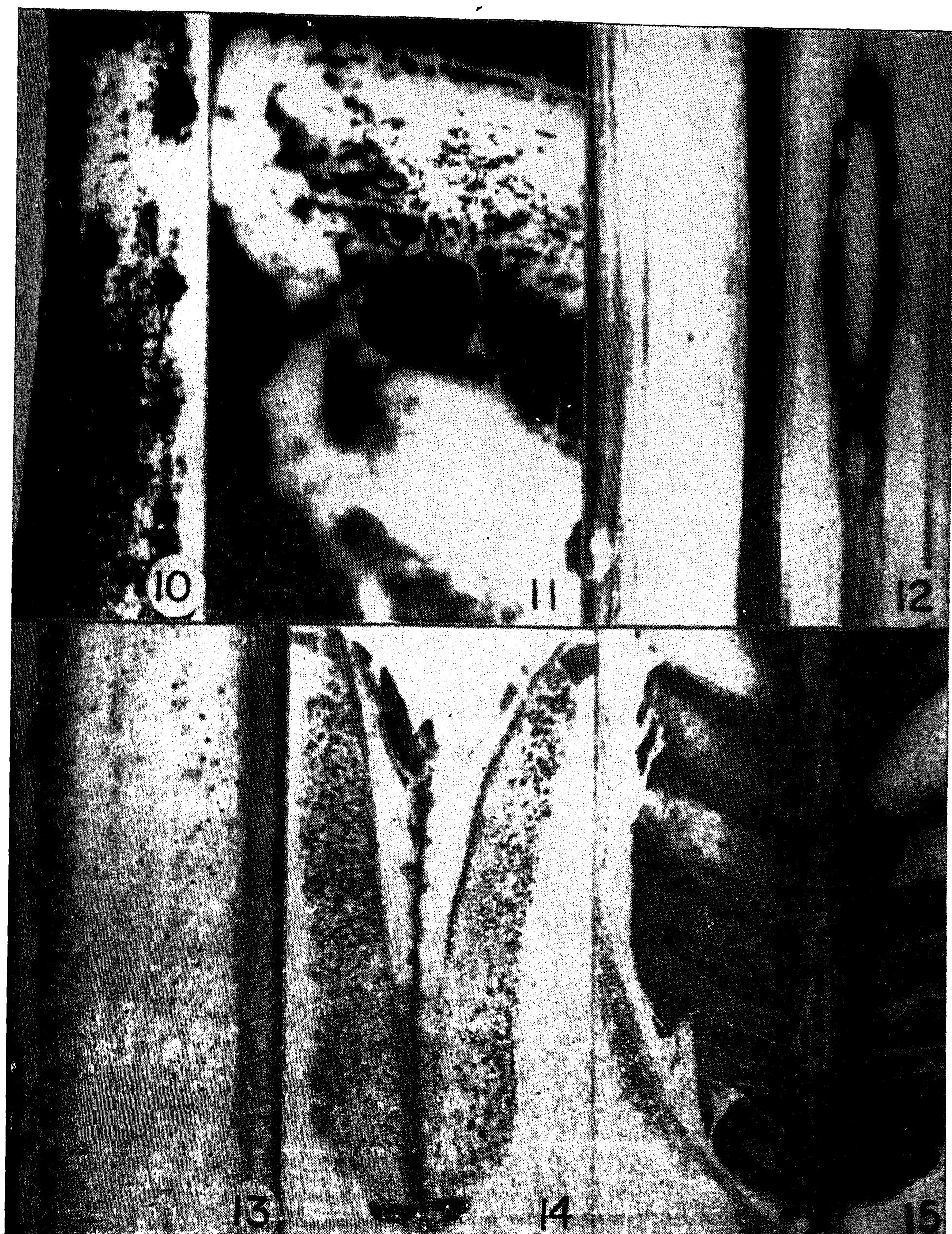
Cleared wholemount preparations of infected leaves showed abundant dark brown, branched and septate mycelium running across the leaf spot (figures 16,17). It traversed mostly parallel to veins. The host cells in infected regions were small, poor in pigments, cytoplasm and food materials. Microsclerotia were not observed.

The wholemount preparations (figure 18), transections and longisections (figures 19,20,21 and 22) showed the presence of mycelium in different tissues including xylem (figures 21,22) and pith ; inter and intracellular mycelium was observed to travel along the vessel length (figure 22). Microsclerotia were also observed in the pith cells (figure 19) and vessels (figure 20).

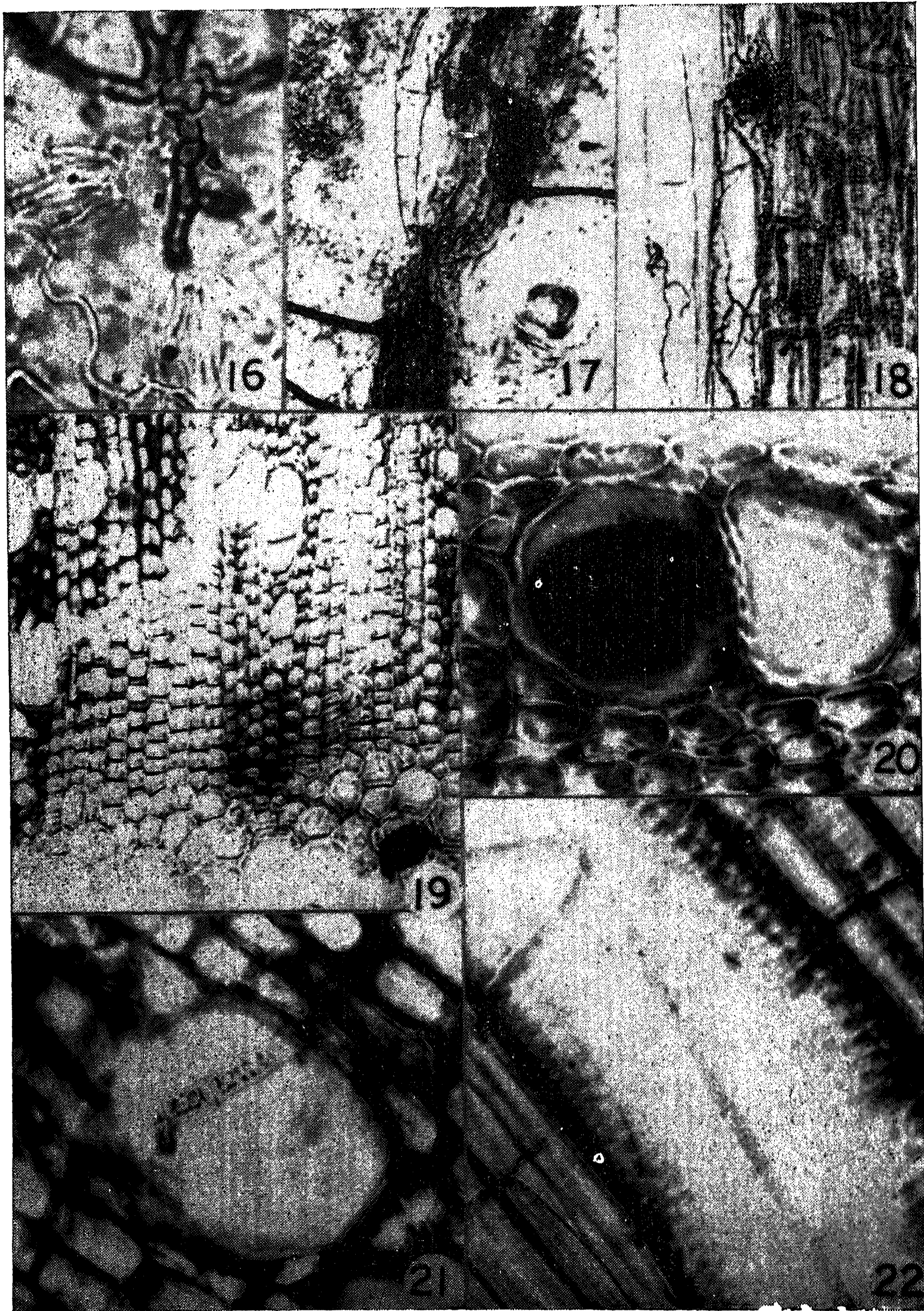
Seeds harvested from experimental plants also showed the occurrence of microsclerotia and mycelium in seed coat, endosperm and embryo as revealed by maceration and sections.

## 4. Discussion

Water agar seedling test and the growth test have revealed the transmission of *M. phaseolina* from seed to plant in all the five sesame seed samples tested presently. The fungus was isolated from rotting, wilting and healthy looking seedlings. The vegetative parts, floral buds, flowers and green fruits of experimental plants also yielded the fungus on incubation. Careful examination of root, stem and fruits from mature and dried plants showed the presence of mycelium and microsclerotia. The fungus was recovered from pedicel, calyx, septum, placenta, inner surface of capsule and seeds attached to the placenta. The infection usually spread from micropylar end onwards in the seeds of infected capsules. The systemic transmission of *M. phaseolina* from infected seeds is strongly supported. The mycelium, running longitudinally as well as horizontally and the microsclerotia, were recorded in pith, cortex and vascular cells in stem. The pith became hollow in infected plants probably due to the production of cellulolytic and pecto-




**Figures 10–15. 10, 11.** Root and flower after incubation on blotter. **10.** Root surface showing pycnidia and microsclerotia. **11.** Bilabiate flower surface of sesame showing microsclerotia. **12–15.** Stem and capsule of sesame showing infection of *M. phaseolina* in dried plants harvested at the end of growing season. **12.** Surface view of healthy and infected stem. **13.** Longitudinally split stem showing microsclerotia. **14.** Partially dehisced capsule; note the microsclerotia on surface. **15.** Interior of an infected capsule. Note the infection of placenta and seeds.




**Figures 16–22. 16–18.** Wholemounts of cleared infected leaf and stem peeling. **16·** Part of leaf surface showing mycelium in stomatal region. **17.** Part of leaf surface showing septate mycelium and its association with vein. **18.** Cleared peeling. from stem surface showing microsclerotia and inter- as well as intracellular mycelium **19–22.** T.S. and L.S. of infected stem. **19.** T.S. stem showing mycelium and microsclerotia. **20–21.** T.S. stem showing mycelium and microsclerotia in vessel, **22.** L.S. stem ; note mycelium traversing in vessel.

**Table 4.** Percentage occurrence of microsclerotia of *Macrophomina phaseolina* or vegetative parts of dried and harvested plants.

| Vegetative parts sample No. | Root | | | | Stem | | | |
|---|---|---|---|---|---|---|---|---|
| | Tap | | Basal | | Middle | | Apical | |
| | Surface | Inside | Surface | Inside | Surface | Inside | Surface | Inside |
| 7 | 86 | 13 | 86 | 53 | 86 | 33 | 86 | 2C |
| 9 | 100 | 10 | 100 | 35 | 100 | 30 | 75 | 15 |
| 15 | 82 | 9 | 76 | 26 | 41 | 5 | 30 | 5 |
| 17 | 78 | 11 | 70 | 24 | 60 | 15 | 40 | 11 |
| 18 | 100 | 14 | 92 | 40 | 82 | 34 | 60 | 10 |

**Table 5.** Percentage occurrence of *Macrophomina phaseolina* on dried capsule parts (after soaking in 70% ethanol for 24 hr)

| Capsule parts sample No. | Stalk | Capsule | | | | Placenta | | Seeds | | Septum |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Outer surface | | Inner surface | | Basal | Distal | Basal | Distal | |
| | | Basal | Distal | Basal | Distal | | | | | |
| 7 | 84 | 82 | 73 | 80 | 40 | 80 | 33 | 93 | 33 | 26 |
| 9 | 80 | 70 | 70 | 65 | 50 | 70 | 42 | 70 | 40 | 30 |
| 15 | 69 | 67 | 67 | 56 | 33 | 60 | 30 | 73 | 45 | 20 |
| 17 | 63 | 52 | 35 | 47 | 40 | 51 | 40 | 50 | 30 | 19 |
| 18 | 92 | 91 | 89 | 85 | 77 | 92 | 79 | 81 | 71 | 67 |

lytic enzymes in large quantities. This also accounts for easy breaking of stem in these plants.

*M. phaseolina* is a well-known soil inhibitant and Cougnèe (1963) reported the disease to be soil borne. Mehta (1951) suspected the possibility of transmission of *Sclerotinia bataticola* by sesame seed in India. Noble *et al* (1958) recorded the seed-borne nature of *M. phaseolina* on the basis of investigation of Mehta in their list of seed borne diseases, but were doubtful. Meiri and Solel (1963) observed that infected seeds of ≪ Ranner 15 ≫ yielded diseased seedlings and also observed the first site of infection in the collar region.

Fakir *et al* (1976) working with sunflower kernels have shown that *M. phaseolina* is highly pathogenic and pathogen could be isolated from seeds of some of the inoculated plants, but seeds of symptomless sunflower plants from naturally infected seeds did not yield *M. phaseolina*. This is in contrast to the present study and does not support the transmission of disease from seed to seedling. However, in their description it is not clearly stated whether these symptomless plants grew out of microsclerotial seeds. The present study not only provides the adequate

evidence of seed transmission of *M. phaseolina* in sesame but also clearly reveals that the seed-borne inoculum plays an important role in the spread as well as in the manifestation of disease.

Pycnidia formation was rare in blotter as well as agar test. Only on rotted seeds and seedlings, and in lesions on stem, pycnidia were frequently formed. Chidambaram and Mathur (1975) reported the production of pycnidia in 20 out of 58 non-sporulating isolates in water agar media. They tested 58 isolates of *M. phaseolina* from 20 plants. Present isolate from *Sesamum indicum* could be described as a moderately sporulating one.

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# Effect of water stress and sucrose on opening and longevity of flowers in gladiolus

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**Abstract.** The percentage of buds opening and flower longevity as affected by the availability of water and sucrose to cut spikes of gladiolus were studied. Uptake of sucrose solution and fresh weight changes in spikes were dependent on sucrose concentration. Marked reduction in uptake and fresh weight occurred when polyethylene glycol (PEG) was used as the stressing agent. In comparison, PEG failed to induce any significant change in the percentage of flower buds opening. Sucrose was essential for opening since the buds that failed to open in the control were caused to open in sucrose. Induced water stress did not curtail flower longevity at any given concentration of sucrose. Thus flower opening and longevity in gladiolus appear to be limited more by the availability of sucrose than water.

**Keywords.** Flower longevity ; flower opening ; gladiolus ; polyethylene glycol ; sucrose ; water stress.

## 1. Introduction

Studies have been carried out on the factors affecting water uptake and vascular blockage in cut flowers on account of their crucial role in maintaining freshness (Durkin and Kuc 1966; Marousky 1969; Gilman and Steponkus 1972; van Meeteren 1978; Rao and Mohan Ram 1982a). Water deficit causes early wilting of flowers (Marousky 1969; Paulin 1972; Mayak *et al* 1974). Mayak *et al* (1974) noted a sharp decline in the water potential of petal tissues in wilting cut roses but not in intact flowers. Water stress has been identified as the cause of failure of flower opening in the spikes of iris stored at low temperature for four days and then for one additional day at 22° C (Mayak and Halevy 1971). Appreciable bud opening has, however, been recorded in gladioli and chrysanthemums stored in cold using sucrose alone or in combination with silver nitrate or gibberellic acid (Kofranek *et al* 1975; Kofranek and Halevy 1976; Rao and Mohan Ram 1979, 1981, 1982b).

In spite of the development of successful techniques to handle cut flowers, our understanding of water requirement of opening flowers and their ability to withstand storage has remained incomplete. The property of sucrose to act as an antidesiccant when supplied before storage, in addition to its metabolic role is

still unclear. For example, gladiolus spikes, given a pulse treatment with sucrose before storage, open satisfactorily on subsequent transfer to water (Mayak *et al* 1973; Bravdo *et al* 1974). However if the spikes are first stored dry, it becomes necessary to provide gibberellin plus sucrose subsequently to ensure full opening (Rao and Mohan Ram 1979, 1982b). This paper discusses the importance of water and sucrose in flower opening and longevity.

## 2. Material and methods

Spikes of *Gladiolus natalensis* Hort. were obtained from a commercial grower in New Delhi at the green-bud stage (harvested one day before the corolla of the lowermost bud emerged from the enveloping bracts and the tip just became visible). The spikes were stored dry for 24 hr after harvest at 20° C to facilitate a larger uptake of the pulsing solution (Rao and Mohan Ram 1981a). Twenty spikes each were pulsed for 48 hr with sucrose solution (0·25 M and 0·5 M) to eliminate any effects caused by a low amount of carbohydrates in the spike. These were then transferred to (i) water (control), (ii) sucrose solution of the same concentration as was used for pulsing and (iii) polyethylene glycol solution (PEG, MW 6000, Sigma Chemical Co., USA) having a water potential similar to the pulsing solution (0·25 M sucrose = −7 bars; 0·5 M sucrose = −15 bars). In all six sets, each with 20 spikes were set up in glass tubes (2·5 × 15·0 cm) containing 40 ml of the test solution. The solutions were prepared using glass-distilled water. One additional set of spikes held continuously in water served as the control. The spikes were kept in a chamber at 20 ± 2° C with 14 hr photoperiod (under cool-white daylight fluorescent tubes giving 500 lux).

The number of flowers opening and withering per spike were recorded daily. The longevity of individual flowers on the spike (the period between flower opening and withering) was recorded. The term 'uptake' used here refers to the volume of the solution taken up and 'fresh weight change' to the differences in fresh weight of a spike over a given period. Tubes containing different solutions but without spikes served as controls to measure loss caused by evaporation. Confidence intervals of the means were determined at $P \leq 0·05$.

## 3. Results

### 3.1. *Rate of uptake*

During the period of pulsing a high initial uptake was recorded for the control (table 1). Spikes kept in 0·25 M sucrose showed a greater uptake than those in 0·5 M during this period. By day 2 the spikes pulse-treated with sucrose and transferred to water took up a larger volume of solution than those continuously held in sucrose and the control. A marked decrease was observed in the amount taken up by the pulsed spikes on day 4 as compared to that on day 2. It was, however, still higher than that for spikes kept continuously in sucrose. A decrease in uptake was noted in the latter during the same period, although it exceeded

**Table 1.** Effect of water stress on rate of uptake (in ml) by gladiolus spikes.

| Treatments | Days after pulse treatment | | | | | | | | | | | | Total uptake | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0* | | 2 | | 4 | | 6 | | 8 | | 10 | | | |
| | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI |
| Control | 24·1 | 2·3 | 8·8 | 1·4 | 5·1 | 0·7 | 5·6 | 2·8 | 1·5 | 2·6 | 2·4 | 0·8 | 47·5 | 2·3 |
| S1W | 18·9 | 1·9 | 13·5 | 1·4 | 11·6 | 1·3 | 6·8 | 0·9 | 4·5 | 1·5 | 3·0 | 1·3 | 58·3 | 2·4 |
| S2W | 12·3 | 0·9 | 14·3 | 1·7 | 7·5 | 0·6 | 6·8 | 0·4 | 5·0 | 0·6 | 5·1 | 0·5 | 51·1 | 3·4 |
| S1C | 15·9 | 1·2 | 10·2 | 1·0 | 8·1 | 1·1 | 3·8 | 1·1 | 2·7 | 1·4 | 0·7 | 1·2 | 41·4 | 3·1 |
| S2C | 12·9 | 0·8 | 7·5 | 1·4 | 5·2 | 1·3 | 2·9 | 1·0 | 1·8 | 0·5 | 1·1 | 0·3 | 31·4 | 3·6 |
| S1P | 16·2 | 1·1 | 0·5 | 0·6 | 1·6 | 0·5 | 0·8 | 0·2 | 0·8 | 0·1 | 0·6 | 0·2 | 20·6 | 1·7 |
| S2P | 11·7 | 0·7 | 0·0 | 0·3 | 0·8 | 0·2 | 0·5 | 0·2 | 0·6 | 0·3 | 0·3 | 0·2 | 14·0 | 0·9 |

* During pulse treatment which lasted 48 hr.
$\bar{X}$ Mean values.
CI Confidence interval calculated at $P \leqslant 0 \cdot 05$.
S1W, S2W Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to water.
S1C, S2C Spikes held continuously in 0·25 M and 0·5 M sucrose.
S1P, S2P Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to PEG at —7 bars and —15 bars, respectively.

the amount in the PEG-treated spikes. Spikes transferred from sucrose (both concentrations) to PEG showed practically no uptake during the first two days. Subsequently, however, a very low uptake was recorded in these spikes; the uptake by spikes held in PEG at −7 bars was slightly higher than that at −15 bars.

During the entire period of experimentation pulse-treatment with sucrose (0·25 M) followed by transference to water resulted in a significantly greater uptake over the control (table 1). However, in the spikes held continuously in sucrose the magnitude of uptake was lower. Treatments with PEG markedly curtailed uptake as compared to the control and other treatments.

### 3.2. *Changes in fresh weight*

As with uptake, the maximal fresh weight increment during the pulsing period was noted in the control, followed by that in spikes treated continuously or pulsed with sucrose at 0·25 M and 0·5 M, respectively (table 2). Whereas after two days the control spikes attained a negative fresh weight change, the spikes which were pulsed with sucrose and transferred to water or sucrose continued to show a positive fresh weight change. Spikes transferred to PEG showed a negative fresh weight value, much lower than that of the control. In general the spikes which were held continuously in 0·5 M sucrose showed a lower fresh weight change as compared to the spikes treated with 0·25 M. In all the treatments, the lowest fresh weight change was recorded on days 6 and 8.

The overall highest fresh weight was observed in spikes pulsed with sucrose at either concentration and transferred to water (table 2). Thus the control exhibited the lowest fresh weight. Low fresh weight was also observed in spikes treated with sucrose (0·5 M) continuously or stressed with PEG at either concentration.

### 3.3. *Percentage of flower buds opening*

A significantly higher percentage of flower buds opened in all the treatments over the control (table 3). Among the treatments the percentage of flower buds opening did not vary except in spikes pulsed with sucrose at both the concentrations and transferred to PEG which showed slightly lower opening as compared with those pulsed with sucrose (0·25 M) and transferred to water.

### 3.4. *Flower longevity*

A study of the longevity of individual flowers at different positions on the spike showed differences between the control and those pulsed with sucrose and transferred to either water or sucrose (table 4). In spikes stressed with PEG at both the concentrations there was a continuous increase in longevity from flowers 1–5. Interestingly when the mean longevity of flowers 1–5 was compared, it turned out that the lowest longevity was recorded for the control (2·8 days). The longevity of flowers in spikes pulsed with a particular concentration of sucrose and

**Table 2.** Effect of water stress on changes in fresh weight (g) by gladiolus spikes.

| | Days after pulse treatment | | | | | | | | | | | | Total fresh weight change | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0* | | 2 | | 4 | | 6 | | 8 | | 10 | | | |
| Treatments | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI | $\bar{X}$ | ± CI |
| Control | 9·8 | 1·3 | —0·8 | 1·3 | —3·7 | 0·8 | —5·8 | 0·8 | —6·2 | 0·6 | —4·0 | 0·7 | —10·6 | 1·6 |
| S1W | 7·7 | 0·6 | 5·6 | 0·6 | 1·3 | 0·3 | —4·2 | 0·7 | —6·5 | 0·7 | —5·9 | 0·3 | — 1·8 | 1·1 |
| S2W | 5·4 | 0·6 | 8·4 | 1·0 | —0·4 | 0·5 | —2·8 | 0·5 | —5·0 | 0·7 | —4·7 | 0·3 | 0·8 | 0·7 |
| S1C | 7·3 | 0·5 | 2·2 | 0·5 | 0·3 | 0·7 | —3·1 | 0·6 | —5·0 | 0·5 | —4·9 | 0·5 | —3·1 | 1·3 |
| S2C | 5·1 | 0·5 | 1·2 | 1·0 | —0·5 | 0·9 | —2·8 | 0·7 | —4·5 | 0·6 | —4·3 | 0·4 | —5·9 | 2·3 |
| S1P | 8·0 | 0·7 | —3·0 | 0·3 | —2·3 | 0·3 | —3·5 | 0·2 | —3·3 | 0·3 | —3·2 | 0·2 | —7·3 | 0·6 |
| S2P | 5·6 | 0·4 | —3·0 | 0·2 | —2·2 | 0·2 | —3·4 | 0·1 | —3·0 | 0·2 | —3·2 | 0·2 | —9·2 | 0·4 |

* During pulse treatment which lasted 48 hr.
$\bar{X}$ Mean values.
CI Confidence interval calculated at $P \leqslant 0·05$.
S1W, S2W Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to water.
S1C, S2C Spikes held continuously in 0·25 M and 0·5 M sucrose.
S1P, S2P Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to PEG at —7 bars and —15 bars, respectively.

**Table 3.** Effect of water stress on the percentage of flower buds opening in gladiolus.

| Treatments | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | | S1W | | S2W | | S1C | | S2C | | S1P | | S2P | |
| $\bar{X}$ | $\pm$ CI | $\bar{X}$ | $\pm$ CI | $\bar{X}$ | $\pm$ CI | $\bar{X}$ | $\pm$ CI | $\bar{X}$ | $\pm$ CI | $\bar{X}$ | $\pm$ CI | $\bar{X}$ | $\pm$ CI |
| 54·0 | 3·0 | 80·0 | 5·0 | 75·0 | 5·0 | 76·0 | 4·0 | 75·0 | 4·0 | 69·0 | 3·0 | 69·0 | 4·0 |

$\bar{X}$ Mean values.

CI Confidence interval calculated at $P \leqslant 0{\cdot}05$.

S1W, S2W Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to water.

S1C, S2C Spikes held continuously in 0·25 M and 0·5 M sucrose.

S1P, S2P Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to PEG at —7 bars and —15 bars, respectively.

**Table 4.** Effect of water stress on flower longevity* in gladiolus.

| Flower number | Treatments | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | S1W | S2W | S1C | S2C | S1P | S2P |
| 1 | 2·6 | 3·8 | 4·2 | 4·0 | 4·2 | 3·2 | 3·8 |
| 2 | 2·6 | 3·6 | 4·2 | 4·0 | 4·2 | 3·4 | 4·0 |
| 3 | 2·6 | 3·4 | 4·2 | 3·8 | 4·6 | 3·6 | 4·6 |
| 4 | 3·0 | 3·6 | 4·2 | 3·8 | 4·8 | 3·8 | 4·8 |
| 5 | 3·2 | 3·8 | 4·4 | 4·0 | 4·6 | 4·0 | 5·0 |
| 6 | 2·6 | 3·8 | 4·4 | 4·4 | 4·4 | 4·6 | ... |
| 7 | ... | 4·0 | ... | ... | ... | ... | ... |
| $\bar{X}$ | 2·8 | 3·6 | 4·2 | 3·9 | 4·6 | 3·6 | 4·4 |

* in days.

$\bar{X}$ Mean longevity of flowers (1–5).

S1W, S2W Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to water.

S1C, S2C Spikes held continuously in 0·25 M and 0·5 M sucrose.

S1P, S2P Spikes pulsed with 0·25 M and 0·5 M sucrose for 48 hr and later transferred to PEG at —7 bars and —15 bars, respectively.

transferred to water, sucrose or PEG was more or less similar. For example, at 0·25 M of sucrose, it ranged from 3·6 to 3·9 days and at 0·5 M of sucrose it varied between 4·2 and 4·6 days in different treatments.

## 4. Discussion

A study of the effect of water stress on gladiolus indicated that uptake of the test solution and fresh weight change during the initial period of pulsing with sucrose were related to the concentration of the solution. Whereas the lower concentration of sucrose itself reduced initial uptake compared to the control, doubling it did not result in a proportional decrease. A similar result has been recorded by Bravdo *et al* (1974) who observed uptake even from a solution with 50% sucrose concentration. The subsequent absorption of liquid was dependent not only on the water potential of the transfer solution but also on the nature of the transfer osmoticum. Thus, when the spikes were held in PEG instead of sucrose of similar water potential, there was a steep drop in uptake.

The fresh weight change of the spikes showed a direct relationship with the water potential of the transfer solution. The sucrose-pulsed spikes which were transferred to water maintained higher fresh weight. Halevy and Mayak (1974) have shown that sucrose decreases the water potential of the petals and enhances their ability to absorb water.

In comparison with water, the uptake of which was markedly curtailed by PEG, the availability of sucrose was found to be a major factor in bud opening. In all the treatments a higher percentage of opening over the control was obtained. Stress pronouncedly affected uptake and fresh weight but not the percentage of flower buds opening. This is quite remarkable in the light of the finding by Goldschmidt and Huberman (1974) that citrus petals have a very large water requirement during opening (highest fresh weight was recorded) and in view of the reported failure of flower bud opening under water stress conditions (Mayak and Halevy 1971). Thus, flower opening in gladiolus appears to be limited more by the availability of sucrose than water, especially because of the ability of the newly opening buds to draw out water from the older open flowers and cause their premature withering (Rao and Mohan Ram 1982a). Our recent study has also shown that green-bud spikes lack adequate reserves of carbohydrates and that this is one of the principal causes of poor opening (Rao and Mohan Ram 1981).

It is significant that in the present work induced water stress did not curtail flower longevity at any given concentration of sucrose. In addition to its role as a respiratory substrate (Coorts 1973), sucrose has been shown to enhance the effect of cytokinins, and counter the deleterious effects of ethylene and abscisic acid (Borochov *et al* 1976a; Mayak and Dilley 1976). Sucrose also reduced the endogenous levels of abscisic acid in cut rose flowers (Borochov *et al* 1976b). Spikes treated continuously with sucrose showed higher longevity than PEG-treated spikes probably because of greater availability of sugar.

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# Petal venation in *Trigonella* (papilionaceae)

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**Abstract.** Petal venation of nine species of *Trigonella* has been worked out. A positive correlation has been found between length or area and the number of dichotomies but no correlation is found with breadth. In all the species corolla is of simple type except *T. polycerata* in which it is of medicagoid type. Among the different types of anastomoses *C* and *D* types are of most frequent occurrence and other types are species specific with a low range of variation.

**Keywords.** Petal venation ; papilionaceae ; trigonella.

## 1. Introduction

The analysis of petal venation received the attention of various workers in tracing phylogeny after the remark that petal venation shows both simplicity and diversity. Petal venation of regular corolla received the attention of various workers (Arnott and Tucker 1963, 1964 ; Banerji and Mukherji 1970 ; Banerji 1972) but that of irregular corolla received only the attention of Datta and Saha (1968) and Subramanyam and Nair (1973). Datta and Saha (1968) reported important differences at specific level in types of anastomoses and their pattern of distribution on standard, wing and keel petals of four species ( *Butea frondosa*, *Cajanus cajan*, *Dolichos lablab* and *Erythrina indica*) belonging to tribe phaseolae. Hence from the perusal of the literature it appears that venation of petals may be significant at specific level and since no work is available on the species of *Trigonella*, therefore, in this paper petal venation of nine species has been worked out.

## 2. Materials and methods

Flower buds for the present study were either collected locally or procured from places as mentioned below :—

| | Species | Place of collection |
|---|---|---|
| 1. | *Trigonella arabica* Dehile | Bet Dagan, Israel. |
| 2. | *T. caerulea* Ser. | Ontario, Canada. |
| 3. | *T. callicerasoites* Fish | Ontario, Canada. |

| Species | Place of collection |
|---|---|
| 4. *T. corniculata* Linn. | Meerut, India. |
| 5. *T. cretica* (L.) Boiss. | Ontario, Canada. |
| 6. *T. gracilis*, Benth. | Nainital, India. |
| 7. *T. polycerata*, Linn. | Meerut, India. |
| 8. *T. stellata* Forsk. | Jerusalem, Israel. |
| 9. *T. suavissima* Lindl. | Canberra, Australia. |

Petals from fully mature flowers were cleaned in lactic acid, stained in 1% aqueous safranin and mounted in glycerine. Twenty-five petals of each species were studied. Drawing of each petal was subdivided equally into basal, central and peripheral regions. Mean number of dichotomies and anastomoses was calculated in each sector. Area of the petal was measured by planimeter. For describing the types of anastomoses classification of Arnott and Tucker (1963) is mainly followed.

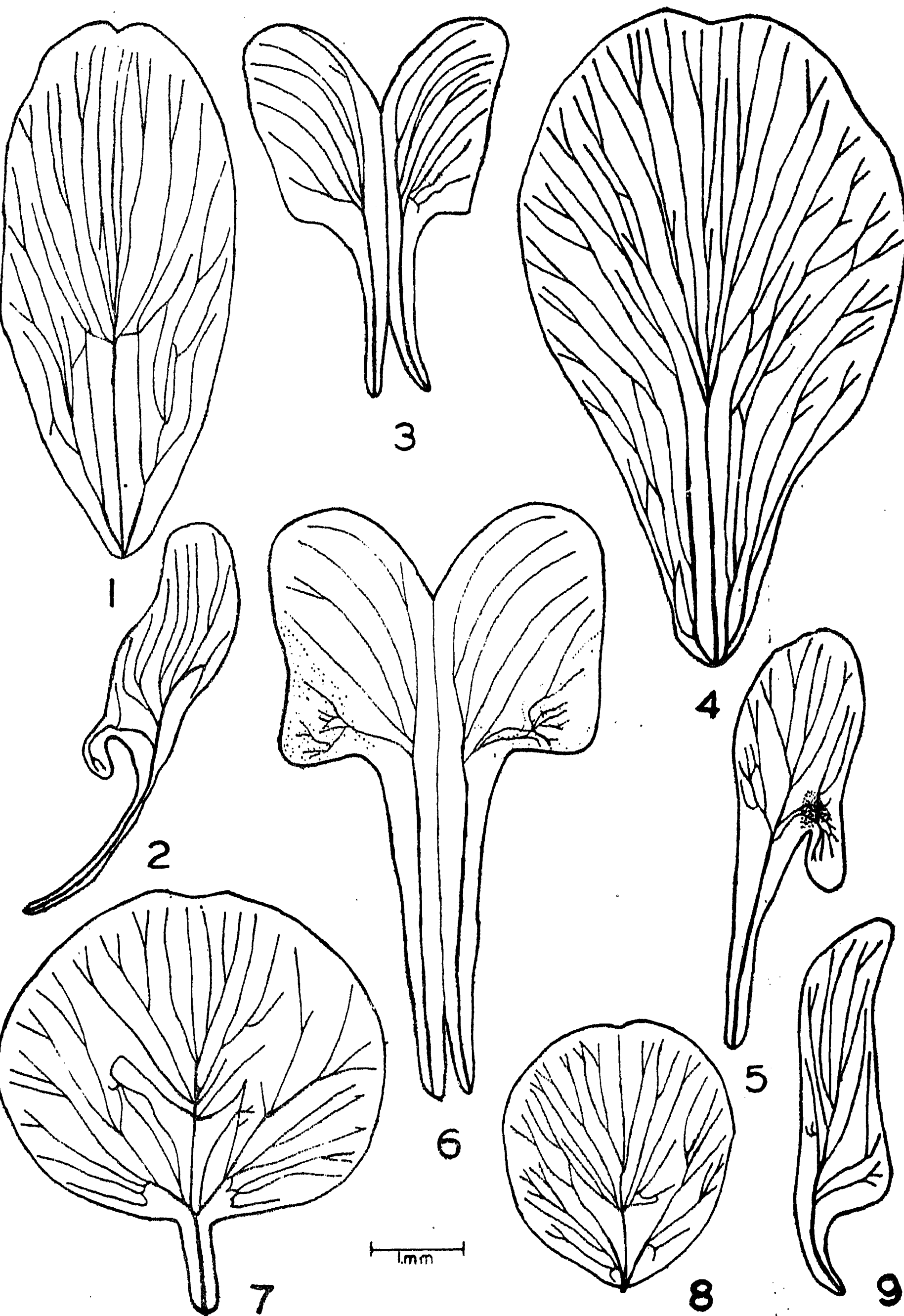
## 3. Observations

Each flower consists of a standard, two keel and two wing petals which vary in shape, size and structure (figures 1–3). Standard petal is symmetrical while the other petals are asymmetrical. Keel and wing petals are clawed (figures 2–3, 5–6), but standard petal is generally nonclawed except *T. arabica*, *T. gracilis* (figure 7) and *T. callicerasoites*. Each petal receives a single trace which branches after the separation of the individual petal (figures 1–2).

Standard petal is generally obovate in shape (figure 4) except *T. gracilis*, *T. cretica* (figure 8) and *T. callicerasoites* in which it is elliptic. Apex is notched in all the species. The bundle that enter the standard petal branches into one median and two costal veins in the basal region (figures 4, 8). However, in *T. gracilis* (figure 7) it branches at the base of the limb. Out of three veins, costal vein branches further dichotomously but the median vein first branches trichotomously and then dichotomously (figures 7–8). However, in few petals of *T. polycerata* median vein shows first dichotomous branching then trichotomous and further dichotomous branching.

The bundle of the keel and wing petals branches dichotomously at the junction of limb and claw (figures 2–3, 5–9). In *T. caerulea* and *T. arabica* wing petals possess ridges and grooves at the distal end and the veins end freely in the groove region. The wing petals of *T. polycerata* (figure 5) and *T. callicerasoites* are serrated at the distal end.

In all the species wing petals at the junction of the limb and claw possess a spur like process which is generally tubular except *T. stellata* and *T. cretica* (figure 9), in which it is flattened. This spur like process receives supply from the main bundle of the petal (figure 3). Wing petal of *T. polycerata* has a tooth like process also at the distal end. This tooth like process is nectariferous and vascular bundles in this region are found to be inversely oriented with respect to the petal. Keel petals of this species has a pocket like structure which receives the tooth like process of the wing petal (figures 5–6). In all the types of petals venation is mainly open dichotomous (figures 1–9).




Figures 1–9. Venation in petals of *Trigonella*. **1–3.** standard, keel and wing petals of *T. caerulea*, **4–6.** standard, wing and keel petal of *T. polycerata*, **7.** standard petal of *T. gracilis*, **8.** standard of *T. suavissima*, **9.** wing petal of *T. cretica*.

Table 1.

| Name of the species/ type of Petal | Mean area in mm² | Mean length in mm ± SE | Mean breadth in mm ± SE | Mean number of dichotomies ± S E | | | |
|---|---|---|---|---|---|---|---|
| | | | | Basal | Central | Peripheral | Total |
| 1. *T. arabica* | | | | | | | |
| Standard | 17·10 | 5·68± ·19 | 4·19± ·13 | 13·60± ·65 | 15·20±1·15 | 14·6 ± ·53 | 44·62±1·07 |
| Wing | 6·06 | 5·53± ·12 | 1·64± ·33 | 5·00± ·95 | 10·60±1·04 | 5·40± ·45 | 21·00±1·02 |
| Keel | 4·87 | 5·24± ·28 | 1·81± ·03 | 2·00± ·69 | 13·00±1·22 | 2·60±0·60 | 14·00±1·96 |
| 2. *T. callicerasoites* | | | | | | | |
| Standard | 14·89 | 5·27± ·27 | 4·21± ·19 | 11·6 ± ·89 | 11·8 ±1·36 | 9·6 ± ·60 | 33·0 ±1·19 |
| Wing | 6·25 | 5·87± ·43 | 1·59± ·33 | 1·40± ·88 | 15·6 ± ·92 | 5·4 ± ·21 | 26·4 ±1·94 |
| Keel | 6·68 | 6·41±1·24 | 1·88± ·65 | 3·0 ±1·28 | 11·8 ± ·99 | 0·8 ± ·25 | 15·6 ±0·91 |
| 3. *T. caerulea* | | | | | | | |
| Standard | 8·90 | 4·69± ·42 | 2·49± ·11 | 7·0 ±1·02 | 15·0 ± ·92 | 3·2 ± ·65 | 25·2 ±1·34 |
| Wing | 2·00 | 4·40± ·39 | 0·87± ·04 | 0 | 9·2 ± ·18 | 1·4 ± ·11 | 10·6 ±0·60 |
| Keel | 2·63 | 4·04± ·21 | 1·24± ·08 | 0 | 8·8 ± ·78 | 1·0 ± ·06 | 8·9 ±0·77 |
| 4. *T. corniculata* | | | | | | | |
| Standard | 15·88 | 6·41± ·35 | 4·16± ·32 | 14·8 ± ·83 | 8·2 ±1·12 | 3·6 ± ·59 | 25·2 ±1·90 |
| Wing | 6·81 | 5·98± ·30 | 1·84± ·07 | 4·6 ± ·96 | 11·2 ±1·17 | 2·2 ± ·28 | 18·0 ±1·01 |
| Keel | 8·50 | 6·63± ·27 | 2·09± ·008 | 5·57± ·74 | 7·75±1·19 | 0 | 13·50± ·43 |
| 5. *T. cretica* | | | | | | | |
| Standard | 6·83 | 3·64± ·22 | 2·72± ·10 | 12·80± ·65 | 9·60±2·0 | 2·2 ± ·52 | 26·6 ±2·26 |
| Wing | 2·31 | 3·81± ·25 | 0·97± ·04 | 4·00±1·04 | 5·00± ·98 | 1·0 ± ·06 | 10·0 ±1·31 |
| Keel | 2·72 | 3·72± ·11 | 1·21± ·02 | 3·00±0·62 | 7·20± ·59 | 0·40± ·31 | 10·60± ·45 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6. *T. gracilis* | | | | | | | |
| Standard | 10·98 | 4·36± ·11 | 4·05± ·04 | 14·00± ·57 | 18·00± ·36 | 12·08± ·06 | 44·06± ·38 |
| Wing | 2·91 | 3·31+ ·41 | 1·35+ ·03 | 2·75+ ·03 | 13·25+ ·73 | 4·25+ ·41 | 20·25+ ·42 |
| Keel | 2·57 | 3·47+ ·36 | 1·36+ ·52 | 0 | 12·00± ·04 | 0 | 12·00± ·04 |
| 7. *T. polycerata* | | | | | | | |
| Standard | 16·24 | 6·97± ·24 | 3·84± ·20 | 10·81±1·21 | 27·20±2·16 | 21·20±1·08 | 61·20±2·36 |
| Wing | 3·37 | 4·87± ·16 | 1·34± ·04 | 0 | 17·20±1·80 | 5·80±0·19 | 23·00±1·76 |
| Keel | 3·06 | 4·78± ·35 | 1·31± ·21 | 0 | 8·40±0·60 | 2·00±0·39 | 10·80±0·59 |
| 8. *T. stellata* | | | | | | | |
| Standard | 7·50 | 4·25± ·03 | 2·69± ·07 | 9·40± ·78 | 14·00±1·33 | 4·40± ·59 | 27·40±1·04 |
| Wing | 2·25 | 3·97± ·07 | 1·07± ·06 | 0·60± ·28 | 10·60±0·45 | 2·00± ·48 | 13·20± ·59 |
| Keel | 2·03 | 3·37± ·25 | 1·15± ·01 | 0 | 8·60± ·78 | 0·80± ·24 | 9·40± ·45 |
| 9. *T. suavissima* | | | | | | | |
| Standard | 5·92 | 3·54± ·40 | 2·40± ·06 | 9·0 ±1·58 | 17·00±1·34 | 15·50±1·57 | 41·50±2·02 |
| Wing | 2·16 | 3·58± ·22 | 0·97± ·03 | 4·20±1·04 | 10·40±1·29 | 3·40±0·35 | 18·40±2·29 |
| Keel | 2·30 | 3·30+ ·02 | 1·14+ ·03 | 0·20+0·16 | 10·40+0·91 | 1·80+0·29 | 12·40+2·34 |

### 3.1. *Dichotomies*

Table 1 shows mean area, mean length and mean breadth of the different types of petals and mean number of dichotomies in different regions with standard error. A positive correlation has been found in all the species except *T. caerulea*, *T. corniculata* and *T. callicerasoites* between area and number of dichotomies (figure 10). Similarly a positive correlation of dichotomies is also found with length except *T. corniculata, T. gracilis* and *T. suavissima* (figure 11). No correlation is found with breadth (figure 12).

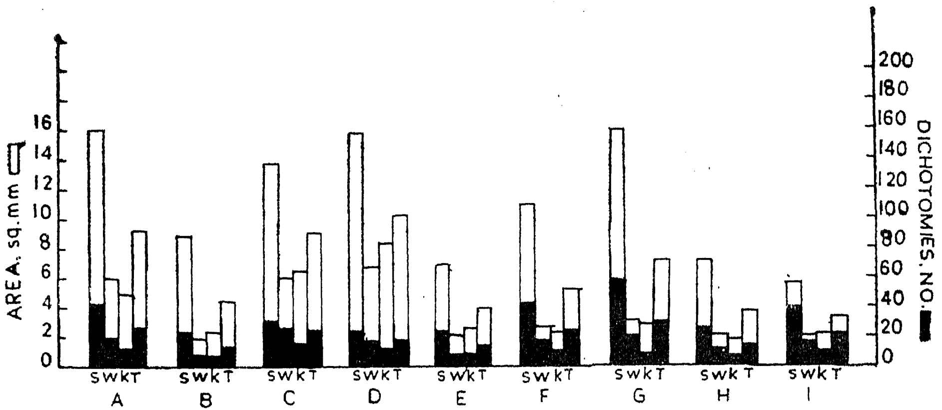



Figure 10. Histogram comparing the average area with mean number of dichotomies of standard (s), wing (w), keel (k) and total corolla (T) in different species.

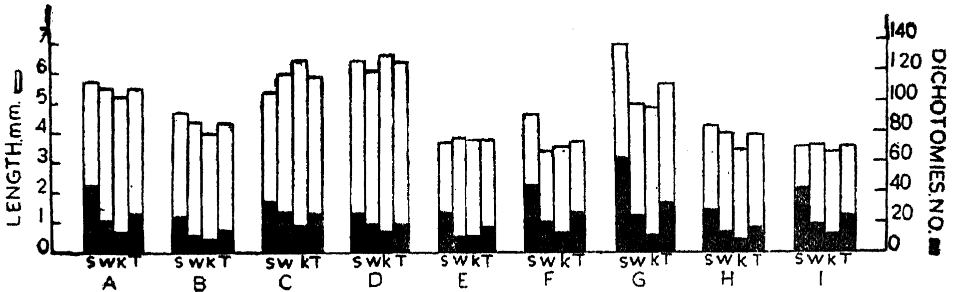



Figure 11. Histogram comparing the average length with the mean number of dichotomies of standard (s), wing (w), keel (k) and total corolla (T) in different species.

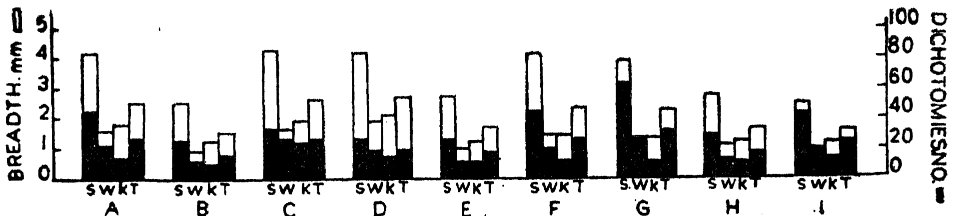



Figure 12. Histogram comparing the average breadth with the mean number of dichotomies of standard (s), wing (w), keel (k) and total corolla (T) in different species.

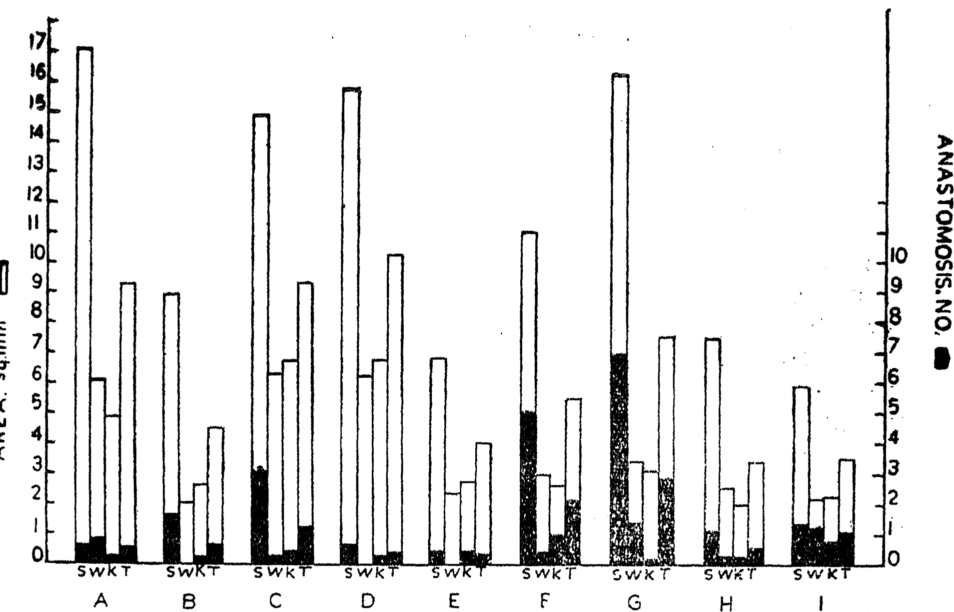



**Figure 13.** Histogram comparing the average area with the mean number of anastomoses of standard (s), wing (w), keel (k) and total corolla (T) in different species.

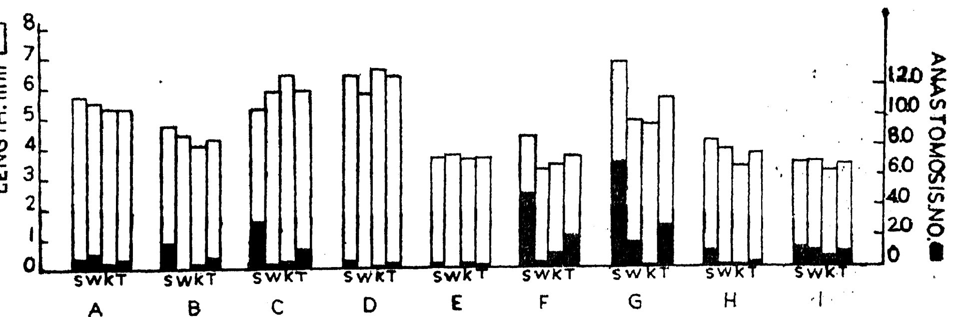



**Figure 14.** Histogram comparing the average length with the mean number of anastomoses of standard (s), wing (w), keel (k) and total corolla (T) in different species.

*Abbreviations* : A = *T. arabica* , B = *T. caerulea* ; C = *T. callicerasoites* ; D = *T. corniculata* ; E = *T. cretica* ; F = *T. gracilis* ; G = *T. polycerata* ; H = *T. stellata* ; I = *T. suavissima.*

## 3.2. *Anastomoses*

The open dichotomous venation becomes complicated at places by vein fusions. The percentage of petals showing vein fusions varies from 20–66·6% (table 2). A slight positive correlation has been observed between the length or area of petals and the number of anastomoses (figures 13–14). In most of the petals anastomosing is generally found at one point. Among the different types of anastomoses *C* and *D* types are of most frequent occurrence in all the species (table 3). In all the species vein anastomoses are generally concentrated in the

**Table 2.** Percentage of petals showing anastomoses.

| Sl. No. | Name of the species | Total (%) | Points of anastomosis | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5–10 |
| 1. | *T. arabica* | 40·0 | 66·6 | 33·34 | .. | .. | .. |
| 2. | *T. callicerassoites* | 53·4 | 37·5 | 25·00 | 25·0 | 12·5 | .. |
| 3. | *T. caerules* | 40·0 | 66·6 | 33·34 | .. | .. | .. |
| 4. | *T. corniculata* | 20·0 | 66·0 | 33·0 | .. | .. | .. |
| 5. | *T. cretica* | 20·0 | 66·0 | 33·0 | .. | .. | .. |
| 6. | *T. gracilis* | 71·4 | 80·0 | 10·0 | .. | 10·0 | .. |
| 7. | *T. polycerata* | 66·6 | 20·0 | 30·0 | .. | 10·0 | 40·0 |
| 8. | *T. stellata* | 33·3 | 80·0 | .. | 20·0 | .. | .. |
| 9. | *T. suavissima* | 66·6 | 60·0 | 20·0 | 10·0 | 10·0 | .. |

**Table 3.** Percentage of different types of anastomoses.

| Sl. No. | Name of the species | *A* | *B* | *C* | *C′* | *C″* | *D* |
|---|---|---|---|---|---|---|---|
| 1. | *T. arabica* | 25·00 | 25·00 | 25·00 | .. | .. | 25·00 |
| 2. | *T. callicerasoites* | .. | .. | 66·60 | .. | .. | 33·34 |
| 3. | *T. caerulea* | .. | 11·11 | 77·78 | .. | .. | 11·11 |
| 4. | *T. corniculata* | .. | 25·00 | 50·00 | .. | .. | 25·00 |
| 5. | *T. cretica* | .. | .. | 25·00 | 75·00 | .. | .. |
| 6. | *T. gracilis* | 11·11 | .. | 44·44 | 11·11 | 11·11 | 22·22 |
| 7. | *T. polycerata* | 6·96 | 6·96 | 41·86 | 4·64 | 2·32 | 37·21 |
| 8. | *T. stellata* | .. | .. | 57·14 | .. | .. | 42·86 |
| 9. | *T. suavissima* | 11·76 | 35·89 | 35·89 | 5·08 | .. | 11·76 |

central region except the standard petal of *T. polycerata* in which they are more in the peripheral region.

## 4. Discussion

The petal venation of this genus has shown some interesting features. Arnott and Tucker (1964) reported significant correlation between size (length, width, area) and number of dichotomies. In this paper a positive correlation is found with the length and area only. They have also given importance to the position of the dichotomies. In the present study maximum number of dichotomies is found in the central region except the standard petal of *T. cretica* and *T. corniculata* in which it is found in the basal region. Generally no correlation is found between the frequency of anastomoses and length of the petals. However, the

petals of *T. arabica*, *T. polycerata* and *T. gracilis* have shown a positive correlation. Just like the length no correlation is found with the area excepting the petals of *T. caerulea*, *T. corniculata* and *T. polycerata*. Thus from above it is clear that frequency of anastomoses is not dependent upon the area or the length of the petals. In all the species highest frequency of anastomoses is found in the central region but the wing petals of *T. arabica* and keel petals of *T. corniculata* and *T. stellata* possess highest frequency in the basal region. Datta and Saha (1968) reported highest frequency in the peripheral region in the members of phaseolae.

Among the different types of anastomoses *C* and *D* types are of most frequent occurrence in all the species studied except *T. cretica* in which *C* and *C'* are common. Earlier Datta and Saha (1968) have also reported the frequent occurrence of these types for the members of phaseolae and Arnott and Tucker (1963) for *Ranunculus repens*. In addition to these types only *B* type is found in *T. caerulea* and *T. corniculata*, *A* and *B* in *T. arabica*, *A*, *B*, *C'* in *T. suavissima*, *A*, *C*, *C''* in *T. gracilis* and all types in *T. polycerata*. Thus in this genus distribution of anastomoses appears species specific. Among the different species more points of fuisons are found in *T. polycerata*, *T. callicerasoites*, *T. gracilis* and *T. suavissima*.

Foster and Arnott (1960), Banerji and Mukherji (1970), and Subramanyam and Nair (1973) are of opinion that open dichotomous venation is primitive while on the other hand Chertek (1962, 1963) views that anastomosed venation is primitive. The present study supports the former view and among the species of this genus *T. callicerasoites*, *T. gracilis*, *T. polycerata* and *T. suavissima* appears to be more advanced as these species have more points of fusions and more number of petals showing vein fusions.

Earlier reports indicate that species of *Trigonella* are characterized by presence of both simple as well as medicagoid type of corolla. In the present investigation simple corolla is found in all the species except *T. polycerata* in which it is of medicagoid type. The wing petal of *T. polycerata* possesses a tooth like structure arising from the inner surface of the petal and receiving supply from the main bundle of the petal. In this region vascular bundles are inversely oriented with respect to the petal bundle. The same inversely oriented supply was reported by Arber (1936) for the nectary of *Ranunculus* and for the corona by *Narcissus* (Arber 1937). Thus this tooth like structure may be regarded as petalline nectary or corona. Earlier Larkin and Graumann (1954) named this structure as horn in *Medicago sativa*, Thus it can be concluded that among the different species of this genus *T. polycerata* is the highest evolved species.

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# Responses of cotton-cultivars to long day conditions

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**Abstract.** Flowering of cultivated varieties of cotton belonging to *G. arboreum*, *G. herbaceum* and *G. hirsutum* was delayed by over 14 hrs of daylength because of increase in number of days for square formation. The long day treatment in general increased height, production of fruiting branches, leaf area and dry weight per plant. The number of fruiting forms, bolls retained, yield of seed cotton and fruiting coefficient decreased under long day conditions. These characters were affected more in upland varieties and short day Cambodia derivatives. The most of *G. arboreum* and *G. herbaceum* varieties became more vegetative in growth but their boll number and yield per plant increased.

Since the varieties 1998 F (*G. hirsutum*) and Gaorani 1111 (*G. arboreum*) were tolerant to long photoperiod and grew satisfactorily, it is suggested that these may be used as donor parents for improving the quality of cottons grown in northern India.

**Keywords.** Photoperiod ; flowering ; long day ; boll ; square ; yield.

## 1. Introduction

The flowering of cultivated and wild species of cotton is governed by both day-length and temperature as shown by Waddle *et al* (1961) and Mauney and Phillips (1963). They found that most of the varieties flower under short days and cool nights. The importance of low night temperature in promoting flowering was further stressed by Mauney (1966) using a non-photoperiodic upland cotton variety. Bhatt (1977) and Bhatt *et al* (1976) found that long days and high temperature singly or in combination delayed flowering of upland cottons of northern India whereas the photosensitive Cambodia varieties did not flower under long days alone or long day plus high temperature. The upland genotypes when grown at latitudes 29° N, 21° and 11° N flowered progressively earlier and at lower nodes at more southern latitudes because of reduction in daylength and temperature.

Though the cotton crops in the northern cotton zone in India are grown under irrigation and thus give higher yields, they are of short and medium staple. An attempt was therefore made to screen some of the promising varieties (belonging to both *G. hirsutum* and *G. arboreum*) for tolerance to long days so as to identify donor parents for improving quality.

## 2. Materials and methods

The cultivated varieties in India representing three species of cotton and an $F_1$ hybrid developed through genetic male sterile line were taken as shown below :

| *Species* | *Variety* | *Area* |
|---|---|---|
| *G. hirsutum* | LSS | Upland type from North zone |
| *G. hirsutum* | 320 F | Upland type from North zone |
| *G. hirsutum* | CP 1998 F | Grown in South zone |
| *G. hirsutum* | 170 Co 2 | An Indo-American type grown in Central zone |
| *G. hirsutum* | MCU 1 | Photosensitive short day derivative of Cambodia cotton from South (used as check) |
| *G. arboreum* | AK 235 | From Central zone |
| *G. arboreum* | G 27 | From North zone |
| *G. arboreum* | K 9 | From South zone |
| *G. arboreum* | Gaorani 1111 | From South zone |
| *G. arboreum* | Gaorani 1187 | From South zone |
| *G. herbaceum* | V 797 | Grown in Central zone |
| *G. herbaceum* | Jayadhar | Grown in South zone |
| | CPH 2 | An intraspecific (*G. hirsutum*) hybrid |

The cotton varieties were raised in large (45 cm diameter and 105 cm depth) pots adequately manured. There were six plants per treatment one in each pot. The daylength around latitude 30°N in northern cotton zone in India when cotton is sown and even thereafter until about 80 days is over 14 hours. The normal daylength in summer at Coimbatore (latitude 11° N) varies from 11·50 hrs to 12·20 hrs. It was extended to 14·50 hrs through 60 watt incandescent lamps. The treatments were (i) Control, *i.e.*, normal daylength of 11·50 hrs to 12·20 hrs, and (ii) long photoperiod of 14·50 hrs. Under both the treatments the day and night temperatures were the same. The day temperature was between 34·0° C to 35·5° C from germination to square formation for about 35 days and thereafter until flowering did not rise above 36·8° C. The night temperature during this period fluctuated between 17·5° C to 21·8° C to 24·6° C and subsequently remained around 23·0° C. The long day treatment was discontinued after 75 days after sowing. Taking cotyledonary node as zero, the node on the main stem producing first sympodium was taken as the first fruiting node. The fruiting coefficient is defined as the yield of seed cotton produced per 100 gm of total dry matter (Crowther 1944).

## 3. Results

The long days delayed formation of flower buds in all the varieties irrespective of the species (table 1). The short day Cambodia derivatives MCU 1 and 170 Co 2 set squares just after the long day treatment was discontinued. The early types CPH 2 and 1998 F took only 4 and 6 days more respectively to square when compared with the delay of three weeks in LSS and 320 F. Among the diploids both the Gaorani types were early whereas in the rest, delay in square formation ranged from 15 to 20 days.

Most of the varieties took significantly more days to flower when photoperiod was extended except CPH 2 and 1998 F with a delay of 4 and 9 days respectively and of 6 days in Gaorani 1111. With a difference of only 2 days Gaorani 1187 appeared to be practically photoinsensitive. The photosensitive types MCU 1 and 170 Co 2 took maximum days to flower followed by the upland types LSS and 320 F, and the Asiatic type AK 235. The square period, *i.e.*, the number of days from initiation of flower bud to opening of the flower remained more or less the same between the treatments except that it increased by 3 days in 1998 F and reduced by 3 days in G 27, 5 days in K 9, 6 days in Gaorani 1187 and 3 days in Jayadhar.

**Table 1.** Flowering behaviour of cotton-cultivars as affected by extended photoperiod.

| Species | Variety | Number of days to square | | Number of days to flower | | First fruiting node | | Days for first boll bursting | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T | C | T |
| *G. hirsutum* | LSS | 34 | 56 | 52 | 75 | 4·3 | 9·0 | 118 | 150 |
| *G. hirsutum* | 320 F | 33 | 54 | 56 | 75 | 4·0 | 6·0 | 120 | 145 |
| *G. hirsutum* | CP 1998 F | 25 | 31 | 46 | 55 | 4·0 | 6·0 | 85 | 95 |
| *G. hirsutum* | 170 Co 2 | 35 | 72 | 54 | 89 | 3·0 | 7·6 | 120 | 147 |
| *G. hirsutum* | CPH 2 | 22 | 26 | 42 | 46 | 6·0 | 6·0 | 86 | 84 |
| *G. hirsutum* | MCU 1 | 39 | 73 | 59 | 95 | 2·3 | 10·3 | 110 | 130 |
| *G. arboreum* | AK 235 | 33 | 52 | 53 | 72 | 3·0 | 7·0 | 122 | 140 |
| *G. arboreum* | G 27 | 35 | 50 | 55 | 67 | 5·3 | 7·7 | 118 | 130 |
| *G. arboreum* | K 9 | 34 | 54 | 59 | 74 | 2·6 | 7·0 | 125 | 142 |
| *G. arboreum* | Gaorani 1111 | 35 | 42 | 53 | 59 | 2·0 | 6·6 | 130 | 139 |
| *G. arboreum* | Gaorani 1187 | 35 | 43 | 53 | 55 | 4·6 | 7·7 | 125 | 132 |
| *G. herbaceum* | V 797 | 45 | 60 | 65 | 82 | 5·0 | 6·0 | 130 | 150 |
| *G. herbaceum* | Jayadhar | 35 | 50 | 65 | 77 | 2·6 | 5·0 | 120 | 135 |
| S.E. for varieties | | | 1·90 | | 8·61 | | 1·40 | | 4·84 |
| S.E. for treatment | | | 1·10 | | 3·51 | | 0·58 | | 1·97 |

C = Control T = Treatment

The node at which the first sympodial branch appeared was also higher, the notable exceptions being CPH 2 and V 797. Other varieties flowered 2–8 nodes higher under long day conditions.

An increase in daylength also increased boll maturation period, *i.e.*, the days from opening of the first flower to dehiscence of carpellary wall or boll bursting thus adding to further lateness. The trend was more or less similar to flowering. The upland type LSS took the maximum number of days whereas CPH 2 was unaffected. It was interesting to note that the Gaorani cultures 1187, 1111 and 1998 F were late by 7, 9 and 10 days respectively.

Except for CPH 2 and Gaorani 1111 plant height increased significantly in all the varieties reaching the maximum in 170 Co 2 and LSS (table 2). The number of sympodia per plant also increased similarly except in CPH 2, V 797 and CP 1998 F. The long day conditions reduced production of fruiting forms with notable exceptions of G 27 and K 9 showing an increase whereas CP 1998 F was not affected significantly. Maximum reduction was found in MCU 1, AK 235 and V 797.

There was a significant increase in leaf area per plant in all the varieties. It was over three times in LSS and over twice the normal area in 320 F, MCU 1 and G 27 followed by 170 Co 2. Increase in leaf area was comparatively less in CPH 2, V 797 and CP 1998 F.

**Table 2.** Effect of extended photoperiod on some growth characters.

| Species | Variety | Height (cm) | | No. of sympodia per plant | | No. of fruiting forms per plant | | Leaf area per plant (cm²) | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T | C | T |
| *G. hirsutum* | LSS | 60·2 | 117·1 | 12 | 20 | 41·6 | 30·7 | 2983 | 11581 |
| *G. hirsutum* | 320 F | 65·3 | 95·2 | 9 | 15 | 33·3 | 39·0 | 2950 | 6170 |
| *G. hirsutum* | CP 1998 F | 40·3 | 60·8 | 13 | 14 | 42·0 | 39·0 | 2507 | 3575 |
| *G. hirsutum* | 170 Co 2 | 55·3 | 120·7 | 12 | 22 | 34·2 | 22·6 | 4210 | 8179 |
| *G. hirsutum* | CPH2 | 54·1 | 54·3 | 11 | 12 | 38·0 | 30·0 | 2668 | 3548 |
| *G. hirsutum* | MCU 1 | 51·3 | 90·1 | 12 | 17 | 35·6 | 17·0 | 6170 | 13217 |
| *G. arboreum* | AK 235 | 97·3 | 144·2 | 22 | 32 | 59·6 | 38·7 | 2850 | 4879 |
| *G. arboreum* | G 27 | 108·0 | 130·8 | 19 | 25 | 65·6 | 70·0 | 2217 | 4717 |
| *G. arboreum* | K 9 | 72·3 | 143·6 | 17 | 25 | 59·3 | 73·3 | 2980 | 5316 |
| *G. arboreum* | Gaorani 1111 | 72·6 | 77·0 | 16 | 18 | 51·3 | 35·0 | 2950 | 4850 |
| *G. arboreum* | Gaorani 1187 | 52·0 | 85·1 | 11 | 15 | 49·0 | 31·0 | 2170 | 3950 |
| *G. herbaceum* | V 797 | 56·0 | 83·3 | 12 | 15 | 62·3 | 40·1 | 2007 | 2970 |
| *G. herbaceum* | Jayadhar | 59·1 | 101·2 | 14 | 20 | 54·3 | 41·6 | 2350 | 4317 |
| S.E. for varieties | | | 7·39 | | 4·28 | | 8·61 | | 159·80 |
| S.E. for treatments | | | 3·02 | | 1·75 | | 3·51 | | 65·24 |

C = Control T = treatment

At maturity dry matter per plant increased in most of the varieties under long day treatment (table 3). But CP 1998 F, AK 235 and Gaorani 1187 were not affected. It was, however, highly significant in CPH 2, LSS 320 F, MCU 1, K 9, Gaorani 1111 and Jayadhar.

In *G. hirsutum* varieties LSS 170 Co 2 and MCU 1, the number of bolls per plant at maturity decreased with consequent decrease in yield of seed cotton. Both 320 F and CP 1998 F were unaffected whereas CPH 2 recorded higher yield under long days. Except Gaorani 1187, all *G. arboreum* varieties and V 797 had more number of bolls and yielded more. Jayadhar remained unaffected. Fruiting coefficient of most of the varieties decreased because of higher dry weights of their vegetative parts when grown under long days.

## 4. Discussion

The enhanced photoperiod delayed flowering because of increase in the number of days required to initiate square formation. The square period was more or less unaffected in *G. hirsutum* varieties confirming the previous findings of Bhatt (1977). *G. arboreum* varieties were similarly affected except Gaorani cultures where flowering was delayed by 2 and 6 days only. The *G. arboreum* variety used by Mauney and Phillips (1963) showed essentially no reaction to the environments in their study whereas flowering was delayed from 12 to 19 days under long day conditions in the present study with the first fruiting node pushed up significantly. Differences in flowering responses of different varieties reported by Mauney and Phillips (1963) and in our experiments may be attributed to differences in day and night temperatures though long day conditions were similar. Day temperatures in their experiments varied from 27° C to 32° C whereas the night temperature was fixed at 15° C or 30° C, while in our experiments day temperatures varied from 34° C to 36° C and night temperatures from 17° C to 24° C.

An increase in leaf and stem dry weight and a decrease in yields of seed cotton of most of the varieties under long days reduced their fruiting coefficients (Bhatt 1970). In *G. hirsutum* group CP 1998 F responded exceptionally well in terms of its vegetative and reproductive growth maintaining high fruiting coefficient. This variety was late in flowering by 9 days under extended photoperiod and took 95 days for the first formed boll to burst as against 150 and 145 days taken by the northern upland varieties LSS and 320 F respectively. Because of its superior fibre characters, better yielding capacity and high degree of tolerance to long day conditions, it may prove as a suitable donor parent for improving the quality of northern *hirsutums* in India. Next in performance was the intra-*hirsutum* hybrid CPH 2. But the short day Cambodia derivatives MCU 1 and 170 Co 2 failed miserably in relation to flowering and growth (Hutchinson 1959; Bhatt 1977).

The *G. arboreum* varieties except Gaorani 1111 took 12 to 19 days more to flower and their bolling period also increased by 12 to 18 days. The new culture Gaorani 1111 in this respect took only 6 to 9 days more for flowering and boll bursting respectively. It compares well with the northern *arboreum* variety G 27 in terms of yield of seed cotton and similar fruiting coefficient. By virtue of its long staple, it may prove useful for improving the quality of northern *arboreums* in India.

Table 3. Effect of extended photoperiod on yield characters.

| Species | Variety | Dry weight per plant (gm) | | No. of bolls per plant | | Yield of seed cotton per plant (gm) | | Weight of lint (gm) | | Fruiting coefficient | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | C | T | C | T | C | T | C | T |
| *G. hirsutum* | LSS | 170·8 | 243·9 | 14·3 | 5·2 | 42·1 | 19·3 | 13·8 | 6·3 | 0·24 | 0·08 |
| *G. hirsutum* | 320 F | 142·4 | 203·6 | 9·0 | 10·0 | 37·3 | 35·0 | 12·3 | 11·5 | 0·26 | 0·17 |
| *G. hirsutum* | CP 1998 F | 79·3 | 80·7 | 16·0 | 17·0 | 49·0 | 49·0 | 17·1 | 17·1 | 0·61 | 0·60 |
| *G. hirsutum* | 170 Co 2 | 196·4 | 211·9 | 10·0 | 6·0 | 39·7 | 24·1 | 13·9 | 8·4 | 0·20 | 0·11 |
| *G. hirsutum* | CPH 2 | 62·6 | 112·7 | 10·0 | 14·0 | 32·8 | 55·2 | 11·5 | 19·3 | 0·52 | 0·49 |
| *G. hirsutum* | MCU 1 | 195·9 | 261·0 | 11·6 | 8·0 | 40·1 | 29·7 | 13·2 | 9·8 | 0·20 | 0·11 |
| *G. arboreum* | AK 235 | 199·9 | 198·8 | 5·6 | 9·1 | 10·2 | 15·3 | 3·4 | 5·2 | 0·08 | 0·07 |
| *G. arboreum* | G 27 | 197·2 | 204·2 | 20·0 | 24·3 | 36·0 | 43·2 | 11·8 | 14·2 | 0·18 | 0·20 |
| *G. arboreum* | K 9 | 178·3 | 222·1 | 13·6 | 23·0 | 26·1 | 45·0 | 8·6 | 14·8 | 0·14 | 0·20 |
| *G. arboreum* | Gaorani 1111 | 142·5 | 203·3 | 12·6 | 21·6 | 23·0 | 38·1 | 7·6 | 12·6 | 0·16 | 0·18 |
| *G. arboreum* | Gaorani 1187 | 172·3 | 169·8 | 17·7 | 9·6 | 30·7 | 15·3 | 10·1 | 5·0 | 0·17 | 0·09 |
| *G. herbaceum* | V 797 | 135·7 | 175·4 | 7·0 | 18·0 | 13·8 | 25·0 | 5·0 | 9·0 | 0·10 | 0·14 |
| *G. herbaceum* | Jayadhar | 115·0 | 164·3 | 16·6 | 15·6 | 32·3 | 28·1 | 11·6 | 10·1 | 0·28 | 0·17 |
| S.E. for varieties | | | 38·76 | | 6·59 | | 10·47 | | | | |
| S.E. for treatment | | | 15·82 | | 69·2 | | 4·27 | | | | |

C = Control T = Treatment

Among the *G. herbaceum* varieties, V 797 though late in flowering and boll bursting appeared tolerant to longer photoperiod as its growth and yield improved. But the fruiting efficiency of Jayadhar was reduced markedly.

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# Seed germination and seedling establishment of two closely related *Schima* species

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**Abstract.** Seed germination of *S. khasiana* from Upper Shillong and *S. wallichii* from Shillong, Umsaw and Burnihat in Meghalaya, north-eastern India, and seedling establishment and growth of these species/populations in reciprocal cultivation were studied. Seeds lost viability and germinability gradually within a year when stored at 5 cm below the soil surface under natural conditions or at 20° C in the laboratory. Storage at 0° C permitted retention of 15–25% viability. Seeds germinated better on the surface layers (0–2 cm) of the soil. *S. khasiana* had a lower temperature optimum (15° C) of germination while the populations of *S. wallichii* had a higher temperature optimum (20/25° C). At a temperature of 30° C, the lower altitude population of *S. wallichii* from Burnihat gave higher germination than the high altitude population from Shillong. A given species/population of *Schima* gave better seedling establishment and growth in its own natural habitat as compared to the introduced populations from the other altitudinal sites. This is indicative of the close adaptation of the natural populations to their habitat and ecotypic differentiation in this species.

**Keywords.** Tree adaptation ; seed germination ; tree seedling establishment ; altitudinal ecotype ; *Schima*.

## 1. Introduction

Germination and establishment represent two critical phases in the life-cycle of a plant species and these two aspects have been related to adaptation and distribution pattern of species in space (Koller *et al* 1962 ; Harper 1965 ; Cohen 1967 ; Ramakrishnan 1972 ; Ross and Harper 1972 ; Thompson 1973 ; Boojh and Ramakrishnan 1981a) and in time (Kapoor and Ramakrishnan 1973). However, this aspect of the problem in relation to adaptive strategy of tree species has received little attention (Kozlowski 1971 ; 1979). Although the size of a single species population is to some extent related to seed supply, it is ultimately determined by favourable conditions available for germination and establishment (Harper *et al* 1970). Further, a large gap often exists between the seeding potential of a species and the actual number of seedlings established in that area, depending upon environmental conditions.

*S. khasiana* Dyer and *S. wallichii* (D C) Korth family Ternstroemiaceae are two closely related and economically important timber tree species of north-eastern hills of India. These species show a distribution pattern on an altitudinal basis where *S. khasiana* is restricted to higher elevations (1800–1900 m), while *S. wallichii* shows a wide distribution ranging from 100 to 1600 m. These two species are early successions and come up in the secondary fallows after slash and burn agriculture (*Jhum*). These are light-demanding and regenerate profusely in the open, through light wind-dispersed seeds. The present study is a comparative investigation of seed germination and seedling establishment of these two species along an altitudinal gradient in the Khasi Hills of Meghalaya.

## 2. Climate

All the three sites are characterised by marked seasonal changes in climate. The year could be divided into 3 distinct seasons : (i) Monsoon season of high temperature and humidity extending from May to October when over 80% of the rainfall occurs, (ii) Winter season (November to February) of lower temperature which is comparatively dry except for a few winter showers, and (iii) A warm, dry and windy summer in March–April (table 1).

## 3. Methods of study

Mature fruits of *S. khasiana* were collected from Upper Shillong and that of *S. wallichii* from 3 sites at Shillong, Umsaw and Burnihat, in the months of February-March, 1978. Seeds were separated out by air drying. The fruit and seed weight measurements were based on 100 fruit/seed with 20 replications.

**Table 1.** Comparison of climatic data at study sites during 1978–79.

| | Upper Shillong | Shillong | Umsaw | Burnihat |
|---|---|---|---|---|
| Location | | | | |
| Latitude (N) | 25·34 | 25·34 | 25·45 | 26·02 |
| Longitude (E) | 91·56 | 91·56 | 91·54 | 91·52 |
| Altitude (m) | 1900 | 1400 | 800 | 100 |
| Temperature (° C) | | | | |
| Mean monsoon maximum | 22 | 24 | 30 | 32 |
| Mean monsoon minimum | 15 | 16 | 22 | 24 |
| Mean winter maximum | 16 | 16 | 20 | 25 |
| Mean winter minimum | 3 | 6 | 10 | 12 |
| Precipitation (mm) | 2400 | 2000 | 1800 | 1600 |

Seeds were stored in the laboratory at 20 ± 5° C and 0° C in BOD incubators in tightly closed plastic bottles. In nature, seeds were similarly stored 5 cm below soil surface. The moisture content of seeds at the time of storage was 10%. Viability and germinability of stored seeds were tested at intervals of 3 months with four replicates of 50 seeds of each species/population. For testing the viability of seeds a freshly prepared 5% aqueous solution of 2, 3, 5–triphenyltetrazolium chloride (TZ) was used. Seeds were first soaked in water for 10–12 hrs, then seed coats were punctured to facilitate entry of the TZ solution and were left in the dark at 30° C for upto 24 hrs. Seeds with completely stained (red colour) embryos were scored as viable. Germinability was tested by placing seeds in petri-dishes over moist filter-paper at a constant temperature of 15° C for *S. khasiana* and 20° C for *S. wallichii*.

Seeds were tested under two conditions, of continuous light under an incandescent fluorescent tube (500–600 1× ) or under continuous darkness by covering the petri-dishes inside thick black paper, at a constant temperature of 20° C. Seed germination in dark was counted under green light. Germination at different constant (15, 20, 25, 30 and 35° C) and alternating (25/15 and 25/20° C) temperature regimes were tried in BOD incubators maintained at these temperatures. The effect of different soil depths of 0, 2, 4, 6, 8 and 10 cm on germination was tested in pots filled with soil, by placing seeds at the appropriate depth.

All germination experiments were replicated 4 times with 50 seeds in each test. The emergence of radicle was taken as an indicator of germination. Tests in all cases were done for 20 days after the seeds were placed for germination.

Ten replicates of 100 viable seeds (viability was ascertained for a given seed-lot on the basis of preliminary germination tests) of each species/population were sown at a depth of 5 cm at all the 4 study sites both in the open and under forested situations, in May 1978. The depth of 5 cm for sowing was chosen in order to avoid washout of seeds under heavy rainfall. Observations on the seedling emergence and establishment were taken at monthly intervals. Seedlings were harvested at the end of one year period and after noting plant height and leaf area using a planimeter, the root and shoot portions were separated and dried to a constant weight at 85 ± 2° C.

## 4. Results

### 4.1. *Fruit and seed characters*

The capsules and seeds of *S. khasiana* were heavier than that of the populations of *S. wallichii*. While the fruit weight of the populations of *S. wallichii* were not very different, significantly higher seed weight was noticed for the Burnihat population of this species compared to that of the two other populations (table 2).

### 4.2. *Germination studies*

4.2a. *Effect of storage* : When seeds were stored in the soil or in the laboratory at a temperature of 20 ± 5° C, both viability and germinability of the seeds of all

the populations decreased markedly with passage of time so that after one year, seeds were totally non-viable or gave very poor germination. However, storage at 0° C maintained better viability and germinability after 1 year storage (figure 1).

4.2*b*. *Depth of burial* : As seen from figure 2, the depth of burial affected both the time and the final percentage of germination. Maximum germination was found to occur at 2 cm depth and it decreased at the depths greater than this for all the species and populations. At soil surface though faster germination occurred the total percentage was lesser than at 2 cm depth.

**Table 2.** Fruit and seed weight of *Schima* species/populations.

| | Fruit weight (g) | Seed weight (mg) |
|---|---|---|
| *S. khasiana* | 1·67±0·09 | 1·18±0·03 |
| *S. wallichii* | | |
| Shillong | 1·13±0·07 | 0·46±0·06 |
| Umsaw | 1·04±0·03 | 0·44±0·02 |
| Burnihat | 1·07±0·05 | 0·53±0·01 |

(± S.E. of the mean)

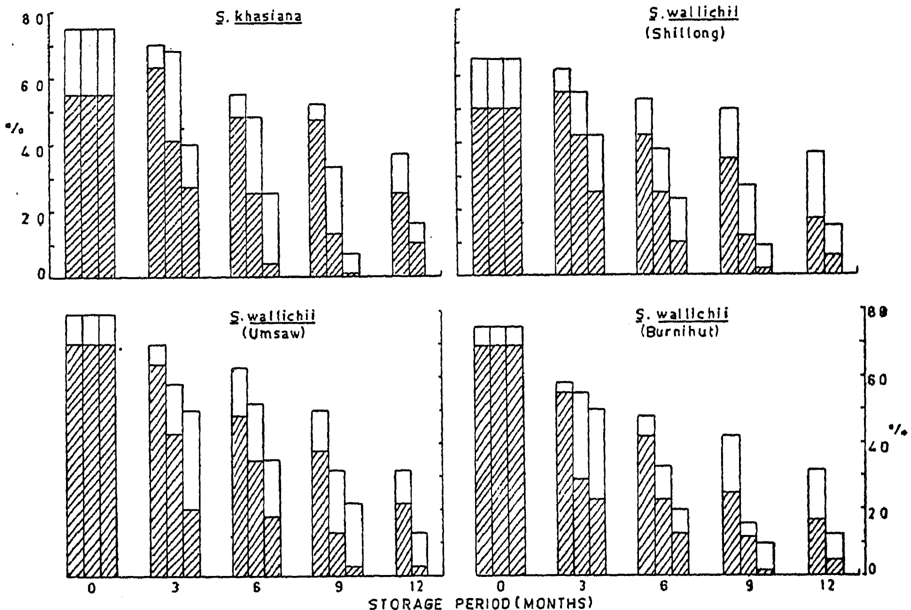


**Figure 1.** Viability (open columns) and germinability (hatched columns) of *Schima* seeds after different storage periods. First column, storage at 0° C ; second column storage at 20 ± 5° C ; and third column, storage under soil.

4.2c. *The effect of light and darkness* : There was germination both in the dark and light and the results obtained were not significantly different under these two conditions (table 3).

4.2d. *The effect of temperature* : Table 4 reveals the effect of various temperature regimes on the germination of the seeds of *Schima* species and populations.

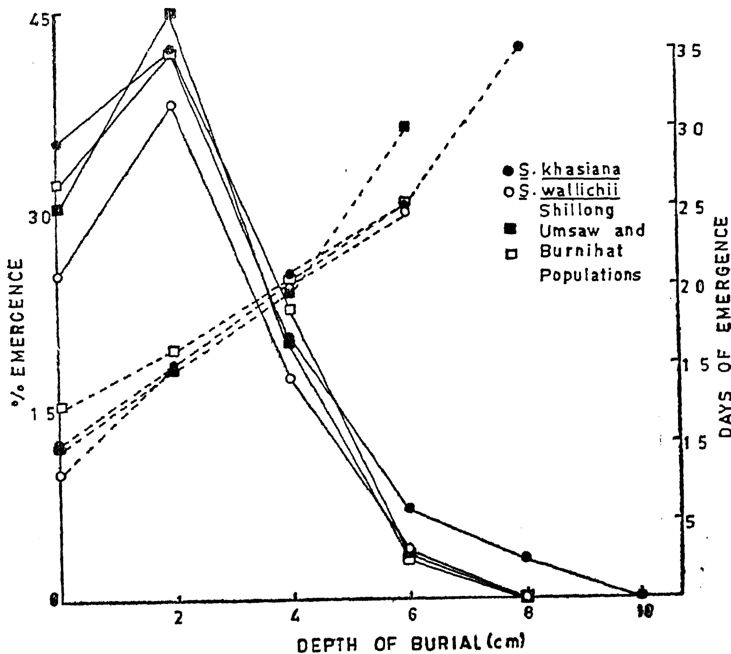


**Figure 2.** The relationship between seed depth, germination and emergence period of seedlings of *Schima* species/populations over a period of 35 days after sowing. Continuous lines represent % emergence of seedlings and broken lines represent number of days taken for emergence.

**Table 3.** The effect of light and dark treatment on seed germination of *Schima* species.

| | Continuous light 20° C | Continuous dark 20° C |
|---|---|---|
| *S. khasiana* | 50 ± 5·4 | 48 ± 3·4 |
| *S. wallichii* | 62 ± 6·4 | 57 ± 1·3 |

(± S.E. of the mean)
The seeds of populations of *S. wallichii* were pooled.

**Table 4.** Germination (%) of *S. khasiana* and *S. wallichii* seeds at various constant and alternating temperatures.

| | Constant temperatures (° C) | | | | Alternating temperatures (° C) | | |
|---|---|---|---|---|---|---|---|
| | 15 | 20 | 25 | 30 | 35 | 25/15 | 25/20 |
| *S. khasiana* | 55 ± 2·7 | 46 ± 3·6 | 44 ± 2·9 | 42 ± 2·2 | 0 | 46 ± 2·3 | 45 ± 5·5 |
| *S. wallichii* | | | | | | | |
| Shillong | 37 ± 4·3 | 48 ± 6·0 | 48 ± 6·8 | 33 ± 4·1 | 20 ± 7·5 | 48 ± 4·1 | 50 ± 4·6 |
| Umsaw | 42 ± 5·3 | 65 ± 3·7 | 58 ± 3·7 | 40 ± 6·8 | 27 ± 2·9 | 61 ± 3·7 | 70 ± 9·9 |
| Burnihat | 38 ± 2·2 | 69 ± 3·7 | 56 ± 5·4 | 50 ± 4·8 | 33 ± 7·6 | 59 ± 3·4 | 64 ± 3·6 |

(± S.E. of the mean)

*S. khasiana* showed maximum germination at constant 15° C, with a gradual decrease with increase in temperature, so that at 35° C no seeds of this species germinated. Populations of *S. wallichii* showed maximum germination at 20 and 25° C with decrease in germination on either side. At 30° C, the lower altitude population of *S. wallichii* from Burnihat gave higher germination than its higher altitude population from Shillong. Two alternating temperature regimes tried were favourable for germination for all the species/populations.

The rate of germination was faster at 15° C for *S. khasiana* and 20 and 25° C or alternating (25/20° C) for *S. wallichii* populations (figure 3).

### 4.3 *Seedling establishment*

4.3a. *Seedling emergence* : Only a small proportion of seedlings could emerge under field conditions at all the study sites. Further the differences in emergence were not significant (at 5% level) between species/populations (table 5).

4.3b. *Survivorship* : No seedlings could survive under forested situations beyond a period of 2 months. Under open grown situations, mortality was generally very high resulting in a steep decline in population upto January–February, at all the sites. At Upper Shillong, however, the rate of decline in population was slower for *S. khasiana* with an ultimately large population size compared to the populations of *S. wallichii*. At the other 3 experimental sites, however, the pattern of survivorship was not very different for the populations of *Schima*, though the local populations showed better survivorship than the introduced ones. *S. khasiana* gave the lowest final survival at these three sites (figure 4).

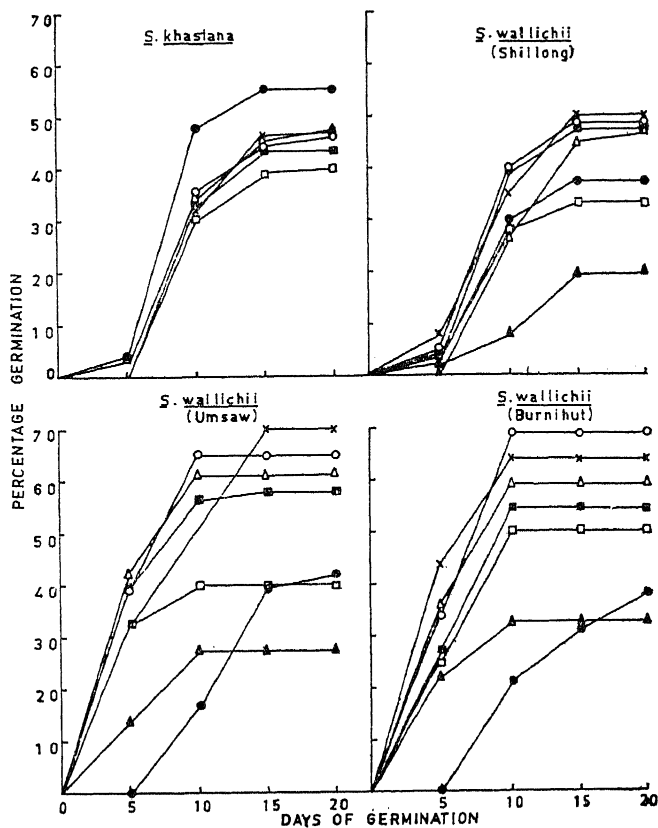


**Figure 3.** Percentage germination of *Schima* species/population at different periods, at constant and alternating temperature of 15° C (●); 20° C (○); 25° C (■); 30° C (□); 35° C (▲); 25/15° C (△) and 25/20° C (×).

**Table 5.** Seedling emergence (%) of *S. khasiana* and *S. wallichii* at different altitudinal sites.

| Field stations | S. *khasiana* | Species/Populations *S. wallichii* | | |
|---|---|---|---|---|
| | | Shillong | Umsaw | Burnihat |
| Jpper Shillong | 20 ± 4·6 | 10 ± 2·3 | 11 ± 1·8 | 14 ± 1·8 |
| hillong | 19 ± 2·4 | 21 ± 3·9 | 10 ± 3·3 | 10 ± 2·1 |
| Jmsaw | 16 ± 1·7 | 13 ± 2·7 | 13 ± 1·2 | 13 ± 2·5 |
| urnihat | 16 ± 3·9 | 12 ± 1·7 | 11 ± 2·3 | 11 ± 0·6 |

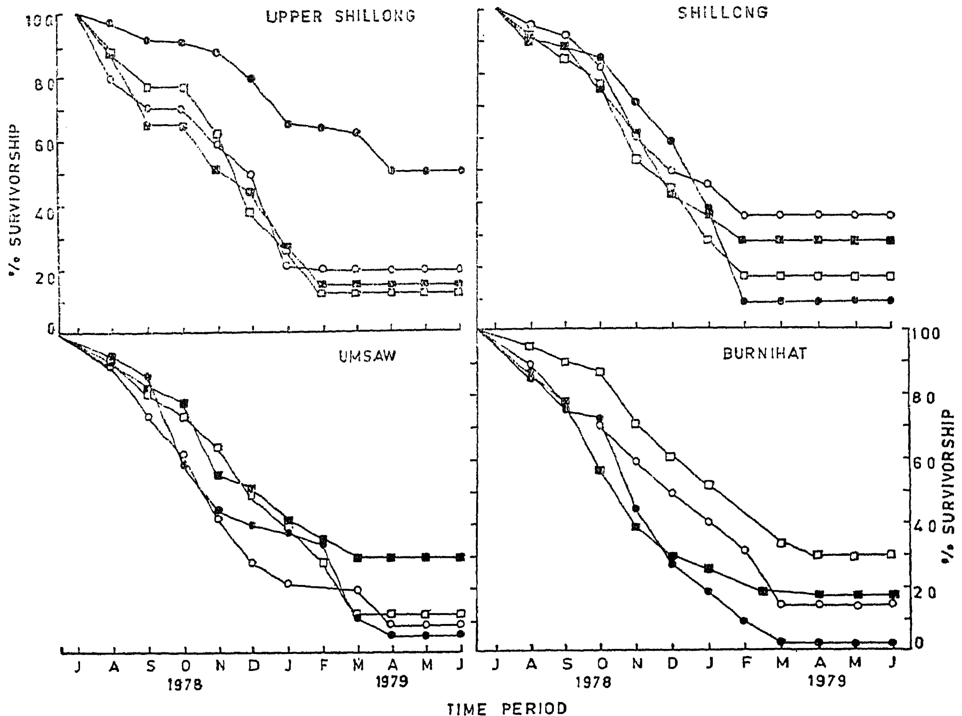


**Figure 4.** Survivorship of *Schima* seedlings under field conditions (in open). No seedlings could survive beyond 2 months under forested situations. *S. khasiana* (●); *S. wallichii*, Shillong (○), Umsaw (■) and Burnihat (□) populations.

4.3c. *Plant performance*: The growth characteristics of the different species/ populations at different sites, given in figure 5, show that the naturalized population for a given site was superior to the other introduced populations. Thus *S. khasiana* gave better growth yield under Upper Shillong site, while the populations of *S. wallichii* from 3 different sites did better in their respective natural habitats.

## 5. Discussion

*Schima* species being early successional colonizers depend for regeneration on the availability of open sites which favour their seed germination, establishment and growth. The most important factor limiting the ability of such species to colonize disturbed sites is the availability of seed, which must come either from a stand in close proximity or from storage in the soil. The latter is not possible for *Schima* as seeds do not remain viable in the soil for an extended period of time as seen from the present study where the viability of seeds is completely lost after storage in soil for one year. Thus, the species is fugitive in nature (Hutchinson 1951), where good dispersal mechanism would play an important role permitting the species to colonize new habitats (Salisbury 1942). *Schima* due to

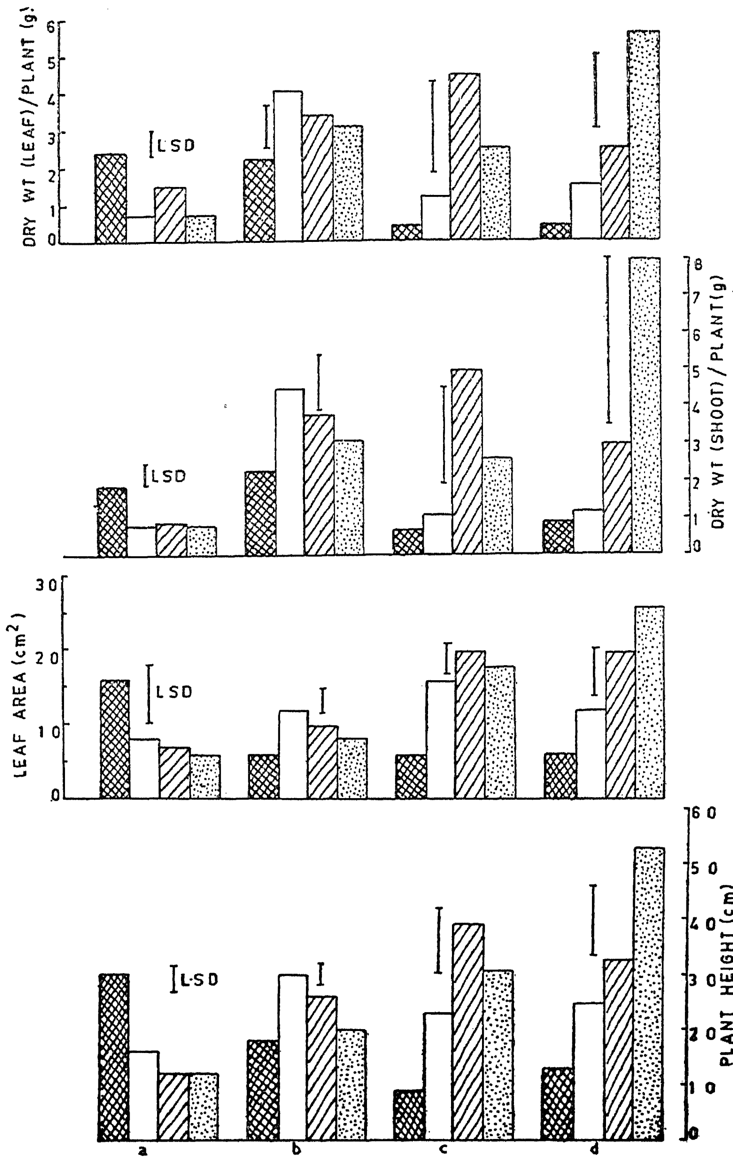


**Figure 5.** Growth performance of *Schima* species/populations at different field conditions. a = Upper Shillong ; b = Shillong ; c = Umsaw and d = Burnihat site ; filled bars for *S. khasiana*, and hollow bars for Shillong ; hatched bars for Umsaw and stippled bars for Burnihat populations of *S. wallichii*.

its light, mobile (winged) seeds often invades highly disturbed areas after slash and burn agriculture in the region. Similarly in temperate forests it has been reported that light seeded species *Fraxinus* and *Betula* play an important role in revegetation after clearcutting (Bormann and Likens 1979). The variation in seed weight in between species/populations may partly be related to climate

(Baker 1972 : Wearstler and Barnes 1977) and partly to ecotypic differences related to altitude which is supported by growth studies of the different *Schima* populations done at different altitudes discussed below.

The differences in germination behaviour in response to temperature as seen in the present case where *S. khasiana* germinated at a comparatively lower temperature compared to *S. wallichii* populations, have often been correlated with climatic conditions and seed source (Callaham 1970 ; Thomson 1973), whereby seeds from colder areas germinate better at lower temperature than those from warmer regions. Grose (1957) has demonstrated that montane species of *Eucalyptus* germinated best at a lower temperature of 16° C, in contrast to somewhat higher temperatures for species of warmer areas. Though the total number of seeds of a species which ultimately germinates at a given temperature is a good indicator of that species potential, however, the time taken to germinate is of much significance since the early germinating individuals enjoy a considerable competitive advantage (Ross and Harper 1972). The germination rate which was higher at 15° C for *S. khasiana* and at 20 or 25° C for *S. wallichii* populations is consistent with temperature optima for their germination. The rapidity of germination in this species without a dormancy mechanism is advantageous in colonizing new areas by producing a profusion of seedlings and this has been reported for a majority of tropical trees which has been termed as biological nomads (Ng 1978).

*Schima* seeds come under microbiotic category (Crocker and Barton 1953) as they normally lose viability and germinability within a year. Small and light seeds are reported to lose their viability faster (Quick 1961) and this has been reported in species of *Salix*, *Populus* and *Ulmus* (Wareing 1963) and *Alnus* (Boojh and Ramakrishnan 1981b). The better retention of viability and germinability under lower temperature storage may be attributed to slow biological and biochemical processes at such temperatures (Kamra 1967).

There exists a large gap between seeding potential of a species and the number of seedlings emerged at a given site. The failure of survival of seedlings under a forest canopy may be attributed to the shade intolerance of the seedlings. The differences in survival pattern for different species/populations under field conditions are suggestive of the adaptation of a given population to the natural climatic conditions in which they grow. This is suggested by the relatively better survival and performance of local species/populations to that habitat compared to the introduced ones. Thus, the lower altitude population of *S. wallichii* which is adapted to longer growing season, higher temperature and frost-free winter is adversely affected at higher altitude.

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# Anther and pollen development in cotton haploids and their parents

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**Abstract.** Development of anther tapetum from premeiotic stages to pollen formation was studied in six x-ray induced haploids of *Gossypium hirsutum*, three interspecific $F_2$ haploids, and one natural haploid of each of *G. hirsutum* and *G. barbadense*, and the observations were compared with those of their respective parents, a genetic male sterile, a male fertile and a cytoplasmic male sterile line of *G. hirsutum*. Significant differences were recorded for number of anthers per flower, pollen size, pollen viability and number of microspores produced by PMC. Anther development in haploids was normal. Anther dehiscence was also normal in some haploids. Non-dehiscent anthers could be mostly attributed to the formation of immature pollen grains. Normal development of anthers and degeneration of tapetum occurred in the parents and in the genetic fertile line. Contrastingly no degeneration of tapetum was noticed in the cytoplasmic male sterile line.

**Keywords.** *Gossypium* spp. ; haploids ; anther tapetum ; male sterile.

## 1. Introduction

Haploids are characterized by significant decrease in size of vegetative plant parts, vigour and fertility (Kostoff 1943) and in diameter of pollen mother cells (Belling and Blakeslee 1923) by half of that in the diploid (Ivanov 1938), and by smaller guard cells (Lamm 1938). Kimber and Riley (1963) indicated a relationship between haploid and diploid guard cells of *G. hirsutum* and *G. barbadense* by a factor of 1·26.

Partial or complete sterility due to halving of chromosome number and several meiotic irregularities in haploids of *G. hirsutum*, *G. barbadense*, their $F_2$ interspecific crosses and x-ray induced haploids have been reported (Mehetre and Thombre 1981b, c, d). Studies were undertaken to investigate the development of anther tapetum and its role in pollen sterility observed in these haploids. The comparative observations made on haploids, their respective parents, genetic male sterile and fertile lines and one cytoplasmic male sterile line have been reported

**Table 1.** Data on pollen and anther development in cotton haploids and their parents.

| Species | Genotype | Chromosome number ($2n$) | Anthers per flower | Microspores per PMC | Pollen size ($\mu m$) | | Pollen viability (%) | Average radial width of tapetal cell ($\mu m$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Range | Av. | | Premeiotic | Prophase-I | Anaphase-I | Tetrad | Pollen |
| | | | | | | | | Stages | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| *G. hirsutum* | HG–108 | $4x = 52$ | 88·00± 9·91 | 4·00 | 116·30–131·20 | 117·30 | 98·2 | 7·62+ 4·02 | 8·12± 0·96 | 6·1± 0·12 | 4·98± 0·19 | 3·89± 0·29 |
| | Haploid 1 ($Hpl_1$) | $2x = 26$ | 56·30± 14·43 | 3·77 | 35·60–86·10 | 69·16 | 2·1 | 6·69± 0·99 | 6·99± 0·23 | 5·51± 0·11 | 5·98± 0·19 | 4·16± 0·24 |
| | $Hpl_2$ | $2x = 26$ | 68·68± 14·08 | 3·90 | 61·20–117·30 | 72·30 | 28·2 | 6·09± 0·16 | 6·51± 0·19 | 6·17± 0·21 | 5·72± 0·21 | 4·97± 0·33 |
| | $Hpl_3$ | $2x = 26$ | 47·10± 17·28 | 3·74 | 35·70–112·20 | 63·14 | 0·0 | 5·99± 0·21 | 6·25± 0·17 | 5·98± 0·23 | 5·16± 0·23 | 4·79± 0·36 |
| | $Hpl_4$ | $2x = 26$ | 26·30± 10·00 | 4·24 | 66·30–112·20 | 74·19 | 32·0 | 6·67± 0·23 | 6·70± 0·19 | 6·39± 0·24 | 6·01± 0·44 | 5·50± 0·49 |
| | $Hpl_5$ | $2x = 26$ | 32·30± 8·62 | 4·15 | 40·80–107·10 | 68·16 | 1·0 | 6·61± 0·31 | 6·74± 0·24 | 5·14± 0·31 | 5·99± 0·34 | 5·16± 0·57 |
| | $Hpl_6$ | $2x = 26$ | 35·70± 10·80 | 4·28 | 40·80–105·10 | 73·25 | 4·1 | 6·91± 0·27 | 7·10± 0·27 | 6·59± 0·44 | 6·10± 0·39 | 5·57± 0·16 |
| | Laxmi (female parent of Varalaxmi) | $4x = 52$ | 62·10± 9·16 | 4·00 | 115·30–137·70 | 129·15 | 94·0 | 6·29± 0·24 | 7·92± 0·12 | 6·16± 0·13 | 4·98± 0·09 | 4·19± 0·13 |
| *G barbadense* | S.B. 289–E (male parent of Varalaxmi) | $4x = 52$ | 70·13± 8·68 | 4·00 | 112·20–137·70 | 131·69 | 95·2 | 6·29± 0·77 | 7·01± 0·19 | 6·98± 0·10 | 4·04± 0·12 | 3·98± 0·39 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $F_2$ of inter-specific cross Varalaxmi | $F_2Hpl_1$ | $2x = 26$ | 64·00± 25·34 | 4·67 | 45·90–117·30 | 63·69 | 3·2 | 5·78± 0·22 | 6·28± 0·22 | 6·01± 0·27 | 5·28± 0·21 | 4·78± 0·43 |
| | $F_2Hpl_2$ | $2x = 26$ | 48·00± 14·83 | 4·60 | 45·90–112·20 | 72·97 | 1·2 | 5·98± 0·39 | 6·51± 0·17 | 5·98± 0·20 | 5·51± 0·39 | 5·28± 0·48 |
| | $F_2Hpl_3$ | $2x = 26$ | 36·16± 16·35 | 4·78 | 45·90–117·30 | 79·17 | 1·8 | 6·22± 0·57 | 6·79± 0·36 | 6·50± 0·19 | 5·78± 0·44 | 5·21± 0·39 |
| *G. hirsutum* | IAH 468 | $4x = 52$ | 103·12± 9·26 | 4·00 | 122·10–130·50 | 122·30 | 94·0 | 6·12± 0·12 | 7·76± 0·21 | 6·29± 0·13 | 4·98± 0·11 | 4·12± 0·21 |
| | Haploid | $2x = 26$ | 63·19± 19·80 | 4·53 | 25·50–112·10 | 66·05 | 2·4 | 5·19± 0·29 | 5·75± 0·34 | 5·25± 0·29 | 5·05± 0·17 | 4·79± 0·29 |
| *G. barbadense* | Giza–45 | $4x = 52$ | 86·00± 7·32 | 4·00 | 122·20–130·00 | 120·26 | 95·2 | 6·98± 0·29 | 7·59± 0·13 | 6·11± 29 | 4·59± 0·12 | 3·29± 0·19 |
| | Haploid | $2x = 26$ | 53·00± 18·17 | 4·13 | 30·60–81·60 | 51·31 | 1·8 | 5·17± 0·31 | 5·79± 0·39 | 5·30± 0·20 | 5·10± 0·27 | 4·88± 0·29 |
| *G. hirsutum* | Gregg genetic male sterile | $4x = 52$ | 86·13± 6·16 | 4·00 | 116·17 129·24 | 119·16 ** | 0·0 | 6·98± 0·17 | 7·25± 0·25 | 7·11± 0·16 | 6·98± 0·39 | 6·28± 0·21 |
| | Gregg male fertile | $4x = 52$ | 89·16± 5·29 | 4·00 | 86·29**–112·12 Non-dehiscent anthers | 96·15 | 96·11 | 7·01± 0·21 | 7·51± 0·17 | 6·27± 0·21 | 5·00± 0·41 | 4·13± 0·31 |
| | Cytoplasmic male sterile GH : 572 | * | 82·00± 9·17 | * | * | * | * | 6·21± 0·17 | 17·25± 0·21 | 27·45± 1·39 | 25·25± 2·17 | 4·13± 0·29 |

* PMCs degenerated before premeiotic stages; therefore chromosome number, microspores/PMC, pollen size and pollen viability could not be studied.

** Significant at 1% (between Gregg male sterile and fertile).

## 2. Material and methods

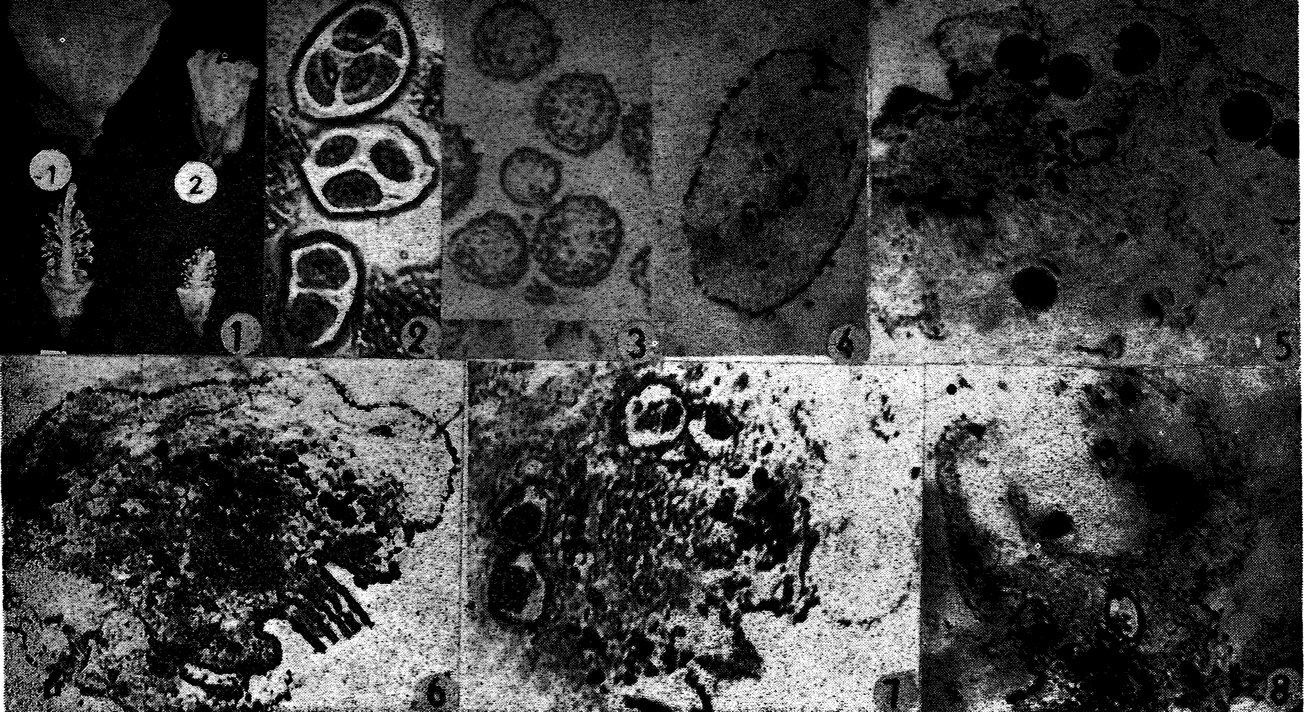
The length of flower bud at various meiotic stages was determined by studying meiosis in the fertile counterparts and haploids. Flower buds of different haploids, their respective parents, genetic male sterile and fertile lines and cytoplasmic male sterile line (table 1) were collected from premeiotic to pollen formation stages and fixed in Randolph's Craf ; after dehydration, the anthers were embedded in paraffin. Sections of 12 $\mu$m were cut and stained with iron alum hematoxylin (Johansen 1940). Pollen viability (fertility/sterility) was tested by differential staining with a solution comprising among other organic components malachite green, acid fuschin, and orange G. (Alexander 1969). The data collected from 25 observations for each of the parameters mentioned in table 1 were analysed statistically (Panse and Sukhatme 1953) and standard deviations and significance of differences between means were calculated.

## 3. Results and discussion

Significant differences were noticed between haploid and diploid plants in size of flowers and androecia, in number of anthers per flower (figure 1), tetrads per microspore (figure 2) and in pollen size and pollen sterility (figure 3). In haploids the average number of microspores resulting from a PMC ranged from 3·77 to 4·78. The large variation observed in pollen size (figure 3) in all the haploids indicated that the pollen grains contained varying number of chromosomes. Although well-developed exine and spines were noticed on some pollen grains, probably microspore mitosis and starch formation had not occurred in them, thus resulting in pollen sterility.

In the cytoplasmic male sterile line the tapetum was well developed and its cells were enlarged. There was normal differentiation of anther wall, but the sporogenous tissue collapsed early during meiosis ; meiosis did not proceed beyond prophase and hence the tapetum remained intact and enlarged (figure 6). Similar observations were recorded by Murthi and Weaver (1974) and Mehetre and Thombre (1981a) for the cytoplasmic and genetic male sterile ($MS_5$ and $MS_6$) stocks of *G. hirsutum*. In the present study on the genetic male sterile stock normal development of anther tapetum was noticed, but the microspores aborted due to development of vacuoles in them (figure 7). In the male fertile counterparts (figure 8), in all the tetraploid parents and in all the haploids (figures 4, 5) the tapetal cells begin to digenerate at the time of separation of microspores from the tetrads.

In the fertile lines the microspores develop a thick exine and a thin intine. Spines develop on the exine and the germ pores become distinct. The microspore nucleus divides to produce the generative nucleus and the vegetative nucleus ; at this stage the pollen grain is considered to be mature and ready for shedding. Similar observations were reported by Murthi and Weaver (1974) and Mehetre and Thombre (1981a) for male fertile anthers and Mehetre (1981) for triploid ($3x = 39$) and tetraploid ($4x = 52$) anthers. In the haploids, however, after separation of the microspores from tetrads the exine may become well developed but the



**Figures 1–8.** **1.** Flowers and androecia of (1) Parent H G 108 and (2) Haploid No. 1 (1/2*x*). **2.** Abnormal "tetrads" of x-ray induced haploid $Hpl_3$ showing different number of microspores (550 ×). **3.** Sterile pollen grains of x-ray induced haploid $Hpl_3$ showing variation in size and underdeveloped spines (450 ×). **4.** Trans-section of anther of haploid $F_2$ $Hpl_1$ prior to initiation of meiosis (20 ×). **5.** LS non-dehiscent anthers of haploid $F_2$ $Hpl_1$ one day before anthesis showing uneven degeneration of tapetum (60 ×). **6.** LS anther of cytoplasmic male sterile line one day before anthesis showing complete intact tapetum (65 ×). **7.** LS anther of genetic male sterile line one day before anthesis showing vacuolated pollen grains (65 ×). **8.** Complete degenerated tapetum of Gregg male fertile line at anthesis (45 ×).

spines are not of uniform size, the germpores are not distinct and the pollen grains do not mature because the mircospore nucleus does not undergo division, as was also observed in the parents of the haploids, such underdeveloped immature pollen does not contain sufficient starch grains.

Decrease in the radial dimensions of tapetal cells occurred in the fertile materials between anaphase I and pollen formation, while in the haploid plants the decrease was observed between anaphase I and tetrad stage only. The extent of decrease was variable from anther to anther, flower to flower and plant to plant.

The data on measurements of radial width of anther tapetum in parents of haploids and in the genetic fertile line indicated that in parents of haploids the degeneration of anther tapetum continues progressively from meiotic anaphase to pollen stages and it ranged from 3 to 6 microns at pollen stage, while in the genetic male sterile line the tapetum remained intact even after microspore tetrad stage. Pollen abortion occurred due to vacuolation of pollen caused presumably by nutritional differences, while in the cytoplasmic male sterile line meiosis did not proceed and hence the tapetum remained intact. A similar behaviour of anther tapetum was also reported by Brooks *et al* (1966) in anthers of different genetic and cytoplasmic male sterile, make fertile and fertility restorer lines of sorghum by Murthi and Weaver (1974) and Mehetre and Thombre (1981a) in cotton.

In all the three groups of haploids a marked variation in the width of tapetal cells from pre-prophase to pollen stage was observed. In some individuals, the degeneration of tapetum was rapid while in some individuals it was slow. Although significant differences in tapetal cell width were noticed in tetraploid and diploid plants, it was not in a 1 : 2 ratio. The pollen abortion and sterility was mainly due to microspores containing variable number of chromosomes and pollen with high variation in size and probably not due to the abnormal development of tapetum.

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# Changes in proteins, amino and keto acids in different seedling parts of *Cyamopsis tetragonolobus* Linn. during growth in light and darkness

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**Abstract.** Comparative changes in protein, free amino and keto acids have been studied in different seedling parts of *Cyamopsis tetragonolobus* plants in light and dark. Endosperm recorded higher level of free amino acids in darkness than in light, while a low concentration of protein was exhibited both in light and dark. The breakdown of soluble protein was more in darkened cotyledon due to higher protease activity. The large increase in the free amino acids in the hypocotyl during seedling growth in the dark may be due to its restricted capacity to incorporate all the amino acids into proteins. Root samples from light recorded higher soluble protein as well as a higher free amino acid pool. $\alpha$-Oxoglutaric acid ($\alpha$-OGA) was recorded in low levels and at few growth stages in both light and dark. In light raised cotyledon samples, the dominating keto acids are phosphoenolpyruvate and pyruvic acid. Low levels of oxaloacetate in light, like $\alpha$-OGA, indicate its rapid utilization during growth, but its accumulation in the dark may suggest sluggish protein synthesis thus sparing the utilization towards the synthesis of amino acids. Utilization of asparagine and glutamine was also affected in dark.

**Keywords.** Seedling parts ; protein ; amino acids ; keto acids ; protease activity ; *Cyamopsis tetragonolobus.*

## 1. Introduction

The correlative changes in amino and keto acids have been studied during seed germination and seedling growth (Fowden and Webb 1955 ; Webb and Fowden 1955 ; Krupka and Towers 1958a, b; Mukherjee 1972 ; Mukherjee and Laloraya 1974, 1979, 1980). Recently, studies have been carried out in our laboratory (Gupta 1981 ; Afria and Mukherjee 1980, 1981) of the comparative changes in aforesaid metabolites along with organic acids and soluble protein in different seedling parts of various plants so that proper assessment could be made of their mobilization and/or breakdown at various growth stages. Leguminous plants can be divided into endospermic and non-endospermic ones depending upon whether the endosperm has been retained into maturity or not. Metabolic

changes during development of endospermic legumes have received less attention in comparison to the other group during seedling growth. For this reason, various biochemical changes with growth in *Cyamopsis tetragonolobus*, an endospermic legume, have been studied here. In this paper the comparative changes in soluble proteins, free amino acids, keto acids and protease activity have been described in endosperm, cotyledon, hypocotyl and root of this endospermic legume, during the early stages of seedling growth in light and dark.

## 2. Material and methods

Seeds of *Cyamopsis tetragonolobus* Linn. were surface sterilized with 0·1% mercuric chloride for 2 to 3 min followed by thorough washing. Acid treatment was given thereafter and washed thoroughly again with sterilized distilled water. Seeds were germinated on filter paper discs moistened with distilled water in Petri dishes and grown in darkness or light (2910 lux provided by fluorescent tubes) in a growth chamber maintained at 30 $\pm 1^\circ$ C. Three replicates of 30 seeds each were taken for each experiment. Every care was taken to select morphologically uniform seeds and to ascertain least variability, experiments on growth in light and darkness (table 1) were repeated thrice. Protein, free amino and keto acids were determined quantitatively in endosperm, cotyledon, hypocotyl and root 48 hr after sowing (termed "initial") as well as 48, 72, 96 and 120 hr after 'initial' of both light and dark grown seedlings.

Soluble proteins from fresh plant material were measured according to the method of Lowry *et al* (1951) using Folin phenol reagent. The plant material was boiled in 80% ethanol for 2 min on a water bath. It was allowed to stand for 15 min at room temperature, ground in the same ethanol and centrifuged at 6000–7000 rpm for 5 min. Supernatant was discarded and the residue was again extracted with 80% ethanol. Supernatant was discarded again and the residue was extracted with 5% perchloric acid, followed by centrifugation at 6000–7000 rpm for 5 min. Supernatant was discarded and the residue was taken out in a test tube containing 1N NaOH and kept for 30 min. in warm water (40–50° C). 0·5 to 1·0 ml of this clear solution was taken and 10 ml of reagent C, which was prepared by adding reagent A (2% sodium carbonate in 0·1 N NaOH) and reagent B (0·5% copper sulphate in 1% sodium–potassium tartarate) in the ratio of 50 : 1 (v/v), was added to it and allowed to stand for 10 min at room temperature. Then added 1 ml of Folin's reagent (diluted twice) rapidly with immediate mixing and allowed to stand for 30 min. The OD was measured at 540 nm in a Bausch and Lomb Spectronic–20 colorimeter. The amount of protein was determined in terms of Bovine Serum Albumin.

The extraction procedure used for amino acids, their chromatographic separation and estimations were the same as recommended by Steward *et al* (1954). For keto acids the extraction procedure of Kaushik (1966) which is a slight modification of the method described by Towers and Steward (1954) has been followed. Free amino and keto acids were quantified as glycine and $\alpha$–oxoglutaric acid equivalents, respectively.

**Table 1.** Changes in fresh weight (in mg), percent dry mass and length in different seedling parts of *Cyamopsis tetragonolobus* in light and dark. Mean ± S.E.

| Stages | Endosperm | | Cotyledon | | Hypocotyl | | Root | | Length in mm | |
|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | Fr. Wt. | % Dry mass | Fr. Wt. | % Dry mass | Fr. Wt. | % Dry mass | Fr. Wt. | % Dry mass | Hypocotyl | Root |
| | | | | | Light | | | | | |
| Initial | 52·91±1·97 | 27·49±0·71 | 29·85±1·14 | 40·53±1·69 | *9·71±0·28 | *22·55±1·29 | ... | ... | ... | ... |
| 48 | 30·33±1·35 | 11·24±0·30 | 41·14±0·71 | 29·31±0·86 | 33·86±1·65 | 8·03±0·11 | 16·40±0·35 | 8·53±0·26 | 15·83±0·71 | 20·60±0·96 |
| 72 | 15·67±0·19 | 10·24±0·15 | 39·17±1·24 | 26·62±1·53 | 36·39±1·27 | 5·66±0·05 | 11·38±0·14 | 11·33±0·19 | 18·40±1·12 | 21·70±1·03 |
| 96 | 11·22±0·45 | 10·07±0·10 | 47·09±0·66 | 21·68±1·23 | 36·78±1·32 | 7·77±0·13 | 12·84±0·21 | 12·53±0·27 | 20·50±1·42 | 24·66±1·40 |
| 120 | 11·22±0·45 | 9·57±0·25 | 47·19±1·69 | 19·54±1·12 | 43·88±1·49 | 7·65±0·20 | 13·40±0·35 | 8·80±0·12 | 22·20±1·11 | 25·73±0·71 |
| | | | | | Dark | | | | | |
| Initial | 64·33±1·92 | 29·50±0·65 | 36·00±1·42 | 41·66±1·98 | *7·90±0·17 | *30·62±1·69 | ... | ... | ... | ... |
| 48 | 29·48±1·33 | 17·46±1·10 | 3(·38±1·27 | 36·99±1·35 | 105·52±1·98 | 6·82±0·10 | 17·39±0·30 | 8·10±0·17 | 37·56±1·35 | 23·56±0·74 |
| 72 | 22·26±0·43 | 17·56±0·25 | 33·27±1·40 | 30·41±1·92 | 129·90±1·72 | 5·67±0·16 | 17·31±0·40 | 6·64±0·26 | 50·68±2·01 | 25·84±1·02 |
| 96 | 11·92±0·05 | 15·83±0·52 | 36·66±1·17 | 29·51±1·45 | 139·73±1·32 | 5·05±0·13 | 20·69±1·11 | 4·69±0·18 | 58·28±1·61 | 30·00±1·40 |
| 120 | 7·94±0·40 | 14·50±0·17 | 30·49±1·80 | 27·68±1·43 | 149·34±1·49 | 4·78±0·13 | 14·59±0·24 | 8·22±0·16 | 62·52±1·29 | 31·12±1·23 |

* Data represent axis as hypocotyl and root did not differentiate.

Protease activity—The method of extraction of the enzyme was a slight modification of that described by Yomo and Varner (1973) and Ihnen (1976). 1% casein solution was prepared in 0·1 N NaOH. 100 mg of each seedling part (at least in 3 replicates) was homogenized in 10 ml of 100 mM phosphate buffer (pH 6·0) and centrifuged at 5000 rpm for 15 min. After filtration the pellet was homogenized with the 5 ml of buffer and the process repeated thrice for maximum recovery. All supernatants were combined so as to make the final volume to 25 ml. Each reaction set received 1 ml of the enzyme extract and 1 ml of casein solution and the pH was 10. The blank set received 1 ml each of enzyme extract, casein solution and 10% Trichloro acetic acid (TCAL) (cold). These sets were incubated at a temperature of 37 ± 2° C for 2.5 hr. 1 ml of 10% TCA (cold) was added to each reaction set after the incubation period was over and both the sets centrifuged. After discarding the residue 1 ml of filtrate was taken from each set and 2 ml of 0·5 N NaOH and 1 ml of 1 N Phenol Folin's reagent were added with immediate mixing. These sets were allowed to stand for 30 min and OD was taken at 540 nm in a ECI Junior Spectrophotometer. Protease activity was expressed in *n* mol of tyrosine equivalent $hr^{-1}$ $g^{-1}$ tissue.

## 3. Results and discussion

Results have been summarized in tables 1–2 and figures 1–3.

### 3.1. *Seedling growth in light and darkness*

Table 1 shows that the growing axis did not differentiate 48 hr after soaking ('Initial' stage) but at 48 hr seedling stage roots and hypocotyls were noticed and the

**Table 2.** *C. tetragonolobus* showing protease activity (n mol) Tyrosine × $10^3$ $hr^{-1}$ $g^{-1}$ (fr. wt.) in different seedling parts in light and darkness.

| Stages (hr) | Endosperm | Cotyledon | Hypocotyl | Root |
|---|---|---|---|---|
| | | Light | | |
| Initial | 2·772±0·036 | 2·592±·249 | *1·726± ·106 | .. |
| 48 | 0·684±0·000 | 2·880±·252 | 2·558± ·249 | 4·464±·200 |
| 72 | 0·900±0·253 | 7·668±·165 | 2·520± ·259 | 3·096±·157 |
| 96 | Trace | 1·332±·259 | 1·296± ·165 | 0·576±·072 |
| 120 | Trace | 1·548±·190 | 1·440± ·252 | 2·916±·225 |
| | | Dark | | |
| Initial | 34·128± ·374 | 59·148±·655 | *16·488±1·590 | .. |
| 48 | 11·592± ·538 | 11·988±·124 | 35·100±0·561 | 40·068±·533 |
| 72 | 8·100± ·272 | 8·532±·409 | 2·772±0·036 | 4·068±·252 |
| 96 | 0·792± ·060 | 1·224±·252 | 0·648±0·000 | 3·060±·095 |
| 120 | 0·432± ·000 | 1·728±·286 | 1·836±0·000 | 1·404±·124 |

* Data represents growing axis as hypocotyl and root did not differentiate.

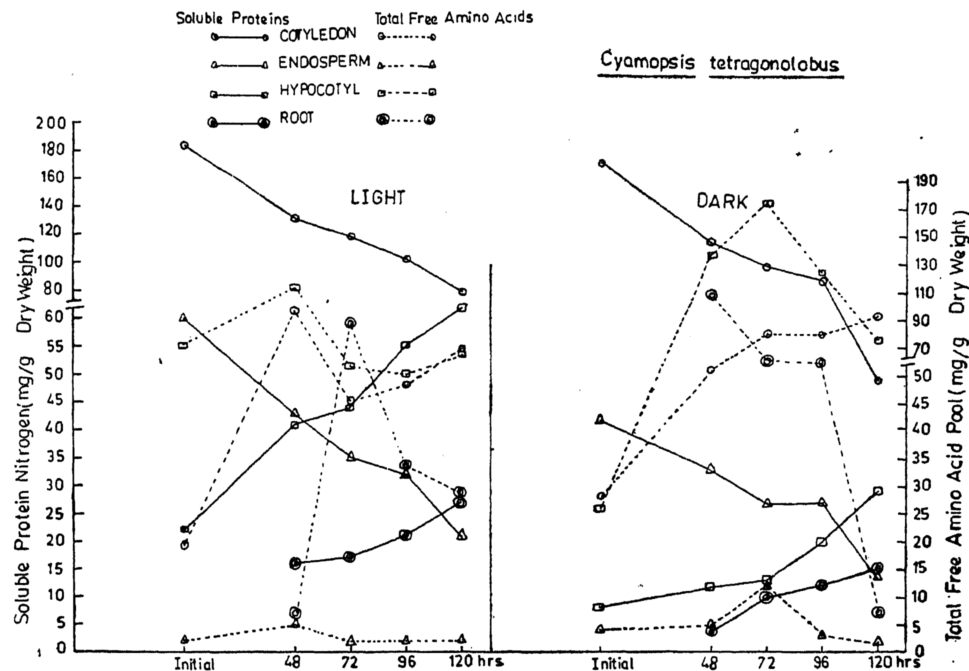


**Figure 1.** *Cyamopsis tetragonolobus*: showing levels of soluble protein—N and total free amino acid pool in light and darkness in different seedling parts at various growth stages.

length of hypocotyls was greater in darkness than in light. At 120 hr stage the hypocotyls length of dark grown seedlings were 181·60% more than those raised in light. Root growth also exhibited the same pattern as recorded for hypocotyls but the increase ranged between 15 to 25% in dark as compared with light.

As regards the fresh weight changes, the endosperm was of greater weight in darkness than in light at 'initial' stage. But with further seedling growth, at 120 hr stage, the value in dark was lower than in light. Changes in percent dry-mass showed a different pattern in that the endosperm from seedlings raised in dark had always a higher value than those in light irrespective of the seedling growth stages (table 1). Cotyledons at 120 hr stage also had more fresh weight in light than in dark. Moreover, percent dry mass although initially more or less of the same value in light and dark decreased much less with further growth in the dark.

Hypocotyls, after differentiation, show more than three-fold increase in fresh weight in dark compared with those in light. However, the dry weight values were slightly lower in the former. Roots exhibited a small decrease in their fresh weight both in light and dark.

Growth data presented here illustrate the two common phenomena of photo-morphogenesis and etiolation in light and dark. Dark-grown seedlings having stimulatory effects on hypocotyl lengthening recorded a linear increase and direct relationship with the fresh weight but percent dry mass exhibited inverse relationship indicating the failure of translocation of the products of reserve hydrolysis to keep pace with the extension growth.

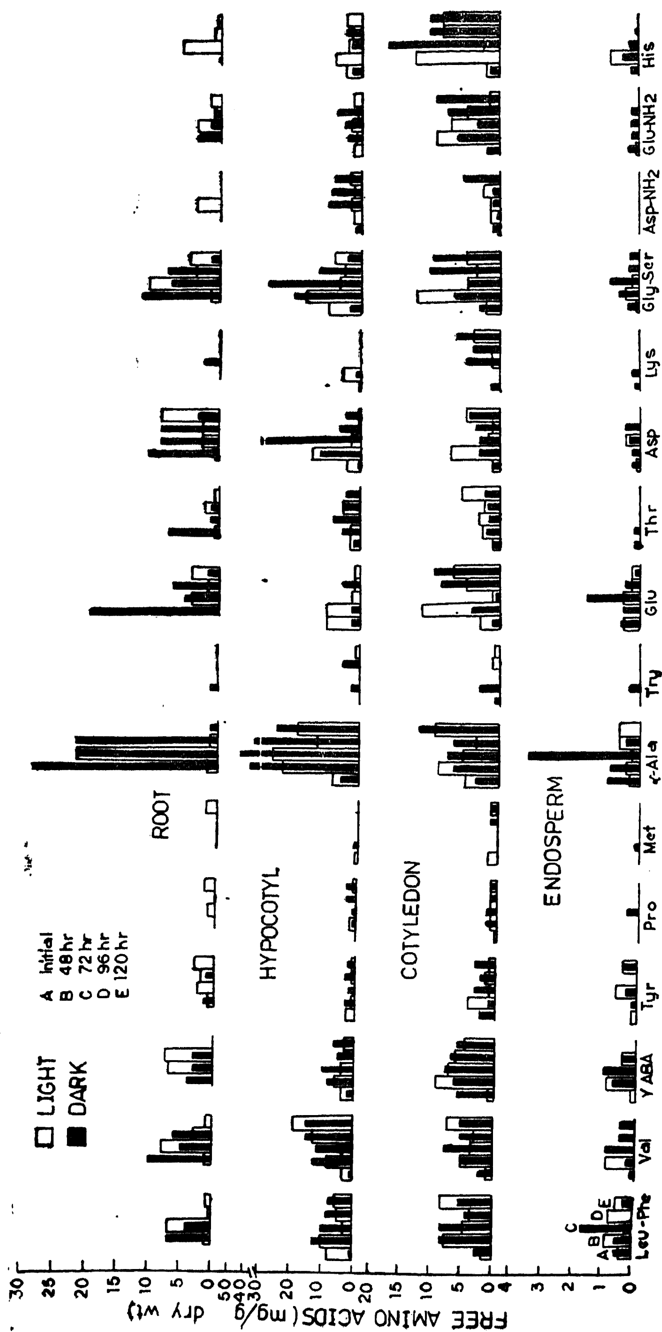


**Figure 2.** *C. tetragonolobus* : Free amino acid changes in different seedling parts at various growth stages.

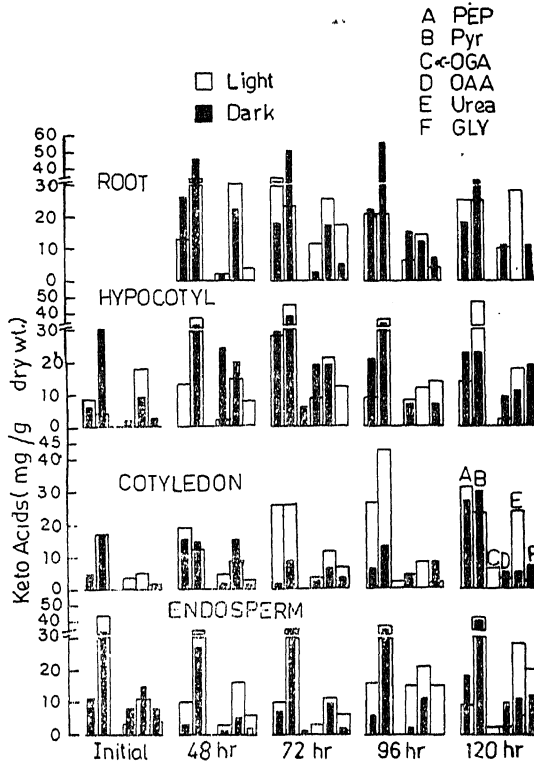


**Figure 3.** *C. tetragonolobus*: Keto acid changes in different seedling parts at various growth stages. *Abbreviations*: PEP, phosphoenolpyruvate; PYR, pyruvic acid; GLY, glyoxylic acid; α-OGA, α-oxoglutaric acid; OAA, oxaloacetic acid.

## 3·2. *Biochemical studies*

The studies with this endospermic legume revealed that the endosperm had low soluble protein values initially which further decreased with seedling growth in both light and dark (figure 1). Endosperm samples from dark treatments recorded a higher number and amount of free amino acids, when compared to corresponding light samples. In light, levels of free amino acids remained low during early stage but dark-raised endosperm samples recorded increasing values up to the 72 hr stage, whereafter they declined (figure 1). Glutamic acid, α-alanine, leucine-phenylalanine, serine-glycine, glutamine and histidine dominated quantitatively in dark-raised endosperm samples (figure 2).

The breakdown of proteins could also be detected in cotyledons of both light and dark grown seedlings. However, the depletion was more in dark since light causes a retention of proteins as mentioned earlier (Rai and Laloraya 1967; Mukherjee and Laloraya 1979). Along with the protein depletion although an enhancement in the free aminoacid pool was expected in endosperm and cotyledons, their marked difference and a large increase in the latter suggests different rates and pattern of accumulation in light and dark and a rapid translocation of these

metabolites (figures 1–2). The hydrolysis of endosperm reserves by enzymes released from the aleurone layer and their absorption by cotyledons followed by the translocation to the growing axis has also been noted by Bewley and Black (1978). Moreover, unequal rate of protein breakdown and free amino acid formation in relation to light and darkness will also influence the transport of amino acids to the growing axes. Further, many-fold increase in the free amino acids in the hypocotyl during seedling growth in dark (figures 1–2) may be due to restricted capacity of dark grown seedlings to convert all the amino acids into proteins as also noticed by Srivastava and Kooner (1972) in *Phaseolus aureus* L. Oota *et al* (1953) while studying the changes in the content of various primary metabolites in germinating *Vigna sesquipedalis* beans showed a decline of these metabolites in the cotyledons while the hypocotyls and roots recorded an increase during early germination for six days. It is also proposed that the growing axes of light grown seedlings are capable of amino acid biosynthesis by amination of carbon skeleton produced in photosynthesis and this process accounts for increase in amino acid levels (Bewley and Black 1978). There is a differential concentration of amino acids in the hypocotyls under the two situations (figures 1–2).

Root samples in the light showed higher protein content in comparison to dark raised samples at all stages and a very marked increase was noticed in the total free amino acid pool at 48 hr stage. Further growth gave a sharp increase in the free amino acid pool in light whereas in dark the decline was more marked and could be observerd from the beginning (figure 2).

Proline and methionine were not widely distributed in different seedling parts of *C. tetragonolobus*. Proline was recorded in cotyledons in both light and dark while endosperm and root contained the same at only few growth stages. Proline may be converted to glutamic acid thus increasing the pool size of this amino acid as reported by Bewley and Black (1978). Cysteic acid was not traced in endosperm and root at any growth stage of light and dark while cotyledon and hypocotyl recorded the same in very low concentrations at few growth stages. Methionine was also traced at few stages. $\gamma$-Aminobutyric acid was found in higher amounts in cotyledons of *C. tetragonolobus* in comparison to other seedling parts although it was of widespread occurrence in different organs. Its higher amount had been reported earlier also (Altschul 1958).

Changes in protease activity in various seedling parts in the light and darkness are shown in table 2. Dark-grown seedlings showed a significantly higher activity of this enzyme than those in light in all parts during growth. Initial stages of all seedling parts were unique in having maximum protease activity. The maximum decline in the activity was noticed in the endosperm and cotyledon which correlated with their protein depletion.

$\alpha$-Oxoglutaric acid, the predominant keto acid, was recorded only at few growth stages mostly in low levels in both light and dark seedling parts of *C. tetragonolobus*. In cotyledons of light grown seedlings, the dominant keto acids were phosphoenolpyruvate and pyruvic acid (figure 3), the levels of which were maintained during seedling growth. Dark raised cotyledon samples had low values which declined further (figure 3). A characteristic feature of the keto acids was a rapid increase in their concentrations followed by later decline. Hypocotyls of light-raised seed-

lings maintained higher levels of phosphoenolpyruvate, pyruvic acid, oxaloacetate and urea up to 72 hr stage followed by a decline and then a small increase, while in dark although above-mentioned keto acids could be detected in higher concentrations, levels of hydrazones recorded a gradual decline after 72 hr stage (figure 3). Higher levels of keto acids could also be found in roots. Low levels, in most of the samples of oxaloacetate, the keto analogue and precursor of aspartic acid like $\alpha$-oxoglutaric acid, can be explained by their rapid utilization during seedling growth. The tendency for accumulation of oxaloacetate especially at 120 hr stage in endosperm, cotyledon and hypocotyl of dark-raised samples may suggest that this keto acid was rapidly utilized in the light-induced growth of seedlings. It has been suggested by Webb and Fowden (1955) that accumulation of keto acids is related to sluggish rate of protein synthesis thus sparing the utilization of keto acids for the synthesis of amino acids.

Higher amounts of keto acid hydrazones of urea in light and dark in *C. tetragonolobus* account for its active role in nitrogen metabolism during seedling growth (figure 3). Asparagine and glutamine, the two common amides, which store excess ammonia to get rid of the toxic compound, recorded higher amounts from hypocotyl of dark grown seedlings in comparison to light while root samples from dark contained no detectable glutamine and asparagine content declined with seedling growth (figure 2). Cotyledons of 120 hr seedling stage were unique in exhibiting accumulation of asparagine and glutamine in dark in comparison to those in light thus sparing the utilization of these compounds in protein synthesis which is affected by dark.

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# Effect of ridge gourd pollen on zoospore germination of *Pseudoperonospora cubensis* and its significance in epidemiology

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MS received 29 October 1981 ; revised 3 June 1982

**Abstract.** Ridge gourd pollen has a stimulatory effect on the germination of *Pseudoperonospora cubensis*. The rate and percentage germination of zoospores increased in the presence of pollen leachates. Spraying of leaves with a mixture of pollen and sporangial suspension enhanced the development of lesions. Early germination of zoospores in the presence of pollen proved advantageous for infection as it provided a prolonged favourable infection period. The results are discussed in relation to the epiphytotics of the disease during flowering period.

**Keywords.** *Pseudoperonospora cubensis* ; ridge gourd ; pollen effect ; zoospore germination ; epidemiology.

## 1. Introduction

*Pseudoperonospora cubensis* (Berk. and Curt.) Rostow, the incitant of cucurbit downy mildew is one of the serious and production limiting diseases of *Luffa acutangula* Roxb. in India. The disease attains serious proportions when the plants start flowering and as a result the susceptible varieties of plants fail to produce fruits. Bains and Jhooty (1975) reported that in Cantaloupes downy mildew appeared during 1972–74, under field conditions only during the flowering and fruiting stage. The effect of host pollen on stimulation of spore germination of fungal pathogens has been worked out in different crops (Chou and Preece 1968 ; Fokkema 1976 ; Preece 1976 ; Meenakshi and Ramalingam 1979 ; Suryanarayana and Ramalingam 1979). So far no report on the effect of host pollen on germination of downy mildew pathogens has been made.

## 2. Materials and methods

### 2.1. *In vitro effect of pollen on zoospore germination*

One of the susceptible varieties of ridge gourd (Pusa Nasdar) was grown in the downy mildew experimental plots to obtain the sporangial inoculum and the host

pollen. Sporangial suspension was prepared by the following method: downy mildew infected leaves were collected at 6 p.m. and the remnants of the downy growth was washed off with moist cotton under running tap water. The leaves were air dried and small bits of leaves with lesions were cut and placed inside petri plates containing a wet blotter with the adaxial surface of the leaf in contact with the blotter. A good crop of sporangia was obtained after incubating the leaf bits for 12 hrs at 22° C in dark. The sporangia were scraped with a blade into a dish containing distilled water. The concentration of sporangial suspension was measured using a haemocytometer and was adjusted to about 10,000/ml. Host pollen was collected and stored at 5° C. Five mg of the pollen was mixed in 10 ml of the sporangial suspension. A suspension of the mixture was placed on glass slides and incubated in moist chambers at room temperature (22–26° C). In controls no pollen was added. Observations for zoospore germination were made under binocular microscope and results were recorded at hourly intervals after fourth hour.

### 2.2. *Effect of pollen on infection of host leaves and lesion development*

Pollen plus sporangial suspension was sprayed on the lower surface of the leaf of 20–30 day old plants and retained inside a glass house. The leaves were covered with moist polythene bags for about 24 hrs and observations for number and size of lesions were made. The effect of pollen in reducing the infection threshold was tested for moisture requirement by covering the leaves with polythene bags for 3, 4, 5 and 6 hrs. After removing the polythene bags the leaf was air dried and left inside the glass house.

### 2.3. *Disease development in the field in relation to age of the crop*

Two varieties of ridge gourd *viz*. Pusa Nasdar, a highly susceptible variety and long variety which is moderately resistant were sown in the month of August 1980 in plots. Disease rating was made at weekly intervals using a 0 to 5 scale as described by Thomas (1977). Fertiliser (NPK 17 : 17 : 17) was applied twice at the age of 20 days and 45 days.

## 3. Observations

### 3.1. *In vitro effect of pollen on zoospore germination*

Sporangial suspension when incubated at room temperature (22–26° C) released zoospores within 1½–2 hrs. Maximum number of zoospores were observed after 2 hrs of incubation. Zoospores remained active in water for 90 to 120 min and then encysted. The data with regard to percentage of zoospore germination and germ tube length with and without pollen are recorded in figures 1 and 2 respectively. Zoospore germination started an hour earlier in the presence of pollen. The percentage of zoospore germination and vigour of the germ tubes in the presence of pollen was greater compared to the zoospore which germinated in the absence of pollen.

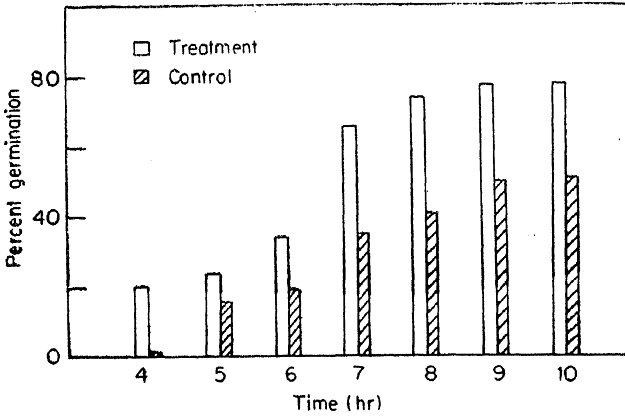


**Figure 1.** Effect of host pollen on the germination of zoospores of *P. cubensis*.

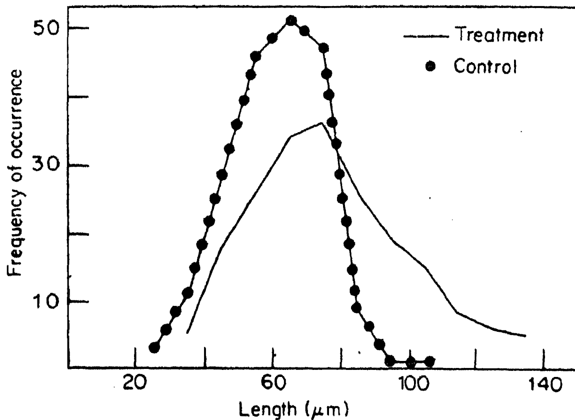


**Figure 2.** Comparison of the germ tube length of zoospores in presence of pollen and in distilled water and their relative frequency of occurrence.

### 3.2. *Effect of pollen on infection of host leaves and lesion development*

Difference in disease reaction was apparent in plants sprayed with the mixture and the sporangial suspension alone. The number and size of lesions were more on leaves sprayed with the pollen mixture. Leaves inoculated with sporangial suspension required a minimum of four hrs of leaf wetness for successful infection under glass house conditions. Only 3 hrs of leaf wetness was needed for infection in the presence of pollen under the same conditions of temperature and inoculum concentration.

### 3.3. *Disease development in the field in relation to age of the crop*

Downy mildew of ridge gourd makes its appearance at the seedling stage. The young true leaves are resistant to downy mildew. When the seedlings attain the

age of 20 days (3–5 true leaf stage) symptoms appear on the true leaves as greenish to yellow lesions. Disease appearance in "Long' is delayed by a week in comparison with Pusa Nasdar. Severity of disease in relation to age is plotted in figure 3. Flowering in both the varieties start 35–40 days after planting. Till then disease severity is less than stage 3 of the 0–5 scale. Soon after flowering the disease reaches severe proportions and it ultimately results in the death of vines in 'Pusa Nasdar' whereas in 'Long' it is not very severe and the vines continue to grow but the yield is significantly reduced.

## 4. Discussion

In saprophytic fungi and facultative pathogens the stimulating effect of pollen on germination of conidia is attributed to carbohydrates (Suryanarayana and Ramalingam 1979 ; Fokkema 1976) but the aggressiveness of such fungi depends on the pollen leachates, rather than the nutrients (Chou and Preece 1968). In *P. cubensis* the zoospores germinate in distilled water thereby showing that it is not nutrient dependent. Hence it is quite probable that pollen leachates provide a stimulatory effect on zoospore germination.

Preece (1976) stated that the effect of pollen on leaf infection may be due to (a) increase in the speed and rate of spore germination. (b) restoring the germinability and infectivity of old spores and (c) reducing the infection threshold by enhancing the speed and virulence of the pathogen. From the present study, it is evident that there is an increase in the number of zoospores germinating and the vigour of germination is enhanced. Under field conditions, sporangial liberation occurs during morning hours (Cohen and Rotem 1971 ; Thomas 1977 ; Bains and Jhooty 1978). It starts at around 6 a.m. and reaches a peak at 8 a.m. For the successful infection of a fresh host leaf a minimum of 4 hrs of leaf wetness is needed. But when the zoospore germinates in presence of host pollen it needs only a period of 3 hrs for infection.

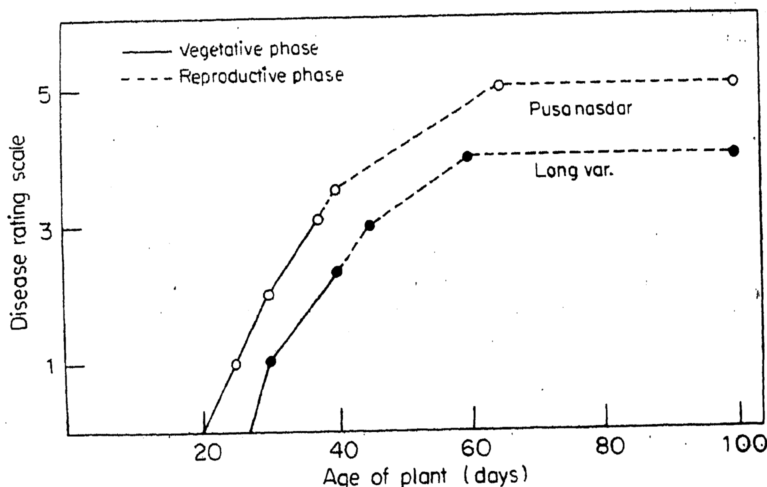


**Figure 3.** Disease progress in two varieties of ridge gourd in relation to age of the plant,

The sporangia liberated during morning hrs are subjected to a period of unfavourable conditions which last until the next dew fall and infection of host plant occurs during night hrs (Cohen and Rotem 1971 ; Cohen and Eyal 1980). From our experiments under Mysore conditions, it is quite probable that those sporangia liberated early in the morning get deposited on the leaves and they start germinating by the production of zoospores. As dew persists at Mysore condition till 9·30 to 10 a.m., infection of the leaf tissue in presence of host pollen can occur successfully during the daytime. In addition, those sporangia which are deposited in later hours of the day, *i.e.*, those sporangia which fail to infect during the morning hours due to the advent of unfavourable period for infection, survive till the next dew fall with a considerable loss in viability of sufficient number of sporangia. Those viable sporangia germinate and cause infection during night hours. As a result, severity of the disease increases significantly during flowering period. This is supported by the results of studies of lesion development and zoospore infection under different leaf wetness periods.

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# Leaf proteinase and nitrate reductase activities in relation to grain protein levels and grain yield in four species of grain amaranth

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**Abstract.** The relationship of leaf nitrate reductase (NR) and proteinase activities to the grain protein level and grain yield was investigated in four species of grain amaranth (*Amaranthus hypochondriacus*, *A. caudatus*, *A. cruentus* and *A. edulis*). A strikingly positive correlation between the leaf proteinase activity and the grain protein content was found. *A. edulis* with higher grain protein level possessed high leaf proteinase activity, while *A. hypochondriacus*, with relatively lower grain protein content had lower leaf proteinase levels. Although there was no definite correlation between the leaf proteinase levels and the grain yield, the integrated leaf NR activity was positively correlated with the grain yield. The total nitrogen content per plant seems to be dependent on the extent of root growth and the levels of NR activity in leaves.

**Keywords.** Grian amaranth ; leaf proteinase activity ; leaf nitrate reductase activity ; grain protein ; grain yield.

## 1. Introduction

Grain amaranth is presently one of the under-exploited crop plants with a considerable economic potential. The grain is regarded to be unique for its high protein content. It is also known to be rich in lysine and sulfur containing amino acids and can therefore be considered superior to the proteins of wheat, corn and rice (Senfit 1980).

Significant correlations were found in the past between the integrated leaf nitrate reductase (NR) activity and grain yield, reduced nitrogen levels of grain and of whole plant in the case of wheat and maize (Abrol and Nair 1978 ; Brunetti and Hageman 1976 ; Hageman 1979). On the other hand Dalling *et al* (1975) found that wheat cultivars with similar levels of NR activity could accumulate variable amounts of reduced nitrogen. Deckard *et al* (1973) identified one maize genotype with relatively low NR activity but a high capacity to accumulate reduced

nitrogen. However, several studies have indicated that NR assays could be a useful predictive selection criterion for grain yield and grain protein levels (Croy and Hageman 1970 ; Fakorede and Mock 1978). Not much work was done on the role of proteinases of leaf in relation to the protein content on grain. That leaf proteinase activities were correlated with grain nitrogen was evident in wheat, rice and maize (Dalling *et al* 1976 ; Perez *et al* 1973 ; Reed *et al* 1980).

In view of meagre work on grain amaranths in general and its nitrogen metabolism in particular, the present study was carried out, leading to an understanding of the relationship between the leaf proteinase activity, grain protein, grain yield and secondly between the leaf NR activity, root growth and the accumulation of reduced nitrogen in four species of grain amaranth.

## 2. Materials and methods

Seeds of *Amaranthus hypochondriacus*, L., *Amaranthus caudatus* L., *Amaranthus cruentus* and *Amaranthus edulis* L. were obtained from National Botanical Research Institute, Lucknow. Plants were grown in 30 cm diameter earthenware pots on soil supplemented with manure (3 parts of red soil + 1 part of farm yard manure) under natural (approximately 12 hr) photoperiod (temperature about 35° C day and 22° C night). Three plants were retained in each pot.

The plants were harvested at three different stages, *viz.*, vegetative stage (30 days after sowing), flowering stage (45 days after sowing) and grain filling stage (60 days after sowing). At each stage, plants from 3 pots were collected and were subdivided into leaf, stem, root and panicle. Fresh and dry weights of these plant parts were determined and the shoot/root ratio was calculated.

Reduced nitrogen content in the dried samples was determined by kjeldahl method using Tecator digestion and distilling systems (Tecator Manual). After anthesis (90 days after sowing) plants were finally harvested to the ground level and the dry weights of the panicle and stover were determined. Grain protein content was calculated by multiplying the grain nitrogen by a factor of 6·25.

### 2.1. *Nitrate reductase* (NR) *assay*

Leaf NR activity was measured at the vegetative, flowering and grain filling stages. Fully expanded young leaves from each pot were collected (at 10 a.m.) into a polyethylene bag placed on an ice bath and were carried into the laboratory. The leaves were deribbed, weighed and were then chopped into pieces. The *in vitro* NR assay was as described by Hageman and Hucklesby (1971).

### 2.2. *Proteinase activity*

The proteinase activity was measured thrice at 15 day intervals after anthesis (60, 75 and 90 days after sowing). The proteinase activity in the middle leaf of the plant was assayed by the modified procedure of Peoples and Dalling (1978). Leaves were homogenised by grinding in a mortar and with a pestle for 90 sec with 5 ml/g extracting medium containing 23 mM sodium citrate ; 155 mM

sodium phosphate ; 5 mM L-cysteine; 5 mM EDTA and 1% PVP, pH 6·8. After straining through cheese cloth the homogenate was centrifuged at 25000 g for 10 min. The supernatant was dialysed at 4° C for 48 hr against 50 mM potassium phosphate buffer, pH 7·0.

1% Bovine serum albumin solution was prepared in 0·05 M Tris–HCl (pH 7·8). 0·1 ml of extract was incubated with 0·5 ml of substrate for 2 hrs at 37° C. The reaction was terminated by adding 0·7 ml of 15% trichloroacetic acid and the soluble nitrogen in the mixture was determined by ninhydrin (Spices 1957). Leucine was used as the amino acid standard.

## 3. Results

### 3.1. *Shoot dry weight*

There was a wide variation in the dry weight of shoot at the vegetative stage between the four species of grain amaranth studied (table 1). Maximum dry matter production during the vegetative stage was in *A. hypochondriacus* followed by *A. edulis*, *A. caudatus* and *A. cruentus*. Though a similar trend was observed at flowering stage, the variation in dry matter content was not significant suggesting that the growth rate during different stages of growth period varied among four species (table 2). The higher dry matter accumulation noticed in *A. caudatus* than that in *A. hypochondriacus* during filling stage may be due to the faster growth rate in *A. caudatus* from flowering stage (table 3). The greater dry matter accumulation in shoots of *A. hypochondriacus* (table 4) than the other three species, might be due to the bigger panicles in the former species.

**Table 1.** Dry weights and leaf reduced nitrogen during vegetative stage (30 days after sowing) of four species of grain amaranth.

| Species | Total dry wt/plant | | | S/R | Leaf reduced N% | Reduced N of stover g/plant |
|---|---|---|---|---|---|---|
| | Shoot g | Root g | Total g | | | |
| *A. hypochondriacus* L. | 9·0 ± 0·4 | 0·80 ± 0·1 | 9·80 ± 0·45 | 11·25 ± 0·25 | 3·85 ± 0·12 | 0·28 ± 0·02 |
| *A. caudatus* L. | 3·5 ± 0·2 | 0·25 ± 0·05 | 3·75 ± 0·26 | 14·00 ± 0·36 | 4·27 ± 0·15 | 0·13 ± 0·02 |
| *A. cruentus* L. | 3·1 ± 0·3 | 0·40 ± 0·04 | 3·5 ± 0·32 | 7·75 ± 0·15 | 4·13 ± 0·08 | 0·11 ± 0·01 |
| *A. edulis* L. | 5·8 ± 0·4 | 0·45 ± 0·03 | 6·25 ± 0·42 | 12·89 ± 0·28 | 3·99 ± 0·07 | 0·19 ± 0·03 |

**Table 2.** Dry weights and leaf reduced nitrogen during flowering (45 days after sowing) of four species of grain amaranth.

| Species | Total dry wt/plant | | | S/R | Leaf reduced N% | Reduced N of stover g/plant |
|---|---|---|---|---|---|---|
| | Shoot g | Root g | Total g | | | |
| *A. hypochondriacus* L. | 44·0 ± 1·8 | 8·2 ± 0·5 | 52·2 ± 2·1 | 5·36 ± 0·25 | 3·92 ± 0·06 | 1·27 ± 0·07 |
| *A. caudatus* L. | 41·5 ± 2·4 | 9·5 ± 0·7 | 51·0 ± 2·0 | 4·37 ± 0·28 | 4·47 ± 0·08 | 1·39 ± 0·06 |
| *A. cruentus* L. | 35·5 ± 1·6 | 8·0 ± 0·3 | 43·5 ± 1·8 | 4·44 ± 0·16 | 3·71 ± 0·04 | 0·09 ± 0·05 |
| *A. edulis* L. | 37·5 ± 1·5 | 7·5 ± 0·2 | 45·0 ± 1·4 | 5·00 ± 0·32 | 3·64 ± 0·05 | 1·02 ± 0·05 |

**Table 3.** Dry weights and leaf reduced nitrogen during grain filling (60 days after sowing) of four species of grain amaranth.

| Species | Total dry wt/plant | | | S/R | Leaf reduced N% | Reduced N of stover g/plant |
|---|---|---|---|---|---|---|
| | Shoot g | Root g | Total g | | | |
| *A. hypochondriacus* L. | 82·0 ± 4·2 | 12·0 ± 0·8 | 94·0 ± 4·5 | 6·80 ± 0·4 | 3·50 ± 0·06 | 2·86 ± 0·21 |
| *A. caudatus* L. | 87·0 ± 3·6 | 15·0 ± 1·2 | 102·0 ± 4·8 | 5·80 ± 0·26 | 3·57 ± 0·04 | 3·29 ± 0·18 |
| *A. cruentus* L. | 66·5 ± 3·8 | 12·0 ± 1·4 | 78·5 ± 4·2 | 5·54 ± 0·32 | 3·44 ± 0·07 | 2·41 ± 0·18 |
| *A. edulis* L. | 69·5 ± 2·3 | 11·5 ± 0·7 | 81·0 ± 3·1 | 6·04 ± 0·22 | 2·94 ± 0·08 | 2·65 ± 0·14 |

### 3.2. *Shoot/Root (S/R) ratio*

The S/R ratio in all the four species was maximum at vegetative stage and decreased gradually during flowering and grain filling stages (tables 1, 2 and 3). *A. caudatus* had the highest S/R ratio among the four species during vegetative phase of growth, but the ratio decreased during flowering and grain filling stages due to increases in root dry weight at later stages of growth.

**Table 4.** Mean NR activity (calculated from figure 1), dry matter at final harvest (90 days after sowing) grain yield, grain protein and mean proteinase activity (calculated from figure 2) of four species of grain amaranth.

| Species | NR activity $\mu$ mol $NO_2^-$ $g^{-1}$ $h^{-1}$ | Dry matter (g/plant) Panicle | Dry matter (g/plant) Stover | Dry matter (g/plant) Total | Grain yield g/plant | Grain protein % | Proteinase activity $\mu$ mol $NH_2$ $g^{-1}$ $h^{-1}$ | Total N g/plant |
|---|---|---|---|---|---|---|---|---|
| *A. hypochondriacus* L. | 26·2 ± 1·4 | 75·0 ± 2·5 | 51·0 ± 1·0 | 126·0 ± 3·4 | 23·6 ± 1·8 | 12·5 ± 0·4 | 21·6 ± 0·8 | 2·42 ± 0·12 |
| *A. caudatus* L. | 23·43 ± 0·8 | 45·0 ± 2·2 | 60·0 ± 1·8 | 105·0 ± 4·0 | 16·8 ± 0·7 | 14·0 ± 0·3 | 31·5 ± 0·6 | 2·96 ± 0·18 |
| *A. cruentus* L. | 20·07 ± 1·2 | 44·0 ± 1·9 | 58·0 ± 2·1 | 102·0 ± 3·8 | 15·6 ± 0·6 | 12·6 ± 0·2 | 28·4 ± 0·7 | 2·15 ± 0·11 |
| *A. edulis* L. | 24·17 ± 0·9 | 69·0 ± 1·7 | 46·0 ± 1·4 | 115·0 ± 3·1 | 21·8 ± 0·7 | 15·0 ± 0·3 | 35·0 ± 1·2 | 2·24 ± 0·09 |

### 3.3. *Levels of leaf reduced nitrogen (%) and grain protein (%)*

Variation in the concentration of leaf reduced nitrogen was observed among the four species of grain amaranth. Maximum protein content was in grain of *A. edulis* while the maximum content of leaf reduced nitrogen was recorded in *A. edulis* (table 4).

### 3.4. NR *activity and grain yield*

The greatest NR activity was at the flowering stage of all the four species of grain amaranth and the activity decreased at grain filling stage (figure 1). The level of NR in *A. hypochondriacus* was more than that in the other three species during vegetative and flowering stages whereas, at grain filling stage, *A. caudatus* had the greatest NR activity. There was a positive correlation between the mean NR activity the three stages and grain yield ($r = +0{\cdot}89$) in all the four species of grain amaranth.

### 3.5. *Proteinase activity*

The results of the mean proteinase activity calculated from figure 2 are given in table 4. The level of leaf proteinase activity was in the decreasing order of *A. edulis* followed by *A. caudatus* ; *A. cruentus* and *A. hypochondriacus*. The leaf proteinase activity was positively correlated with the percentage of protein

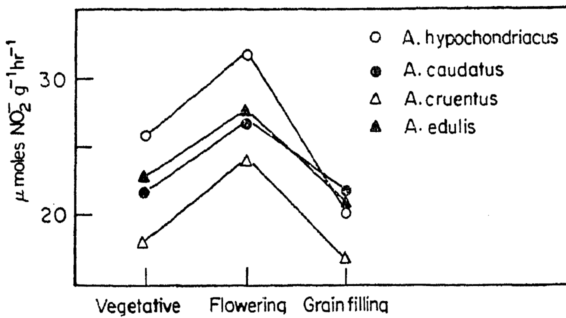


**Figure 1.** The leaf *in vitro* nitrate reductase activity of four species of grain amaranth during different stages of growth.

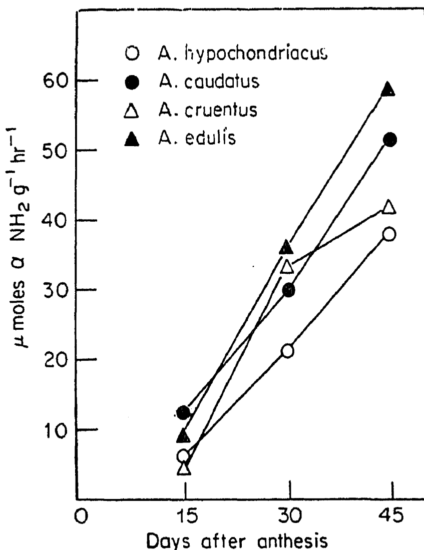


**Figure 2.** The leaf proteinase activity of four species of grain amaranth.

in grain ($r = + 0{\cdot}85$) in all the four species. Leaf proteinase activity increased during grain development (figure 2).

## 4. Discussion

The total reduced N per plant was positively correlated with leaf NR activity ($r = + 0{\cdot}86$) during the vegetative phase. The leaf NR activity was positively correlated with the total reduced N per plant ($r = + 0{\cdot}86$) but not with leaf reduced nitrogen ($r = + 0{\cdot}19$). There was also significant correlation between root growth and the total reduced N per plant ($r = + 0{\cdot}87$) and NR activity ($r = + 0{\cdot}65$). These data indicate that total reduced nitrogen per plant was

influenced by NR activity as well as by the root growth. Therefore the higher NR activity and root growth in *A. hypochondriacus* and *A. edulis* than those in *A. caudatus* and *A. cruentus* resulted in more total reduced nitrogen per plant in the former two species.

During the flowering stage also no positive correlations could be traced between NR activity and leaf reduced nitrogen or ($r = +0 \cdot 16$) or total reduced nitrogen per plant ($r = +0 \cdot 42$) (table 2). *A. caudatus* had high N levels in leaf and in total plant during flowering stage, but its NR activity was less than that of the other three species. Reed and Hageman (1980) observed that accumulation of reduced nitrogen was dependent more on nitrate uptake than on the relative levels of NR activity per plant. Raper *et al* (1977a, b) suggested that nitrogen uptake was positively related to the rate of root growth in tobacco, cotton and soybean. The remarkable increase in root growth of *A. caudatus* during the flowering stage might have resulted in its comparatively high reduced nitrogen per plant (table 2).

*A. caudatus* retained more reduced nitrogen per plant during grain filling stage. Again the NR activity was not related with either leaf reduced nitrogen ($r = +0 \cdot 14$) or total reduced nitrogen per plant ($r = +0 \cdot 34$) in all the four species (table 3). The high root growth of *A. caudatus* could have facilitated the accumulation of more reduced nitrogen per plant either through mobilisation of reduced N or by augmenting the uptake of nitrate and efflux. While there was always a positive correlation between the root growth and total reduced nitrogen per plant ($r = +0 \cdot 78$), the NR activity was not correlated with the total reduced nitrogen per plant even at the harvest (table 4). Thus, our observations confirm that at any stage of growth period in the grain amaranths studied, the leaf NR activity alone is not an index of total reduced nitrogen per plant but the extent of root growth along with the NR levels would together influence the nitrogen content per plant.

The average nitrate reductase activity in the leaf was positively correlated with the grain yield ($r = +0 \cdot 89$) as well as total dry matter accumulation at harvest ($r = 0 \cdot 86$) in all the four species of grain amaranth (table 4). These findings confirm the observations of earlier workers where NR activity was correlated with grain yield (Blackwood and Hallam 1979 ; Deckard *et al* 1977 ; Dalling and Loyn 1977 ; Johnson *et al* 1976).

On the other hand, the protein levels in the grain were not related with the NR activities ($r = -0 \cdot 37$), but were strongly correlated with proteinase activity ($r = +0 \cdot 85$) in all the four species (table 4). This agrees with the results of Deckard *et al* (1977) who concluded that the NR activities were not correlated with grain protein. Differences in nitrogen translocation efficiency could reduce the correlation between NR activity and grain protein (Croy and Hageman 1970 ; Eilrich and Hageman 1973). Leaf proteinase activities were related more closely to accumulation of grain nitrogen than leaf NR activity (Reed *et al* 1980). In the present study, *A. edulis* contained greater proteolytic activity in the leaf and accumulated more protein in its grain than that in *A. hypochondriacus* in spite of high NR activity in the latter species.

Although the number of observations are limited, the present investigation nevertheless suggests that in grain amaranths the level of leaf proteinase activity

is an important determinant of the grain protein content whereas the leaf NR activity and root growth modulate the total reduced nitrogen per plant.

**Acknowledgements**

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# Cell division in *Staurastrum gracile* Ralfs. under the scanning electron microscope

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MS received 3 December 1981 ; revised 14 August 1982

**Abstract.** *Staurastrum gracile* Ralfs. was grown in Chu's No. 10 culture medium, in a culture cabinet at 18–20° C with 16 hrs light and 8 hrs dark period. The cells exhibited polymorphism. The cells were fixed and their division and growth was examined under the scanning electron microscope.

**Keywords.** Desmids ; *Staurastrum gracile* Ralfs.

## 1. Introduction

The process of cell division is unique in desmid biology, with special reference to placoderms, and differs from the other algal groups. Some of the problems it solved, may help in providing a better understanding of some of the principles of morphogenesis and the control of the shape of cells in general. With each division of these, often elaborately-shaped desmids two daughter semicells are produced, which then acquire the typical complex and symmetrical shape of the parent semicells.

Having studied the morphological features, surface ornamentation and polymorphism under the scanning electron microscope (Vidyavati 1981), it was thought desirable to study the division of the cells also.

Previous work on cell division, under TEM and SEM, were mainly contributed by Dodge (1963), Drawert and Kalden (1967), Drawert and Mix (1961), Pickett-Heaps and Fowke (1969, 1970), Pickett-Heaps (1973, 1974, 1975), Schülle (1975) and Brook (1981).

## 2. Material and methods

*Staurastrum gracile* Ralfs. 679/3 was obtained from the culture collection of Algae and Protozoa, Cambridge, U K. The work was carried out at the Botany Department, Royal Holloway College, University of London, U K.

From the cultures thus obtained, unialgal isolations were made following Pringsheim's method and these cultures were maintained in Chu's (1942) 10 medium, at 18–20° C temperature, subjected to alternate light and dark conditions for 16 and 8 hrs, respectively.

Fixation from healthy cultures in exponential growth were made at hourly intervals in order to study the cells at various stages of division and follow the change in shape of the new semicells. The cells were fixed in 1% glutaraldehyde, made up in the culture medium (Chu's 10) for about 1 hr at room temperature, after washing in culture medium, they were then post-fixed for about 1 hr in 1% Osmium tetroxide also made up in the culture medium. They were then washed 3 times in culture medium. The cells were dehydrated in acetone of 30%, 50%, 70%, 90% and 100 %. Fixation, washing and dehydration were all carried out in the centrifuge tubes and each time the cells were centrifuged discarding the supernatant. The cells were then passed through critical point drying procedure. The dried specimens were moved from the CPD apparatus and were mounted on specimen stubs, using transfer on double-sided sticky tape. These were then coated quite heavily with carbon and gold. Specimens were examined at 15 KV in a jeel-JSM-25 S scanning electron microscope.

## 3. Observations and results

*Staurastrum gracile* Ralfs. is known for its polymorphic form, cells are variable, medium in size (length 27–60 $\mu$ ; breadth, including processes, 44–110 $\mu$ ; breadth of isthmus 5·5–13 $\mu$), constriction slight, usually an acute notch ; semicells variable, upper angles produced to from long, slender processes of variable length each with 3 or 4 minute spines and provided with denticulations. The vertical view is usually triangular, sometimes quadrangular, angles are produced to form long processes, chloroplasts are axile with a central pyrenoid in each semicell (West and West 1923).

For many placoderm desmids cell division seems to be the only means of reproduction. Sexual reproduction is rarely observed in nature or under laboratory conditions. During division, the cell symmetry is completely destroyed by a wall that grows around the narrow isthmus joining semicells. During the process of division, the cell enlarges at the isthmus region, and elongates, as a result the semicells are pushed further apart. The median septum then forms and the walls push out to produce the new semicells. As the semicell enlarges lobe formation proceeds and finally the arms of the typical species will be formed by further wall elongation.

When the primary wall is almost fully expanded, the outer secondary wall begins to be laid down with its pattern of ornamentation matching that already established, in the primary wall. The secondary wall also acquires its system of mucilage pores, the position of which are indicated very early in wall deposition which penetrate the entire secondary wall.

The daughter cells remain joined to one another, with their apices, until the shedding of the primary wall. These newly formed daughter cells move apart probably due to the extrusion of mucilage.

It was found that cell division always occurred at a definite time after the beginning of the light period, when the light and dark periods were alternated regularly. This suggests that the onset of illumination triggers the events, which set cell division in motion. Schülle (1975) reported that the total period of development

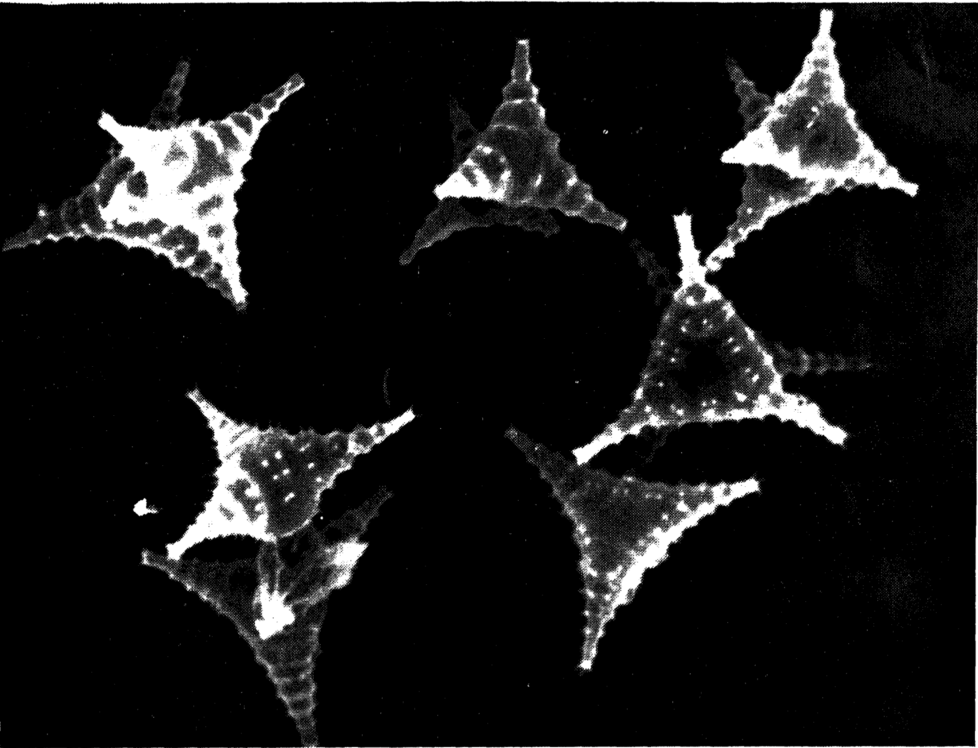
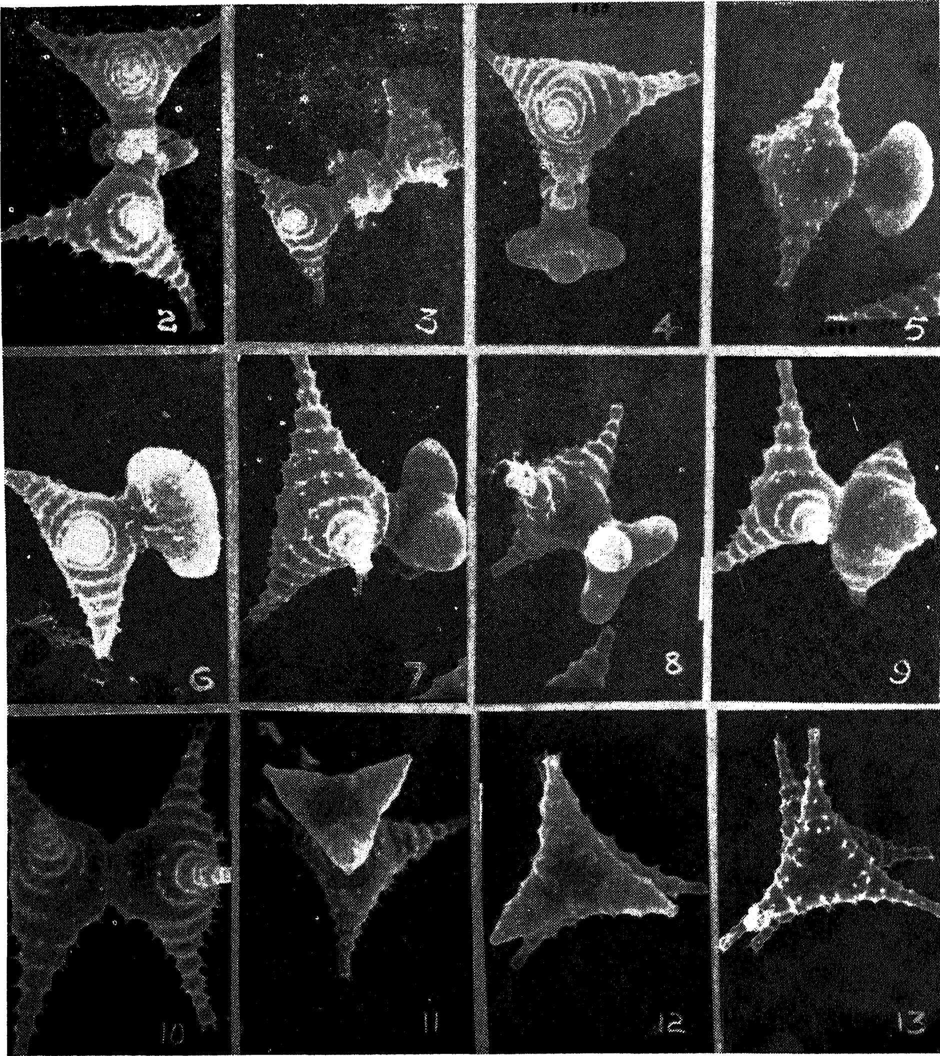


**Figure 1.** *Staurastrum gracile* Ralfs. showing triradiate form ( × 980).




**Figures 2–13.** *Staurastrum gracile* Ralfs. cell division. **2.** Isthmus region becoming elongated. **3** and **4.** Semi cells becoming separated. **5, 6** and **7.** The young semi cell showing bulged and lobed condition. **8.** The lobes elongating. **9.** The development of the typical ornamentation and shape of the semicell. **10.** Mature cell, in side-view. **11** and **12.** Development of the semicell in a triradiate form. **13.** Mature cell viewed from above. [(**2, 3, 11** end **13** (× 840) **4–10** and **12** ( × 1120)]

f newly-formed semicells was from 2–3 hrs. Their development was complete fter this interval of time, but the actual separation of the newly formed cells usually ook another 3 hrs. Under these conditions, cells divide only once every 24 hrs.

Scannnig electron microphotographs were taken at various stages of division. igure 1 illustrates cells in a population, mostly, in a triradiate form. Figures 2, and 4 show enlargement of the isthmus region. Figure 5 shows one smooth oung semicell. Figures 6, 7 and 8 show young semicells bulged and lobe formaon. Figure 9 shows the development of the typical ornamentation and shape f the semicell. Figure 10 shows mature cell in side-view. Figures 11 and 12 iow development of the semicell in a triradiate form and figure 13 shows mature ell viewed from above. Thus figures 2–13 illustrate the various stages in the ell division of the species, under investigation.

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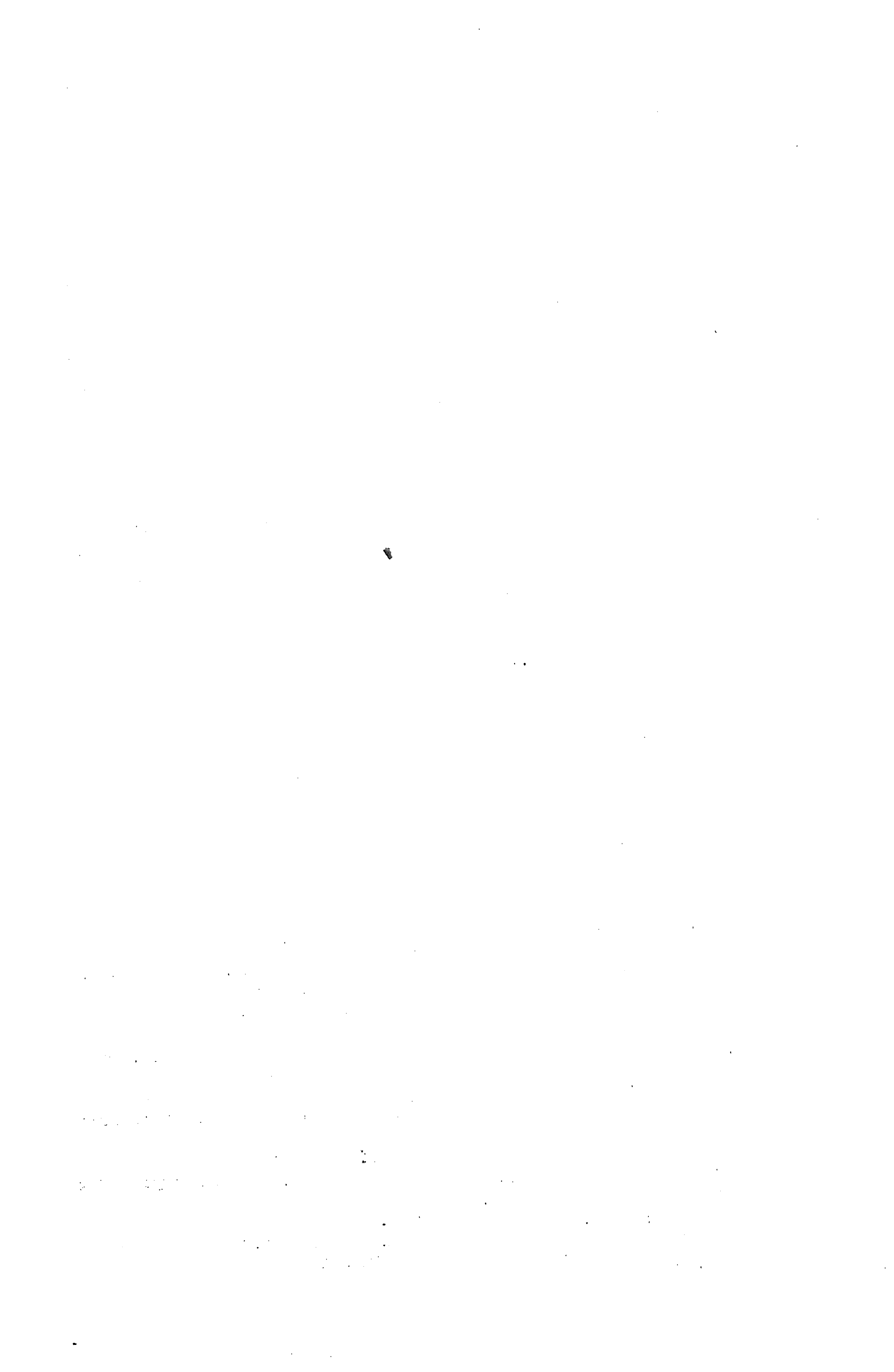

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# Leaf surface studies of some medicinal salvias*

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**Abstract.** Scanning electron microscopic studies on the leaves of 8 medicinal salvias comprising mainly surface ornamentation of the various epidermal cells and the appendages, provide useful parameters to distinguish one species from another. Some of the distinguishing features of the species studied are : *Salvia cabulica* Benth.—Striated lower epidermis, stomatal ledges broad and smooth ; *S. lanata* Roxb.—Abaxial side completely covered over by a thick coat of trichomes ; *S. macrosiphon* Boiss.—verrucose trichomes with constricted joints ; *S. moorcroftiana* Wall.—longitudinal folds on basal cells of trichomes ; *S. officinalis* Linn.—curved cylindrical trichomes, cells over veins with characteristic longitudinal ridges, gland stalk very long ; *S. plebeia* R. Br.—basal cells of trichomes transversely striated ; *S. pratensis* Linn.—verrucose trichomes and series of irregular folds on lower epidermis ; *S. spinosa* Linn.—smooth collapsible hairs, folds on general surface similar to *S. pratensis*.

**Keywords.** Salvia ; Lamiaceae ; leaves ; SEM studies.

## 1. Introduction

A large number of species of the Lamiaceae are presently in medicinal use, particularly in the Indian Systems of Medicine mainly for their essential oils. However, like most crude drugs these are subject to substitution and adulteration. Determination of reliable criteria for distinguishing the genuine drugs is, therefore, greatly important.

Very often the marketed drugs consist of small broken pieces of different organs which are, therefore, difficult to identify. Ultra morphology of the surfaces of diffferent plant organs offers a useful, simple and reliable procedure for authentication and standardization of herbal drugs where often only minute surfaces are available for study (Cappelletti and Casadoro 1977).

*Salvia* (commonly known as Sage in European countries) is one of the most important genera in this respect, of which 9 species are medicinal (Chopra *et al*

* NBRI Research Publication No. 148 (New series).

1956). The genus, comprising of ornamental herbs and shrubs, is distribute mostly in the temperate regions. Some 24 species of *Salvia* are reported fror the Indian subcontinent (Anonymous 1972).

## 2. Materials and methods

The present study deals with scanning electron microscopy of the leaf surfac of 8 *Salvia* species, *viz.*, *S. cabulica* Benth. (Afganistan–Kunar Prov. *S. lanata* Roxb. (Himachal Pradesh–Panjain), *S. macrosiphon* Boiss. (Afganistan Kandhar), *S. moorcroftiana* Wall. (Afganistan–Maidan), *S. officinalis* Lin (W. Germany–Garmisch), *S. plebeia* R. Br. (Afganistan–Kunar Prov.), *S. pratens* Linn. (West Germany–Bavaria), and *S. spinosa* Linn. (Iraq–Basra) collected fro the Herbarium of the Institute for Systematic Botany and Botanical Garden University of Munich, West Germany. Material of *S. lanata* Roxb. fro Herbarium, NBRI, was also used for confirmation.

The dried leaves were first soaked in hot water and after thorough washin 1 cm square strips were cut from the middle portions of the lamina midwa between the midrib and the margin and dehydrated through ethyl alcohol seri followed by critical point drying procedure using liquid $CO_2$. This was part cularly necessary as the oil glands presented a distorted picture on simple dryin 2 mm square pieces were cut from the dried material and one piece each of t adaxial and abaxial surfaces were mounted on to the specimen stubs usir double sided adhesive tapes.

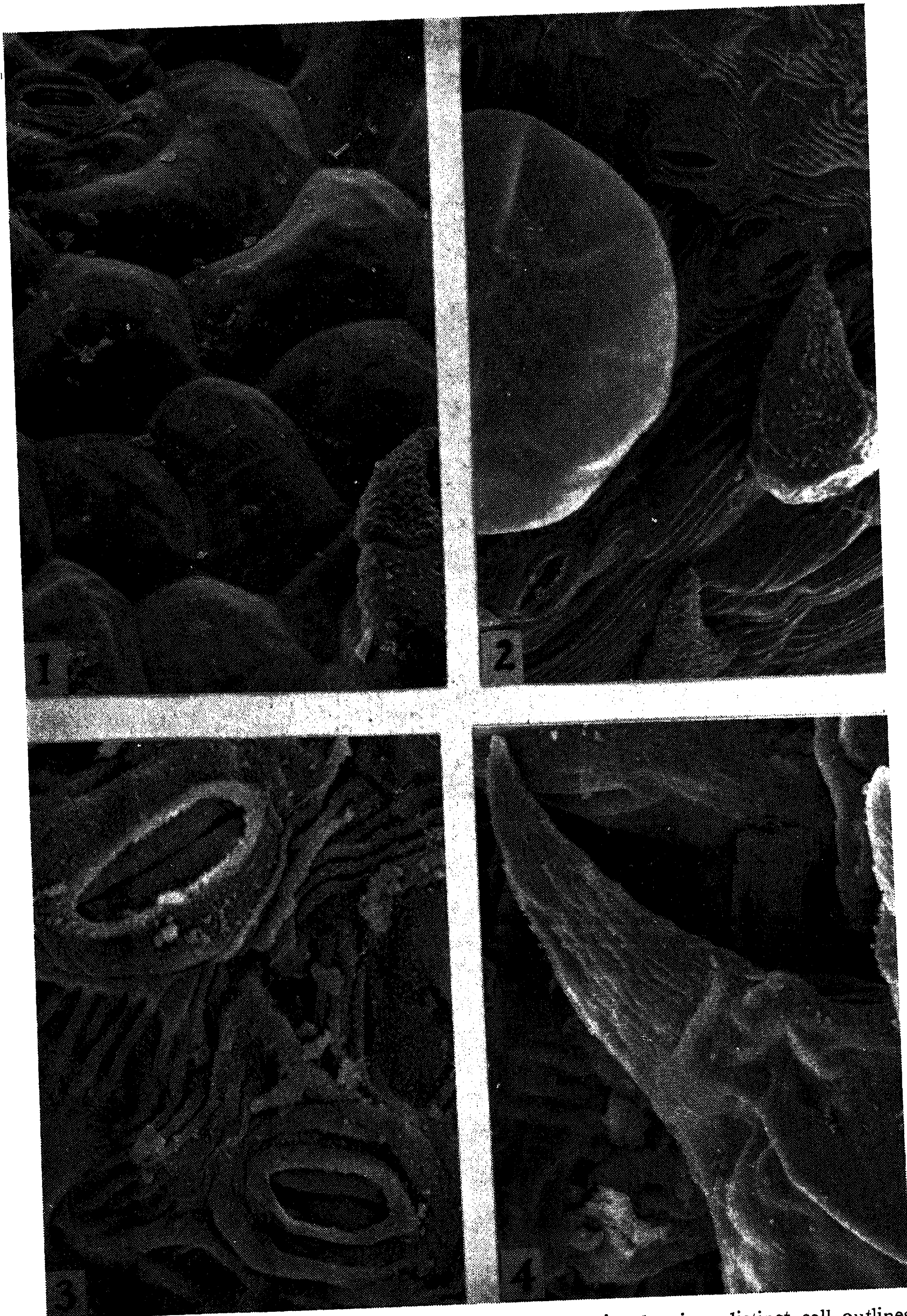
Gold coating of the specimens, about 200 Å thick, was carried out in an ic sputter coater (JFC-1100). The specimens were examined under a JEOL-JSM-35 scanning electron microscope at an accelerating voltage of 10 kV and tilt of 3 incident to the electron beam at an aperture 100 $\mu$m. The image was observed magnifications ranging from $\times 200$–$\times 5000$ and photographs recorded on ORW 120 films.

## 3. Observations

### 3.1. *Salvia cabulica Benth.* (*figures* 1–3)

3.1*a*. *Adaxial surface* : Sparsely hairy, trichomes small, 1–2 celled, extremely thi walled having narrow lumina, basal cells bulbous showing a distinct girdle disc at the base and at the joint; upper cell swollen just above the joint, curv and pointed, more warty than the basal one, warts mammilate, irregularly arrang (figure 1). Epidermal cells polygonal, mostly straight walled or smoothly curve without epicuticular extrusions. Stomata few, mostly diacytic or with four su sidiary cells and flush with the surface. Guard cells longitudinally striat parallel to the pore (figure 1). Glandular hairs very few.

3.1*b*. *Abaxial surface* : Hairs more profuse, up to 4-celled, otherwise simil to the upper ones, stomata also profuse, raised above the surface, lips ridg




**Figures 1–4.** 1. *Salvia cabulica*—Upper epidermis showing distinct cell outlines, a stoma on the left and a bicelled uniseriate trichome on the right (× 600). **2.** Lower epidermis showing distinct cell outlines, epicuticular striations, part of a large glandular trichome on the left and a bicelled trichome on the right (× 600). **3.** A surface view of two stomata with prominent ledges and broad lips (× 1800). **4.** *Salvia lanata*—Trichome from the upper epidermis with the basal cell showing epicuticular folds and a beaked apical cell (× 1300).

ıpward forming a rim round the pore. Epidermal cells wavy in outline and urface rough and profusely striated; striations on each cell sparse except for ıose over the veins (figures 2 and 3). Glandular hairs frequent, all of the same ype, thin walled, heads very large (figure 2).

.2. *Salvia lanata Roxb.* (*figures* 4–7)

.2*a*. *Adaxial surface* : Thickly hairy, hairs of three types (besides the glandular airs) : (i) in some of the 2-celled hairs the basal cells taper upward and appear istinctly cuticularised with broad epicuticular folds. The apical cell is also ıickly cuticularised, has short longitudinal wavy wrinkles and pointed curved r beaked tips (figure 4). (ii) Short, 2–3 celled, basal cells broad at the base nd flattened at the top with edges forming a broad rim. From the centre of ıis arises the next upper cell which is distinctly narrower and gradually tapers pward (figure 5). (iii) The other types are very long, thin, flattened, woolly and ollapsible forming a thick mat which has to be removed to permit a glimpse f the epidermal characters (figure 4).

The epidermal cells are wavy in outline and have raised cuticular rims all along ıe periphery. The central part of the cell surface is again convex upwards. 'urther, parallel cuticular striations are also found on the epidermal surface igure 6). The glands are stalked as usual and the heads are very small, one-elled and capitate.

The lower epidermis is completely hidden under the woolly hairs (figure 7).

.3 *Salvia macrosiphon Boiss.* (*figures* 8–9)

.3*a*. *Adaxial surface* : Hairs upto 6– celled, long, thin-walled, cylindrical but ollapsible on drying, closely tuberculate, tubercles laterally flattened, arranged ı continuous longitudinal ridges (figure 8), ground cell of the hair swollen and aised above the epidermal surface, basal cell shorter and broader than the ıbsequent one. Joints constricted with annulus type thickening on either side. ;pidermal cells smaller than those of other species, outline wavy; intercellular artitions depressed but the general surface raised and striated; striations longi-ıdinal and discontinuous. Stomata on both surfaces equally profuse, distinctly aised above in a broad dome-like manner. Stomatal ledges wavy but compara-.vely narrow (figure 8). Glandular hairs infrequent.

.3*b*. *Abaxial surface* : Hairs similar to those of the upper surface. Cell out-nes marked by longitudinal folds; surface thrown into fine wrinkles and folds ll over. Stomata on the lower side similar to those of the upper surface except ıat the subsidiary cells have broad longitudinal folds along their outer periphery igure 9). Glandular hairs of two types, 1 and 8 celled.

.4 *Salvia moorcroftiana Wall.* (*figures* 10–11)

.4*a*. *Adaxial surface* : Hairs sparse, verrucose, 2–6 celled, comparatively thinner; varts minute, sparse, arranged on longitudinally flattened ridges; surface wrinkled

with loose cuticular folds; joints constricted with upper and lower cells swollen just above and just below the constriction respectively; tips narrow and pointed, sometimes curved (figure 10). Long, thin-walled collapsible type hairs also present. Anticlinal walls of epidermal cells sinuous, but sinuosities obscured by irregular loose cuticular folds all over surface of cells (figure 10); stomata scanty. Glandular hairs scarce.

3.4*b*. *Abaxial surface* : Hairs similar to the upper ones. Long thin collapsible hairs also present. Cell margins appear ridged; stomata almost similar to *S. plebeia* but distinctly smaller and flush with the surface (figures 10 and 15); ledges narrow, margins of the ledges wavy (figure 11). Glandular hairs are of two types: (i) small, one-celled with one-celled stalk and (ii) larger 8-celled, sessile.

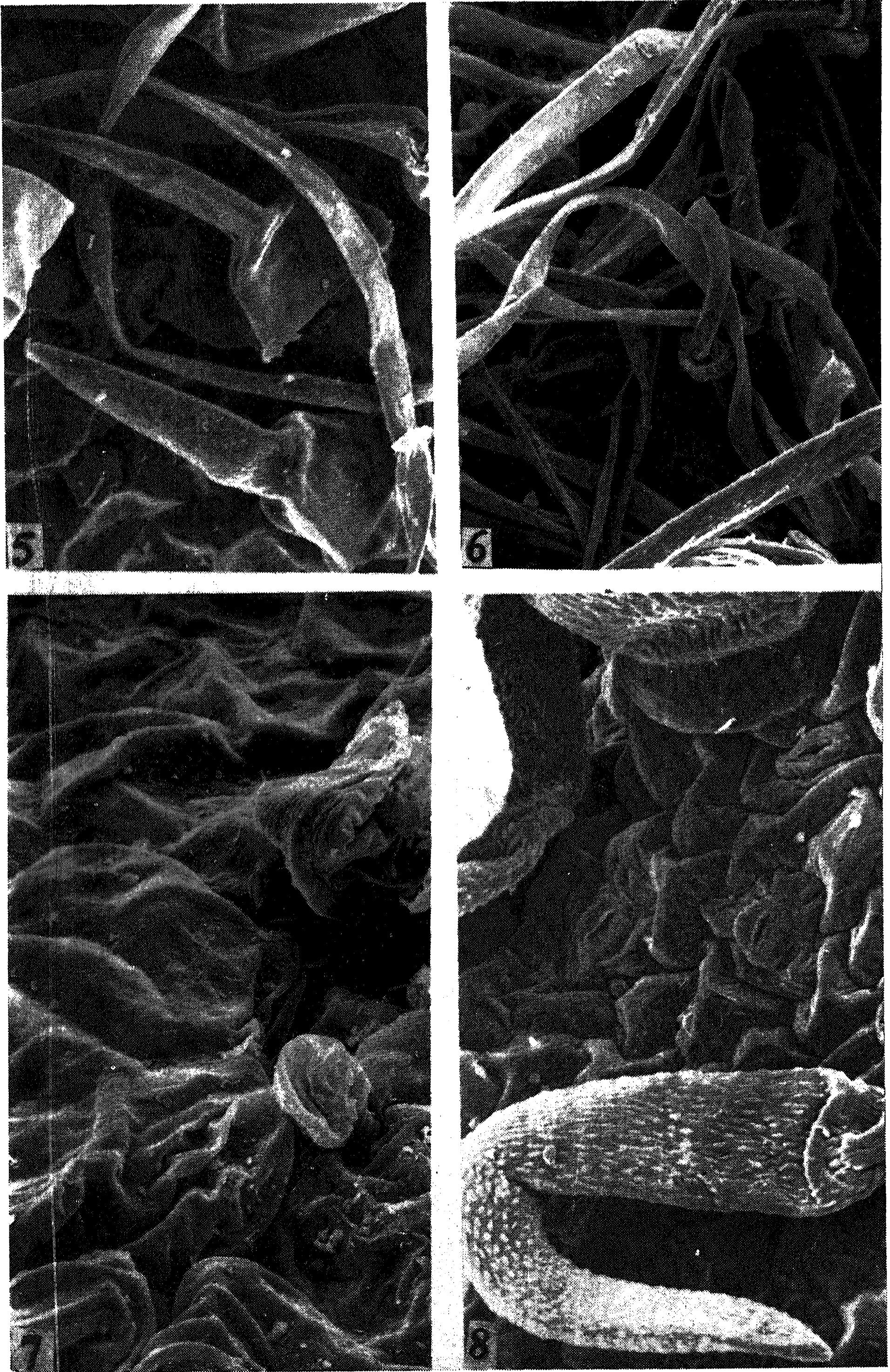
3.5 *Salvia officinalis Linn.* (*figures* 12 *and* 13)

3.5*a*. *Adaxial surface* : Hairy, hairs 2- to several-celled, thin and cylindrical, apex obtuse, usually psilate; basal cell bulbous at the lower end and psilate with very faint transverse markings, upper ones with longitudinal ridges; joints slightly swollen. Upper cells of some hairs over midrib sometimes having a few sparsely arranged warts. Two-celled glandular hairs having long stalks present. Anticlinal walls of epidermal cells sinuate, sometimes sharply so; intercellular partitions depressed; cell surface with broad depressions and epicuticular folds (figure 12). Cells over the midrib elongated having characteristic prominent longitudinal ridges involving even the stalk cells of the secretory hairs. Stomata diacytic, cuticular ridges of subsidiary cells converging on stomata, outer rim of guard cells raised but their central portion depressed; inner ledges wavy; surface with a series of wavy cuticular folds parallel to the opening. Transverse cuticular folds also prominent (figure 12).

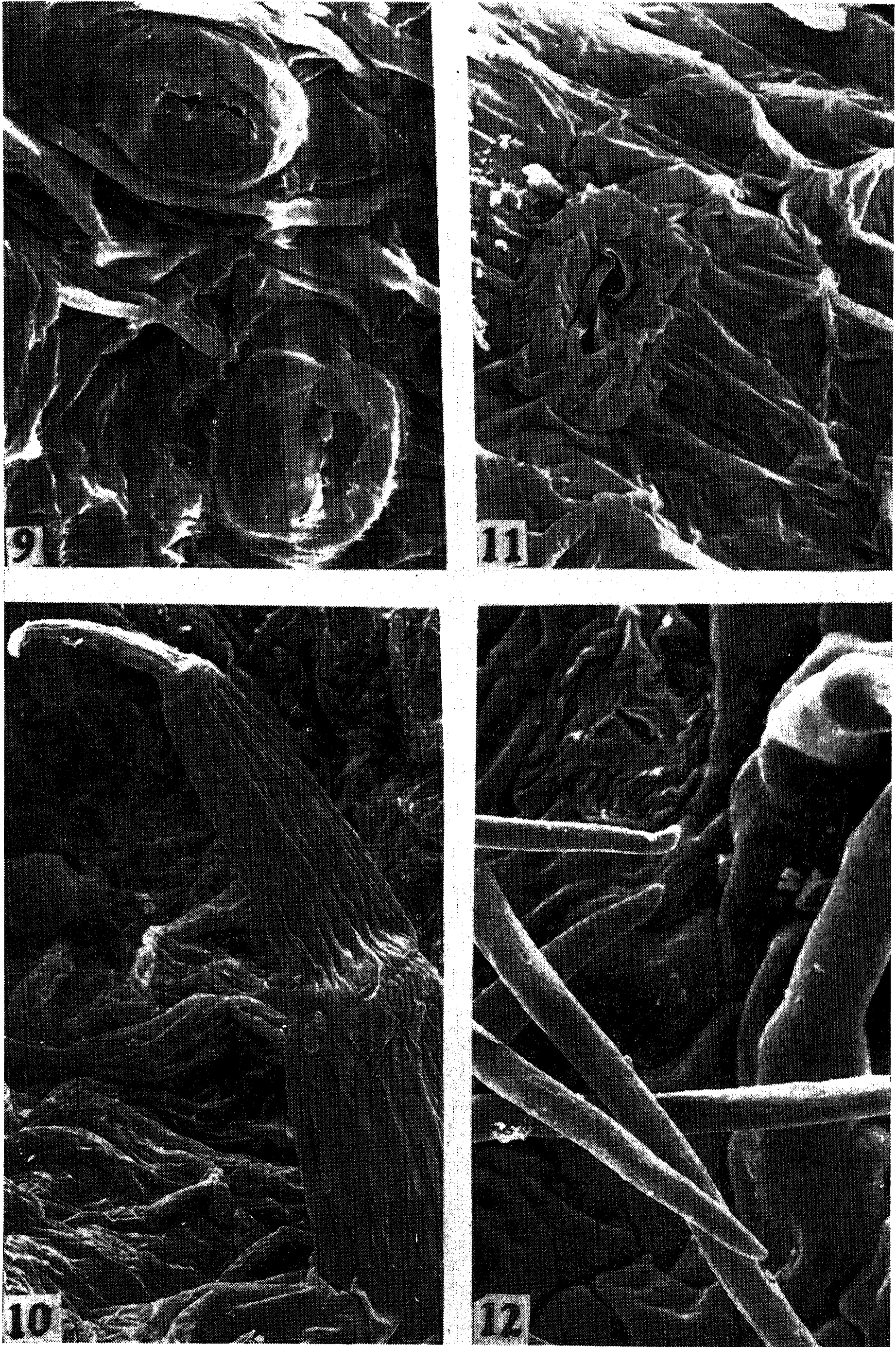
3.5*b*. *Abaxial surface*: Densely hairy, hairs similar to those of the upper epidermis, those over the veins turned backward; characteristic minute, club-shaped hairs also present in this region. Their basal cells are depressed laterally and the upper cells are broader, blunt and flattened (figure 13). Epidermal cells over the veins elongated and very deeply furrowed longitudinally (figure 13). Large 8 celled glandular hairs with small stalks present.

3.6. *Salvia plebeia R.Br.* (*figures* 14–15)

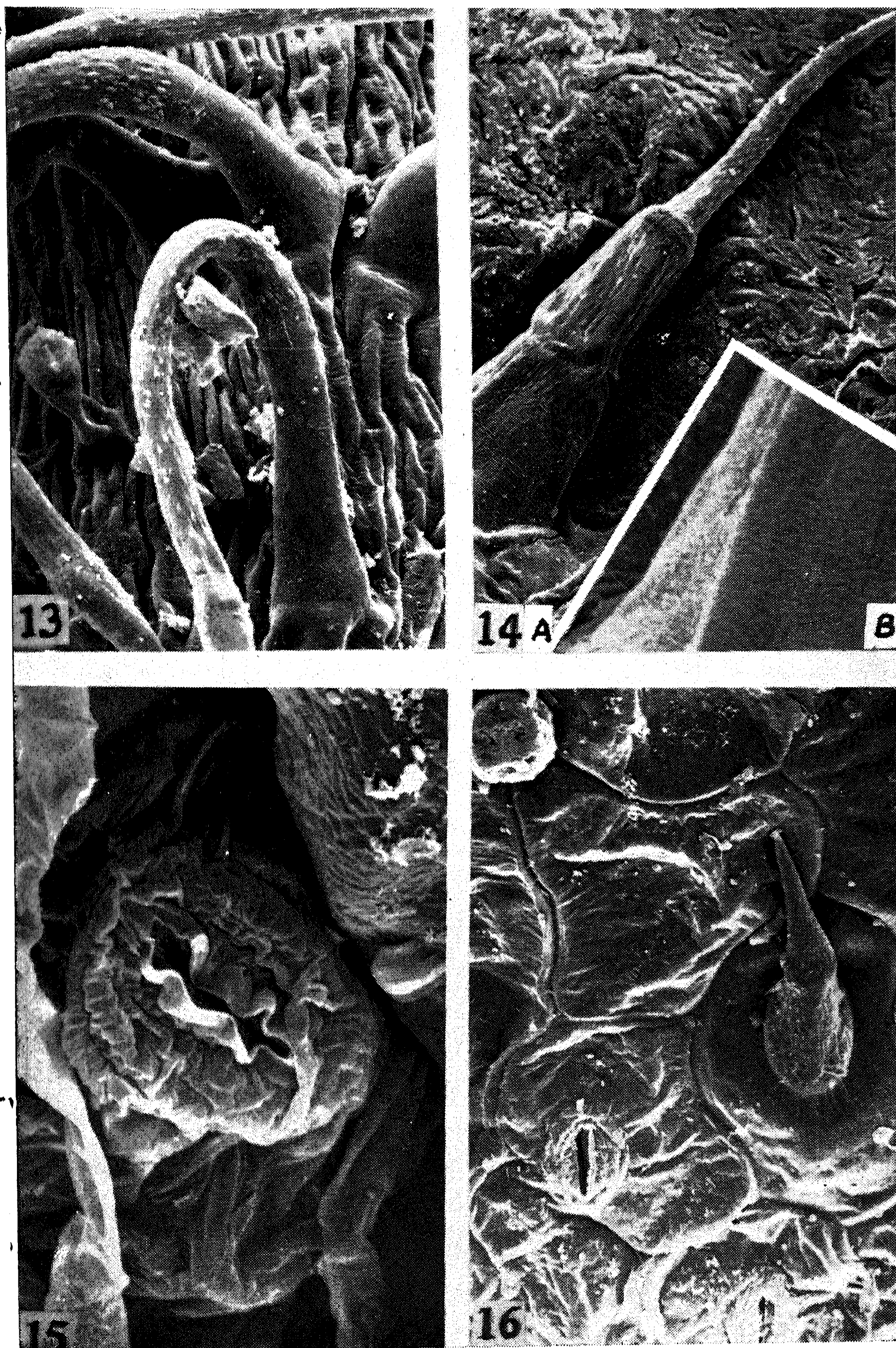
3.6*a*. *Adaxial surface* : Hairs sparse, evenly distributed, stout, thick-walled and verrucose, basal cell broad at the distal end and tapering upward; lower cells polygonal; the apical one cylindrical, very narrow and elongated with pointed tip; joints greatly constricted; tubercles sparse on the two lower cells but much denser on the apical one. Horizontal striations on the basal cell of trichomes noticeable (figure 14 A, B). Stomatal guard cells raised upward, ledges quite prominent and sinuous. Epidermal cells also striated ; striations longitudinal. Glandular hairs occur on both surfaces, heads usually small, 1- and 4-celled; stalk also 2-celled.



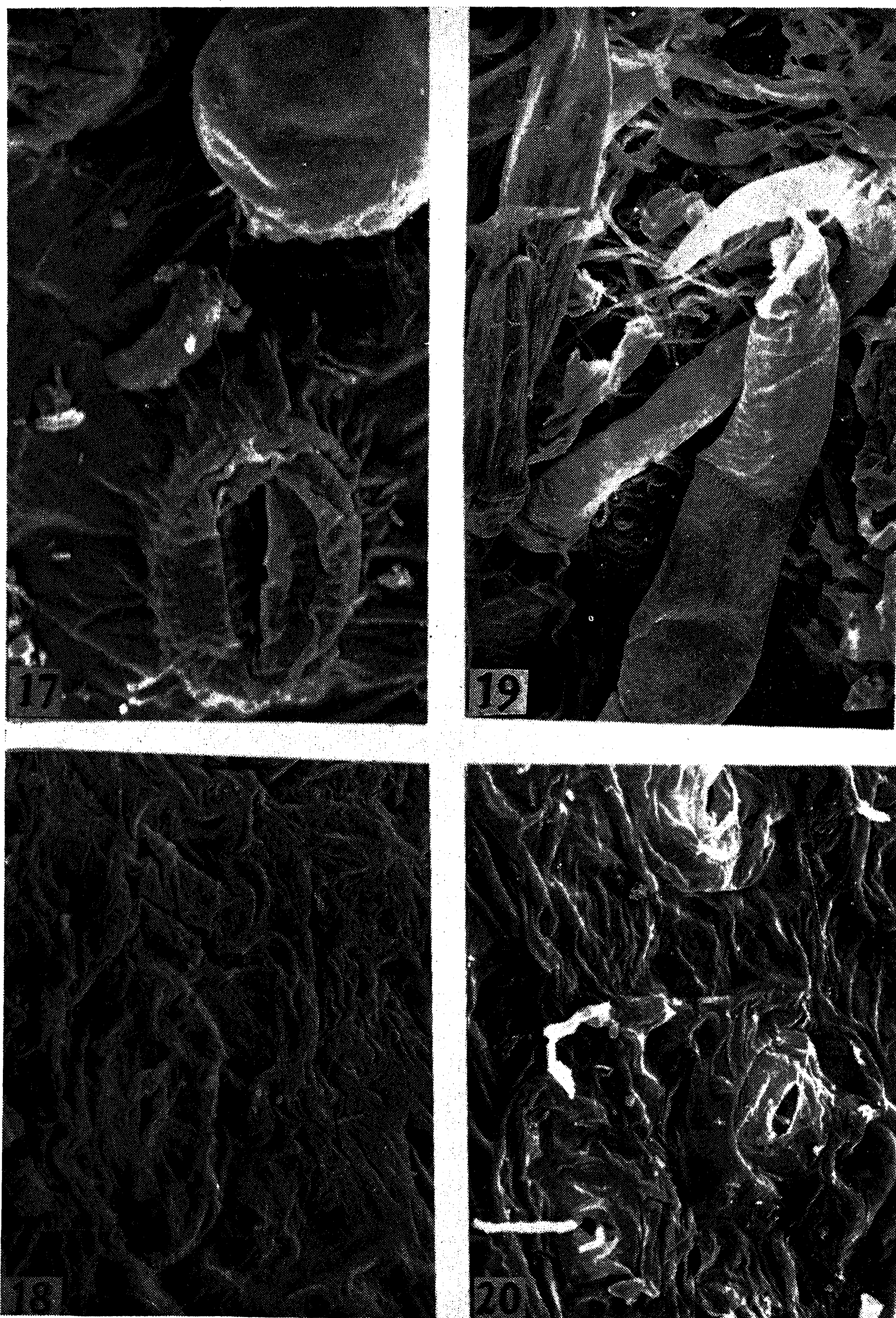

**Figures 5–8. 5.** *Salvia lanata*—Trichomes from the upper epidermis with a broad rounded basal cell and much narrower upper cell (× 600). **6.** Upper epidermis showing epicuticular folds and glandular hairs. Cell outlines indistinct (× 1000). **7.** Lower epidermis covered with a thick mat of collapsible multicellular trichomes (× 600). **8.** *Salvia macrosiphon*—Upper epidermis showing cells with distinct cell outlines, verrucose trichomes showing a constricted joint with annular type thickening and stomata with wavy ledges (× 600).




**Figures 9–12. 9.** *Salvia macrosiphon*—Lower epidermal cells showing broad longitudinal folds and stomata with wavy ledges (× 2000). **10.** *Salvia moorcrofliana*—Upper epidermis showing wrinkled surface with loose epicuticular folds, trichome with longitudinal folds on the basal cell and a glandular hair on the left (× 600). **11.** Lower epidermal cells showing a stoma with wavy ledges (× 2000). **12.** *Salvia officinalis*—Upper epidermis showing trichomes and a stoma (× 1000).




**Figures 13–16. 13.** *Salvia officinalis*—Lower epidermis showing cylindrical trichomes over the vein cells having characteristic epicuticular ridges (× 260). **14 A.** *Salvia plebia*—Upper epidermis showing a trichome with its basal cell having fine transverse striations (× 400). **14 B.** A portion of trichome from figure 14a enlarged to show transverse striations (× 960). **15.** Lower epidermis showing collapsible trichomes and a stoma with wavy ledges (× 2000). **16.** *Salvia pratensis*—Upper epidermis with distinct cell outlines, small glandular hairs, stomata and a trichome (× 600).




**Figures 17–20.** **17.** *Salvia pratensis*—Lower surface showing wrinkled epidermis, a stoma and glandular hair (× 2000). **18.** *Salvia spinosa*—Upper surface of the leaf showing wrinkles all over (× 660). **19.** Lower epidermis showing a large number of trichomes (× 600). **20.** Lower epidermis showing stoma with fungal mycelium coming out of one of the stomatal pores (× 860).

Table 1. Comparative surface character of the leaves of medicinal salvias.

| Parameters | | | *Salvia cabulica* Benth. (1) | *Salvia lanata* Roxb. (2) | *S. macrosiphon* Boiss. (3) | *S. moorcroftiana* Wall. (4) | *S. officinalis* Linn. (5) | *S. plebeia* R. Br. (6) | *S. pratensis* Linn. (7) | *S. spinosa* Linn. (8) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Upper epidermis** | | | | | | | | | | |
| Surface ornamentation | Present | Striations | | * | * | | | * | * | |
| | | Loose folds | | | | * | * | | | * |
| | Absent | | * | | | | | | | |
| Cell partitions | | Distinct | * | * | * | | | | * | |
| | | Indistinct | | | | * | * | * | | * |
| **Lower epidermis** | | | | | | | | | | |
| Surface ornamentation | Present | Striations | * | | | | | * | | |
| | | Loose folds | | Not seen | * | * | | | * | * |
| | Absent | | | | | | * | | | |
| Cell partitions | | Distinct | * | Not seen | | | * | | | |
| | | Indistinct | | | * | * | | * | * | * |
| **Stomata** | Flush on both surfaces | | * | * | | * | | | | |
| | Raised on both surfaces | | | | * | | | * | * | |
| | Flush on the upper but raised on the lower surface | | | | | | | | | * |
| | Flush on the upper but depressed on the lower surface | | | | | | * | | | |
| **Trichomes** | | | | | | | | | | |
| Types | Single type (short) | | * | | * | | | | | |
| | Two types ; short and thick ; long and thin | | | | | * | | | * | * |
| | Three types : short two types ; thin and long | | | * | | | | | | |

| Character | | | – | – | – | – | – | – | – | – |
|---|---|---|---|---|---|---|---|---|---|---|
| Ornamentation of short trichomes | Psilate | | | * | | | | | | *† |
| | Entire surface verrucose/ | | * | | * | | | * | * | |
| | Partly verrucose | | | | | * | * | | | |
| Glandular hairs | | | | | | | | | | |
| Distribution | Occurring on one surface | Upper | | | * | | | | | |
| | | Lower | | | | * | | | | |
| | Occurring on both surfaces | Distribution equal | | * | | | * | * | * | * |
| | | Distribution unequal | * | | | | | | | |
| Heads | Single type | All large | * | | | | | | | |
| | | All small | | * | | | | | | |
| | Mixed | Smaller one | | | | | | | | |
| | | 1-celled | | | * | * | | * | * | * |
| | | 2-celled | | | | | * | | | |
| | | Larger one | | | | | | | | |
| | | 4-celled | | | | | | * | | |
| | | 8-celled | | | | | * | | * | * |

†Occasionally tuberculate. *Character positive.

3.6*b*. *Abaxial surface*: Beside the stout hairs, thin several-celled collapsible hairs also present, particularly over the veins. Epidermal cells wavy and thick walled. Stomata raised and characterised by cuticular ledges which are wavy and overhang the stoma on either side. Beside these the cuticle over the guard cells thrown into two sets of folds, one set wavy and arranged more or less parallel to the stomatal opening while the other set is represented by a fine series of wrinkles at right angles to the long axis of the opening (figure 15). The cuticle of subsidiary cells is also thrown into a series of folds perpendicular to and converging towards the guard cells.

3.7. *Salvia pratensis Linn.* (*figures* 16 *and* 17)

3.7*a*. *Adaxial surface* : Hairs on the lamina very sparse, short, cylindrical, thick-walled and sharply pointed at the tip, 1-3-celled; basal cell very broad at the lower end forming a disc ; upper one swollen just above the joint, verrucose, warts denser on the upper cell and arranged in vertical rows (figure 16). Hairs on midrib and petiole very long, more dense, thin walled and collapsible in dried material. Ordinary epidermal cells sinuate and striated, striations very fine; anticlinal partitions depressed and forming a groove with edges of the adjacent cells raised (figure 16). Stomata slightly raised, lips with raised longitudinal ledges. Glandular hairs very few.

3.7*b*. *Abaxial surface* : Hairs of two types : (i) longer collapsible type over the midrib and (ii) thick cylindrical types elsewhere. The latter 1–4 celled, but otherwise similar to those on upper surface, faint horizontal wrinkles present on the hairs. Stomata diacytic, slightly raised, ledges on stomatal lips quite prominent (figure 17). Epicuticular ornamentation in the form of irregular wrinkles present particularly along the edges of the guard cells (figure 17). Glandular hairs frequent, stalk 1–3 celled, gradually expanding upward, head globular and composed of eight cells.

3.8. *Salvia spinosa Linn.* (*figures* 18–20)

3.8*a*. *Adaxial surface*: General surface undulating and greatly wrinkled all over, wrinkles short, irregular and wavy (figure 18). Peripheral margins of epidermal cells raised, stomata diacytic, similar on both surfaces, outer and inner margins of the guard cells raised. Outer margin broadly ridged, central part concave and wrinkled, one set at right angles to the stomatal pore and the other discontinuous but more or less parallel to it (figure 19). Stomatal ledges prominent (figures 18 and 19). Glandular hairs with large, smooth, 8-celled heads.

3.8*b*. *Abaxial surface* : Surface similar to the upper one but hidden under a thick mat of multicellular hair. Hairs long, thin, smooth with faint longitudinal cuticular markings and a few transverse wrinkles (figure 20); joints constricted. Stomata similar to those on the upper surface but smaller and more dense. A large number of fungal mycelia observed entering through stomatal pores (figure 20).

## 4. Discussion

Comparative morphology of epidermal surfaces using SEM has proved valuable in elucidating taxonomic problems (Atwood and Willams 1979; Ayensu 1974; Cutler and Brandham 1977; Dehgan 1980; Ehler 1974; Rollins and Banerjee, 1975).

Cappelletti and Casadoro (1977) distinguished *Atropa bella-donna* (family Solanaceae) from its adulterants—*Ailanthus altissima* (Miller) Swingle (family—Simurubaceae) and *Phytolacca americana* L. (family—Phytolaccaceae)—with the help of ultra morphology of leaf surfaces.

The present study was undertaken with a view to determine such parameters as could help characterise the different medicinal *Salvia* spp. even if the surfaces available are too small to be of much diagnostic value otherwise.

The study has shown that ultra morphology of the leaf surface provides a useful tool in authentication of vegetable drugs, at least in the case of salvias. The epidermal surface on both sides have epicuticular ornamentation in the form of striations or loose folds in all species except for the adaxial surface in *S. cabulica* and the abaxial one in *S. officinalis*. Of the remaining, *S. plebeia* has striations on both surfaces while loose folds occur on both sides in *S. moorcroftiana* and *S. spinosa*. In all others the two surfaces are dissimilar. Similarly, there are differences in the stomatal characters. Another important feature which helps to characterise the different species studied is the type of trichomes and surface ornamentation (table 1). For instance, whereas only a single type of hair (besides the glandular hair) were observed in three species, *S. cabulica*, *S. macrosiphon* and *S. officinalis*, all others have long, psilate, thin-walled collapsible type of hair as well. In case of *S. lanata* even the short hairs are of two types. Again, except *S. cabulica* and *S. spinosa* all others have verrucose to tuberculate ornamentation all over the trichome surface. However, of the latter ones *S. moorcroftiana* has tubercles on the uppermost cell and *S. officinalis* on the middle one.

Scanning electron microscopic studies have, thus, proved to be extremely useful in characterisation of the different species of the same genus *Salvia* and a positive advantage in standardization of herbal drugs.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# Morphological and histochemical changes in the egg and zygote of *Lagerstroemia speciosa*. I. Cell size, vacuole and insoluble polysaccharides*

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**Abstract.** In *Lagerstroemia speciosa*, the decrease in size of the egg and its micropylar vacuole immediately after fertilization is followed by a progressive and marked expansion of the cell. The PAS-positive cell wall material in egg is confined to the micropylar half. Soon after fertilization, but before completion of decrease in size of the zygote, its cell wall grows in thickness. A complete wall is not formed around the zygote. The bulk of the insoluble polysaccharides in the cytoplasm is localized at the chalazal pole of the egg and zygote. Following fertilization, the size and number of starch granules in the egg cytoplasm significantly increased followed by a decrease and again an increase during zygote development. The morphological changes in the egg following fertilization are probably related to the osmolarity of the cell and of the vacuole which would account for the change in cell size.

**Keywords.** *Lagerstroemia speciosa*; variation in vacuolar size; cell wall; insoluble polysaccharides.

## 1. Introduction

The zygote is the fundamental structural and functional unit which constitutes a new beginning and affords opportunities for investigating growth, development, differentiation, assumption of form and functional activities. Ultrastructural changes have been described in the fertilized egg of a few plants such as cotton (Jensen 1968), *Capsella bursa-pastoris* (Schulz and Jensen 1968), *Zea mays* (Diboll 1968), *Petunia hybrida* (van Went 1970b), flax (D'Alascio-Deschamps 1972), barley (Norstog 1972) and *Quercus gambelii* (Mogensen 1972; Singh and Mogensen 1975). But little is known about the metabolic changes accompanying the formation of the zygote. The present study deals with the structural and certain histochemical changes that result on fertilization of the egg of *Lagerstroemia speciosa* and its subsequent development up to the first division of the zygote.

This paper is dedicated to late Professor B. G. L. Swamy.
* Part of the thesis of PR approved by the University of Calicut for the Ph.D. degree.

## 2. Material and methods

Ovules and seeds of *Lagerstroemia speciosa* (Linn.) Pers. at different stages in development were collected at weekly interval from areas in and around Calicut University campus, fixed instantaneously in the field in formalin-acetic-alcohol or Carnoy's fluid. Conventional method of dehydration through tertiary butyl alcohol series was employed and serial microtome sections (10–15 $\mu$m) were prepared. Insoluble polysaccharides were demonstrated by PAS-reaction (Jensen 1962). Tissue oxidation was carried out in 0·5% periodic acid in distilled water for 20–30 min. Response to the stain was the same by sections fixed by either fixatives. Treatment of tissues fixed in Carnoy's fluid with 1% aqueous mercuric chloride solution gave a negative reaction with Schiff's reagent. Confirmatory test carried out with potassium iodide–iodine stain indicates that the materials stained with PAS is constituted of starch grains and that these occur in plastids. Cellular, nuclear and vacuolar areas were calculated from camera lucida drawings of the stained preparations.

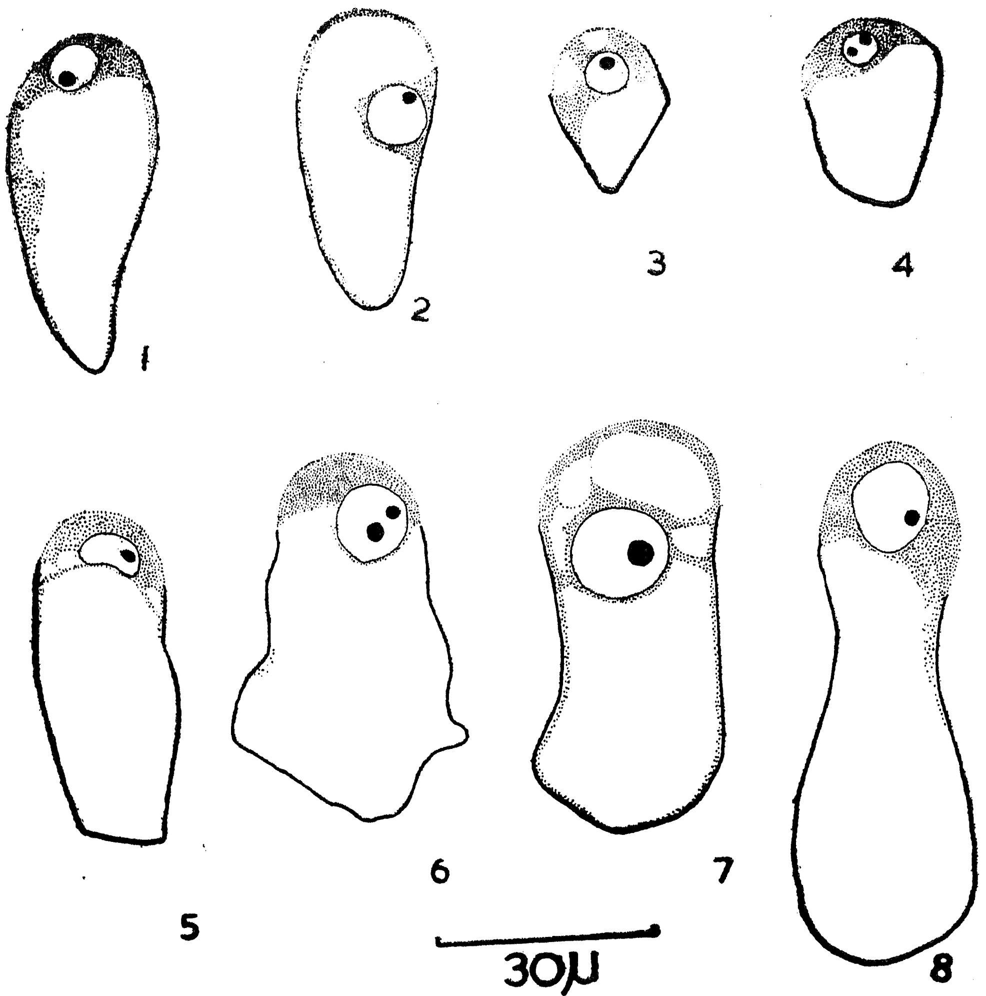
## 3. Results and discussion

### 3.1. *Change in size of the cell and its vacuole*

The pear-shaped egg cell with its broad chalazal and tapering micropylar poles ranges from 457 $\mu$m to 632 $\mu$m in length. It contains a thin layer of cytoplasm surrounding a large vacuole which reaches almost the cell wall at the micropylar pole. The cytoplasm is concentrated in the chalazal half. The nucleus is confined to either the chalazal pole (figure 1) or to a lateral position in the chalazal half (figure 2).

Immediately after fertilization the cell shrinks to about 240 $\mu$m and the vacuole also decreases (figure 3). This decrease in size is followed by a progressive and marked expansion of the cell (figures 4–8), so that the maximal size attained is about five-fold the initial size of the zygote and about two and a half-fold the size of egg cell (figure 8). Furthermore, a progressive flattening of the micropylar region of the zygote occurs. In few preparations the nucleus was seen displaced towards the centre of the cell due to the appearance of two smaller vacuoles at the chalazal pole (figure 3). Subsequently these two apical vacuoles expanded disproportionately to the remainder of the cell and displaced the nucleus to a more central position (figure 7). In 75% of the zygotes examined, only the micropylar vacuole was present which enlarged retaining the nucleus at the chalazal pole itself.

The observed size changes in the egg cell and zygote of *Lagerstroemia* cannot be accounted for merely by biological variation among the preparations examined. In cotton also a prominent shrinkage of the zygote occurs (Jensen 1964, 1968), but not in *Capsella* (Schulz and Jensen 1968) and barley (Norstog 1972). Cell enlargement and cell elongation are considered as general features of the zygote preparing for division. Assuming that cell shrinkage is due to loss of water, the process is reversible because water from the exterior can be abstracted by the maturing zygote. The underlying osmotic changes in the zygote and the




**Figures 1–8.** *Lagerstroemia speciosa*— changes in size of egg, zygote and their vacuole. All are longisections ; micropylar pole towards bottom of page. **1.** Egg cell ; the major part of cytoplasm is confined to chalazal pole. **2.** Egg cell showing lateral disposition of nucleus and cytoplasm at chalazal half. **3.** Zygote ; note decrease in cell volume, and formation of two smaller vacuoles at the chalazal pole. **4–8.** Zygote at later stages in development. Note the progressive increase in size and the flattening of micropylar region.

surrounding milieu result from metabolic changes at these two sites. One of these changes is the reversible soluble carbohydrate/polysaccharide transformation in zygote to be discussed in § 3.3.

### 3.2. *Cell wall*

Both in the egg and in the zygote the extent of cell wall present is variable. Electron microscopic studies have shown that in mature eggs of cotton (Jensen

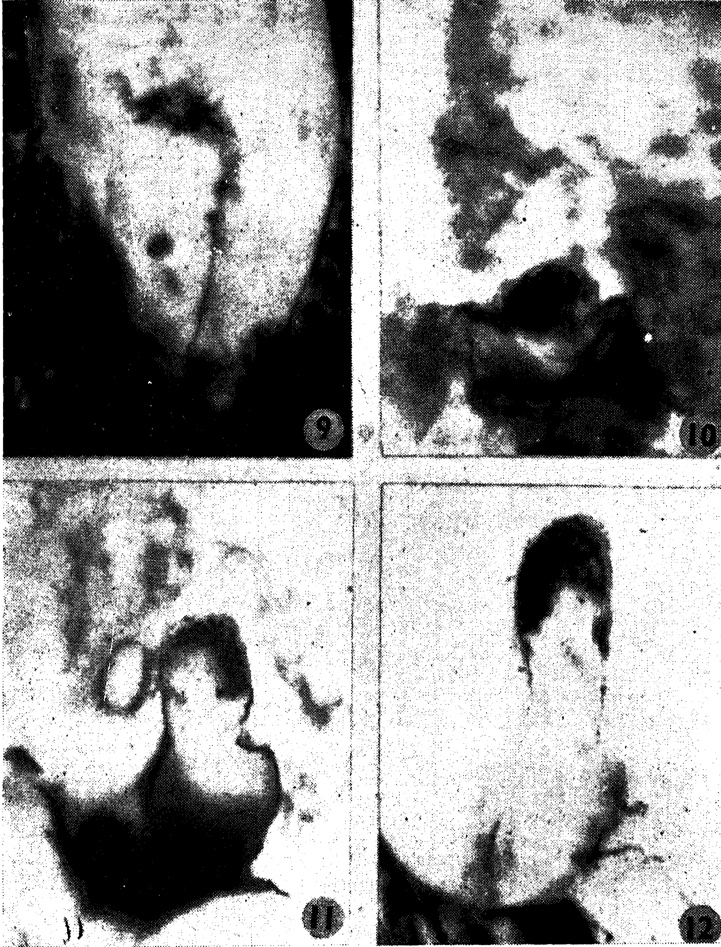
1964, 1965), *Torenia fournieri* (van der Pluijm 1964), maize (Diboll and Larson 1966) and *Petunia hybrida* (van Went 1970a, b) the cell wall extends to only half way up the micropylar pole. However, in the egg cell of *Capsella bursa-pastoris* the wall extends almost over the entire cell ; at the chalazal pole the structure is honey-combed with large gaps (Schulz and Jensen 1968). Thus, in all the species investigated the egg cell shows regions of the plasma membrane at the chalazal pole in direct contact with the embryosac, a feature which possibly enables the egg to derive nutrition directly from the central cell. In general, in angiosperms the micropylar region of the zygote is anchored to the wall of the embryosac, and during development wall formation extends over the open chalazal region and envelops the zygote all round. In *Capsella bursa-pastoris* simultaneously with the deposition of the wall material in the gaps in the chalazal region, the wall in the micropylar portion of the zygote becomes thickened (Schulz and Jensen 1968). In barley the wall of the zygote is thicker at the micropylar region than elsewhere (Norstog 1972).

In *Lagerstroemia speciosa* a positive periodic acid–Schiff reaction was obtained both in the cell periphery and in the cytoplasm of the developing embryo. But in the egg the reaction was confined to the micropylar half, resembling in this respect, cotton, maize, *Torenia* and *Petunia*. But, unlike in cotton, in *Lagerstroemia* a complete cell wall is not formed around the young zygote. In the egg the reaction product is visible as a fine film extending to 60% of the perimeter of the cell from the micropylar pole (figure 9). Soon after fertilization, but before completion of decrease in size of the zygote, the cell wall grows further in length covering 70% of the cell perimeter measuring 56 $\mu$m (figure 10). Subsequently, however, the addition of wall material does not appreciably increase the thickness, and the percentage of wall material to the perimeter of zygote remains unchanged (figure 11). In a nearly mature zygote the wall extends to 112 $\mu$m from the micropylar pole covering 80% of the cell perimeter (figure 12), and its thickness is slightly less than that in the two earlier stages but more than that in the egg.

Street and Öpik (1970) have pointed out that during cell expansion (elongation), the cell wall does not thin out, but there occurs a proportionate increase in cell wall synthesis. It is not clear how a cell would respond to a decrease in cell size. Because in both the egg and the zygote of *Lagerstroemia speciosa* the cell wall is present only at the micropylar half, a decrease in size of the young zygote will not presumably impose as much strain as it would have been if the zygote had an entire cell wall.

### 3.3. *Insoluble polysaccharides* (*starch granules*)

Histochemical staining with periodic acid–Schiff reagent showed, besides cell wall, numerous granules of insoluble polysaccharides in the cytoplasm of both egg and zygote, especially localized in their chalazal pole. In all stages of development beginning from egg, the starch granules are heterogeneous in size and varied in number. The egg cell contains about 50 tiny starch granules (figure 9). Following fertilization, there is a significant increase in their size and number. In the young zygote, which showed a decrease in size, the number of starch granules increased to 90–100 (figure 10). As the zygote enlarged, but before attaining



Figures 9–12. *Lagerstroemia speciosa*—median longisections of egg and zygote after treatment with periodic acid-Schiff reagent. **9.** Egg with PAS-positive wall covering 60% of its perimeter from the micropylar pole. Note the size and number of starch granules at the chalazal pole. **10.** Zygote as seen immediately after formation; note further growth of wall in thickness, decrease in cell size, and number of starch granules. **11.** Zygote at a later stage, showing decrease in size and number of starch granules but with no appreciable change in wall material deposition. **12.** Nearly mature zygote with the wall covering 80% of the cell perimeter. × 850.

maturity, the number of granules decreased to about 60 ; their size also decreased (figure 11). In the nearly mature zygote, a second increase in number to about 100 granules can be noticed (figure 12), accompanied by an increase in size.

In cotton the egg cell contains one or two small starch granules per plastid and there is no specific association of the plastids with other organelles in the cell. Following fertilization, when cell size is decreasing, the plastids accumulate along with mitochondria, around the nucleus (Jensen 1968). At this stage, starch begins to accumulate in the plastids. The close association of the nucleus, plastids and ribosomes presumbly facilitates the ready elaboration of the biosynthetic system (s) and the transfer of the enzyme concerned to the granule or the cytoplasm, as the case may be. During maturation of the zygote, when additional wall material is being formed so as to complete the wall around the entire cell, the number of plastids remains unaltered, but their size increases. Also, the number of starch grains per plastid increases so that the plastids become filled with starch.

The occurrence of starch grains in the egg cell of *Lagerstroemia speciosa*, the increase in their number soon after fertilization and their presence in large amounts in the mature zygote, are in accord with the findings in cotton (Jensen 1968), but the decrease in number and size of starch granules which occurs in the intermediate stage is distinctive of *Lagerstroemia*. The increase in number and/or size of the starch granules following fertilization is accompanied by the decrease in the vaculoar volume. Similarly, a decrease in the number and size of starch granules is sometimes associated with increased vacuolar size, as when the early zygote reaches the intermediate stage of development. Such a formation of starch granules at the expense of soluble sugars stored in the vacuole would reduce the osmotic pressure and result in a diminution of cell and vacuolar size. This would account for the morphological changes, namely, a marked decrease in size, on fertilization of the egg. The reverse process, namely starch degradation and transfer of soluble sugars to the vacuoles, would increase the osmolarity of the cell and vacuole. This would account for the increase in size of the early zygote and its vacuole. Contrary to expectation, the second increase in number and size of starch granules, which occurs in the mature zygote is associated with an actual increase both in cell and vacuolar size.

A significant point is that cell wall synthesis in the zygote occurs at the micropylar half, even though the protoplasm is concentrated at the chalazal half. Evidently, the polarity of the cell does not extend to the metabolic activity of the cell relative to wall synthesis.

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# The floral anatomy of *Puya spathacea* Mez. (Bromeliaceae) with special reference to nectaries

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**Abstract.** The floral anatomy of *Puya spathacea* Mez. is described in detail. The outer floral whorls are united to develop a short hypanthium which is adnate to the base of the ovary. The sepals are five-traced and the petals, three-traced. The placentation is axile. The occurrence of numerous ovules in more than two rows as well as the extension of the carpellary ventrals into the style are less advanced features. The ovarian nectary is extensively developed and shows a transition between typical septal and epigynous nectaries of certain monocotyledonous taxa.

**Keywords.** *Puya spathacea*; floral anatomy; nectaries.

## 1. Introduction

The Bromeliaceae are a fairly large family with about sixty genera and about 2000 species. Hutchinson (1959) considers the family to be a homogeneous taxon representing the 'climax of a line of descent wherein the calyx and corolla have remained distinct or fairly distinct from each other'. He treats it as related to, but more advanced than the Commelinales.

Smith's extensive studies (1934) point out that the family has strongest affinities with the Rapateaceae and that both families probably arose from a common ancestral stock. Within the family, *Puya* is treated as probably the source of ancestral types from which the other sub-families developed. According to Pittendrigh (1948), *Puya* is the most primitive of living bromeliad genera.

The genus *Puya* is a native of the Andes with unique habit and habitat. Some species of the genus are considered to be the largest and most interesting of the bromeliads, *e.g.*, *Puya raimondii* Harms, a rare monument of the Peruvian Andes threatened with extinction. Plants of this species attain a height of 9·5 m and bear thousands of flowers with sugar-poor honey and pollinated by birds. The tiny, winged seeds may number even a billion per plant. The plants are mono-

carpic and hapaxanthic (blooming once in their life and dying out thereafter, and propagated exclusively by seed). *Puya spathacea* Mez. is a much smaller plant attaining a height of 1 m.

Studies in the floral anatomy and morphology of this interesting group of plants are meagre. An extensive investigation on the vascular anatomy of the flower of the group is, therefore, undertaken in this laboratory, and the results on one of the most unique genera are presented in this paper. Amongst the noteworthy earlier contributions on the family is a paper by Budnowski (1922) who is of the opinion that probably no Bromeliaceae lack septal glands. Some work on the group is in progress at Professor Rauh's laboratory in West Germany.

## 2. Materials and methods

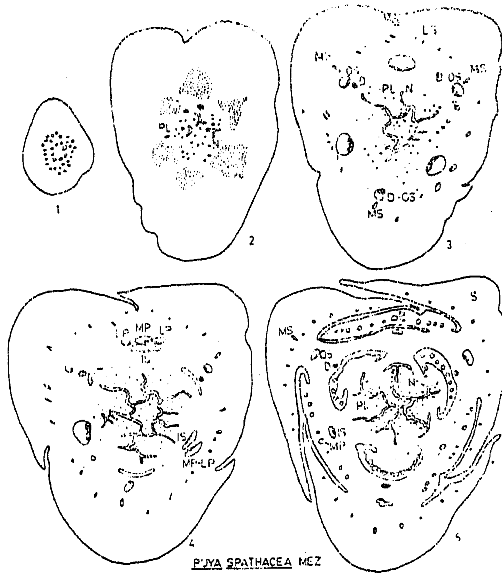
The fixed flowering material was received from Prof. H Merxmuller, West Germany, collected from his University Botanic Gardens. The usual paraffin method has been followed. Serial transections (10–12 $\mu$ in thickness) and longisections were stained with crystal violet using erythrosin as a counter stain.

## 3. Observations

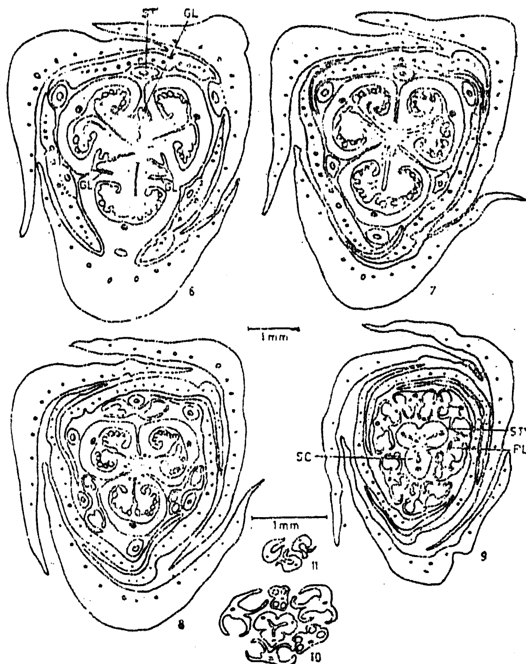
The pedicel contains a ring of six to eight prominent bundles surrounded by many discrete smaller strands (figure 1). All these bundles divide and form numerous strands which resolve into a large number of centrally placed placental bundles and six outer groups of strands from which the principal bundles of the floral whorls emerge out (figure 2). From each of the posterior and antero-lateral groups of strands three LS strands are derived. While the laterals amongst the three branch and extend into the sepals on either side, the median one bifurcates and branches into the margins of both the sepals (figure 3). The remainder of these groups resolve into an inner IS bundle and an outer strand which splits into an MP bundle and two LP strands (figure 4). The three groups in the postero-lateral and anterior positions resolve into the MS, OS and D strands (figure 4)

The nectary is developed from the very base of the ovary beneath the level of the loculi (figure 4). It is extensively developed with many canal-like passage ways and surrounded by the repeatedly dividing placental bundles (figures 3, 5). This gives the appearance of 'processes' of axile tissue of the ovary lined by glandular cells. Upwards, the nectary is closed in the centre to result in three glandular clefts and some of the 'processes' persist in the form of lobes of carpellary tissue enclosed in septal cavities or canals. These open to the outside towards the middle of the length of the ovary (figure 6).

The ovary is trilocular with the placentation axile (figure 6). Upwards, the margins of the carpels meet only in the centre to continue syncarpy (figures 6, 7). Some of the placental bundles arrange themselves opposite to the loculi and these bear traces into the placentae and the ovules (figures 6, 7). The ovules are numerous and borne in many rows on each placenta. Most of the placental bundles end in bearing traces to the ovules and towards the nectary. Six placental




**Figures 1–5.** *Puya spathacea*, serial transections of the flower from the base upwards ; D, carpellary dorsal ; IS, inner staminal strand ; LP, lateral bundle of petal ; LS, lateral bundle of sepal ; MP, median bundle of petal ; MS, median bundle of sepal ; N, nectary ; P, petal ; PL, placental bundles ; OS, outer staminal strand ; S, sepal.



**Figures 6–11.** *Puya spathacea*, serial transections of the upper part of the flower ; GL, gland ; PL, placental bundles ; SC, stylar canal ; ST, stamen ; STY, style,

bundles (carpellary ventrals) continue upwards into the base of the style (figure 8). The ovarian loculi continue in the form of three canals which merge into a triradiate canal in the style (figure 9). The carpellary dorsals continue up to the tip of the style and into the three shallow stigmatic lobes.

The outer floral whorls form a short hypanthium which is adnate to the base of the ovary. The sepals, petals and stamens separate out simultaneously (figure 6). The sepals are twisted to the left and the petals to the right (figure 7). At the level of insertion, the sepals and the petals receive 8 or 9 traces (figure 5). The median bundles of the sepals and the petals bear lateral branches, some of which may divide further.

The six stamens are one-traced. The staminal bundle extends upwards into the connective and ends at the base of the short crest of the anther (figure 10). It bears a lateral branch towards either anther lobe (figure 9). The anther is two-celled. The outer stamens are longer (figure 10).

## 4. Discussion

The vascular anatomy of the flower of *Puya* presents several features of interest and importance. The outer floral whorls are united into a short hypanthium which is adnate to the ovary for some length. This is a trend in the development of an inferior ovary which is characteristic of the allegedly advanced bromeliads, *e.g.*, *Aechmea*, *Billbergia*, *Cryptanthus*, *Neoregelia*. The adnation is, however, not of a marked degree which is also reflected by the absence of fusion of the principal strands of the floral whorls. The placentation is axile. The occurrence of numerous ovules borne in many rows is a primitive feature. The extension of the carpellary ventrals into the style is also a less specialised condition.

The development and the structural details of the nectary are very significant. Initially, the nectary is in the form of a central crater with canal-like passageways which proliferate profusely. The placental bundles repeatedly divide and are lodged in what appear as 'processes' of carpellary tissue lined by glandular cells. The placental bundles have to be associated with the nectary in its function (Agthe 1951 ; Budnowski 1922 ; Frei 1955 ; Pai and Tilak 1965 ; Tilak and Pai 1974). Upwards, the nectary is closed in the centre and the 'processes' of carpellary tissue appear in the form of distinct glandular outgrowths lodged in septal cavities. These are vascularised by the placental bundles. The glandular lobes open to the outside. In *Costus* and *Tapeinochilus* of the Costaceae a more or less similar initial condition is observed which probably prompted Brown (1938) to describe the nectaries in *Costus* as septal. Rao *et al* (1954) describe them as not septal nectaries but as vascularised outgrowths of carpellary tissue which extend upwards in ovarian canals along the septal radii and open at the top of the ovary. In *Kaempferia rosea* (Pai 1966) these glandular lobes appear on the septal radii in similar but epiovarian canals at about the level where those in *Costus* end, and extend above the ovary. In the majority of zingibers, the nectaries are epigynous.

It may be noted that in monocotyledonous taxa with extensively developed septal nectaries with canal-like passageways, vascularised 'processes' of carpellary tissue do occur, *e.g.*, *Musa* and *Ensete* (Tilak and Pai 1974). However, these

do not develop into glandular outgrowths. This would seem to indicate that these 'processes' may persist and extend upwards as glandular lobes or outgrowths in some taxa as in *Puya*, *Costus* and *Tapeinochilus*. While they are short in *Puya* and open to the outside, they proliferate and extend upwards and above the ovary in the latter two genera. The function of nectar secretion is taken over by the lobes or outgrowths so that the canals in which they appear lodged do not have the secretory lining layer. The lining layer in *Puya* is secretory in the basal half and the function of secretion is taken over by the glandular outgrowths upwards.

Anatomical evidence is also significant in this context. The glandular outgrowths in *Puya*, *Costus*, *Tapeinochilus* and *Kaempferia rosea* are vascularised by the placental bundles. As the glands are elevated to an epigynous position, the placental bundles are replaced by the vascular tissue derived from an anastomosing vascular plexus which is generally developed at the top of the ovary in the zingibers (*cf.* Pai 1966).

The condition in *Puya* may, therefore, be considered as transitory ; rather the origin of epigynous nectaries of most zingiberaceous taxa may have to be sought from extensively developed septal glands.

The sepals receive basically five traces while the petals are three-traced. The differential twisting of the two whorls of the perianth is characteristic of many bromeliads.

The stamens are one-traced and the outer whorl of the stamens is longer than the inner. This is a feature observed in many petaloid monocotyledonous taxa (Kulkarni 1973 ; Markandeya 1978 ; Vaikos 1974 ; Vaikos *et al* 1978) and demonstrates the trend in differentiation of the two androecial whorls and the ultimate reduction of either whorl. In the bromeliads, this has not made much headway as is revealed by a study of many genera of the family (Kulkarni, unpublished data). There is a short crest for the anther which is not described in most taxonomic accounts. However, it does not appear to be of pertinent phylogenetic significance.

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# Cytological studies on certain Acanthaceae from Central India

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**Abstract.** Cytological studies have been made on 19 species of Acanthaceae from Pachmarhi hills in Central India. Present studies reveal the first count of chromosome numbers for four species, namely, *Dyschoriste depressa* Nees, $n = 30$ ; *Lepidagathis fasciculata* Nees, $n = 10$ ; *L. hyalina* Nees, $n = 10$ and *Justicia diffusa* Willd. var. *prostrata* Roxb., $n = 9$. New cytotypes have been located in three species as *Hemigraphis latebrosa* Nees, $n = 28$ ($4x$) ; *Rungia parviflora* Nees, $n = 13$ ($2x$) and *R. pectinata* Nees, $n = 13$ ($2x$). Diploid cytotypes of three species, *viz.*, *Blepharis maderaspatensis* (Linn.) Roth, $n = 15$ ; *Justicia betonica* Linn., $n = 17$ and *Thunbergia alata* Bojer ex. Sims, $n = 9$ have been detected for the first time from India. An analysis of the worked out species reveals the existence of only 10·53% polyploid species.

**Keywords.** Cytology ; polyploidy ; cytotypes ; Acanthaceae.

## 1. Introduction

Acanthaceae is a large pantropical family of about 250 genera and 2,500 species (Airy Shaw 1973). The members are chiefly distributed in tropics and sub-tropics but are also found in the Mediterranean regions. There is great diversity in habit and habitat of the members of the family which are usually herbs or shrubs and very rarely small sized trees. Economically, the family is important due to the presence of large numbers of ornamentals and some medicinal plants.

In India the family is represented by about 427 species falling in 81 genera (Santapau and Henry 1973). Although the members are widely represented in our flora and are of economic importance yet very little attention has been paid towards their cytological analysis. The only contributions are by Narayanan (1951); De (1964; 1966); Joseph (1964) ; Kaur (1965a,b, 1966, 1970; Kaur and Nizam (1970); Verma and Dhillon (1967); Datta and Maiti (1968, 1970), Sareen and Sanjogta (1976) and Krishnaswami and Menon (1974). In spite of these, the members of the family from Pachmarhi hills, Central India by and large have not been worked out. Therefore, as a part of our project of cytological studies on the flora of Central India, the present work was taken up in 1978.

**Table 1.** Chromosome numbers in members of Acanthaceae from Central India.

| Name of Taxon | Locality | PUN Accession number/s | Chromosome number | Ploidy level |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| *Adhatoda vasica* Nees | Pachmarhi (M.P.) Jata Shankar, 1,000 m. | 24240 | $n = 17$ | Diploid |
| *Blepharis maderaspatensis* (Linn.) Roth (= *B. boerhaaviaefolia* Pers) | Pachmarhi Little Fall, 950 m. | 20847 | $n = 15$ (figure 11) | Diploid |
| *Crossandra infundibuliformis* Nees | Pachmarhi Raj Bhawan, 1,000 m. | 24235 | $n = 19$ | Diploid |
| *Dicliptera bupleuroides* Nees | Pachmarhi Jata Shankar, 1,000 m. | 24207 | $n = 13$ (figure 2) | Diploid |
| *D. roxburghiana* Nees | Pachmarhi . Pathar Chata, 900 m. | 24208 | $n = 13$ (figure 1) | Diploid |
| **Dyschoriste depressa* Nees | Pachmarhi : Raj Bhawan, 1,000 m. | 20841 | $n = 30$ (figure 12) | Tetraploid |
| *Gendarussa vulgaris* Nees (= *Justicia gendarussa* Linn.) | Pipariya (M.P.) : 200 m. | 24223 | $n = 15$ (figure 3) | Diploid |
| ***Hemigraphis latebrosa* Nees | Pachmarhi : Jata Shankar, 1,000 m. | 20832 | $n = 28$ (figure 13) | Tetraploid |
| *Hygrophila auriculata* (Schum.) Heine (= *H. spinosa* T. And.) | Pachmarhi : 1,050 m. | 24228 | $n = 16$ (figure 4) | Diploid |
| *Justicia betonica* Linn. | Pachmarhi : Bee Fall, 950 m. | 20840 | $n = 17$ (figure 5) | Diploid |
| *J. diffusa* Willd. | Pachmarhi : Mandadeo Caves, 900 m. | 24232 | $n = 9$ (figure 7) | Diploid |
| **J. diffusa* Willd. var. *prostrata* Roxb. | Pachmarhi : Mandadeo Caves, 900 m. | 20839 | $n = 9$ (figure 6) | Diploid |

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| *Lepidagathis cuspidata* Nees | Pachmarhi : Dhupgarh, 1,200 m. | 24239 | $n = 11$ (figure 14) | Diploid |
| *L. fasciculata* Nees | Pachmarhi : 1,000 m. | 20830 | $n = 10$ (figure 16) | Diploid |
| **L. hyalina* Nees | Pachmarhi : Jambu Dwip, 950 m. | 20838 | $n = 10$ (figure 15) | Diploid |
| *Petalidium bralerioides* Nees | Pachmarhi : Pagara, 800 m. | 24237 | $n = 16$ (figure 8) | Diploid |
| ***Rungia parviflora* Nees | Pachmarhi : Jata Shankar, 1,000 m. | 20833 | $n = 13$ (figure 17) | Diploid |
| ***R. pectinata* (Linn.) Nees | Pachmarhi : Polo garden, 950 m. | 20842 | $n = 13$ (figure 9) | Diploid |
| *Thunbergia alata* Bojer ex Sims | Pachmarhi : 1,050 m. | 24226 | $n = 9$ (figure 10) | Diploid |

* Reported for the first time.
** Reports of new chromosome number. For previous reports reference is made to Darlington and Wylie (1955), Love and Love (1961, 1964, 1975) Fedorov (1969). Index to Plant Chromosome numbers (1956–1974) and IOPB chromosome number reports (1964 onwards).

## 2. Material and methods

Materials for the present investigations were collected from different populations of wild as well as cultivated plants from Pachmarhi and its surroundings. The specific locality and altitude of each taxon are indicated in table 1. For meiotic studies anthers from young flower buds were squashed in 1% acetocarmine after fixing for 24 hrs in carnoy's fluid. Slides were made permanent in the usual manner and mounted in cuparal. Voucher specimens have been deposited in the Herbarium, Punjabi University, Patiala (PUN).

## 3. Observations

Information about chromosome numbers of the presently worked out 19 species belonging to 13 genera of the family has been provided in table 1 (figures 1–17). Course of meiotic division was found to be normal in all the worked out taxa.

## 4. Discussion

*Hemigraphis latebrosa* Nees with $n = 28$ is a new record for the species and is tetraploid. Earlier Sareen and Sanjagta (1976) recorded a diploid species with

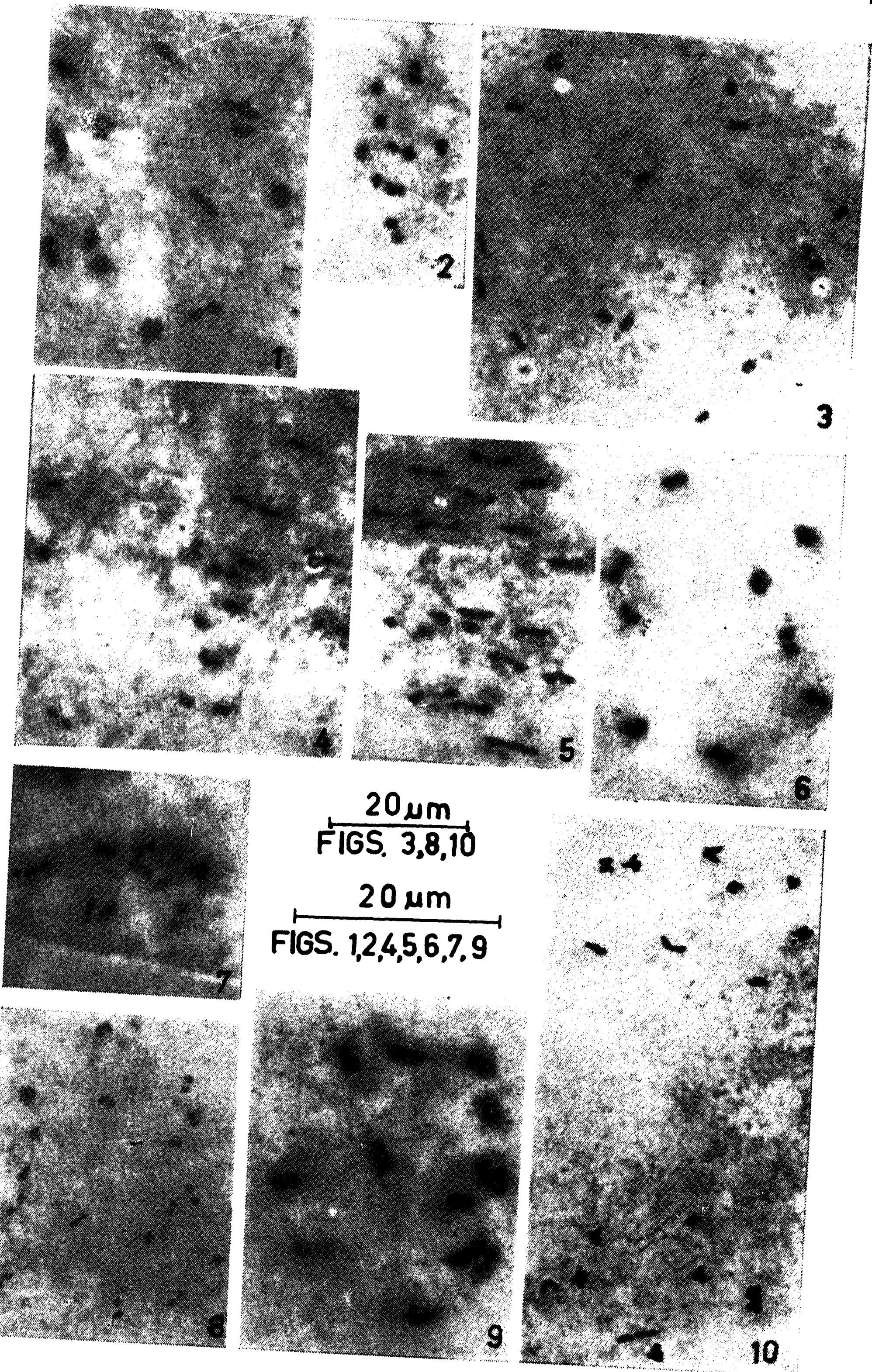
$n = 14$ from North India. Both the presently worked out species of *Rungia* namely, *R. parviflora* Nees and *R. pectinata* Nees are diploid with $n = 13$. Record of $n = 13$ for *R. parviflora* Nees is new because the earlier reports are of $n = 8$ (Baquar 1967–68), $n = 15$ (Datta and Maiti 1970) and $n = 26$ (Mehra and Vasudevan 1972). Similarly, count of $n = 13$ for *R. pectinata* Nees is different from that of $2n = 50$ by De (1966) for the same species. So far only 3 species of *Rungia* are known cytologically. Datta and Maiti (1970) suggested $x = 10$ to be the base number for the genus, but the possibility of polybasic nature of genus with numbers $x = 8, 10\ 13, 15, 25$ cannot be ruled out although $x = 15, 25$ would naturally be secondary basic numbers.

Cytotype of *Blepharis maderaspatensis* (Linn.) Roth with $n = 15$ has been worked out for the first time from India. Earlier, Kaur (1966) recorded $n = 13$ from South India. From Africa, $n = 15$ had earlier been reported by Miege (1960). Apparently, the present species is diploid. Cytological information about *Blepharis* is meagre since only 5 out of a total of 100 species have been worked out so far. Somatic members reported for the genus are $2n = 24, 26, 30, 34$, clearly indicating polybasic nature with base numbers $x = 12, 13, 15, 17$.

*Dyschoriste depressa* Nees ($n = 30$) has presently been worked out for the first time and is a tetraploid. Base number $x = 15$ is well established in all the cytologically worked out species. *Justicia diffusa* Willd. var. *prostrata* Roxb. with $n = 9$ has been worked out for the first time. *Justicia diffusa* Willd. with $n = 9$ and *J. betonica* Linn. with $n = 17$ confirms the earlier reports of $n = 9$ by Mehra and Vasudevan (1972) and Bir and Sidhu (1974) for the former and $n = 17$ by Ellis (1962) for the latter. Narayanan (1951), however, recorded $2n = 28$ for *J. betonica* Linn. A perusal of literature reveals the polybasic nature of the genus with $x = 9, 13, 14, 15, 16, 17$. Base numbers $x = 9$ and 14 are of common occurrence. Thus all the three presently worked out species of *Justicia* are at diploid level. *Lepidagathis fasciculata* Nees ($n = 10$) and *L. hyalina* Nees ($n = 10$) have been worked out for the first time. The other species, *L. cuspidata* Nees with $n = 11$ confirms the earlier counts by Verma and Dhillon (1967). All the three present species are diploid.

The basic numbers in the family range from $x = 8$–$25$ but the commonest numbers are $x = 9, 14, 16, 17$. A number of genera show polybasic nature as *Barleria* ($x = 15, 16, 19, 20$), *Blepharis* ($x = 12, 13, 15, 17$), *Thunbergia* ($x = 9, 10, 14, 16$), *Rungia* ($x = 8, 10, 13, 15, 25$), *Justicia* ($x = 9, 13, 14, 15, 16, 17$) and *Strobilanthes* ($x = 8, 9, 10, 11, 13, 14, 15$). The variability in the base numbers and polybasic nature of several genera clearly indicate that cytologically the family is a highly evolved one and all this could possibly be the result of aneuploidy operative at generic level. This has led to the evolution of morpho-

**Figures 1–10.** Meiosis in pollen mother cells. **1.** *Dicliptera roxburghiana*, M–I with 13II. **2.** *D. bupleuroides*, $n = 13$ at M–I. **3.** *Gendarussa vulgaris*, M–I with 15II. **4.** *Hygrophila auriculata*, M–I with 16II. **5.** *Justicia betonica*, 17II at M–I. **6.** *J. diffusa*, $n = 9$ at diakinesis. **7.** *J. diffusa* var. *prostrata*, $n = 9$. **8.** *Patalidium barlerioides*, 16II at M–I. **9.** *Rungia pectinata*, 13II at M–I. **10.** *Thunbergia alata*, M–II with $n = 9$.




Figures 1–10.

**Figures 11–1**:. Meiosis in pollen mother cells. **11.** *Blepharis maderaspatensis*, $n = 15$ at diakinesis. **12.** *Dyschoriste depressa*, 30II at M–I. **13.** *Hemigraphis latebrosa*, diakinesis showing 28II. **14.** *Lepidagathis cuspidata*, $n = 11$. **15.** *L. hyalina* showing 10 + 10 chromosomes. at M–II. **16.** *L. fasciculata* with 10II at M–I. **17.** *Rungia parviflora*, M–I showing $n = 13$.

ogical variations. Out of presently investigated 19 species of Acanthaceae only wo (10·53%) are polyploids and both at tetraploid level only. Taking an overall icture on the basis of the world-wide cumulative data, it is seen that only 17·81%* f the worked out species are polyploids. Intraspecific polyploidy is so ar reported in only eight species, namely, *Crossandra infundibuliformis* (2x, 6x) ; *C. nilotica* (4x, 6x) ; *Dicliptera elagans* (2x, 4x), *Hemigraphis latebrosa* (2x, 4x) ; *Rungia parviflora* (2x, 4x) and *Thunbergia grandiflora* (2x, 4x). It appers that olyploidy has not been as potent a factor of cytological evolution in the family s aneuploidy.

Out of 2500 species in Acanthaceae, only 219 are worked out and amongst these 180 species e diploid and 39 polyploids (for details see footnote to table 1).

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# Heterotrophic bacteria associated with seaweed

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**Abstract.** The heterotrophic bacterial population associated with seaweeds (*Enteromorpha* sp., *Chaetomorpha* sp. and *Hypnea* sp.) and water of the Vellar Estuary, Porto Novo, were estimated. Total heterotrophic bacteria associated with the seaweeds were found to be more abundant than in water samples. Representative cultures were isolated and their morphological and biochemical characteristics were studied. In addition, production of amylase, lipase and proteinase of the isolates was also studied. *Bacillus*, *Corynebacterium*, *Vibrio*, *Alcaligenes* and *Pseudomonas* were the genera commonly encountered. The role of these bacteria associated with seaweeds is discussed.

**Keywords.** Heterotrophic bacteria; seaweed; *Enteromorpha* sp.; *Chaetomorpha* sp. *Hypnea* sp.; Vellar estuary; Porto Novo.

## 1. Introduction

Available information on micro-organisms in an estuarine environment concerns water and sediment predominantly. The epiphytic bacterial flora of seaweed appears to have been neglected. Bacteria adhere to many types of solid surfaces, probably by means of several mechanisms and in some cases in a selective manner (Daniel 1972; Gibbons and van Houte 1975; Marshall 1976). Currently increasing attention has been paid to bacterial adhesion and its ecological significance. Some are adhesive to rocks in flowing streams (Geesey *et al* 1977), to suspended matter and solid surfaces in an estuary (Goulder 1976, 1977; Austin *et al* 1979a), to animal surfaces (Gibbons and van Houte 1975) and to sea weed (Sieburth 1962, 1968; Kong and Chan 1979; Sjoblad and Mitchell 1979; Shiba and Taga 1980). Much interest has not been shown on the epiphytic bacteria associated with seaweed in Indian estuaries, except by Chandramohan (1971). The purpose of the present study is to describe heterotrophic bacteria present in water and in association with seaweed (*Enteromorpha* sp.; *Chaetomorpha* sp.; and *Hypnea* sp.) at a particular location in a tropical estuary such as Vellar.

## 2. Material and methods

Seaweed samples were collected and transferred to sterile polyethylene bags after draining completely. Water samples were collected in sterile glass bottles. The samples were stored in a small insulation container (5° C) and brought to the laboratory. All the samples were plated within an hour of collection.

A portion of the seaweed was washed separately in sterile water and transferred to 100 ml sterile estuarine water blank in a 500 ml flask shaken on a reciprocal shaker (120 strokes per min) for 30 min at room temperature. The bacterial population was estimated by serial dilution plate method using Estuarine Peptone Yeast Extract Agar (EPYA; Bacto Peptone 1%, Yeast extract Difco 0·3%, Bacto Agar 2%) and the bacterial population was expressed as numbers/g dry weight of the seaweed and per ml of water. Bacterial strains were randomly isolated, purified by repeated streaking and identified to various genera using the taxonomic key of Simidu and Aiso (1962). Amylolytic, proteolytic and lipolytic ability of the isolates were also tested (Harrigan and McCance 1972).

## 3. Results

The total aerobic heterotrophic bacterial population from all the samples were estimated and the results are presented in table 1. The epiphytic bacterial population associated with *Chaetomorpha* sp. varied from 1·16 to 9·22 × $10^6$/g, *Enteromorpha* sp. from 1·13 to 18·63 × $10^6$/g and *Hypnea* sp. from 1·14 to 13·75 × $10^6$/g dry weight. Estimates of bacterial populations in the water sample ranged from 3·9 × $10^3$ to 1·53 × $10^4$/ml. The maximum bacterial population among the samples was recorded in October. Higher (18·63 × $10^6$/g) number of epiphytic bacteria was found to be associated in *Enteromorpha* sp. followed by *Hypnea* sp. and *Chaetomorpha* sp. Since this year experienced a heavy rainfall in monsoon season, no seaweed was found in the estuarine environment in November 1978 through February 1979, and this might be due to heavy freshwater inflow and lower salinity. They appeared again in March. So, no data could be collected between November 1978 and February 1979.

The total heterotrophic bacterial flora of water in Vellar estuary was found to consist of *Bacillus* (13·33%), *Corynebacterium* (20%,) *Micrococcus* (10%), *Vibrio* (16·67%), *Pseudomonas* (16·67%), *Alcaligenes* (10%), *Flavobacterium–Cytophaga* group (10%), and Enterobacteriaceae (3·33%) as shown in table 2. In general, gram-negative bacteria were more common (56·67%) than gram-positive. However gram-positive groups (*Bacillus*, *Corynebacterium* and *Micrococcus*) were abundant in April than the gram-negative groups. *Bacillus*, *Vibrio*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium–Cytophaga* were recorded in all the samples. The maximum number of *Corynebacterium* was recorded in March. The results suggest that the dominant epiphytic flora associated with seaweed, *Chaetomorpha* sp., *Enteromorpha* sp. and *Hypnea* sp., is *Vibrio*. Various genera associated were *Alcaligenes*, *Flavobacterium–Cytophaga* group, *Pseudomonas*, Enterobacteriaceae, *Corynebacterium* and *Micrococcus*. The domination of gram-negative bacteria was observed in all collections. The epiphytic *Vibrio* peak was recorded in October in association with all three seaweeds and a reduction in number was

**Table 1.** Total aerobic heterotrophic bacterial population.

| Month | *Chaetomorpha* sp. ($10^6$/g) | *Enteromorpha* sp. ($10^6$/g) | *Hypnea* sp. ($10^6$/g) | Water ($10^3$/ml) |
|---|---|---|---|---|
| September 1978 | 3·51 | 3·19 | 4·32 | 4·9 |
| October | 9·22 | 18·63 | 13·75 | 15·3 |
| March 1979 | 3·81 | 2·93 | 4·10 | 15·1 |
| April | 1·76 | 1·36 | 1·49 | 3·9 |
| May | 1·16 | 1·13 | 1·14 | 4·2 |

**Table 2.** Total number of isolates assigned to various genera.

| | *Choetomorpha* sp. | *Enteromorpha* sp. | *Hypnea* sp. | Water |
|---|---|---|---|---|
| Total number of isolates tested | 72 | 74 | 73 | 60 |
| *Bacillus* | ... | ... | ... | 8 (13·33) |
| *Corynebacterium* | 2 (2·78) | 3 (4·05) | 1 (1·37) | 12 (20·00) |
| *Micrococcus* | 2 (2·78) | 1 (1·35) | ... | 6 (10·0) |
| *Vibrio* | 42 (58·33) | 38 (51·35) | 40 (54·8) | 10 (16·67) |
| *Pseudomonas* | 8 (11·11) | 11 (14·86) | 14 (19·18) | 10 (16·67) |
| *Alcaligenes* | 4 (5·56) | 3 (4·05) | 5 (6·85) | 6 (10·0) |
| *Flavobacterium–Cytophaga* group | 8 (11·11) | 10 (13·51) | 9 (12·33) | 6 (10·0) |
| Enterobacteriaceae | 6 (8·33) | 8 (10·81) | 4 (5·48) | 2 (3·33) |

(Values in parentheses are percentages in total number of isolates tested).

noticed in March. Members of genus *Vibrio* could be isolated throughout the period of study and formed major portion of the flora, followed by *Flavobacterium–Cytophaga* group, *Pseudomonas* and *Alcaligenes*.

The bacterial flora in water and in association with seaweed were assigned to various physiological groups (table 3). Majority of heterotrophs in water appeared proteolytic (63·33%), lipolytic (61·67%) and a low percentage (43·33%) amylolytic. Bacterial populations associated with *Chaetomorpha* sp., was dominated by proteolytic (56·16%) followed by lipolytic (48.61%).

## 4. Discussion

Results of this study on the bacterial populations in water are comparable to those reported for Long Island Sound, Chesapeake Bay and Tokyo Bay. Altschular and Riley (1967) and Murchelano and Brown (1970) reported $10^3$ to $10^4$ bacteria per ml in Long Island Sound and $8·4 \times 10^1$ to $2·0 \times 10^4$/ml in Chesapeake Bay and $1·8 \times 10^1$ to $9·1 \times 10^4$/ml in Tokyo Bay (Austin *et al* 1979b).

The maximum bacterial populations were recorded in October and minimum in April, within the period of study. According to Velanker (1969), the numerical magnitude of heterotrophs broadly parallels the distribution of other living organisms and the dissolved organic matter in the sea. Murchelano and Brown (1970) suggested that the annual bacterial cycle in Long Island Sound coincided with that of phytoplankton. Phytoplankton constitutes a locus for bacterial attachment and produces organic substrates for bacterial utilization. Ecological parameters, *viz.*, physical, chemical and biological can have a definite influence on the bacterial population in an estuary. The annual bacterial cycle can be studied by repetitive sampling at one geographic locus, along with environmental parameters. Since this study was done for only five months, no definite conclusions could be reached regarding the annual cycle. The density of bacterial populations associated with seaweeds varied considerably, from 1·3 to $18·63 \times 10^6$/g. The epiphytic populations may result from availability of vitamins, growth factors or

**Table 3.** Physiological groupings of the bacterial isolates.

| Sample | Total number of isolates | Proteolytic | Amylolytic | Lipolytic |
|---|---|---|---|---|
| *Chaetomorpha* sp. | 72 | 39 (54·16) | 12 (16·67) | 35 (48·61) |
| *Enteromorpha* sp. | 74 | 42 (56·75) | 9 (12·16) | 37 (50·0) |
| *Hypnea* sp. | 73 | 35 (47·94) | 13 (17·81) | 35 (47·94) |
| Water | 60 | 38 (63·33) | 26 (43·33) | 37 (61·67) |

(Values in parantheses are percentages of isolates tested).

other external metabolites extruded by actively growing algal filaments which nourish the epiphytic flora (Fogg 1966 ; Sieburth 1968 ; Wetzel 1969 ; Wetzel and Allen 1972 ; Bell *et al* 1974 ; Morishita *et al* 1978). Chandramohan (1971) reported $1 \cdot 26 \times 10^6$/g bacterial population associated with *Enteromorpha intestinalis* in the Vellar estuary. Actively growing algal filaments were reported to carry high numbers of epiphytic bacteria (Sieburth 1968 ; Ramsay and Fry 1976). Mary (1977) recorded high bacterial counts when mullets were fed on large quantities of green filamentous alga *Enteromorpha* sp., which might account for the epiphytic flora associated with the algae in the same area. Quantitative data for bacteria were significantly high when there was an abundance of phytoplankton, *Navicula* sp.

The generic composition of the bacteria in water showed the presence of various groups in Vellar estuary. In general, gram-negative bacteria were predominant compared with gram-positives. On a generic basis, *Corynebacterium* were dominant (20%), followed by *Vibrio* (16·67%), *Pseudomonas* (16·67%) and *Bacillus* (13·33%). Murchelano and Brown (1970) reported that *Pseudomonas* was the dominant bacterial group in Long Island Sound and *Pseudomonas, Achromobacter* and *Flavobacterium* composed of 92·3% of the bacteria isolated. *Vibrio, Bacillus, Micrococcus* and *Cytophaga* accounted for only 7·3%. The various studies carried out using water samples showed a domination of *Vibrio* and *Achromobacter* in Chesapeake Bay, *Vibrio* in Kanagawa Bay, *Pseudomonas* in Long Island Sound and *Flavobacterium* in Naragansett Bay (as cited by Murchelano and Brown 1970). Austin *et al* (1979b), in their comparative study, found that the prominent aerobic heterotrophic bacterial flora of the water column of Chesapeake Bay consisted of *Vibrio, Achromobacter, Pseudomonas* and *Corynebacterium*, but in Tokyo Bay predominantly *Acinetobacter–Moraxella*-like species, *Caulobacter* and *Pseudomonas*. It is difficult to quantify the bacterial genera diversities unless considerable data are collected.

It may be inferred from the study reported here that members of seven genera were found associated with seaweeds. *Vibrio* was found dominating over *Pseudomonas, Flavobacterium* and *Alcaligenes*. Abundance of *Vibrio* and *Alcaligenes* during luxurious growth of algae (*Enteromorpha* sp.) suggested that these genera were probably associated with algal bloom. The recovery of large population of *Vibrio* throughout the study suggests that they form part of indigenous flora of seaweed. Presence of epiphytic flora of marine algae has been reported by Sieburth (1968) who examined species of *Polysiphonia* and *Sargassum* for possible generic specificity of epiphytic flora and noticed *Vibrio* as the dominant species in both. Kong and Chan (1979) reported seven genera to be associated with marine algae and similar study was conducted by Shiba and Taga (1980) who found that *Flavobacterium* was the dominant microflora. *Vibrio* were present in minimum numbers, suggesting metabolites being toxic for *Vibrio* sp., a phenomenon not observed in this investigation. No other taxonomic study has been carried out to date on epiphytic bacteria associated with seaweed in Indian estuaries. The generic composition, seasonal variation and interrelationships of associated heterotrophic bacteria remain to be defined.

The existence of bacterial populations on seaweed suggests that there exists a beneficial relationship between the seaweed and the epiphytic bacteria. The bacteria belonging to the genus *Vibrio* were predominant on the seaweed. It is

reported that the extract of seaweed acted as attractant for the marine bacterium, *Vibrio alginolyticus,* by Sjoblad and Mitchell (1979). Hence it may be suggested that a beneficial relationship exists between seaweed and their epiphytic bacteria *Vibrio.*

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# Association of chlorophyll content, phyllotaxy, photosynthesis and B group vitamins in some $C_3$ and $C_4$ plants

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**Abstract.** The photosynthetically efficient $C_4$ plants *viz Amaranthus viridis*, *Euphorbia hirta* and a $C_3$ plant, *Acalypha indica* with mosaic leaf pattern showed the maximum amount of *B* vitamins when compared to the other $C_3$ plants. It is observed that photosynthesis and vitamin synthesis go hand-in-hand showing close correlation. The results also indicate that there is a close relation between chlorophyll content and vitamin content. However, there appears to be no relation between phyllotaxy and photosynthesis. Between the two $C_3$ plants, *viz.*, *Acalypha* and *Carica*, the photosynthetic inefficiency of the latter might be due to more of chlorophyll *b* and less of chlorophyll *a* as seen from chlorophyll *a*/chlorophyll *b* ratios.

**Keywords.** Chlorophyll ; B vitamins ; phyllotaxy ; photosynthesis ; $C_3$ and $C_4$ plants.

## 1. Introduction

The chlorophyll content of the cell must be closely associated with photosynthetic activity because the photosynthetic rate is proportional to the chlorophyll concentration (Maksymowych 1973), but there are contrary reports also. Black (1972) concluded that the high rate of photosynthesis/mg chlorophyll is directly related to the low chlorophyll content. The photosynthetic rates of different chlorophyll mutants of pea, soybean, cotton and tobacco were studied by Benedict (1972). The photosynthetic rate/mg of chlorophyll in mutants is 2–11 times faster than in the wild type leaves. This phenomenon is compared to the photosynthetic rate of the yellow sectors of variegated leaves. These yellow sectors although containing a reduced chloropyhll content, have a much higher photosynthetic rate/mg chlorophyll than the green sectors. Bonner and Bonner (1948) found that thiamine synthesis in seedlings is often light-dependent and mature leaves of full grown tomato plants are the centre of production. Gustafson (1948) suggested that light stimulates thiamine biosynthesis. It was stated that the photosynthetic unit and not the chlorophyll content determines the rate of photosynthesis (Black 1972).

The present investigation has been designed to understand the relationship between chlorophyll content, vitamin content, phyllotaxy and photosynthesis in some $C_3$ and $C_4$ plants. Work on the relationship between phyllotaxy and photosynthesis is scanty. Earlier work (Evans 1975) indicates that canopies with more vertically inclined leaves have a higher photosynthetic rate than those with horizontal leaves.

## 2. Materials and methods

Young and fully expanded leaves from various plant species, *viz*., (1) *Acalypha indica*, L., (2) *Amaranthus viridis*, L., (3) *Carica papaya*, L., (4) *Commelina benghalensis*, L., (5) *Euphorbia hirta*, L., (6) *Euphorbia pulcherrima*, Willd., (7) *Ervatamia coronaria*, Stapf., (8) *Werium odorum*, Soland., (9) *Nyctanthus arbor-tristis*, L., (10) *Petunia hybrida*, L., (11) *Sida acuta*, Burn, (12) *Tridax procumbens*, L., growing in the university Botanical Garden under natural photoperiod constitute the experimental material.

Chlorophylls were extracted with 80% acetone and estimated according to the method of Arnon (1949). The chloroplasts were isolated using N/15 phosphate buffer (pH 7·3) containing sucrose (0·33 M), disodium salt of EDTA ($2 \times 10^{-3}$ M) dithiothreitol ($5 \times 10^{-3}$ M), $MgCl_2$ ($1 \times 10^{-3}$ M), $MgSO_4$ ($1{\cdot}5 \times 10^{-3}$ M) and 5% (w/v) polyvinylpyrolidene as isolation medium following the procedure of James and Das (1957). The method of Jagendorf and Evans (1957) was used to assay Hill reaction activity of chloroplast preparation. $^{14}CO_2$ fixation studies were done using a technique similar to that described by Berry *et al* (1970), using $^{14}C$ sodium bicarbonate (specific activity, 27 mci/m mole) and the net photosynthesis was expressed as mg $CO_2$ fixed $dm^{-2}hr^{-1}$.

The different vitamins of the *B* group, *viz*., thiamine ($B_1$), riboflavin ($B_2$), pyridoxin ($B_6$), niacin and folic acids were extracted and estimated colorimetrically following the methods given by Manzur-ul-Haque Hashmi (1973) and the results were expressed as $\mu$g/g dry wt. All the results were averages of three individual experiments.

## 3. Results and discussion

The results in table 1 indicate that *Amaranthus viridis*, *Euphorbia hirta* which are $C_4$ photosynthetic plants and *Acalypha indica*, a $C_3$ plant with mosaic pattern of phyllotaxy have maximum Hill activity, net photosynthesis and chlorophyll content. *Commelina benghalensis*, *Euphorbia pulcherrima*, *Ervatamia coronaria* and *Sida acuta* occupy the next position in order in this respect. *Nerium odorum* shows the minimum activity.

It is tempting to note that there is a close correlation between the photosynthetic parameters (table 1) and vitamins of the *B* group (table 2) *viz*., thiamine ($B_1$), riboflavin ($B_2$), pyridoxin ($B_6$), niacin and folic acid in the plants mentioned above. *Nerium odorum* shows the minimum amount of vitamins of the *B* group. The data thus gives a circumstantial evidence to show that photosynthesis and vitamin synthesis go hand-in-hand showing a close correlation (table 3). The photosynthetically efficient $C_4$ plants *viz*., *Amaranthus veridis*, *Euphorbia hirta*

**Table 1.** The pattern of chlorophylls and the rate of photosynthesis in different plant species.

| | Plant species | Phyllotaxy | Plant type | Total chloro-phylls * | Chlorophyll *a/b* ratio * | Hill activity + | Net photo-synthesis ** |
|---|---|---|---|---|---|---|---|
| 1. | *Acalypha indica* L. | mosaic | $C_3$ | 4·62 ±0·84 | 1·16 ±0·04 | 163·5 + 6·21 | 38·4 ± 2·22 |
| 2. | *Amaranthus viridis* L. | alternate | $C_4$ | 3·58 ±0·22 | 1·21 ±0·12 | 186·2 ± 10·6 | 45·8 ± 5·16 |
| 3. | *Carica papaya* L. | mosaic | $C_3$ | 1·90 ±0·31 | 0·18 ±0·01 | 121·1 ± 7·81 | 26·5 ± 1·76 |
| 4. | *Commelina benghalensis* L. | alternate | $C_3$ | 2·43 ±0·14 | 1·17 ±0·06 | 148·4 ± 3·42 | 31·6 ± 4·32 |
| 5. | *Euphorbia hirta* L. | opp-super-imposed | $C_4$ | 3·96 ±0·86 | 1·25 ±0·22 | 169·5 ± 12·31 | 42·0 ± 1·78 |
| 6. | *Euphorbia pulcherrima* Willd. | opp-decussate | $C_3$ | 3·47 ±0·86 | 1·28 ±0·08 | 142·6 ± 16·2 | 36·8 ± 6·52 |
| 7. | *Ervatamia coronoria* Stapf. | opp-decussate | $C_3$ | 2·02 ±0·10 | 1·25 ±0·12 | 136·0 ± 5·61 | 29·1 ± 1·34 |
| 8. | *Nerium odorum* Soland | Whorled | $C_3$ | 1·69 ±0·21 | 1·63 ±0·34 | 84·3 ± 7·52 | 17·2 ± 2·88 |
| 9. | *Nyctanthes arbortristis* L. | opp-super-imposed | $C_3$ | 1·98 ±0·28 | 1·26 ±0·07 | 117·5 ± 11·6 | 21·6 ± 1·06 |
| 10. | *Petunia hybrida* L. | alternate | $C_3$ | 1·76 ±0·11 | 1·31 ±0·26 | 104·2 ± 18·2 | 19·0 ± 3·82 |
| 11. | *Sida acuta* Burm. | alternate | $C_3$ | 3·13 ±0·16 | 1·17 ±0·34 | 126·1 ± 7·01 | 23·2 ± 2·17 |
| 12. | *Tridax procumbens* L. | alternate | $C_3$ | 1·65 ±0·81 | 1·24 ±0·12 | 100·8 ± 9·44 | 21·8 ± 4·61 |

* mg $g^{-1}$ fresh wt.

** mg $CO_2$ fixed $d^{-2}m$ $hr^{-1}$.

\+ $\mu$ moles of DCPIP reduced $mg^{-1}$ chl $hr^{-1}$.

(Values are means ± S.E. of three individual experiments).

and *Acalypha indica*, a $C_3$ plant with mosaic leaf pattern showed the maximum amount of vitamins. *Commelina benghalensis*, *Eurphorbia pulcherrima*, *Ervatamia coronaria* and *Sida acuta* form the second group in their vitamin contents quite parallel to their photosynthetic parameters.

The results categorically indicate that there is a close relation between chlorophyll content and vitamin content (table 3). The maximum amount of chlorophyll (total) is seen in *Acalypha indica* (4·62 mg/g fresh wt.). Nevertheless,

**Table 2.** Vitamin content ($\mu g\ g^{-1}$ dry wt.) in the leaves of different plant species.

| Plant spp* | Thiamine ($B_1$) | Riboflavin ($B_2$) | Pyridoxin ($B_6$) | Niacin | Folic acid | Total |
|---|---|---|---|---|---|---|
| 1. | 369·0 ± 12·1 | 124·0 ± 8·9 | 215·0 ± 16·2 | 152·0 ± 20·4 | 134·0 ± 6·2 | 994 |
| 2. | 280·0 ± 21·3 | 136·0 ± 12·4 | 198·0 ± 17·4 | 169·0 ± 11·2 | 149·0 ± 13·1 | 932 |
| 3. | 114·0 ± 16·2 | 82·0 ± 3·2 | 128·0 ± 15·8 | 97·0 ± 8·6 | 78·0 ± 4·4 | 499 |
| 4. | 232·0 ± 31·0 | 64·0 ± 5·7 | 176·0 ± 21·1 | 160·0 ± 11·1 | 52·0 ± 2·3 | 684 |
| 5. | 291·0 ± 18·6 | 99·0 ± 2·8 | 195·0 ± 8·8 | 141·0 ± 6·1 | 130·0 ± 7·6 | 856 |
| 6. | 207·0 ± 26·2 | 76·0 ± 4·9 | 180·0 ± 11·2 | 122·0 ± 10·1 | 104·0 ± 11·2 | 689 |
| 7. | 197·0 ± 16·6 | 108·0 ± 13·1 | 209·0 ± 6·8 | 135·0 ± 14·2 | 126·0 ± 13·6 | 775 |
| 8. | 74·0 ± 8·1 | 42·0 ± 3·6 | 96·0 ± 2·4 | 28·0 ± 3·8 | 49·0 ± 6·4 | 289 |
| 9. | 139·0 ± 11·2 | 34·0 ± 2·7 | 126·0 ± 7·6 | 47·0 ± 1·2 | 61·0 ± 3·4 | 407 |
| 10. | 98·0 ± 14·1 | 51·0 ± 2·6 | 113·0 ± 3·2 | 62·0 ± 5·6 | 93·0 ± 2·8 | 417 |
| 11. | 156·0 ± 21·7 | 78·0 ± 1·7 | 174·0 ± 14·6 | 103·0 ± 2·8 | 82·0 ± 4·1 | 593 |
| 12. | 124·0 ± 6·4 | 46·0 ± 1·1 | 101·0 ± 9·9 | 94·0 ± 2·1 | 76·0 ± 3·6 | 441 |

* Plant names as represented in table 1 serially.
(Values are means ± S.E. of three replications).

**Table 3.** Statistical analysis.

1. Correlation coefficient (for tables 1 and 2)

| | between a set of parameters | | correlation coefficient ($r_{xy}$) |
|---|---|---|---|
| (a) | Total chlorophylls | × hill activity | + 0·822 (4·564)* |
| (b) | ,, | × photosynthesis | + 0·826 (4·635)* |
| (c) | ,, | × thiamine ($B_1$) | + 0·895 (6·346)* |
| (d) | ,, | × riboflavin ($B_2$) | + 0·730 (3·378)* |
| (e) | ,, | × pyridoxin ($B_6$) | + 0·209 (0·676) |
| (f) | ,, | × niacin | + 0·676 (2·901)* |
| (g) | ,, | × folic acid | + 0·907 (6·809)* |
| (h) | ,, | × total vitamins | + 0·849 (5·083)* |

2. Analysis of variance (for table 2)

| | | Vitamins | Plant species |
|---|---|---|---|
| F calculated | ... | 26·765* | 10·515* |
| F from table at 5% level | ... | 2·594 | 2·152 |
| C.D. at 5% level | ... | 15·802 | 8·972 |

* Significant at 5% level.

Values in the parantheses represent the calculated values of *t*-statistic for testing the significance of correlation coefficients.

chlorophyll *a*/*b* ratio is not the highest in *Acalypha indica* (table 1). Chlorophyll *a*/*b* ratio is maximum (1·63) in *Nerium odorum*, but the vitamin content is at a minimum level indicating that neither chlorophyll *a* nor *b* has anything to do with vitamin synthesis, but it is the total chlorophyll content that is associated with vitamin content, both being at a lower level (tables 1 and 3).

It is noted that there is no relationship between photosynthesis and phyllotaxy. Mosaic pattern of phyllotaxy is assumed to help light to fall directly on all the leaves without any impediment, thus making the plant photosynthetically more efficient. But, if a comparison is made between *Acalypha* and *Carica* (both show mosaic pattern) the latter is not at all efficient (table 1). Similarly *Amaranthus veridis* has alternate leaves, while *Euphorbia hirta* which is also a $C_4$ plant possesses opposite superimposed leaf arrangement thus indicating that there is no relation between phyllotaxy and photosynthesis. In the case of opposite decussate pattern in *Ervatamia* and *Euphorbia pulcherrima* where overshadowing is avoided, the photosynthetic efficiency is somewhat better than that of *Carica papaya* with mosaic pattern.

On the basis of computations it can be inferred that there is a highly positive correlation between total chlorophyll content and other parameters such as Hill activity, net photosynthesis, thiamine, riboflavin, niacin, folic acid and the total vitamins (table 3). The correlation coefficients of all the parameters are significant at 5% level except with pyridoxin. Hence there is an association of chlorophyll content and other parameters (except phyllotaxy).

Even an analysis of variance (for table 2) reveals a close association among the parameters. *F*-values calculated for vitamins and plant species are significant at 5% level.

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# Effect of morphactin, AMO-1618 and DPX-1840 on the endogenous levels of hormones and its implication on apical dominance in *Glycine max* Linn.

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**Abstract.** Application of morphactin, AMO-1618 and DPX-1840 to 20 day old plants of soybean caused the sprouting of almost all lateral buds. However, the follow-up growth of newly ensued buds was manifested only with morphactin and not with AMO-1618 or DPX-1840. The quantitative estimations of growth promoters, carried out 20 days after the application of inhibitors, revealed that the possible mechanism through which these substances exerted their influence on apical dominance varied with the type of regulator. It appeared that morphactin lifted the apical dominance mainly through the increase in endogenous levels of cytokinin and partly through lowering the levels of auxins. AMO-1618 checked the gibberellin turnover while DPX-1840 suppressed significantly the levels of auxins. The data show that apical dominance and not the subsequent growth of newly evocated buds is regulated by the ratio of cytokinins (CK) to auxins (Au) plus gibberellins (GA) and any factor(s) which enhance(s) CK (like morphactin) or suppress(es) GA (like AMO-1618) or Au (like DPX-1840 or morphactin) would play a key role in the abolition of apical dominance.

**Keywords.** Apical dominance ; hormones ; growth retardants ; soybean.

## 1. Introduction

Ever since Thimann and Skoog (1934) showed that an application of auxin preparation from fungus *Rhizopus* onto the cut stump of decapitated *Vicia faba* prevented the growth of lateral buds, many workers have put forward a plethora of chemicals which could be utilised for abolition or induction of apical dominance in intact plants. In spite of these successful reports by such widely diverse substances like IAA (Thimann *et al* 1971), $GA_3$ (Bradley and Crane 1960), kinetin (Ali and Fletcher 1970), B-995 (Brooks 1964), AMO-1618 (Ruddat and Pharis 1966), morphactin (Tognoni *et al* 1967 ; Schneider 1970), TIBA (Morey and Dahl 1975) and various ethylene releasing compounds (Morgan and Durham 1972), there has hardly been an attempt to divulge the underlying mechanism through

which these regulators might be controlling this phenomenon. Dua *et al* (1978) and Dua and Dhuria (1980) reported that morphactin-induced growth of lateral buds was associated with a concomitant increase in the levels of endogenous cytokinins. However, a comprehensive hormonal appraisal of apical dominance with regard to morphactin *vis-a-vis* a few other retardants, though unequivocally linked with this process, is still lacking. The present investigation attempts to study this aspect beside probing into the mechanism of apical dominance in *Glycine max* Linn.

## 2. Materials and methods

*Glycine max* Linn. cv 'Bragg' was raised in pots of 25 cm height and 22·5 cm in diameter under optimum conditions and 20 day old seedlings were sprayed with different inhibitors with an atomiser. The treatments consisted of morphactin (methyl-2-chloro-9-hydroxy-fluorene- (9)-carboxylate-chlorofluornol-methyl ester-IT 3456), DPX–1840 (3, 3*a*-dihydro-2-[*p*-methoxyphenyl]8H-pyrazolo-[5,1]-*a* isoindol-8-one) and AMO–1618 (ammonium (5-hydroxycarvacryl) trimethyl chloride piperidine carboxylate) of 200, 1000 and 1000 ppm respectively. The three regulators were selected on the basis of previous work and have been used frequently in relation to studies on apical dominance (see introduction for references). Twenty days after the spray, the data for various morphological characters were recorded. For extractions of different endogenous plant growth substances, the samples from 20-day old treated or control seedlings were kept in methanol for 48 hrs at 0° C in a refrigerator. The extract was evaporated under suction to remove ethanol and residue (pH 3·0) was utilised for the extraction of various growth regulatory substances. Extraction procedures described by Nitsch (1956) for auxins, by Murakami (1966) for gibberellins and by Dua and Jandaik (1979) for different cytokinins were adopted. Estimates for auxin activity were carried out on the basis of growth test of 'Kent' oat (*Avena sativa* Linn.) coleoptiles as described by Mer *et al* (1962), gibberellins by the modified technique of Ogawa (1963) using 'Tainan-3' rice (*Oryza sativa* Linn.) and cytokinin by *Xanthium* leaf disc senescence method of Osborne and McCalla (1961). Standard series of cytokinins (zeatin, 2iP, zeatin riboside, zeatin ribotide), gibberellins ($GA_3$, $GA_4$, $GA_5$, $GA_7$) and auxins (IAA, IAN) were run separately for their Rf values. The data were statistically analysed according to the variance method.

## 3. Results

### 3.1. *Morphological changes*

The application of various growth retardants alone or in combinations brought a number of morphological changes within 20 days of treatment (table 1). The plant height *vis-a-vis* internodal length was reduced drastically. The maximum reduction in length (56·1% over control) was recorded when all the three regulators were present together. The most conspicuous morphological change was the growth of lateral buds in all treatments and 50–65% buds sprouted as compared

**Table 1.** Effect of morphactin, AMO–1618 and DPX–1840 on the various morphological characters in *Glycine max* Linn. after 20 days of treatment (average of 5 replications).

| Treatment | Plant height (cm) | Internodal length (cm) | Number of sprouted lateral buds | Length of the lateral buds (cm) |
|---|---|---|---|---|
| Control | 56·21 | 18·25 | 2·00 | 2·23 |
| Morphactin | 42·25 | 13·16 | 13·00 | 8·76 |
| AMO–1618 | 34·65 | 11·25 | 10·00 | 3·52 |
| DPX–1840 | 38·75 | 12·30 | 11·00 | 3·89 |
| Morphactin + AMO–1618 | 30·25 | 11·03 | 16·00 | 3·25 |
| Morphactin + DPX–1840 | 34·25 | 11·32 | 16·00 | 3·25 |
| AMO–1618 + DPX–1840 | 28·25 | 10·97 | 15·00 | 3·30 |
| Morphactin + AMO–1618 + DPX–1840 | 26·23 | 10·81 | 17·00 | 4·25 |
| Critical difference at 5% level of significance | 3·63 | 2·12 | 1·50 | 1·93 |

to control where only 10% buds were growing. The effect of each chemical, in promoting the growth of lateral buds, was further augmented in the presence of the second retardant (75–80% buds growing) and maximum effect was detectable when all the three regvlators were present simultaneously (85% buds initiated). Another striking revelation was the growth of lateral buds, following their release from apical dominance (table 1). Though all the regulatory substances significantly abolished apical dominance, to almost the same degree, the subsequent growth of the newly ensued buds was insignificant and poor in all the treatments except morphactin. It was only in the later treatment (morphactin) that a significant follow-up growth of the axillary branches occurred (approximately 2·8 times more than control or other treatments). Surprisingly, in the treatments where morphactin was combined with other regulators, the morphactin's promotory effect on the growth of lateral branches was also nullified.

### 3.2. *Behaviour of different endogenous hormones*

3.2a. *Auxins*: The estimates of auxins revealed the presence of three auxins at Rfs 0·1–0·2, 0·6–0·7 (IAA) and 0·9–1·0 while IAN (Rf 0·4–0·5) was not discernible. Application of morphactin and DPX–1840 resulted in significantly lowering the total auxins (table 2 and figure 1) whereas AMO–1618 was ineffective in bringing about any significant change. In combined treatments, DPX–1840 or morphactin also checked the levels of auxins and minimum auxin activity was noticed when both of them were present simultaneously.

3.2b. *Gibberellins* : The data (table 2 and figure 1) showed that two gibberellins at Rfs 0·4–0·5 ($GA_3$) and 0·7–0·8 ($GA_5$) were detectable and $GA_3$ contributed the major component (80%) of the total gibberellins. The only treatment to significantly check the levels of endogenous gibberellins was AMO–1618 singly or in combination with other retardants which themselves were ineffective in causing any such change.

3.2c. *Cytokinins* : Bioassays of different extracts deciphered the presence of four different cytokinins, namely, zeatin riboside (Rf 0·1–0·2), zeatin (Rf 0·4–0·5), zeatin ribotide (Rf 0·8–0·9) and an unknown cytokinin (Rf 0·7–0·8) The treatment of morphactin led to an increase of total cytokinins (table 3 and figure 1).

**Table 2.** Effect of morphactin, AMO–1618 and DPX–1840 on the endogenous level of auxins and gibberellins ($\mu$g/100 g fresh weight) (average of 3 replications).

| Treatments | Auxins (Rf) | | | Gibberellins (Rf) | |
|---|---|---|---|---|---|
| | 0·1–0·2 | 0·6–0·7 | 0·9–1·0 | 0·4–0·5 | 0·7 0·8 |
| Control | 2·53 | 12·51 | 1·52 | 1·20 | 0·30 |
| Morphactin | 0·85 | 7·28 | 0·85 | 1·15 | 0·30 |
| AMO–1618 | 2·12 | 10·87 | 1·35 | 0·51 | Tr |
| DPX–1840 | 0·56 | 5·33 | 0·80 | 1·08 | Tr |
| Morphactin + AMO–1618 | 0·84 | 7·35 | 0·86 | 0·49 | 0·25 |
| Morphactin + DPX–1840 | 0·51 | 3·84 | 0·60 | 1·02 | Tr |
| AMO–1618 + DPX–1840 | 0·58 | 5·52 | 0·85 | 0·44 | Tr |
| Mcrphactin + AMO–1618 + DPX–1840 | 0·52 | 3·91 | 0·65 | 0·43 | Tr |
| Critical difference at 5% level of significance | 0·47 | 1·81 | 0·56 | 0·39 | 0·13 |

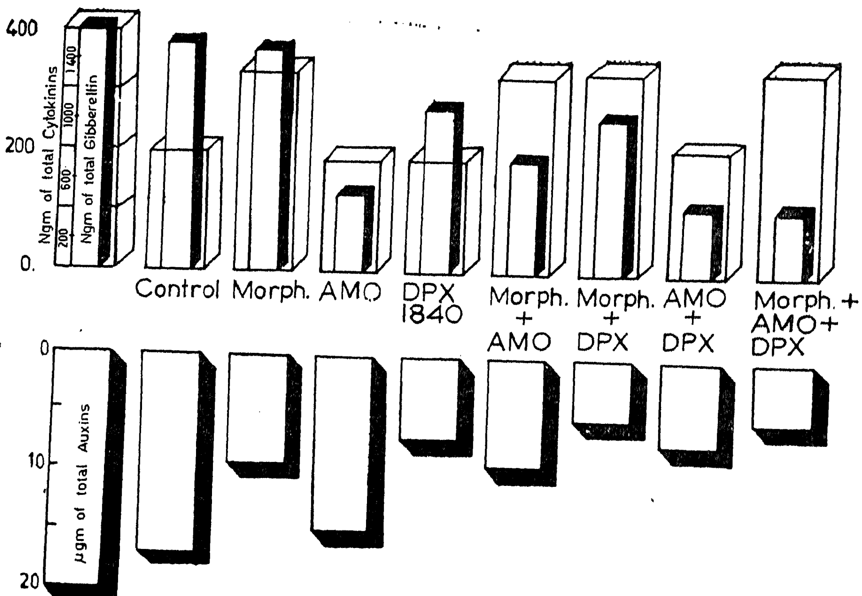


**Figure 1.** Effect of morphactin, AMO–1618 and DPX–1840 on the endogenous levels of total CK and GA (small shaded blocks inside the bigger blocks) in ngm and auxins (in $\mu$g) per 100 g of fresh weight (average of 3 replications).

Table 3. Effect of morphactin, AMO–1618 and DPX–1840 on the endogenous levels of cytokinin μg/100 g fresh weight of kinetin equivalent (average of 3 replications).

| Treatment | Cytokinins Rf | | | |
|---|---|---|---|---|
| | ZR 0·1–0·2 | Z 0·4–0·5 | UC 0·7–0·8 | ZRT 0·8–0·9 |
| Control | 0·058 | 0·035 | 0·021 | 0·061 |
| Morphactin | 0·091 | 0·083 | 0·062 | 0·096 |
| AMO–1618 | 0·053 | 0·056 | 0·023 | 0·053 |
| DPX–1840 | 0·057 | 0·059 | 0·028 | 0·041 |
| Morphactin + AMO–1618 | 0·089 | 0·081 | 0·079 | 0·073 |
| Morphactin + DPX–1840 | 0·093 | 0·082 | 0·078 | 0·076 |
| AMO–1618 + DPX–1840 | 0·061 | 0·063 | 0·028 | 0·058 |
| Morphactin + AMO–1618 + DPX–1840 | 0·089 | 0·084 | 0·080 | 0·079 |
| Critical difference at 5% level of significance | 0·008 | 0·01 | 0·01 | 0·009 |

Both AMO–1618 and DPX–1840 were ineffective in multiplying the level of individual or total cytokinins. In combined treatments, the cytokinin enhancing capacity of morphactin was not impaired by the presence of other substances.

## 4. Discussion

The habit of growth displayed by herbaceous and woody plants or control of the plagiotropic position of the lateral branches are examples of correlative effects where the stem apex influences the growth and development of the other parts of the plant. The way in which this phenomenon is mediated has been a matter of controversy and different postulations, for different species, have been envisaged. Some of the early investigators (Goebel 1900 ; Loeb 1918 ; Dostal 1926) and a few of the late reports (McIntyre 1968 ; Wagner and Michael 1971) have emphasised the importance of inorganic and organic nutrition. However, a majority of researches from mid-sixties were concentrated on the possibility that a correlative signal from the apex was hormonal in nature (Phillips 1969 ; Kung Woo *et al* 1974). Though endogenous auxins (Au) was linked with apical dominance quite early (Wickson and Thimann 1960) and later on confirmed by more workers (Phillips 1975) the evidences favouring gibberellins (GA) involvement with the same have been very scanty and rare. In a variety of reports, gibberellins have been found to be stimulatory (Brain *et al* 1959 ; Marth *et al* 1956 ; Ruddat and Pharis 1966), inhibitory (Bruinsma and Patil 1963) or indifferent (Sachs and Thimann 1964) to the growth of lateral buds. As compared to GA, there has been

more convincing evidence linking cytokinin (CK) to apical dominance (Guern and Usciati 1972 ; Thimann 1972). Unfortunately, most of the above correlations were deduced by studying the growth of lateral buds following exogenous application of some synthetic or natural regulators and there had been a lack of effort to estimate quantitatively the endogenous hormones following the evocation of lateral buds. The present investigations show that lifting of apical dominance, through growth retardants, coincided with a shift in the balance of various hormones. The data revealed that in *Glycine max* this phenomenon is probably regulated by a ratio of CK to Au plus GA. The growth retardants morphactin, DPX–1840 or AMO–1618 lifted apical dominance by increasing CK or decreasing Au or GA respectively. The role of these retardants in successfully managing these changes was apparent from the studies on the changes of endogenous hormones under different treatments. This also draws support from the work of Ruddat and Pharis (1966) and Baldev *et al* (1965) on AMO–1618 ; Morey and Dahl (1975) and Morey (1974) on DPX–1840 ; and Bednar and Linsmaier-Bednar (1971), Dua *et al* (1978), Dua and Dhuria (1980) on morphactin.

The present findings further point that release from the apical dominance and the follow-up growth of the newly sprouted buds are independent of each other and probably have their own specific requirements. It was seen that while inhibitors of auxins or gibberellins lifted the apical dominance by lowering the denominator in CK/Au + GA ratio, the subsequent growth was impaired owing to the reduction of the same. It seemed that for this latter growth, GA or Au presence or relatively higher levels were obligatory. This inkling was further supported from the observation on the treatment where morphactin was in combination with DPX–1840 or AMO–1618. In this case though the number of buds released from apical dominance was significantly higher (by virtue of increase in CK), the follow-up growth of lateral buds was insignificant (probably due to the non-availability of sufficient Au or GAS).

In essence, it seems that apical dominance in soybean is regulated by a balance of cytokinins to auxin and gibberellin and any factor which can affect this balance is likely to influence the manifestation of growth of lateral buds. On the contrary, the follow-up growth of newly ensued buds is independent of the above domag and for this the presence of auxin and/or gibberellins seems mandatory.

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# Taxonomic importance of epidermal characters in the Indian *Thespesia* Corr. (Malvaceae)

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**Abstract.** In *Thespesia lampas* and *T. populnea*, the foliar stomata are anisocytic, anomocytic and tetracytic, the first type being dominant. Further in *Thespesia lampas* and *T. populnea* altogether eight trichome types are recognisable mostly on the basis of structure. The two species can be distinguished from each other by the presence of multiseriate aseptate stellate hair in the former and that of multiseriate aseptate peltate hair in the latter. *T. populnea* is also distinct from that of *T. lampas* due to curved to wavy epidermal walls, striated surface, absence of mucilaginous cells on the leaf abaxial. The present evidence also supports treatment of *T. lampas* under *Thespesia* rather than in *Hibiscus*.

**Keywords.** Epidermal characters ; taxonomy ; *Thespesia* ; Malvaceae.

## 1. Introduction

Taxonomic importance of epidermal characters in general and those of trichomes in particular in angiospermous plants is now widely recognised (Prat 1932 ; Tomlinson 1961 ; Stace 1965 ; Ghose and Davis 1973 ; Verhoeven and Schijff 1973). However, previous studies on the epidermis are limited to few taxa and only to certain aspects in the Malvaceae (Solereder 1908 ; Metcalfe and Chalk 1950 ; Inamdar and Chohan 1969; Ramayya and Shanmukha Rao 1976 ; Shanmukha Rao and Ramayya 1977a, b). Hence, the present investigation was undertaken which deals with foliar epidermal characters along with structure and organographic distribution of trichomes in whole plant and their taxonomic importance in the two Indian *Thespesia*.

## 2. Material and methods

The material of *Thespesia lampas* Dalz. & Gibs. was collected from Caramjol, Goa, whereas *T. populnea* (L.) Sol. ex Corr. from plants growing at Sardar Patel

College campus, Secunderabad. Mature trichomes were studied either from epidermal peelings or those isolated by scraping the plant parts. These micropreparations were stained either with anilin blue in lactophenol or safranin, hematoxylin and then mounted in 70% glycerine. Boiling the material with 5–10% glacial acetic acid was useful in obtaining the peels. Microtome sections of shoot apices were cut at 10–14 μm thickness and stained with hematoxylin and basic fuchsin. The foot of the different trichomes was determined in freehand and microtome sections of various parts of the species investigated. The walls of the different trichomes were tested with phloroglucin and 2% HCl for lignin (Johansen 1940).

The terms describing stomatal types are after Metcalfe and Chalk (1950) and as redefined by Shanmukha Rao and Ramayya (1977a) and for trichome types, after Ramayya (1972) and Shanmukha Rao and Ramayya (1977b).

## 3. Observations

### 3.1. *Structure and distribution of epidermal and stomatal complexes on the leaf*

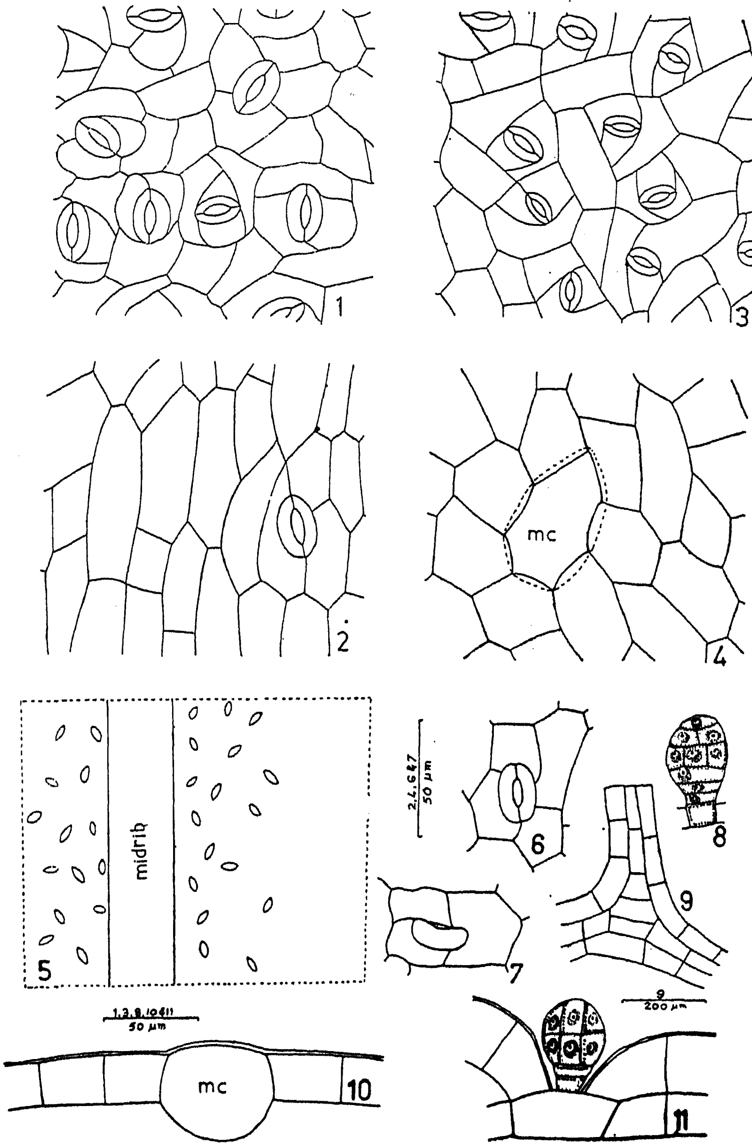
3.1a. *Epidermal cell complex*: Epidermal cells: 4–6-sided, anisodiametric; contents dense, brownish in *T. populnea*; sides thin, straight (leaf abaxial and adaxial of *T. lampas* and leaf adaxial of *T. populnea*) or curved to wavy (leaf abaxial of *T. populnea*); surface smooth (*T. lampas*) or striated (*T. populnea*), striations wavy, prominent and continuous. Distribution: Confined to the interstices, variously oriented (figures 1–4). Costal cells: 4-sided, linear; contents scanty or mostly brownish (*T. populnea*); sides moderately thick, straight or curved (leaf abaxial of *T. populnea*); surface smooth (*T. lampas*) or striated (*T. populnea*). Distribution: Oriented parallel to the veins, diffuse. Mucilaginous cells: Similar to the epidermal cells but enlarged below with opaque contents; sides thin, straight or curved; surface smooth. Distribution: Occasional either in leaf abaxial (*T. lampas*) or adaxial (*T. lampas* and *T. populnea*) (figures 4 and 10).

3·1b. *Stomatal complex*: Stomata mostly anisocytic, occasionally anomocytic or tetracytic. Subsidiaries 3 or 4, monocyclic unequal, similar to the epidermal cells except that cuticular striations are absent over stomata. Distribution: Amphistomatic but adaxially stomata confined to margins of the midrib (figures 1–6).

Data on the epidermal and stomatal complexes are given in table 1. In both the species, single guard cells as stomatal abnormality are occasionally observed in the leaf abaxial whereas they are totally absent in the adaxial (figure 7).

### 3.2. *Structure and distribution of trichome complex on vegetative and floral parts*

Eight trichome types could be recognised in the two species, *viz.*, (i) unicellular conical hiar, (ii) uniseriate filiform clavate hair, (iii) uniseriate filiform pyriform hair, (iv) multiseriate aseptate peltate hair, (v) multiseriate aseptate stellate hair, (vi) multiseriate aseptate 4-armed stellate hair, (vii) multiseriate aseptate 3-armed stellate hair and (viii) biseriate aseptate V-shaped hair. The details of the structure of the trichome types are as follows:

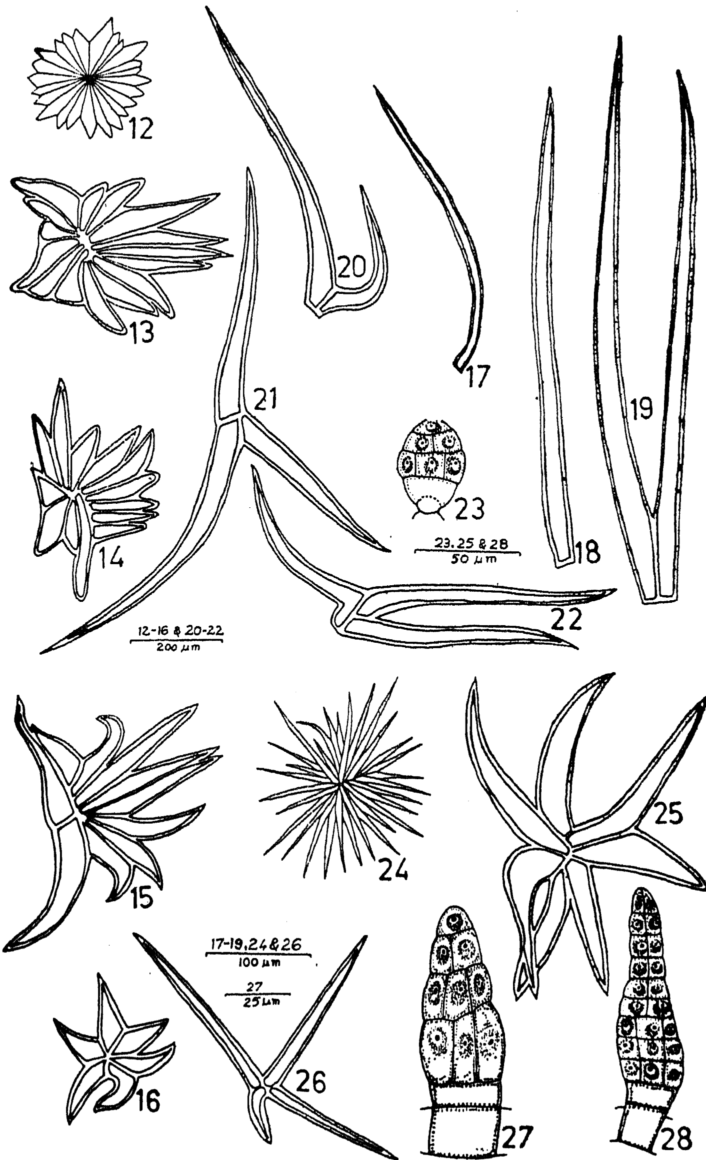



**Figures 1–11.** Epidermis of *Thespesia. T. populnea* ; **1** and **2.** Surface views of leaf abaxial and adaxial respectively (cuticular striations not drawn). **7.** Surface view of stomatal abnormality with single guard cell from leaf abaxial. **10.** Mucilaginous cell from leaf abaxial epidermis in T.S. **11.** Oblipue seotion showing sunken uniseriate filiform clavate hair from T.S. leaf (foot cell not visible). *T. lampas* : **3** and **4.** Surface views of leaf abaxiial and adaxial respectively. **5.** Semi-diagrammatic representation of stomatal distribution confined to margins of midrib from leaf adaxial. **6.** Surface view of anomocytic stoma from leaf adaxial. **8.** Uniseriate filifoirm clavate hair from T.S. pedicel. **9.** Side view of emergence from pedicel. (mc = mucilaginous cell).

**Table 1.** Data of foliar epidermal cells and stomata in the Indian *Thespesia*

| Species | | Epidermal cells | | | | Stomata | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Shape | Sides | Surface | Epidermal cell complex per sq. cm. | % of Anisocytic | % of Anomocytic | % of Tetracytic | Stomatal frequency per sq. cm. | Stomatal index |
| *Thespesia lampas* | leaf abaxial | Anisodiametric | Straight | Smooth | 1,28,890 | 85·9 | 8·5 | 5·6 | 31,550 | 19·7 |
| | leaf adaxial | Anisodiametric | Straight | Smooth | 1,10,222 | +* | +* | +* | +* | +* |
| *Thespesia populnea* | leaf abaxial | Anisodiametric | Curved or wavy | Striated | 1,33,330 | 83·3 | 11·1 | 5·6 | 27,000 | 13·8 |
| | leaf adaxial | Anisodiametric | Straight or Curved | Striated | 1,06,220 | +* | +* | +* | +* | +* |

+* = Stomatal complex is confined only to the margins of the mid rib, hence only epidermal cell frequency provided.




**Figures 12–28.** Epidermis of *Thespesia*. *T. populnea* : **12.** Multiseriate aseptate peltate hair from leaf abaxial. **13–15.** Intermediates between multiseriate aseptate peltate hair and multiseriate aseptate stellate hair from petal abaxial. **16.** Multiseriate aseptate stellate hair from petal abaxial. **17.** Isolated unicellular conical hair from sepal adaxial. **27.** Uniseriate filiform pyriform hair of nectary from sepal adaxial. *T. lampas* : **18.** Unicellular conical hair from sepal adaxial. **19, 20.** Biseriate V-shaped aseptate hair from sepal adaxial and petal abaxial respectively. **21, 22.** Multiseriate aseptate 3-armed stellate hair from petal abaxial. **23.** Surface view of uniseriate filiform clavate hair from pedicel. **24, 25.** Multiseriate aseptate stellate hair from leaf abaxial and pedicel respectively. **26.** Multiseriate aseptate 4-armed stellate hair from ovary. **28.** Uniseriate filiform pyriform hair of nectary from sepal adaxial.

3.2a. *Unicellular conical hair*: Foot consisting of the basal end of the body cell, indistinct from the body, embedded in the epidermis; neighbouring cells of foot annular and striated; contents absent; wall thick. Body representing extension of the foot, conical, apically pointed; contents absent; wall thick and nonlignified; surface smooth (figures 17 and 18).

3.2b. *Uniseriate filiform clavate hair*: Foot 1-celled, square to linear or trapezoidal, embedded or projected above the epidermis; contents absent; wall thin. Stalk 1-celled, rectangular to linear; contents scanty; wall thin; surface smooth. Head clavate, 2-6-tiered, each tier 2-6-celled; cells square to linear; contents dense; walls thin; surface smooth (figures 8, 11 and 23).

3.2c. *Uniseriate filiform pyriform hair*: Foot 1-celled, linear, embedded or projected above the epidermis; contents absent; wall thin. Stalk 1-celled, rectangular to linear; contents scanty; wall thin; surface smooth. Head pyriform, multiseriate, 4–10-tiered, each tier 2–6-celled, generally terminated by a pair of rounded cells; cells rectangular to linear; contents dense; walls thin; surface smooth (figures 27, 28).

3.2d. *Multiseriate aseptate peltate hair*: Foot narrow, consisting of the juxtaposed basal ends of the 20-35-body cells, embedded in the epidermis, polygonal in outline in peels mounted upside down, occasionally subtended by an emergence; contents absent; walls thin, nonlignified. Body peltate, nearly circular or uneven, serrate or crenulate at margin, 20–35-armed, representing continuation of the foot; arms broadened near the centre of the body but tapering towards distal end, parallel to the epidermis; contents brown; walls thin, nonlignified; surface smooth (figure 12).

3.2e. *Multiseriate aseptate stellate hair*: Foot as in the above. Body stellate, 5–40-armed, representing continuation of the foot; arms tapering towards the distal end, unequal in length, generally parallel to the epidermis, some obliquely raised above; contents absent; walls thin to moderately thick, nonlignified; surface smooth (figures 9, 16, 24 and 25).

Multiseriate aseptate 4-armed stellate hair, multiseriate aseptate 3-armed stellate hair and biseriate aseptate V-shaped hair types are similar to the multiseriate aseptate stellate hair described above except for the difference in number of arms of the body as indicated by the names of trichome types (figures 19–22 and 26). The details of distribution of the various trichome types are given in table 2.

## 4. Discussion

Inamdar and Chohan (1969) recorded anisocytic and anomocytic stomata in *Thespesia populnea*, which is presently confirmed. Further, tetracytic stomata (as defined by Shanmukha Rao and Ramayya 1977a) have also been presently noted in the leaves of the two species of *Thespesia* studied. Among the three stomatal types, the anisocytic is dominant (table 1).

**Table 2.** Organographic distribution of trichome types in the Indian *Thespesia.*

| Plant part | *Thespesia lampas* | *Thespesia populnea* |
|---|---|---|
| Leaf abaxial | B, E | B, D |
| Leaf adaxial | B, E | B, D |
| Leaf margin | A, E | B, D |
| Petiole | B, E | B, D |
| Stipule abaxial | B, E | B, D |
| Stipule adaxial | B, E | B, D |
| Stipule margin | B, E | D |
| Stem | B, E | B, D |
| Peduncle | B. E | B. D |
| Bracteole abaxial | B, E | B, D |
| Bracteole adaxial | B | B, D |
| Bracteole margin | B, E | D |
| Sepal abaxial | ... | B, D |
| Sepal adaxial | A, C, H | A, C |
| Sepal margin | A | A |
| Petal abaxial | A, E–H | B, E–H |
| Petal adaxial | ... | ... |
| Petal margin | A, E–H | A |
| Staminal tube | ... | ... |
| Style | ... | ... |
| Ovary | A, E–H | D |

A, Unicellular conical hair ; B, Uniseriate filiform clavate hair ; C, Uniseriate filiform pyriform hair ; D, Multiseriate aseptate peltate hair ; E, Multiseriate aseptate stellate hair ; F, Multiseriate aseptate 4-armed stellate hair ; G, Multiseriate aseptate 3-armed stellate hair ; H, Biseriate aseptate V-shaped hair.

According to Youngman and Pande (1929) the following trichome types occur in *Thespesia* : (i) single unbranched hairs, (ii) stellate hairs, (iii) peltate scales and (iv) club shaped bodies. The first and third trichome types quoted above are the same as the unicellular conical and multiseriate aseptate peltate hair types respectively, described by us. On the other hand, we consider the "stellate hair" type quoted above, to be a trichome complex rather than representing a specific trichome type. On comparison with the trichome types delimited in the present investigation, the stellate hair is resolvable into the following four types : (i) multiseriate aseptate stellate hair, (ii) multiseriate aseptate 4-armed stellate hair, (iii) multiseriate aseptate 3-armed stellate hair and (iv) biseriate aseptate V-shaped hair. Similarly the club shaped body is distinguishable into two of the presently described trichome types : (i) uniseriate filiform clavate hair and (ii) uniseriate filiform pyriform hair. In the two species of *Thespesia* investigated thus in all eight trichome types are presently recognisable as described in the text.

Though, in *T. lampas*, the multiseriate aseptate stellate hair on pedicel and sepal abaxial are 5 to many armed (figure 25), those on the leaf lamina, petiole and stem are 20-40-armed with thin walls (figure 24). Likewise in *T. populnea* the arms of the multiseriate aseptate peltate hair are 20–35 with thin walls on all the parts of its occurrence (figure 12). Many intermediate forms connecting the peltate and stellate types also occur on petals (figures 12–16). The characters which distinguish the multiseriate aseptate stellate hair of *T. lampas* and the multiseriate aseptate peltate hair of *T. populnea* from each other include the separate and tapering nature of the arms in the former and the connate condition and rounded ends of the arms in the latter (figures 12 and 24). As shown by Ramayya and Shanmukha Rao (1976) the above differences in the two trichome types are due to early onset of apical intrusive growth instead of symplastic growth in the development of arms in the multiseriate aseptate stellate hair. Thus the differences described are deep in origin and hence are significant in justifying the separation of the two trichome types.

In the two species of *Thespesia*, all the vegetative parts are trichiferous whereas in the floral parts, the surface of the petal adaxial, staminal tube and style are non-trichiferous (table 2). Further, in each of the species, all the trichiferous parts show at least two trichome types (*viz.*, multiseriate aseptate peltate hair and uniseriate filiform clavate hair in *T. populnea* and multiseriate aseptate stellate hair and uniseriate filiform clavate hair in *T. lampas*) except the abaxial surface of the petal and the ovary which possess several trichome types each (table 2).

Out of the eight trichome types now described, all of them occur in *T. populnea* whereas in *T. lampas* there are seven types, the multiseriate aseptate peltate hair being absent (table 2). Thus the two species are distinguished from each other due to the presence of the multiseriate aseptate peltate hair in the former and that of the multiseriate aseptate stellate hair in the latter. Further, the leaves of *T. populnea* are distinct from those of *T. lampas* by curved epidermal cells with striations and absence of mucilaginous cells on the abaxial surfaces (table 1).

In the systematic accounts, *Thespesia lampas* (Masters 1874 ; Borssum 1966 ; Rakshit and Kundu 1970 ; Saldanha and Nicolson 1976) is considered conspecific with *Hibiscus lampas* (Schumann 1890 ; Hochreutiner 1900 ; Gamble 1957) and *Azanza lampas* (Babu 1977). The tribe Gossypieae (includes *Thespesia*) differs from Hibisceae (includes *Hibiscus*) by the gossypol glands and conduplicate embryos (Fryxell 1968). The presence of gossypol glands on different parts of *Thespesia lampas*, *T. populnea* and other two species (Standford and Viehoever 1918 ; Lukefahr and Fryxell 1967) supports the separation of *Thespesia* from *Hibiscus*. Further, *Thespesia lampas* stands out distinct in other Malvaceae (with thick-walled non-lignified stellate hair) by the multiseriate aseptate thin-walled stellate hair, thus providing further evidence to treat *Thespesia lampas* distinct from *Hibiscus lampas*.

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# Embryological studies in *Launaea nudicaulis* Hook.*

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**Abstract**. The ovule is anatropous, unitegmic and tenuinucellate. Their funicular vascular strands extend almost to the base of the ovule. A large hypodermal archespoial cell functions directly as the megaspore mother cell. It divides to form a linear tetrad. The upper three megaspores degenerate while the chalazal develops into an 8-nucleate embryo sac of the Polygonum type. Endothelium differentiates at the megaspore tetrad stage. Fertilization is porogamous. Syngamy and triple fusion take place almost simultaneously. The endosperm is nuclear but it becomes cellular subsequently. The embryo is the *Senecio* variation of Asterad type. Occurrence of polyembryony has been recorded only in one ovule.

**Keywords.** *Launaea nudicaulis* ; embryology ; Compositae.

## 1. Introduction

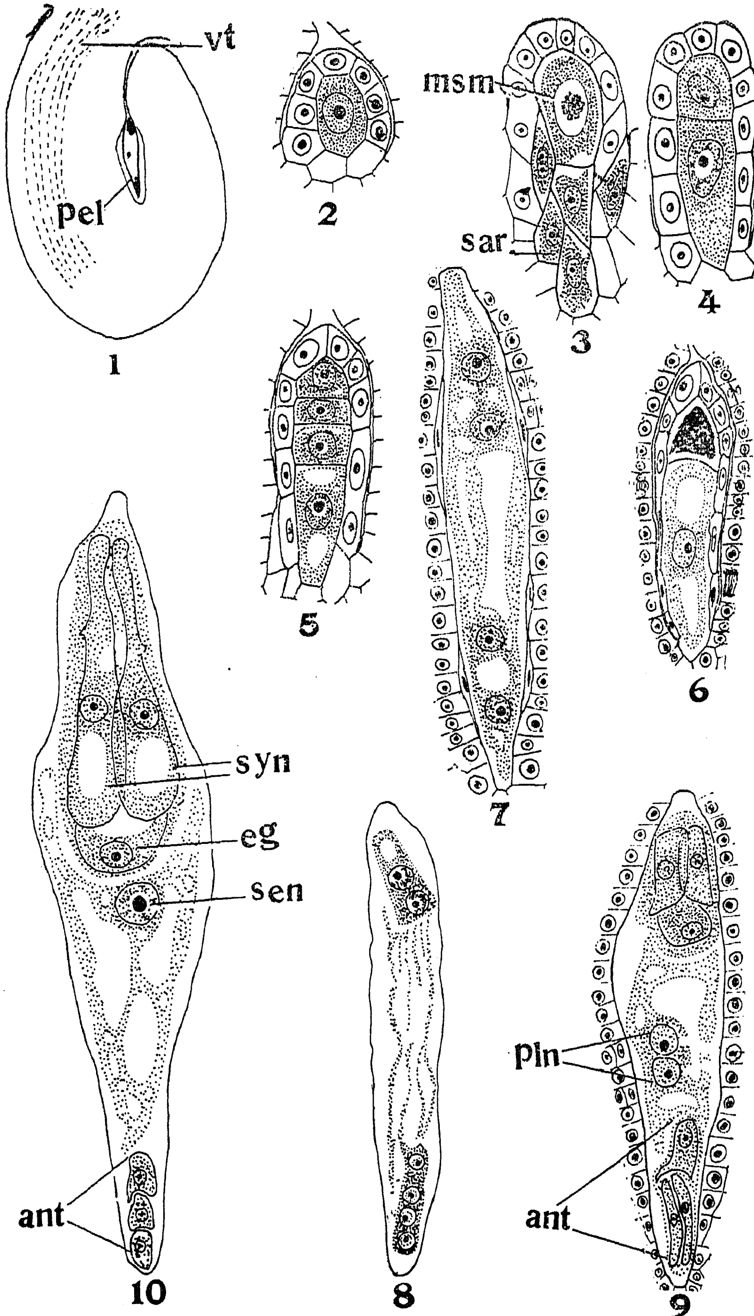
The Compositae is one of the largest families of flowering plants consisting of more than 20,000 species, yet the embryological work in the family is scanty. Davis (1966) has reviewed the previous embryological literature on the family Compositae. Venkateswarlu and Maheswari Devi (1955a) have made embryological studies on *Launaea nudicaulis*, which is widely distributed and also has cytotypes, only a note has appeared so far (see Venkateswarlu 1939).

The cytotypes are diploid ($2n = 18$) and tetraploid ($2n = 36$). Attempt is made here to present some aspects of comparative embryological studies of both diploid and tetraploid taxa.

## 2. Material and methods

Young heads were fixed as such but old heads were cut symmetrically into two halves before they were fixed in formalin-acetic alcohol. Following customary methods, the material was dehydrated in ethyl alcohol series and embedded in

* Part of the Ph.D. thesis submitted by the Junior author (B S Hiremath) to the Karnatak University, Dharwad.




**Figures 1–10.** L.S. of *Launaea nudicaulis* ovules. **1.** Showing anatropous condition (× 150). **2.** Single hypodermal archesproial cell (× 350). **3.** Megaspore mother cell and supernumerary archesporial cells (× 350). **4, 5.** Dyad and a tetrad of megaspores. **6–8.** Uni, four and six nucleate embryo sacs (× 350). **9, 10.** Organised and mature embiyo sacs (× 350). *ant*, antipodal cells ; *pel*, periendothelial zone ; *sar*, supernumerary archesporial cells ; *ser*, secondary nucleus ; *syn*. synergid cells ; *vt*, vascular traces.
Figures 1, 2, 4–7, 9–25 of 2*n* species. Figures 3, 8, 26 and 27 of 4*n* species.

paraffin. Serial sections were cut from 6 to 15 microns and were stained in Heidenhains iron-alum haematoxylin using sometimes erythrocin in 90% alcohol as counter stain.

## 3. Observations

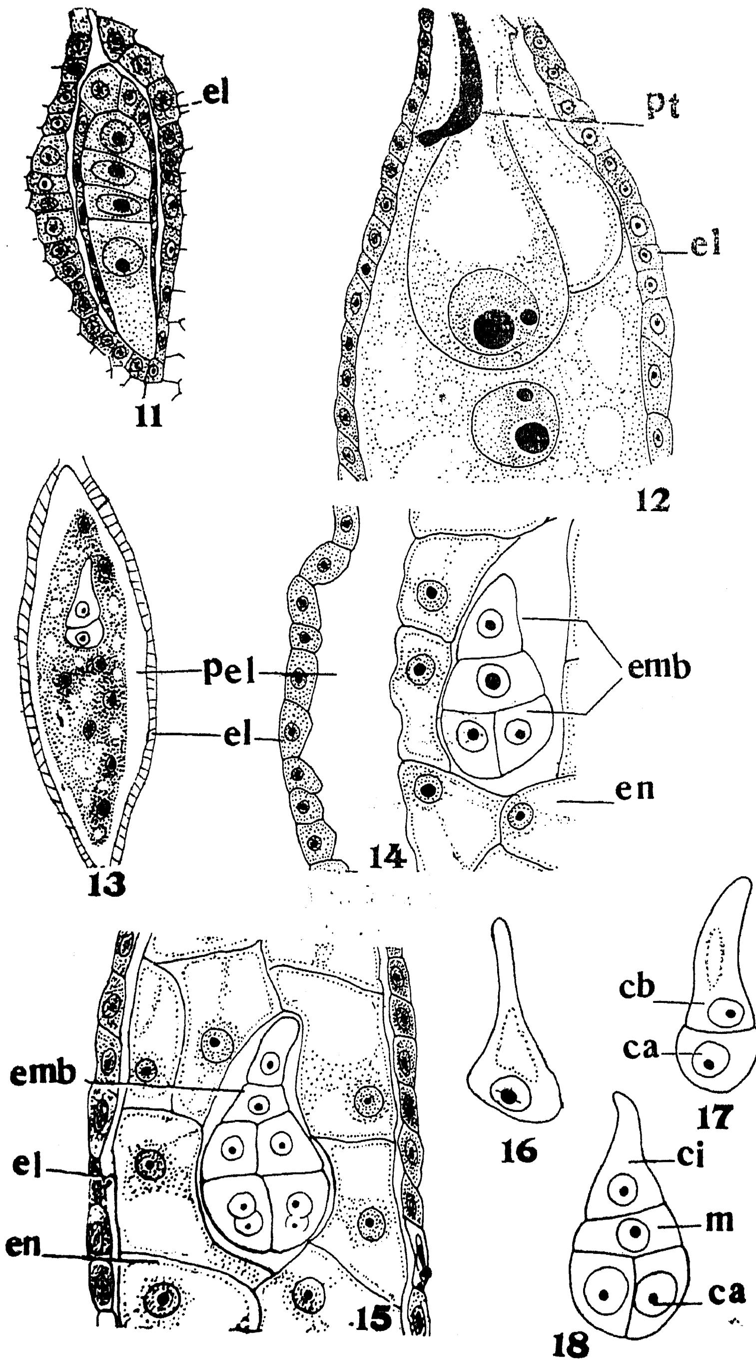
Detailed observations in the diploid taxon and only variations in the tetraploid species are described. The inferior, bicarpellary and unilocular ovary bears a single ovule at its base. The ovule is anatropous, unitegmic and tenuinucellate (figure 1). The funicular vascular strands extend almost to the base of the ovule. There is generally a large hypodermal archesporial cell which functions directly as the megaspore mother cell (figure 2). In tetraploid taxon supernumerary archesporial cells are usually observed (figure 3). Their number varies from 2 to 8. An archesporial cell undergoes meiotic divisions and forms a linear tetrad (figures 4, 5). Further development of supernumerary archesporial cells in tetraploid taxon is not observed. Subsequently, the upper three megaspores degenerate while the chalazal megaspore organises into an 8-nucleate embryo sac of the polygonum type (figures 6, 7, 9). In tetraploid taxon the divisions at both chalazal and micropylar ends are not synchronous. At the chalazal end the third mitotic division has already resulted in four nuclei while at the micropylar end the two nuclei are yet to divide (figure 8).

The organised embryo sac is spindle-shaped and its chalazal end is narrow. The egg apparatus is at its micropylar and consists of two synergids and a centrally placed egg cell (figure 9). Synergids are hooked (figure 10). There are three antipodal cells which are placed in triangular or linear fashion in the narrow chalazal end of the embryo sac. The two polar nuclei are in the centre and are very near to each other (figure 9). Later the two polar nuclei, just below the egg apparatus, form a secondary nucleus (figure 10).

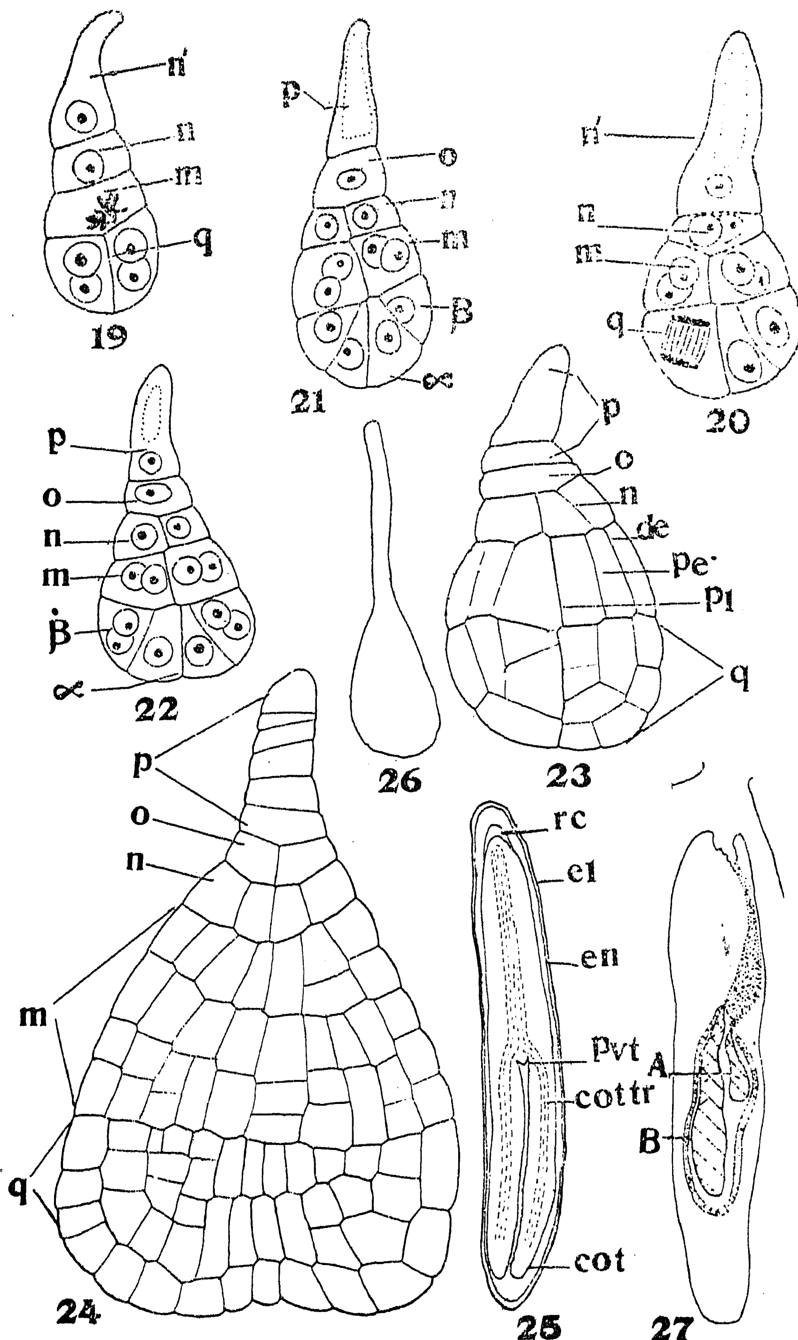
Endothelium appears at the megaspore tetrad stage and presents its characteristic glandular appearance (figure 11). It arises from the inner epidermis of the integument enclosing the nucellus (figures 7, 9 and 12). It consists of rectangular cells and the cells are rich in cytoplasm (figure 11). Endothelium nourishes the growing embryo sac. Gradually it starts losing its compactness (figures 12 to 15). Its peripheral layer called periendothelial zone loses its cell contents finally forming an empty layer (figures 13, 14). The endothelium persists as a thin layer around mature embryo (figure 25).

Fertilization is porogamous. The pollen tube, after reaching the base of the egg, bursts open at its base discharging the two male gametes. One male gamete fuses with the egg and the other with the secondary nucleus (figure 12). Syngamy and triple fusion take place almost simultaneously (figure 12).

The division of the endosperm nucleus precedes that of the zygote. The first division of the primary endosperm nucleus results in the formation of two free nuclei. As many as eight free nuclei have been observed around the two celled proembryo (figure 13). Later it becomes cellular. Hence the endosperm is of nuclear type. The developing embryo absorbs most of the endosperm tissue so




**Figures 11–18.** L.S. *L. nudicaulis* nucellus. **11.** Endothelium at megaspore tetrad stage. **12.** Syngamy and double fertilization (× 350). **13.** 8-nucleate endosperm (× 350). **14, 15.** Stages in the development of endosperm and endothelium (× 350). **16.** Zygote (× 350). **17, 18.** 2- and 4-celled embryos.




**Figures 19–27.** L.S. ovules of *L. nudicaulis*. **19–24.** Stages of development of embryo (× 350). **25.** L.S. of mature seed (× 20). **26.** L.S. of globular embryo showing long suspensor (× 350). **27.** L.S. of seed showing two embryos A and B (× 20). *cot,* cotyledon ; *cot tr,* cotyledonary traces ; *de,* dermatogen initials ; *el,* endothelium ; *en,* endosperm ; *pe,* periblem initials ; *pl,* pleurome initials ; *pvt,* stem tip ; *rc,* root cap.

that the mature seed has a thin layer of endosperm surrounding the embryo (figure 25).

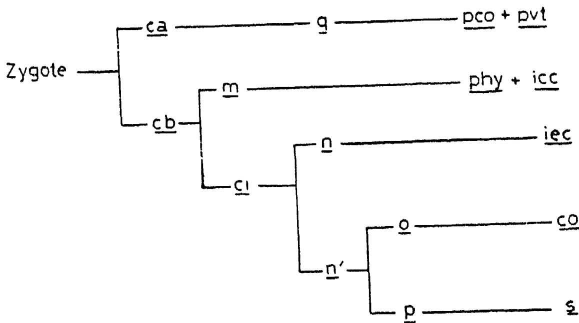
The zygote is pyriform (figure 16). The first division of zygote is transverse resulting in the formation of a terminal cell *ca* and a basal cell *cb* (figure 17). The next transverse division occurs in *cb* forming two tiers *m* and *ci* of one cell each (figure 18) while the cell *ca* divides longitudinally forming two juxtaposed cells resulting in a *T*-shaped proembryo. Further, the tier *ca* divides longitudinally in a plane perpendicular to the first forming a quadrant *q* (figures 18–20).

Simultaneously, the tier *m* divides vertically into two cells while the tier *ci* divides transversely into two tiers *n* and *n'* of one cell each (figures 19, 20). Generally, the transverse division in the tier *ci* precedes that of the longitudinal division in the tier *m* (figure 19). Thus, a proembryo of seven cells disposed in four tiers is formed (figure 19). The tier *ca* consists of four cells, *m* of one cell, *n* and *n'* of one cell each. The walls in the quadrant are placed obliquely dividing them into central cells $\alpha$ and peripheral cells $\beta$ (figures 21, 22). The peripheral cells $\beta$ are very active and they form two cotyledons (*pco*) while the central cells $\alpha$ are less active initially, but contribute to stem apex (*pvt*) (figures 22, 24). By this time *m* has undergone another vertical division to form four circumaxial cells (figures 21, 22). Meanwhile the tier *n'* has divided transversely forming two tiers *o* and *p* of one cell each (figures 21, 22) The cell *p* is highly elongated and vacuolated. The proembryo consists of 14 cells arranged in five tiers (figure 22). The terminal tier *q* consists of 6-cells, the tier *m* of four cells, the tier *n* of two cells and the tiers *o* and *p* of one cell each. Subsequently the periblem and pleurome initials are cut off from the tier *q* (figure 23).

Each one of the four circumaxial cells in the tier *m* divides periclinally giving rise to dermatogen initials which become continuous with the dermatogen formed in the apical tier (figure 23). Likewise, the periblem initials which are differentiated in tier *q* also extend into tier *m*. Later, longitudinal and transverse walls are laid down in this tier *m* which finally gives rise to hypocotyl (*phy*) and radical (*icc*) (figure 24).

The two juxtaposed cells in the tier *n* undergo periclinal division cutting off epidermal initials. The cells in the centre further divide both longitudinally and transversely to contribute to root tip (*iec*). The tier *o* divides periclinally and anticlinally to form a root cap (*co*). The tier *p* contributes to the suspensor (*s*). In tetraploid taxon there is a long suspensor (figure 26). It perists till the heart-shaped embryo stage and is totally absent in the mature embryo. The mature embryo is dicotyledonous and somewhat straight (figure 25). It consists of an elongated hypocotyl-root axis. The root apex is protected by the root-cap. The stem apex is centrally placed between the two cotyledons having abundant starch. The vascular strands run from the hypocotyl into the cotyledons. The following sequence represents the development of the embryo of *Launaea nudicaulis.*

A rare instance of polyembryony has been observed in tetraploid taxon based on its position, the adventitious embryo seems to have been originated from one of the synergids (figure 27).




## 4. Discussion

ntegumentary vascular traces have been reported in some Compositae (Misra 1965 ; Chopra and Singh 1976). In *Launaea nudicaulis* funicular vascular strands are present which extend only to the base of the chalaza. Although pluricellular archesporium has been reported earlier (Harling 1951a ; Sehgal 1966), in *Launaea nudicaulis* (2*x*) there is only one archesporial cell. However, in tetraploid plants supernumerary archesporial cells are also observed. In most of the Compositae, a linear tetrad of megaspores is formed in which the chalaza develops into an eight nucleate embryo sac of the polygonum type (Pullaiah 1977a, b ; Sharma and Murthy 1978). *T*-shaped tetrads have also been reported in many members (Deshpande 1964a, b ; Kaul *et al* 1975). Invariably in *Launaea nudicaulis* only linear tetrad of megaspores is observed.

There are many interesting features in the embryo sac development in the Compositae. Several divergences from its normal development occur (Davis 1966). An Allium type in *Ammobium* spp. (Davis 1962), in *Chrysanthemum* spp. and *Erigeron* spp. (Harling 1951a, b) has been described. Three variations of the tetrasporic development of the embryo sac, a *Fritillaria* type in *Gaillardia picta* (Venkateswarlu and Maheswari Devi 1955b), *Ratibida tagetes* (Howe 1964), *Drusa* type in *Chrysanthemum* spp., *Erigeron* spp. (Harling 1951a, b) and *Adoxa* type in *Rudbeckia hirta* (Palm 1934) have been reported. In *Launaea nudicaulis* the *Polygonum* type of embryo sac has been recorded. Large haustorial synergids, often extending into the micropyle, appears to be a common feature in the Compositae (Davis 1961b). Pullaiah (1977a, b) recorded hooked synergids in *Solidago canadensis* and *Achillea squarrosa.* In *Launaea nudicaulis,* hooked synergids with blunt ends are observed. There is a great variation in the number of antipodal cells and in the number of nuclei in them, a feature found in many members of the Compositae. Secondly, antipodal region has been found to be haustorial persisting as vermiform appendage (Davis 1961a). Taigi and Taimni (1963) have also noted the haustorial antipodals in *Vernonia sinerscens.* Murthy and Sharma (1976) and Sharma and Murthy (1978) have observed basically three antipodals in all the three species, *Felicia bergariana, Conyza stricta* and *Erigeron bonariensis* while in *Bellia perennis* they have noticed an increase in the number of antipodal cells up to 21. In *Launaea nudicaulis* there are three antipodal cells placed in a triangular fashion and persist till the mature proembryo stage. But they do not show any haustorial nature.

Endothelium is present in the Compositae (Venkateswarlu and Maheswari Devi 1955a; Kaul *et al* 1975 ; Deshpande and Kothare 1976 ; Chopra and Singh 1976 ; Sharma and Murthy 1978 ; Sehgal 1979). It appears in the early development of the ovule at megaspore mother cell stage in *Vernonia anthelmintica* (Misra 1972) or at megaspore tetrad stage in *Guizotia abyssinica* (Chopra and Singh 1976). In the present study differentiation of the endothelium takes place at the megaspore tetrad stage. It is usually uniseriate but sometimes biseriate. Nutritive activity of the endothelium becomes evident during the development of the embryo sac. The layers of cells surrounding the endothelium show some marked changes, especially with respect to size, shape, form and stainability. This layer is called periendothelial zone (Misra 1972). In the material under study the nutritive activity is at its peak at the time of fertilization.

Both cellular and nuclear types of endosperm are known to occur in the Compositae, the former being more frequent (Davis 1966). Endosperm is of nuclear type in the taxa under study. Persistence of the endosperm as a thin layer around the embryo is in line with the observations of Misra (1965, 1972), Kaul *et al* (1975) and Sharma and Murthy (1978).

Deshpande (1961) has shown that in *Eclipta prostrata* obliquely oriented walls are laid down in the octant which is characteristic of Onagrad type. He, further, commented that these features constitute intermediate stages of the Onagrad type and the Asterad type. However, the present study does not indicate any such variations.

## Acknowledgement

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# Quantitative profile structure of certain forests in the Kumaun Himalaya

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**Abstract.** The structure of forests occurring within the north-western catchment of the river Gola in Kumaun Himalaya is quantitatively described. All the forestt indicated a total of four strata ; two upper strata represented by trees, the third stratum represented mainly by shrubs, and the fourth of herbs. The tree heighs of the $A_2$ (top most) stratum decreased with an increase in altitude. On the other hand, the proportion of trees devoted to the canopy in the $A_1$ and $A_2$ strata increased with an increase in altitude. In all forests, the crowns of the $A_1$ and $A_2$ strata were more deep than wide. In general, the shrub layer in three oak forests was comparatively dense and the crowns of the shrubs overlapped with each other. The canopy index, a relative measure of canopy coverage, of tree and shrub layers was maximum for *Quercus floribunda* forest and minimum for *Pinus roxburghii* forest. Further, the cooler aspects develcped a greater canopy index for these layers as compared to the warmer aspects. Oak forests exhibited a poor development of their herb layers. The trees in the *Quercus lanuginosa* forest weere more stable, while in *Pinus roxburghii* forest they were specially susceptible to wind effect. In general the warmer aspects had more stable trees, while the cooler aspects showed a lower tree stability. The different forest types, presently studied, could be graded, as follows, in a decreasing order of potential for soil protection : *Quercus floribunda* > *Quercus leucotrichophora* > *Quercus lanuginosa* > mixed > *Pinus roxburghii*.

**Keywords.** Himalayan forests ; profile structure ; canopy index.

## 1. Introduction

The Himalaya offers an array of forest types below the timber line, and is the cradle of major rivers of India, harbouring (thus) a net work of catchment areas. Growing human interference with the vegetation cover of the catchment of rivers has generally led to substantial reduction of forest cover which in turn has led to serious ecological disasters, such as, soil erosion, loss of soil fertility and catastrophic floods. As the catchment efficiency depends on the type, quantity and stratification of vegetation, a quantitative evaluation of its vegetation is a pre-requisite. However, such data are few (Ralhan *et al* 1982; Saxena and Singh

1982; Saxena *et al* 1982; Tewari and Singh 1981). Earlier studies, mainly qualitative, have been reviewed by Puri (1960) and Champion and Seth (1968). The present study describes the structure of the forests occurring within the north-western catchment of the river Gola in Kumaun Himalaya.

Due to the presence of a large variety of growth forms, forests are generally highly stratified (Smith 1974). Tropical forests usually have a total of five strata above the soil surface, while the temperate forests have only two or three strata (Richards 1952). Upto some extent, light and moisture determine the various strata which in turn modify the environment from the canopy to the forest floor. The amount of light received in various strata of a community varies, depending upon the density of different strata, type of vegetation, opening of forest crown, etc. (Knight 1965). The canopy closure also plays a major role n the regeneration of forests by conditioning the light intensity reaching the forest floor. According to Richards (1952) and Holdridge (1968), an excellent visual representation of the structure of a forest community can be communicated by constructing a profile diagram. Several workers, including Beard (1941, 1955), Burges and Johnston (1953), Dansereau (1957), Keay (1957), Webb (1959), Fosberg (1961) and Legris (1961) have recognized the usefulness of profile diagrams and their structural-functional information. Ashton and Brunig (1975) and Whitmore (1975) recently reviewed the structural variation in the humid tropical forests and in the forests of south-east Asia, respectively.

## 2. Study area and methods

### 2.1. *Location*

A total of 14 sites, located in the north-western catchment of river Gola in Kumaun Himalaya (29° 19′ to 29° 27′ N lat. and 79° 32′ to 79° 42′ E long.) were selected for the present study. The sites are at different altitude, aspects ands lope angles (table 1).

**Table 1.** Aspect, altitude and slope angle of the selected locations.

| Forest type | Aspect | Altitude (m) | Slope angle (°) | Locality |
|---|---|---|---|---|
| *Pinus roxburghii* | NE, E, SW | 1280–1320 | 30 65 | Champhi, Sattal |
| Mixed | E, NW, S | 1320–1365 | 25-50 | Sattal |
| *Q. leucotrichophora* | NE, E, SW | 1950-2025 | 40–50 | Maheshkhan |
| *Q. lanuginosa* | S | 2010 | 55–65 | Maheshkhan |
| *Q. floribunda* | N, NE, E, S | 2100–2227 | 35–55 | Maheshkhan |

Under the forest settlement of 1911 to 1915, some of the new reserves were grouped into 'settlement blocks'. The right and concessions to the villagers were given whenever possible for building-timber, wood for agricultural implements, grazing for a limited number of cattle, lopping, collection of fuel, grasses, etc. In 1921, the new reserve forests were divided into class I and class II forests and the rules regarding rights were modified. Class I forests retained their status as reserve forests but their management was under the civil authorities, while the class II forests were kept under the direct management of the Forest Department. According to the rules, in class I forests all *bonafied* residents of this region were allowed to graze cattle without any limit, fell and lop trees, cut grasses, etc., but the felling or lopping of timber trees were restricted. In the class II forests also, all *bonafied* residents of this region could graze cattle, lop *Quercus* and miscellaneous species, cut grasse and collect fallen fuel woods. The exceptions were the regeneration areas, fuel and fodder reserves and plantation areas in which some or all of these concessions were restricted. Timber trees were also reserved and not allowed to be cut or lopped. In 1964, the management of class I forests, except in those in which forest Panchayats had been formed, was transferred back to the Forest Department. The present sites are located in the forests of 'new reserve' category notified after 1915 and represent the original class II forests. Thus the rules regarding utilization were liberal and were not related to the carrying capacity of the area. The broad leaved trees, especially *Quercus* spp., were frequently lopped. To stop this, in 1974, the Government banned the felling by public, of all the trees of *Quercus leucotrichophora* and *Rhododendron arboreum* except for the dead, diseased and overmature trees.

### 2.2. *Climate, soil and geology*

The sites are characterized by a climate which shows three distinct seasons, *viz.*, rainy (June to September), winter (October to February) and summer (March to May). The average annual rainfall at Naini Tal is 2820 mm, 88% of which occurs between June to September. The mean maximum temperature ranges from 10·0 to 30·0° C and the mean minimum from 0·2 to 19·8° C.

The soil has been derived from parent materials comprising mainly of quartzite, quartz porphyry and schists (Raina and Dungrakoti 1975). The soil is dominated by sand particles. Proportion of sand is lower in oak forests compared to the mixed and *Pinus roxburghii* forests. The clay percentage differs little in different soils but in contrast to the pattern shown by sand, silt percentage is higher in the oak forests compared to other forests. The soil under all forests is slightly acidic. Details of climate and soil are given in Saxena and Singh (1980).

### 2.3. *Methods*

Sampling was done on four topographic situations (*viz.*, hill base, lower and upper slopes and hill top) for each aspect (site). Each sample consisted of 12 randomly placed 10 × 10 m quadrats. The size and number of quadrats were determined, respectively by species area curve (Oosting 1958) and the running mean method (Kershaw 1973). Detailed phytosociology of the woody species in these forests is described elsewhere (Saxena and Singh 1982).

In each quadrat, diameter at breast height (dbh at 1·37 m from the ground) of all trees > 10 cm dbh was measured and recorded individually by species. Each quadrat was subdivided into four 5 × 5 m plots for analysing shrubs, saplings and seedlings. All individuals of 3·3 to 10·0 cm dbh were tallied either as sapling or shrub, as appropriate. The herb layer was studied through tiller analysis (Singh 1967, 1969) by using 50 × 50 cm harvest plots. The sampling was done when the herbaceous vegetation was at its peak, *i.e.*, during last week of September to the first week of October. It is not intended to compare the density values for herbs with those of the trees, however.

The other measurements included total tree height, height to first branch and canopy width for each species. A rough sketch of the trees was made in the field. Height of the tree and canopy depth were measured by a hypsometer (Forbes 1961).

Profile diagrams were prepared for each topographic situations on each aspect following Knight (1963). In order to include a maximum number of species in the diagrams, an area equivalent to 20 × 10 m was found suitable. The number of trees, saplings and shrubs to be included in the diagram was calculated on the basis of their density (Curtis and McIntosh 1950). Trees of each species were selected for inclusion from among all trees of that species actually measured in the stand by using a random table (Campbell 1974).

The canopy indexes for tree and shrub layers were calculated by dividing the sum of the lengths of the strip covered by canopies by the total length of the profile strip. The resulting value was then multiplied by 100. These calculated values give only a relative measure of canopy coverage.

The quotient of slenderness (SG) for the top canopy trees, in each stand, was calculated by the formula given by Brunig and Heuveldop (1976) :

$$\text{SG} = h/d,$$

where, $h$ = average height of the tree (m) ; $d$ = average diameter of tree at breast height (m).

Spatial distribution, abundance and stratification of vegetation are summarized in vegetational formulae (Christian and Perry 1953). Letters and figures were assigned to the trees, shrubs and herbs, to their component layers, and to the density of each. Thus, $A_1$ was used for low trees and $A_2$ for tall trees. The letters, $B$ and $C$ were used for shrubs and herbs, respectively. Heights were recorded for each stratum as mean values. Thus, $A_2^{20}$ represents the trees with an average height of 20 m. Density was sim'larly treated by prefixing $x$, $y$ or $z$ for dense, average, or sparse and $xx$ or $zz$ for very dense and very sparse, respectively. Thus, a stand with two tree layers, one shrub and one herb layer at varying densities is expressed as :

$$A_2^{20}\, z,\ A_1^{10}\, y,\ B^{1\cdot 2}\, xx,\ C^{0\cdot 5}.\, x$$

This would indicate that the vegetation has two tree layers, one with average height of 20 m, the other with average height of 10 m, one shrub layer with mean height of 1·2 m and one herb layer with an average of 0·5 m. Further, the tall trees are sparse, low trees have an average density, the shrub layer is very denset and the herb layer is dense. The ranges for $x$, $y$, $z$, $xx$ and $zz$ are given in table

Table 2. Ranges of plant density for density symbols used in vegetational formulae.

(Individuals 200 $m^{-2}$)

| Density Symbols | $A_2$ and $A_1$ strata (trees + saplings) | $B$ stratum (Shrubs) | $C$ stratum (herbs + seedlings) |
|---|---|---|---|
| *xx* | 81–100 | 801–1000 | 279033–347800 |
| *x* | 61–80 | 601–800 | 210265–279032 |
| *y* | 41–60 | 401–600 | 141497–210264 |
| *z* | 21–40 | 201–400 | 72729–141496 |
| *zz* | 1–20 | 1–200 | 3960–72728 |

2. The range of the stratal height was set as : stratum $A_2$ = 15 to 30 m ; stratum $A_1$ = 2 to 15 m and stratum $B$ = 0·5 to 2 m.

The herbaceous stratum ($C$) is not shown in the diagrams although the information is included in the vegetational formulae based on the earlier description by Saxena and Singh (1980).

## Results

The profile diagrams for various forests are given in figures 1–5 and the vegetational formulae in table 3.

### 3.1. *Pinus roxburghii forest*

On all aspects, the average height of the $A_2$ stratum was higher on the hill top compared to other topographic situations (table 3). The $A_2$ stratum, on all topographic situations and aspects was represented by trees of *P. roxburghii* as illustrated by the profile diagram of this forest in figure 1. At the SW hill base and NE lower slope, the $A_1$ stratum was represented only by *P. roxburghii*. On the other hand the $A_1$ stratum at the hill base of east aspect consisted of *Pyrus pashia* and *Cocculus laurifolius*, while the same at the upper slope of this aspect had *Quercus leucotrichophora* and *Engelhardtia spicata*.

The crowns of *P. roxburghii* in the $A_2$ and $A_1$ strata were more deep than wide, and were conical in shape. The canopy of the $A_2$ stratum was comparatively denser and almost continuous at the NE hill base, lower slope and hill top, at the SW hill base and at the E lower slope. On other topographic situations and aspects, the canopy in this stratum was markedly broken. The canopy of the $A_1$ stratum on all topographic situations and aspects, was remarkably discontinuous. The trees in the $A_2$ and $A_1$ strata were very sparse (table 3).

The total combined canopy index of the $A_1$ and $A_2$ strata was highest at the NE lower slope and lowest on the E upper slope and hill top (table 4).

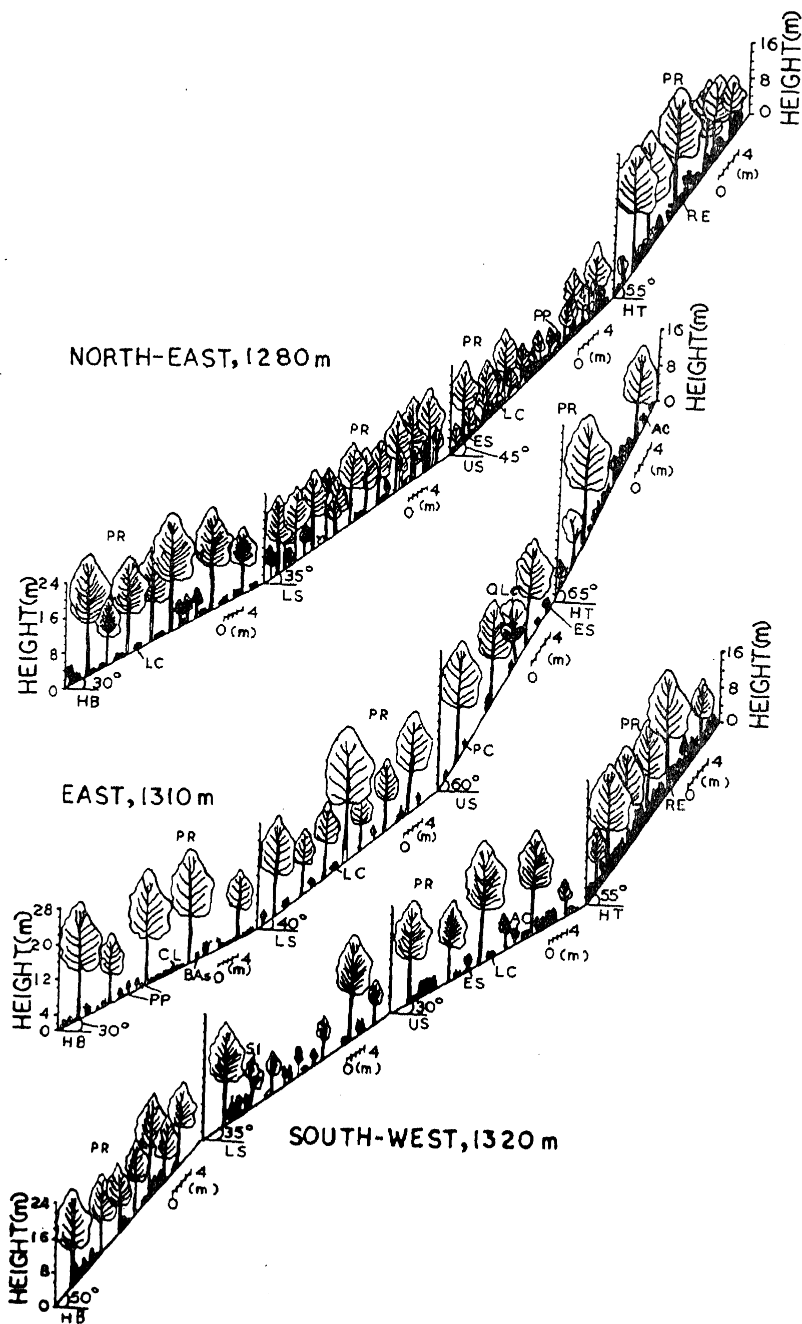


**Figure 1.** Profile diagram for *Pinus roxburghii* forest on north-east, east and south-west aspects. The scale on the *y*-axis is for the height and the scale on *x*-axis represents width of canopy and dbh. Each profile diagram is made up of four sections as follows : HB = hill base, LS = lower slope, US = upper slope, HT = hill top. Each section represents an area of 200 m². PR = *Pinus roxburghii* Sarg., ES = *Engelhardtia spicata* Leschen ex B$_l$. Var. *Colebrookiana* (Lindl. ex Wall.) Ktze, SI = *Sapium insigne* Benth., PP = *Pyrus pashia* Buch-Ham ex D.Don., QLe = *Quercus leucotrichophora* A. Camus, AC = *Adina cordifolia* (Roxb.) HK.f. ex Brandis, CL = *Cocculus laurifolius* DC., LC = *Lantana camara* Linn., RE = *Rubus ellipticus* Smith., PC = *Pyracantha crenulata* (D.Don) Roem., BAS = *Berberis asiatica* Roxb. ex DC.

Figure 2. Profile diagram for mixed forest on east, north-west and south aspects. For rest of the explanation see figure 1. The additional species are : BR = *Bauhinia retusa* Buch-Ham. ex DC., PO = *Persea odoratissima* (Nees) Kosterm., PI = *Pistacia integerrima* Stewart, CE = *Celtis eriocarpa* Decaisne, GO = *Grewia oppositifolia.* Buch-Ham. ex Roxb., RT = *Rhamnus triqueter* Wall., CC = *Cedrela ciliata* Roem, PS = *Parthenocissus semicordata* Wall., ER = *Euphorbia royleana* Boiss, SCu = *Syzygium cumini* (L.) Skeels., MP = *Murraya paniculata* (L.) Jack., BVr = *Bauhinia variegata* Linn., BVh = *Bauhinia vahlii* Wight & Arn., AO = *Acer oblongum* Wall. ex DC., MAu = *Millettia auriculata* Baker ex Brand., MB = *Myrica esculenta* Var. *sapida* Buch-Ham., GS = *Grewia subinaequalis* DC., CF = *Cassia floribunda* car., MK = *Murraya koenigii* (L.) spreng., AT = *Aechmanthera tomentosa* Nees.

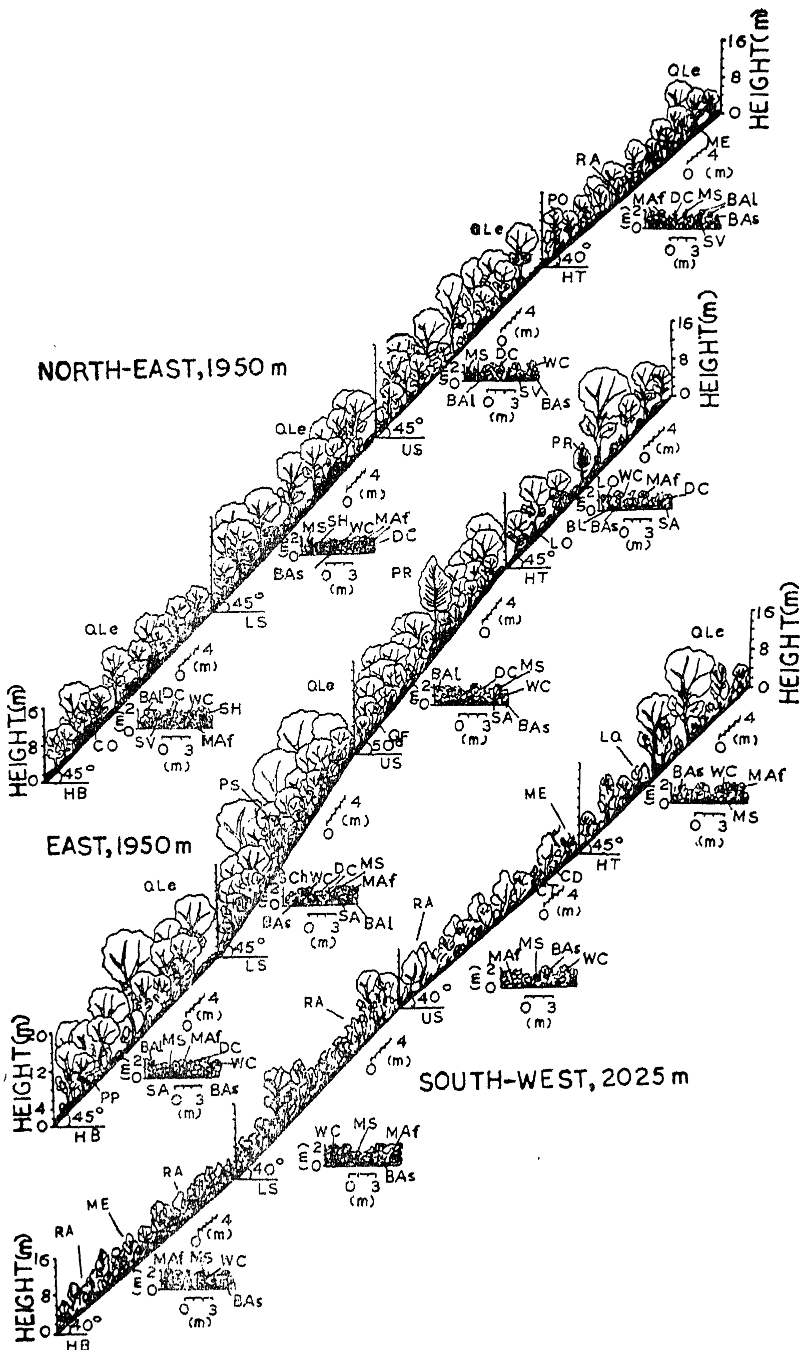


**Figure 3.** Profile diagram for *Quercus leucotrichophora* forest on north-east, east and south-west aspects. For rest of the explanation see figure 1. The inset diagram represents mainly the shrub layer magnified from the main diagram for an area equivalent to 15·7 m². The additional species are: RA = *Rhododendron arboreum* Sm., CO = *Cornus oblonga* Wall., LO = *Lyonia ovalifolia* (Wall.) Drude, QF = *Quercus floribunda* Rehder, sch = *Symplocos chinensis* (Lour) Druce, LQ = *Lonicera quinquelocularis* Hardw., CT = *Cupressus torulosa* D.Don., CD = *Cedrus deodara* (Roxb. ex Lambert) D.Don., MAf = *Myrsine africana* Linn., MS = *Myrsine semiserrata* Wall., SH = *Sarcococca hookeriana* Baill., BAl = *Boenninghausenia albiflora* (Hook.) Reichenb., SV = *Smilax vaginata* Decaisne., SA = *Smilax aspera* Linn., WC = *Wikstroemia canescens* Meissn., DC = *Daphne cannabina* Sensu Hook. f., BL = *Berberis lycium* Royle.

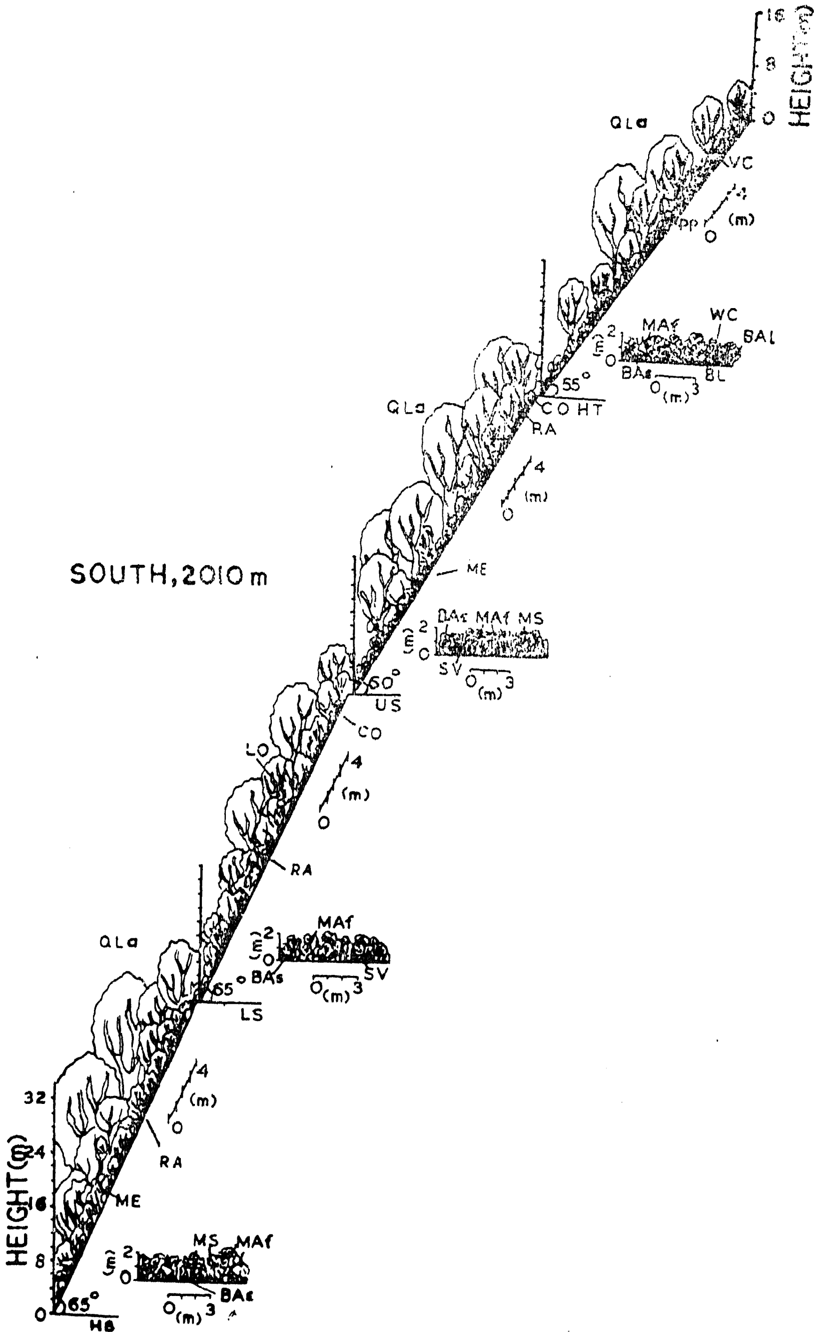


**Figure 4.** Profile diagram for *Quercus lanuginosa* forest on south aspect. For rest of the explanation see figures 1 and 3. The additional species are : QLA = *Quercus lanuginosa* D.Don., VC = *Viburnum cotinifolium* D.Don.

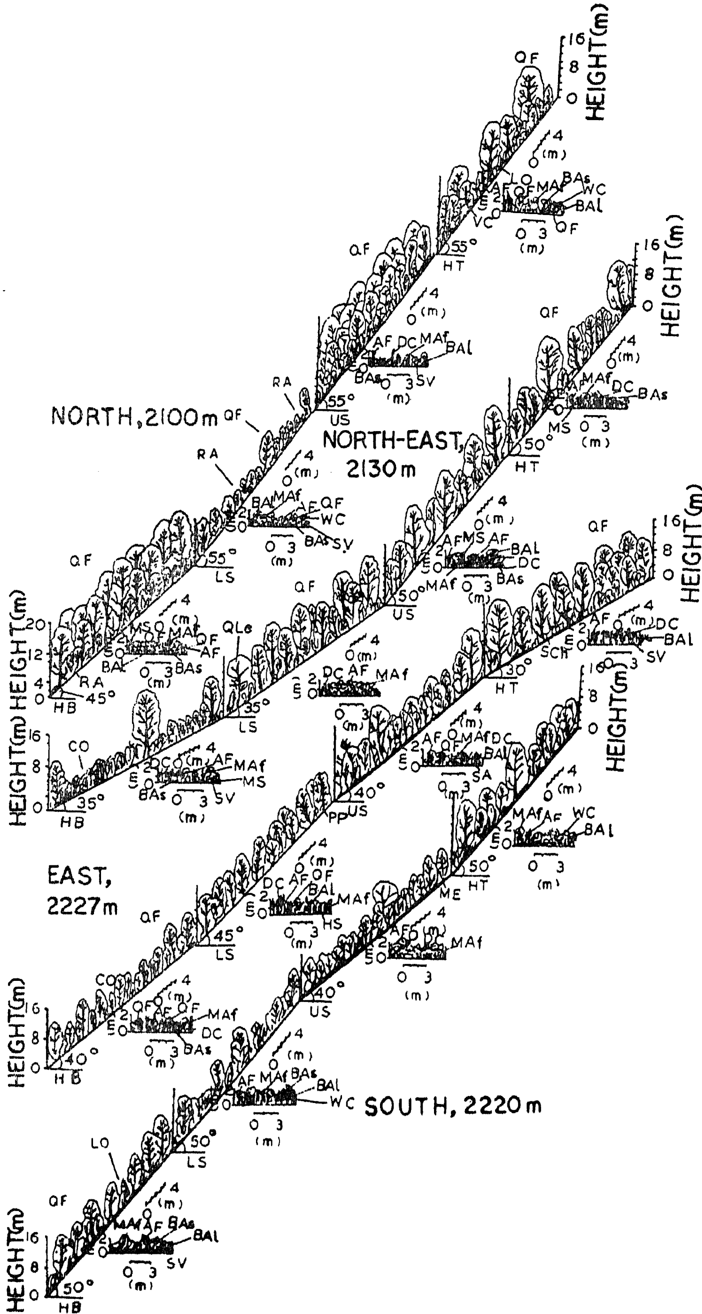


**Figure 5.** Profile diagram for *Quercus floribunda* forest on north, north-east, east and south aspects. For rest of the explanation see figures 1 and 3. The additional species is : AF = *Arundinaria falcata* Nees.

**Table 3.** Vegetational formulae for the stands examined. For explanation see text.

| Forest type | Aspect | Position | Vegetation formula |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| *Pinus roxburghii* | North-east | Hill base | $A_2^{21\cdot9}zz$, $A_1^{5\cdot9}zz$, $B^{1\cdot2}zz$, $Cy$ |
| | | Lower slope | $A_2^{17\cdot4}zz$, $A_1^{7\cdot9}zz$, $B^{1\cdot2}zz$, $Cy$ |
| | | Upper slope | $A_2^{16\cdot5}zz$, $A_1^{7\cdot6}zz$, $B^{1\cdot0}zz$, $Cy$ |
| | | Hill top | $A_2^{25\cdot0}zz$, $A_1^{4\cdot5}zz$, $B^{1\cdot5}zz$, $Czz$ |
| | East | Hill base | $A_2^{22\cdot4}zz$, $A_1^{2\cdot8}zz$, $B^{1\cdot1}zz$, $Cx$ |
| | | Lower slope | $A_2^{21\cdot2}zz$, $A_1^{5\cdot0}zz$, $B^{1\cdot5}zz$, $Cy$ |
| | | Upper slope | $A_2^{25\cdot9}zz$, $A_1^{8\cdot8}zz$, $B^{1\cdot5}zz$, $Cy$ |
| | | Hill top | $A_2^{26\cdot6}zz$, $A_1^{5\cdot5}zz$, $B^{1\cdot5}zz$, $Cz$ |
| | South-west | Hill base | $A_2^{19\cdot6}zz$, $A_1^{4\cdot9}zz$, $B^{1\cdot5}zz$, $Cy$ |
| | | Lower slope | $A_2^{23\cdot7}zz$, $A_1^{5\cdot8}zz$, $B^{1\cdot4}zz$, $Cxx$ |
| | | Upper slope | $A_2^{23\cdot2}zz$, $A_1^{3\cdot8}zz$, $B^{1\cdot2}zz$, $Cx$ |
| | | Hill top | $A_2^{24\cdot9}zz$, $A_1^{4\cdot3}zz$, $B^{1\cdot5}zz$, $Cxx$ |
| Mixed | East | Hill base | $A_2^{19\cdot1}zz$, $A_1^{9\cdot3}zz$, $B^{1\cdot6}zz$, $Cxx$ |
| | | Lower slope | $A_2^{15\cdot7}zz$, $A_1^{12\cdot5}zz$, $B^{5\cdot1}zz$, $Cy$ |
| | | Upper slope | $A_2^{17\cdot3}zz$, $A_1^{7\cdot6}zz$, $B^{1\cdot7}zz$, $Cy$ |
| | | Hill top | $A_2^{16\cdot5}zz$, $A_1^{7\cdot8}zz$, $B^{1\cdot2}zz$, $Cy$ |
| | North-west | Hill base | $A_2^{0}$, $A_1^{8\cdot5}zz$, $B^{1\cdot1}zz$, $Cz$ |
| | | Lower slope | $A_2^{26\cdot7}zz$, $A_1^{8\cdot5}zz$, $B^{1\cdot2}zz$, $Czz$ |
| | | Upper slope | $A_2^{23\cdot2}zz$, $A_1^{7\cdot0}zz$, $B^{0\cdot9}zz$, $Cz$ |
| | | Hill top | $A_2^{20\cdot9}zz$, $A_1^{7\cdot9}zz$, $B^{1\cdot0}zz$, $Cz$ |
| | South | Hill base | $A_2^{19\cdot8}zz$, $A_1^{7\cdot0}zz$, $B^{1\cdot5}zz$, $Cy$ |
| | | Lower slope | $A_2^{19\cdot0}zz$, $A_1^{8\cdot7}zz$, $B^{1\cdot6}zz$, $Cz$ |
| | | Upper slope | $A_2^{18\cdot5}zz$, $A_1^{10\cdot8}zz$, $B^{1\cdot6}zz$, $Cz$ |
| | | Hill top | $A_2^{16\cdot8}zz$, $A_1^{11\cdot7}zz$, $B^{1\cdot4}zz$, $Cz$ |
| *Quercus leucotrichophora* | North-east | Hill base | $A_2^{15\cdot6}zz$, $A_1^{6\cdot6}zz$, $B^{1\cdot0}x$, $Cz$ |
| | | Lower slope | $A_2^{16\cdot8}zz$, $A_1^{5\cdot9}z$, $B^{0\cdot9}z$, $Czz$ |
| | | Upper slope | $A_2^{16\cdot5}zz$, $A_1^{5\cdot8}zz$, $B^{0\cdot9}x$, $Czz$ |
| | | Hill top | $A_2^{15\cdot3}zz$, $A_1^{6\cdot9}z$, $B^{1\cdot2}z$, $Czz$ |
| | East | Hill base | $A_2^{18\cdot2}zz$, $A_1^{5\cdot1}zz$, $B^{1\cdot2}y$, $Czz$ |
| | | Lower slope | $A_2^{19\cdot1}zz$, $A_1^{7\cdot1}z$, $B^{1\cdot1}x$, $Czz$ |
| | | Upper slope | $A_2^{17\cdot2}zz$, $A_1^{8\cdot1}z$, $B^{0\cdot8}y$, $Czz$ |
| | | Hill top | $A_2^{22\cdot2}zz$, $A_1^{3\cdot9}z$, $B^{0\cdot9}x$, $Czz$ |
| | South-west | Hill base | $A_2^{0}$, $A_1^{6\cdot1}z$, $B^{1\cdot3}zz$, $Czz$ |
| | | Lower slope | $A_2^{0}$, $A_1^{6\cdot2}z$, $B^{1\cdot0}zz$, $Czz$ |
| | | Upper slope | $A_2^{0}$, $A_1^{5\cdot8}z$, $B^{1\cdot0}zz$, $Czz$ |
| | | Hill top | $A_2^{19\cdot9}zz$, $A_1^{4\cdot7}z$, $B^{0\cdot9}zz$, $Czz$ |
| *Quercus lanuginosa* | South | Hill base | $A_2^{19\cdot6}zz$, $A_1^{8\cdot9}xx$, $B^{1\cdot3}z$, $Cz$ |
| | | Lower slope | $A_2^{17\cdot5}zz$, $A_1^{4\cdot8}y$, $B^{1\cdot8}z$, $Czz$ |

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| | | Upper slope | $A_2^{17\cdot7}zz$, $A_1^{3,6}z$, $B^{1\cdot2}zz$, $Czz$ |
| | | Hill top | $A_2^{17\cdot4}zz$, $A_1^{4\cdot1}y$, $B^{1\cdot2}zz$, $Cz$ |
| *Quercus floribunda* | North | Hill base | $A_2^{17\cdot9}zz$, $A_1^{5\cdot5}y$, $B^{1\cdot5}x$, $Czz$ |
| | | Lower slope | $A_2^{0}$, $A_1^{4\cdot0}y$, $B^{1\cdot3}xx$, $Czz$ |
| | | Upper slope | $A_2^{18\cdot8}zz$, $A_1^{7\cdot7}y$, $B^{1\cdot6}z$, $Czz$ |
| | | Hill top | $A_2^{17\cdot4}zz$, $A_1^{5\cdot5}y$, $B^{1\cdot2}z$, $Czz$ |
| | North-east | Hill base | $A_2^{18\cdot2}zz$, $A_1^{3\cdot6}xx$, $B^{1\cdot6}xx$, $Czz$ |
| | | Lower slope | $A_2^{15,5}zz$, $A_1^{6\cdot1}z$, $B^{1\cdot0}y$, $Czz$ |
| | | Upper slope | $A_2^{16\cdot5}zz$, $A_1^{4\cdot4}x$, $B^{1\cdot4}x$, $Czz$ |
| | | Hill top | $A_2^{17\cdot7}zz$, $A_1^{4\cdot5}xx$, $B^{1\cdot3}y$, $Czz$ |
| | East | Hill base | $A_2^{0}$, $A_1^{4\cdot5}x$, $B^{1\cdot0}y$, $Czz$ |
| | | Lower slope | $A_2^{0}$, $A_1^{6\cdot3}x$, $B^{0\cdot9}y$, $Czz$ |
| | | Upper slope | $A_2^{15\cdot8}zz$, $A_1^{6/4}xx$, $B^{1\cdot3}x$, $Czz$ |
| | | Hill top | $A_2^{16,4}zz$, $A_1^{6\cdot1}y$, $B^{1\cdot1}xx$, $Czz$ |
| | South | Hill base | $A_2^{0}$, $A_1^{4\cdot4}xx$, $B^{1\cdot4}z$, $Czz$ |
| | | Lower slope | $A_2^{0}$, $A_1^{4\cdot7}xx$, $B^{1\cdot1}y$, $Czz$ |
| | | Upper slope | $A_2^{0}$, $A_1^{5\cdot2}x$, $B^{0\cdot9}y$, $Czz$ |
| | | Hill top | $A_2^{16\cdot0}zz$, $A_1^{4\cdot3}xx$, $B^{0\cdot8}xx$, $Czz$ |

The *B* stratum, being very sparse was not well defined on any topographic situation and aspect (table 3). *Lantana camara* was the only occupant of this stratum on most of the topographic situations and aspects. This stratum had the highest canopy index at the E hill base and the lowest on the SW hill top (table 4).

The herb layer in most stands indicated an average density and was composed of *Chrysopogon serrulatus-Desmodium polycarpum* community (table 3).

The trees in the $A_2$ stratum, on the NE hill top were highly resistant to swaying and bending by wind (SG = 52·2) (table 4). On the other hand, tree stability was lowest at the SW lower slope (SG = 71·6).

### 3.2. *Mixed forest*

The profile structures of the mixed forest stand are shown in figure 2. On S and E aspects, the hill base position indicated the highest average height of trees in $A_2$ stratum (table 3). On NW aspect, the maximum average height of the $A_2$ stratum was recorded on the lower slope, while at the hill base this stratum was altogether absent. Except for the S hill base where *Q. leucotrichophora* was the sole occupant of the $A_2$ stratum and the NW upper slope where *P. roxburghii* alone formed the $A_2$ stratum, this stratum was constituted by a mixture of species such as *Bauhinia retusa*, *Persea odoratissima*, *Celtis eriocarpa*, *Bauhinia variegata*, *Cedrela ciliata*, *Grewia subinaequalis*, etc. The canopy of this stratum was fairly irregular and discontinuous and trees were very sparse. The crowns

**Table 4.** Canopy depth, canopy index and quotient of slenderness for different forests in north-western part of Gola catchment.

| Forest type | Aspect | Position | Average canopy depth (m) | | Canopy index (%) | | | Quotient of slenderness (SG) |
|---|---|---|---|---|---|---|---|---|
| | | | strata | | strata | | | stratum |
| | | | $A_2$ | $A_1$ | $A_2+A_1$ | $B$ | Total | $A_2$ |
| 1 | 2 | 3 | 4 | | 5 | | | 6 |
| *inus oxburghii* | North-east | Hill base | 12·7 | 3·5 | 128·6 | 37·6 | 166·2 | 57·9 |
| | | Lower slope | 10·5 | 4·4 | 190·6 | 14·9 | 205·5 | 61·9 |
| | | Upper slope | 9·3 | 4·4 | 144·3 | 40·4 | 184·7 | 62·3 |
| | | Hill top | 14·3 | 3·0 | 115·7 | 18·4 | 134·1 | 52·2 |
| | East | Hill base | 13·1 | 2·0 | 98·8 | 40·8 | 139·6 | 56·3 |
| | | Lower slope | 12·0 | 3·1 | 110·6 | 10·6 | 121·2 | 60·9 |
| | | Upper slope | 14·3 | 5·9 | 68·2 | 13·7 | 81·9 | 62·6 |
| | | Hill top | 15·3 | 3·2 | 69·4 | 11·4 | 80·8 | 59·8 |
| | South-west | Hill base | 11·2 | 3·5 | 108·6 | 14·5 | 123·1 | 66·4 |
| | | Lower slope | 13·5 | 3·6 | 81·6 | 18·4 | 100·0 | 71·6 |
| | | Upper slope | 13·4 | 2·8 | 110·2 | 18·4 | 128·6 | 57·6 |
| | | Hill top | 14·4 | 3·1 | 155·3 | 9·4 | 164·7 | 65·0 |
| Iixed | East | Hill base | 13·6 | 6·0 | 116·9 | 256·0 | 372·9 | 39·5 |
| | | Lower slope | 9·8 | 6·2 | 91·8 | 151·4 | 243·2 | 50·3 |
| | | Upper slope | 11·6 | 5·1 | 126·7 | 397·3 | 524·0 | 46·6 |
| | | Hill top | 12·7 | 5·1 | 123·5 | 483·5 | 607·0 | 60·2 |
| | North-west | Hill base | 0 | 6·3 | 82·0 | 16·9 | 98·9 | 0 |
| | | Lower slope | 17·3 | 5·0 | 169·0 | 27·5 | 196·5 | 48·3 |
| | | Upper slope | 13·4 | 3·8 | 100·8 | 60·8 | 161·6 | 57·9 |
| | | Hill top | 13·3 | 4·8 | 111·4 | 154·9 | 266·3 | 57·9 |
| | South | Hill base | 13·6 | 5·1 | 147·8 | 512·2 | 660·0 | 45·4 |
| | | Lower slope | 12·2 | 5·8 | 169·8 | 450·2 | 620·0 | 40·6 |
| | | Upper slope | 12·0 | 6·7 | 128·2 | 491·8 | 620·0 | 41·6 |
| | | Hill top | 12·0 | 8·4 | 139·2 | 408·2 | 547·4 | 56·6 |
| *Quercus eucotricho-hora* | North-east | Hill base | 10·6 | 4·6 | 156·5 | 974·1 | 1130·6 | 45·6 |
| | | Lower slope | 11·2 | 3·7 | 206·3 | 394·1 | 600·8 | 47·9 |
| | | Upper slope | 11·0 | 4·0 | 191·8 | 760·0 | 951·8 | 44·4 |
| | | Hill top | 10·4 | 4·6 | 167·0 | 398·8 | 565·8 | 47·7 |
| | East | Hill base | 12·3 | 3·3 | 230·6 | 807·5 | 1038·1 | 41·3 |
| | | Lower slope | 13·1 | 4·6 | 335·7 | 896·0 | 1231·7 | 47·6 |
| | | Upper slope | 11·3 | 5·3 | 328·2 | 630·6 | 958·8 | 44·8 |
| | | Hill top | 15·0 | 2·6 | 168·6 | 1020·4 | 1189·0 | 42·5 |
| | South-west | Hill base | 0 | 4·2 | 131·4 | 165·9 | 297·3 | 0 |
| | | Lower slope | 0 | 4·2 | 161·2 | 151·4 | 312·6 | 0 |
| | | Upper slope | 0 | 4·2 | 144·7 | 208·6 | 353·3 | 0 |
| | | Hill top | 13·6 | 3·2 | 142·0 | 116·5 | 258·5 | 49·6 |

**Table 4.** (*Contd.*)

| 1 | 2 | 3 | 4 | | 5 | | | 6 |
|---|---|---|---|---|---|---|---|---|
| *Quercus lanuginosa* | South | Hill base | 14·3 | 2·6 | 333·7 | 418·8 | 752·5 | 30·2 |
| | | Lower slope | 12·7 | 3·4 | 218·8 | 368·6 | 587·4 | 30·5 |
| | | Upper slope | 12·7 | 2·5 | 209·0 | 255·3 | 464·3 | 30·8 |
| | | Hill top | 17·8 | 2·8 | 182·4 | 218·8 | 401·2 | 35·7 |
| *Quercus floribunda* | North | Hill base | 14·1 | 4·1 | 277·3 | 777·6 | 1054·9 | 52·8 |
| | | Lower slope | 0 | 3·0 | 160·8 | 344·7 | 1505·5 | 0 |
| | | Upper slope | 14·6 | 6·0 | 350·6 | 360·0 | 710·6 | 58·0 |
| | | Hill top | 13·5 | 4·3 | 214·9 | 316·0 | 530·9 | 69·3 |
| | North-east | Hill base | 15·0 | 2·8 | 237·3 | 1165·5 | 1402·8 | 42·3 |
| | | Lower slope | 13·0 | 4·6 | 176·0 | 673·7 | 849·7 | 56·0 |
| | | Upper slope | 13·2 | 3·4 | 260·4 | 868·6 | 1129·0 | 56·1 |
| | | Hill top | 14·0 | 3·4 | 255·7 | 539·6 | 795·3 | 56·2 |
| | East | Hill base | 0 | 3·9 | 205·5 | 608·6 | 814·1 | 0 |
| | | Lower slope | 0 | 4·9 | 241·6 | 556·1 | 797·7 | 0 |
| | | Upper slope | 13·0 | 4·9 | 320·8 | 833·3 | 1154·1 | 58·7 |
| | | Hill top | 13·6 | 4·7 | 275·3 | 814·1 | 1089·4 | 59·4 |
| | South | Hill base | 0 | 3·6 | 280·0 | 402·7 | 682·7 | 0 |
| | | Lower slope | 0 | 3·8 | 247·5 | 600·0 | 847·5 | 0 |
| | | Upper slope | 0 | 4·1 | 224·7 | 896·5 | 1121·2 | 0 |
| | | Hill top | 12·8 | 3·4 | 256·0 | 1574·0 | 1830·0 | 53·0 |

of trees were usually deeper than wide. On S and E aspects the maximum average canopy depth occurred at the hill base and on the NW aspect at the lower slope (table 4).

The trees in the $A_1$ stratum were also very sparse (table 3). However, the canopy was comparatively better developed at the NW hill base than on other topographic situations and aspects. Almost all trees in this stratum were young individuals of species which reach the $A_2$ stratum upon maturity. Like the $A_2$ stratum, the crowns tended to be more deep than wide on all topographic situations and aspects.

On S and NW aspects, the canopy index ($A_2$ + $A_1$ strata) was highest at the lower slope, while on E aspect it was highest on the upper slope (table 4).

With the exception of NW aspect, the *B* stratum was well developed with almost a continuous canopy. This stratum was dominated by *L. camara*, which despite a low density developed a spreading, close canopy, except for NW hill top where it was dominated by *Aechmanthera tomentosa*. The canopy index of the shrub layer was maximum at the S hill base and minimum at the NW hill base (table 4).

The herb layer on most of the positions of NW and S aspect was sparse, while on E aspect it indicated an average density (table 3). This layer was composed of *Dicliptera bupleuroides—Oplismenus burmanii* community.

The trees of the $A_2$ stratum were more slender on the S and E hill tops (SG = 56·6 and 60·2, respectively), and on the NW upper slope and hill top

(SG = 57·9) (table 4). These results indicate lower stability of trees at these positions, hence they are more susceptible to wind damage.

### 3.3. *Quercus leucotrichophora forest*

The canopy in the $A_2$ stratum was almost continuous at the E hill base, lower slope and upper slope and at the NE lower slope (figure 3). At the SW hill base and lower and upper slopes, the $A_2$ stratum was absent. Where present, it was composed of only *Q. leucotrichophora* with the exception of the E upper slope, where one tree of *P. roxburghii* (per 200 m$^2$) occurred with *Q. leucotrichophora*. The crowns of the trees were more deep than wide in this stratum. The canopy depth was maximum on the E hill top and minimum on the NE hill top (table 4). Stratum $A_1$ was dominated by *Q. leucotrichophora* on all positions of E and NE aspect (figure 3). On the other hand this stratum on all the four situations of SW was dominated either by *Myrica esculenta, Rhododendron arboreum* or by *Lyonia ovalifolia.* The E hill top had one tree of *P. roxburghii* per 200 m$^2$. The canopy in this stratum was more or less continuous on almost all positions and aspects. Most of the gaps in the $A_2$ stratum were closed by trees in the stratum $A_1$, thus, strata $A_2$ and $A_1$ together formed a good cover on all positions and aspects. The crowns in this stratum were more deep than wide.

The trees on all positions and aspects in the $A_2$ stratum were very sparse, while on most of the positions and aspects in $A_1$ stratum they were sparse (table 3).

The shrub stratum (*B*) was well defined on all positions and aspects (figure 3). With the exception of NE and E upper slope, all other positions on different aspects were dominated by the shrub *Myrsine africana*. On the E and NE upper slope *Boenninghausenia albiflora* was the dominant shrub. The shrub layer on all positions of SW was very sparse, while on the NE lower slope and hill top the same was sparse (table 3). This layer on the E lower slope and hill top and on the NE hill base and upper slope was dense. The canopy index of this stratum was maximum on the E hill top (table 4).

On most topographic situations and aspects, the plants in the herb layer were very sparse (table 3). This layer was comprised of *Arundinella nepalensis—Carex nubigena* community.

Tree stability was lowest on the SW hill top (SG = 49.6) where only two trees of *Q. leucotrichophora* per 200 m$^2$ occurred (table 4).

### 3.4. *Quercus lanuginosa forest*

At the S hill base and upper slope, the canopy of the $A_2$ stratum was fairly dense and at places the crowns touched each other (figure 4). On the other hand at the lower slope and hill top positions, canopies were broken ; only two trees of *Q. lanuginosa* were present per 200 m$^2$ on each of these positions. This stratum consisted of only *Q. lanuginosa* trees on all positions. The crowns were deeper than wide. The average canopy depth was maximum at the hill base (table 4).

The canopy of the $A_1$ stratum was more dense as compared to that of the $A_2$ stratum on all positions (table 3). Though, this stratum was dominated by *Q. lanuginosa*, other species such as *R. arboreum*, *M. esculenta*, *L. ovalifolia*,

*Cornus oblonga*, etc., were also present. Like $A_2$ stratum, the crowns of this stratum were also deeper than wide.

The canopy index of $A_2 + A_1$ strata was maximum at the hill base and minimum on the hill top (table 4).

The plants in the stratum $B$ were very sparse on the upper slope and hill top positions, and sparse at the hill base and lower slope positions (table 3). The dominant shrub on all positions was *M. africana*. The shrub canopy index was maximum at the hill base and minimum on the hill top (table 4).

The herb layer on the hill base and hill top positions was sparse and on the lower and upper slopes very sparse (table 3). This layer was dominated by the *Apluda mutica—Themeda anathera* community.

The quotient of slenderness for the $A_2$ stratum trees was highest on the hill top and lowest at the hill base (table 4).

### 3.5. *Quercus floribunda forest*

The profile diagrams for the *Quercus floribunda* forest stands are illustrated in figure 5. The $A_2$ stratum was well defined only at the N hill base and upper slope. The trees of this stratum on all positions and aspects belonged to *Q. floribunda*.

The crowns of the $A_2$ stratum tended to be deeper than wide. The average canopy depth was the highest at the NE hill base and the lowest on the S hill top (table 4).

The stratum $A_1$ had a remarkably dense canopy, the individual crowns usually touched each other. A majority of trees in this stratum was represented by young individuals of *Q. floribunda*. Other species in this stratum were : *R. arboreum*, *L. ovalifolia*, *C. oblonga*, *Pyrus pashia*, *Q. leucotrichophora*, etc. The crowns were deeper than wide.

The trees in the $A_2$ stratum on all positions and aspects were very sparse, while in $A_1$ stratum the trees on most positions and aspects were either dense or very dense (table 3). The canopy index of the upper two strata ($A_2 + A_1$) was the highest on the N upper slope and the lowest at the N lower slope (table 4).

Below the two storeys of trees, the stratum $B$ consisting chiefly of shrubs was well defined in this forest. The plants were very dense at the NE hill base, N lower slope and on the E and S hill top (table 3). On all S positions, at the E hill base and at the N lower slope, *M. africana* was the dominant shrub while *M. semiserrata* played the vicariant role on the N hill top. On rest of the positions and aspects *Arundinaria falcata* showed its dominance. The canopy index of this stratum was on the whole higher than that of the corresponding stratum in other forests of the study area (table 4).

The herb layer on all positions and aspects was very sparse (table 3) and was composed of *Muehlenbergia duthieana-Helictotrichon asperum* community.

The tree stability in $A_2$ stratum was the highest at the NE hill base (SG = 42·3) and the lowest on the N hill top (SG = 69·3) (table 4).

## 4. Discussion

The structure of the forests varied from stand to stand. Such inter-tand variations in tree stature, crown geometry and canopy architecture are common (Anderson 1961 ; Ashton 1964 ; Brunig 1970, 1976 ; Brunig and Heuveldop 1976).

In these forests there was a total of four strata ; two upper strata represented by trees, the third stratum represented mainly by shrubs, and the fourth by herbs. The maximum average tree height (across positions and aspects) in the $A_2$ stratum was recorded for *P. roxburghii* forest (22·4 m) and the minimum for *Q. floribunda* forest (17.1 m). *Q. leucotrichophora* and *Q. lanuginosa* forests had almost equal average tree height (about 18·0 m) in this stratum. Further, with increasing altitude the tree height of the $A_2$ stratum decreased ($Y = 27{\cdot}3008 - 0{\cdot}0047\,X$ ; $r = -0{\cdot}6799$, $P < 0{\cdot}001$ ; where $Y$ = tree height in m and $X$ = altitude in m). Brown (1919) suggested that the decrease in plant height with increasing altitude is due to the combined effects of decreased temperature and decreased illumination (due to increased cloudiness). Richards (1952) pointed out that this dwarfing of the vegetation may be partly due to exposure to strong wind.

In all forests, the crowns of the $A_2$ stratum were deeper than wide. On an average, across positions and aspects, about 80% length of the trees in *Q. floribunda*, 72% in *Q. lanuginosa*, 68% in *Q. leucotrichophora*, 67% in mixed and about 57% in *P. roxburghii* forests was covered by the canopy. Contrary to the tree height, the proportion of the tree devoted to canopy in the $A_2$ stratum increased with an increase in the altitude ($Y = 36{\cdot}6628 + 0{\cdot}0185\,X$ ; $r = 0.8395$, $P < 0{\cdot}001$ ; where $Y$ = percent length of the tree devoted to canopy and $X$ = altitude in m). Thus the tree compensated for decrease in height by allocating more of its length to development of photosynthetic canopy.

The canopies in the $A_1$ stratum were comparatively denser than those in $A_2$ stratum in all forests except those of *P. roxburghii.* The plants on most positions and aspects in $A_1$ stratum were very sparse in *P. roxburghii* and mixed forests, sparse in *Q. leucotrichophora* forest, average in *Q. lanuginosa* forest and dense to very dense in *Q. floribunda* forest. Most of the individuals in this stratum belonged to the species which constituted the $A_2$ stratum. However, some other trees such as, *R. arboreum, L. ovalifolia, C. oblonga, S. insigne, M. esculenta, C. laurifolius. Rhamnus triqueter,* etc., were confined only to this stratum.

The average tree height (across positions and aspects) $A_1$ stratum was the highest in the mixed forest (8·9 m) and the lowest in *Q. lanuginosa* forest (4·1 m). In this stratum also, almost all trees in all forest types had their crowns deeper than wide. As the altitude increased, the percent of tree height covered by canopy also increased ($Y = 45{\cdot}2850 + 0{\cdot}0134\,X$ ; $r = 0{\cdot}806$, $P < 0{\cdot}001$ ; where $Y$ = percent length of the tree devoted to canopy and $X$ = altitude in m) ; maximum (78·8%) being in the *Q. floribunda* forest and minimum (62·2%) in the *P. roxburghii* forest. The proportion of the tree devoted to canopy depth was greater in the $A_2$ stratum compared to the $A_1$ stratum.

There was usually no clear vertical discontinuity between the canopies of $A_2$ and $A_1$ strata because of the occurrence of a variable number of layers in each stratum. The exceptions were the E hill base and hill top and SW upper slope of

*P. roxburghii* forest, where a clear vertical discontinuity between the $A_2$ and $A_1$ strata occurred.

The shrub density on most positions and aspects in *Q. floribunda* forest was average, while it was very sparse in *P. roxburghii* and mixed forests. In *Q. leucotrichophora* forest, SW aspect exhibited very sparse density, while on most positions of NE and E, the plants were dense. In general, the shrub layer (*B* stratum- in three oak forests was comparatively dense and the crowns of the shrubs over) lapped each other. In the *P. roxburghii* forest, this stratum was not well developed.

The canopy index, a crude and relative measure of canopy cover, of both tree and shrub layers was maximum for *Q. floribunda* forest and minimum for *P. roxburghii* forest. On categorizing the aspects into cooler (N, NE, E, and NW) and warmer (S and SW), the cooler aspects developed a greater canopy index for tree ($\bar{X} = 183 \cdot 7\%$) and shrub ($\bar{X} = 431 \cdot 5\%$) layers as compared to the warmer aspects (canopy index for tree layer, $\bar{X} = 170 \cdot 4\%$ and for shrub layer, $\bar{X} = 318 \cdot 9\%$).

About 50% of the stands each in *P. roxburghii* and mixed forests had, respectively, an average and sparse plant density in the herb layer, while in *Q. leucotrichophora* and *Q. floribunda* forests the herbaceous plants were very sparse. The plants in this layer were sparse to very sparse in *Q. lanuginosa* forest. Poor development of herbaceous plants under the oak forests may be because of a tendency of inverse relationship between the canopy cover (of tree + shrub layers) and the development of the herb layer (Richards 1952; Smith 1956 ; Zobel *et al* 1976 ; Killingbeck and Wali 1978). Naturally, relatively open overhead canopies would induce the development of the herb layer.

The data on the quotient of slenderness (SG) indicates the stability of trees ; the lower the SG value the higher is the stability (Brunig and Heuveldop 1976). In the present area the trees in *Q. lanuginosa* forest were more stable, while in the *P. roxburghii* forest trees were specially susceptible to wind effect. Since *P. roxburghii* is a rich source of resin, trees are tapped heavily in this region (Saxena 1977). As the tapping reduces the diameter, the resistence of the heavily tapped trees to wind is greatly reduced (Assmann 1970). Because of the characteristic low wind stability the tapping of this species for resin should be very cautious and perhaps should be avoided in those forests which are exposed to greater wind velocities or having poor stocking density.

A majority of trees in the present forests were more wind resistant as compared to those in the humid tropical forests (Brunig and Heuveldop 1976). It may be pointed out that a mountainous country, due to varied nature of slopes, is characterized by a higher degree of wind turbulence compared to a non-mountainous region, and therefore, lower values of quotient of slenderness in the present trees may be an adaptational feature.

In general the warmer aspects had more stable trees, *i.e.*, lower SG value ($\bar{X} = 50 \cdot 5$), while the cooler aspects showed lower tree stability, *i.e.*, high SG value ($\bar{X} = 55 \cdot 1$). In comparison to cooler aspects, the warmer aspects experience greater wind velocities (Smith 1974). The low SG values of trees, thus growing on warmer aspects may be an adaptational feature.

The canopies in the different strata also influence the soil condition of a site. Packer 1951) observed that overland flow and erosion decrease with increase in cover. A dense cover of vegetation is the most powerful weapon for reducing erosion. According to Lull (1964), the drops that drip from the leaves are generally larger than the rain drops and their terminal velocity is reached by the time they have fallen 7·5 m. Trimble and Weitzman (1954) concluded that a high tree canopy has a limited value in reducing the erosion potential of rainfall intensity, but a forest with a canopy that reaches close to the ground can effectively reduce the erosion potential. Thus a site with trees confined only to the $A_2$ stratum and having their canopies concentrated on the top will be relatively less protective for the soil. But when the trees in the $A_2$ stratum are supported by deep and dense canopies in $A_1$ or $B$ strata, the vegetation becomes more protective for the soil. A forest with a multilayered canopy with a high canopy index and a well developed forest floor will, thus, have a greater protective value as compared to a forest which has fewer layers and a lower canopy index (Kittredge 1948).

In this region, the rainfall is concentrated in a short monsoon period (June to September). This is preceded by a long dry period (winter and summer seasons) during which the herbaceous cover dries up and shatters. Additionally during this dry period, grazing, herbage removal for animal feed and ground fires, particularly in pine forests further decimate the herb-litter cover, leaving often a semibare floor. At the culmination of this dry period, the monsoon breaks with a high rainfall intensity. Under such situations, if the tree canopy is thin or high or monolayered with little or no shrubby undergrowth, soil erosion and runoff are remarkably accelerated. Keeping the above in mind the present forest could be graded in a decreasing order of potential for soil protection : *Q. floribunda* > *Q. leucotrichophora* > *Q. lanuginosa* > mixed > *P. roxburghii*. In sensitive catchment areas maintained for soil and water conservation, *Q. floribunda* and *Q. leucotrichophora* forests should, therefore, be encouraged.

## Acknowledgement

The financial support from Indian Space Research Organisation, Bangalore is gratefully acknowledged. Dr Uma Pandey and Mr O P Chaturvedi assisted in the preparation of figures.

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(SG = 57·9) (table 4). These results indicate lower stability of trees at these positions, hence they are more susceptible to wind damage.

### 3.3. *Quercus leucotrichophora forest*

The canopy in the $A_2$ stratum was almost continuous at the E hill base, lower slope and upper slope and at the NE lower slope (figure 3). At the SW hill base and lower and upper slopes, the $A_2$ stratum was absent. Where present, it was composed of only *Q. leucotrichophora* with the exception of the E upper slope, where one tree of *P. roxburghii* (per 200 m$^2$) occurred with *Q. leucotrichophora*. The crowns of the trees were more deep than wide in this stratum. The canopy depth was maximum on the E hill top and minimum on the NE hill top (table 4). Stratum $A_1$ was dominated by *Q. leucotrichophora* on all positions of E and NE aspect (figure 3). On the other hand this stratum on all the four situations of SW was dominated either by *Myrica esculenta, Rhododendron arboreum* or by *Lyonia ovalifolia*. The E hill top had one tree of *P. roxburghii* per 200 m$^2$. The canopy in this stratum was more or less continuous on almost all positions and aspects. Most of the gaps in the $A_2$ stratum were closed by trees in the stratum $A_1$, thus, strata $A_2$ and $A_1$ together formed a good cover on all positions and aspects. The crowns in this stratum were more deep than wide.

The trees on all positions and aspects in the $A_2$ stratum were very sparse, while on most of the positions and aspects in $A_1$ stratum they were sparse (table 3).

The shrub stratum (*B*) was well defined on all positions and aspects (figure 3). With the exception of NE and E upper slope, all other positions on different aspects were dominated by the shrub *Myrsine africana*. On the E and NE upper slope *Boenninghausenia albiflora* was the dominant shrub. The shrub layer on all positions of SW was very sparse, while on the NE lower slope and hill top the same was sparse (table 3). This layer on the E lower slope and hill top and on the NE hill base and upper slope was dense. The canopy index of this stratum was maximum on the E hill top (table 4).

On most topographic situations and aspects, the plants in the herb layer were very sparse (table 3). This layer was comprised of *Arundinella nepalensis—Carex nubigena* community.

Tree stability was lowest on the SW hill top (SG = 49.6) where only two trees of *Q. leucotrichophora* per 200 m$^2$ occurred (table 4).

### 3.4. *Quercus lanuginosa forest*

At the S hill base and upper slope, the canopy of the $A_2$ stratum was fairly dense and at places the crowns touched each other (figure 4). On the other hand at the lower slope and hill top positions, canopies were broken ; only two trees of *Q. lanuginosa* were present per 200 m$^2$ on each of these positions. This stratum consisted of only *Q. lanuginosa* trees on all positions. The crowns were deeper than wide. The average canopy depth was maximum at the hill base (table 4).

The canopy of the $A_1$ stratum was more dense as compared to that of the $A_2$ stratum on all positions (table 3). Though, this stratum was dominated by *Q. lanuginosa*, other species such as *R. arboreum*, *M. esculenta*, *L. ovalifolia*,

*Cornus oblonga*, etc., were also present. Like $A_2$ stratum, the crowns of this stratum were also deeper than wide.

The canopy index of $A_2 + A_1$ strata was maximum at the hill base and minimum on the hill top (table 4).

The plants in the stratum *B* were very sparse on the upper slope and hill top positions, and sparse at the hill base and lower slope positions (table 3). The dominant shrub on all positions was *M. africana*. The shrub canopy index was maximum at the hill base and minimum on the hill top (table 4).

The herb layer on the hill base and hill top positions was sparse and on the lower and upper slopes very sparse (table 3). This layer was dominated by the *Apluda mutica—Themeda anathera* community.

The quotient of slenderness for the $A_2$ stratum trees was highest on the hill top and lowest at the hill base (table 4).

### 3.5. *Quercus floribunda forest*

The profile diagrams for the *Quercus floribunda* forest stands are illustrated in figure 5. The $A_2$ stratum was well defined only at the N hill base and upper slope. The trees of this stratum on all positions and aspects belonged to *Q. floribunda*.

The crowns of the $A_2$ stratum tended to be deeper than wide. The average canopy depth was the highest at the NE hill base and the lowest on the S hill top (table 4).

The stratum $A_1$ had a remarkably dense canopy, the individual crowns usually touched each other. A majority of trees in this stratum was represented by young individuals of *Q. floribunda*. Other species in this stratum were : *R. arboreum, L. ovalifolia, C. oblonga, Pyrus pashia, Q. leucotrichophora*, etc. The crowns were deeper than wide.

The trees in the $A_2$ stratum on all positions and aspects were very sparse, while in $A_1$ stratum the trees on most positions and aspects were either dense or very dense (table 3). The canopy index of the upper two strata ($A_2 + A_1$) was the highest on the N upper slope and the lowest at the N lower slope (table 4).

Below the two storeys of trees, the stratum *B* consisting chiefly of shrubs was well defined in this forest. The plants were very dense at the NE hill base, N lower slope and on the E and S hill top (table 3). On all S positions, at the E hill base and at the N lower slope, *M. africana* was the dominant shrub while *M. semiserrata* played the vicariant role on the N hill top. On rest of the positions and aspects *Arundinaria falcata* showed its dominance. The canopy index of this stratum was on the whole higher than that of the corresponding stratum in other forests of the study area (table 4).

The herb layer on all positions and aspects was very sparse (table 3) and was composed of *Muehlenbergia duthieana-Helictotrichon asperum* community.

The tree stability in $A_2$ stratum was the highest at the NE hill base (SG = 42·3) and the lowest on the N hill top (SG = 69·3) (table 4).

## 4. Discussion

The structure of the forests varied from stand to stand. Such inter-stand variations in tree stature, crown geometry and canopy architecture are common (Anderson 1961 ; Ashton 1964 ; Brunig 1970, 1976 ; Brunig and Heuveldop 1976).

In these forests there was a total of four strata ; two upper strata represented by trees, the third stratum represented mainly by shrubs, and the fourth by herbs. The maximum average tree height (across positions and aspects) in the $A_2$ stratum was recorded for *P. roxburghii* forest (22·4 m) and the minimum for *Q. floribunda* forest (17.1 m). *Q. leucotrichophora* and *Q. lanuginosa* forests had almost equal average tree height (about 18·0 m) in this stratum. Further, with increasing altitude the tree height of the $A_2$ stratum decreased ($Y = 27{\cdot}3008 - 0{\cdot}0047\,X$ ; $r = -0{\cdot}6799$, $P < 0{\cdot}001$ ; where $Y$ = tree height in m and $X$ = altitude in m). Brown (1919) suggested that the decrease in plant height with increasing altitude is due to the combined effects of decreased temperature and decreased illumination (due to increased cloudiness). Richards (1952) pointed out that this dwarfing of the vegetation may be partly due to exposure to strong wind.

In all forests, the crowns of the $A_2$ stratum were deeper than wide. On an average, across positions and aspects, about 80% length of the trees in *Q. floribunda*, 72% in *Q. lanuginosa*, 68% in *Q. leucotrichophora*, 67% in mixed and about 57% in *P. roxburghii* forests was covered by the canopy. Contrary to the tree height, the proportion of the tree devoted to canopy in the $A_2$ stratum increased with an increase in the altitude ($Y = 36{\cdot}6628 + 0{\cdot}0185\,X$ ; $r = 0.8395$, $P < 0{\cdot}001$ ; where $Y$ = percent length of the tree devoted to canopy and $X$ = altitude in m). Thus the tree compensated for decrease in height by allocating more of its length to development of photosynthetic canopy.

The canopies in the $A_1$ stratum were comparatively denser than those in $A_2$ stratum in all forests except those of *P. roxburghii*. The plants on most positions and aspects in $A_1$ stratum were very sparse in *P. roxburghii* and mixed forests, sparse in *Q. leucotrichophora* forest, average in *Q. lanuginosa* forest and dense to very dense in *Q. floribunda* forest. Most of the individuals in this stratum belonged to the species which constituted the $A_2$ stratum. However, some other trees such as, *R. arboreum, L. ovalifolia, C. oblonga, S. insigne, M. esculenta, C. laurifolius. Rhamnus triqueter*, etc., were confined only to this stratum.

The average tree height (across positions and aspects) $A_1$ stratum was the highest in the mixed forest (8·9 m) and the lowest in *Q. lanuginosa* forest (4·1 m). In this stratum also, almost all trees in all forest types had their crowns deeper than wide. As the altitude increased, the percent of tree height covered by canopy also increased ($Y = 45{\cdot}2850 + 0{\cdot}0134\,X$ ; $r = 0{\cdot}806$, $P < 0{\cdot}001$ ; where $Y$ = percent length of the tree devoted to canopy and $X$ = altitude in m) ; maximum (78·8%) being in the *Q. floribunda* forest and minimum (62·2%) in the *P. roxburghii* forest. The proportion of the tree devoted to canopy depth was greater in the $A_2$ stratum compared to the $A_1$ stratum.

There was usually no clear vertical discontinuity between the canopies of $A_2$ and $A_1$ strata because of the occurrence of a variable number of layers in each stratum. The exceptions were the E hill base and hill top and SW upper slope of

*P. roxburghii* forest, where a clear vertical discontinuity between the $A_2$ and $A_1$ strata occurred.

The shrub density on most positions and aspects in *Q. floribunda* forest was average, while it was very sparse in *P. roxburghii* and mixed forests. In *Q. leucotrichophora* forest, SW aspect exhibited very sparse density, while on most positions of NE and E, the plants were dense. In general, the shrub layer (*B* stratum- in three oak forests was comparatively dense and the crowns of the shrubs over) lapped each other. In the *P. roxburghii* forest, this stratum was not well developed.

The canopy index, a crude and relative measure of canopy cover, of both tree and shrub layers was maximum for *Q. floribunda* forest and minimum for *P. roxburghii* forest. On categorizing the aspects into cooler (N, NE, E, and NW) and warmer (S and SW), the cooler aspects developed a greater canopy index for tree ($\bar{X}$ = 183·7%) and shrub ($\bar{X}$ = 431·5%) layers as compared to the warmer aspects (canopy index for tree layer, $\bar{X}$ = 170·4% and for shrub layer, $\bar{X}$ = 318·9%).

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# Contributions to our knowledge of Indian algae-III. Euglenineae-Part-I. The genus *Euglena* Ehrenberg*

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**Abstract.** An account of 29 taxa comprising 24 species and five varieties of the genus *Euglena* collected from various localities in north-east, central and south India during 1937–76 is given. Of these, two, *viz.*, *E. vaginicola* and *E. pseudo-ehrenbergii* have been considered as new species, one, *viz.*, *E. viridis* var. *maxima* a new variety and one, *viz.*, *E. tuba* var. *pseudotuba* f. *minima* a new form of a new combination variety. Four species, *viz.*, *E. vagans*, *E. helicoideus*, *E. granulata* and *E. hemichromata* and three varieties, *viz.*, *E. tripteris* var. *klebsii*, *E. oxyuris* var. *playfairii* and *E. caudata* var. *minor* appear to be new records for the Indian region.

By studying the taxa from different ecological habitats it is shown that there is considerable variation within species in *E. acus* and *E. tuba* and to a limited extent in *E. oxyuris*. Since *E. ehrenbergii* as known at present is a composite species, *E. srinagari* has been separated from it, following Huber–Pestalozzi in this respect. *E. tuba* Carter which had been incompletely known so far and considered by most authors as doubtful, was studied in detail and shown to be a well defined species needing an emended description. *E. tuba* Johnson, which shows some essential differences from Carter's species, is treated as a variety of *E. tuba*, *viz.*, var. *pseudotuba* with an Indian form which is new. There is also a possibility of polymorphism in *E. tuba*.

Meteorological and water conditions under which some of the species dominated are given. It is also shown that some species are characteristic of particular habitats.

The need to give all relevant details including proper illustrations while describing the taxa of this difficult genus is stressed. The author has made an attempt to fill the gaps in existing descriptions of the Indian taxa given in this paper to help in proper identifications. A key to these taxa is also given.

A list of 29 other species reported from India by other workers is given with references and localities.

**Keywords.** Chromatophores; flagellum; haematochrome; paramylum; gullet; moat.

## 1. Introduction

Though there have been records of species of *Euglena* from the Indian region comprising India, Pakistan, Afghanistan, Nepal, Bangladesh, Burma and

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Sri (Lanka) from 1856 onwards, our information on the genus from this region is still quite meagre. The earliest record of *Euglena* from the region appears to be that of Carter (1856) who described *E. agilis* from Bombay. This species is better known as *E. pisciformis* Klebs since Carter's description was considered inadequate. He also recorded four other species, *viz. E. acus, E. spirogyra E. deses* and *E. viridis* during the same year along with a fifth one *E. texta* (Duj.) Huebner under the name of *Crumenula texta* Dujardin. In 1858 Carter again referred to the occurrence of *E. viridis* and *E. acus* in his collections from Bombay. The same author's (1859) *E. fusiformis* and *E. zonalis* are actually *Lepocinclis fusiformis* (Carter) Lemm. and *L. ovum* (Ehr). Lemm. respectively.

In 1869, Carter described an interesting species, *E. tuba*, from Bombay. Since his description was inadequate and the connection between the free living organism and the characteristic encysted individual was not clearly established, subsequent authors (see Kent 1881 ; Gojdics 1953 ; Pringsheim 1956) expressed doubts about its real identity, the latter two authors considering it as probably a *E. sanguinea.* Hansgirg (1908) recorded *E. agilis* again from Igatpuri, Bombay. Kashyap (1908) reported a bloom of *Euglena* resembling *E. tuba* from Lahore, to which Walton (1915) gave the name of *E. orientalis.* Since Kashyap's description was incomplete and without accompanying figures, the real identity of his species is unknown.

Bhatia (1930) described four species of *Euglena* and a fifth under the name of *Amblyophis srinagari* sp. nov., from Kashmir. Banerji (1936) reported *E. viridis* from Lower Bengal. Skvortzov (1937) recorded four species from Rangoon. Philipose (1940) gave accounts of eleven species from Museum Pond, Madras, and Gonzalves and Joshi (1943, 1943a, 1946) listed and illustrated five species from Bombay. Skuja (1949) reported seven species and two varieties from Burma. Biswas (1949) stated that *E. viridis* and *E. sanguinea* are quite common in Indian and Burmese ponds. Suxena (1955) described four and Zafar (1959) recorded three species from Hyderabad, Philipose (1960) enumerated a number of algae including species of *Euglena* occurring commonly in Indian inland fishery waters while Singh (1960) recorded two species from Uttar Pradesh.

Iyengar (1962) described a new species, *viz. E. pringsheimii* in which there are " inner " pyrenoids. He also emended the description of *E. oblonga* Schmitz after detailed study of material from Madras.

Kamat (1961–62, 1963, 1964, 1967, 1968, 1968a, 1974 and 1975) and Kamat and Frietas (1976) recorded twenty-seven species (including a new one) and a number of varieties from Maharashtra, Gujarat, Rajasthan and Himachal Pradesh, while Naidu (1962, 1966) gave accounts of nineteen taxa covering sixteen species from Andhra Pradesh. Hortobágyi (1969) described seven species and two varieties from three reservoirs on the banks of River Jamuna. Suxena *et al* (1973) recorded three species from Kerala. Dodkundi *et al* (1973) observed six species in a pond at Dharwar. Pandhol and Grover (1976) refer to one species from Ludhiana. Hosmani (1977) and Hosmani and Bharati (1975) reported blooms of *E. sanguinea* and *E. elastica* respectively from Dharwar. Venkateswarlu (1976) observed four species in River Moosi, Hyderabad.

With the overlapping in various records, the total number of species (excluding varieties and synonyms) so far recorded from the Indian region come to about forty-seven. In spite of this sizeable number, including descriptions of some, it

cannot be considered that due attention has been given to the genus from the Indian region.

According to Pringsheim (1956) quite a large number of species of *Euglena* have been described inadequately or illustrated unsatisfactorily with the result that these species cannot be recognised or they have to be treated as synonyms of well-known species. Even records attributed to well-known species cannot always be considered accurate when unaccompanied by important details. This, he states, is particularly applicable to groups other than "Rigidae" and "Lentiferae" (see under Systematic Account) especially species of the "Sanguinea" group. Records from the Indian subcontinent are no exception to this, quite a number of records being limited to lists or with incomplete descriptions and figures. This is particularly true of most taxonomic records other than those of Bhatia (1930); Skvortzov (1937); Skuja (1949); Suxena (1955); Iyengar (1962) and Hortobágyi (1969). It has, therefore, been considered necessary to give full details of the taxa of this difficult genus encountered by the author in a wide variety of habitats in India, inlcuding considerable variation within species in several instances. Species of *Lepocinclis*, *Phacus*, *Trachelomonas* and *Strombomonas* from these habitats will be dealt with in later communications.

In this paper twenty-nine taxa belonging to twenty-four species, including those from Museum Pond, Madras (Philipose 1940) are given making the total number of species for the subcontinent to about fifty-three. These were collected during year round ecological studies of some water bodies or during general surveys of inland fishery waters of north-east, central and south India during 1937-76.

For a better understanding of the taxa discussed here, a key to the species and varieties is given at the outset, following the classification into main groups proposed by Pringsheim (1956). The class name Euglenineae as given by Fritsch (1935), is adopted in preference to Euglenophyceae and Euglenoidina used by some authors.

## 2. Localities and dates of collection

Plankton collections were made from a number of States covering north-east and south India and a few from central India. Most of the collections were from general surveys conducted by the staff of the Central Inland Fisheries Research Institute, Barrackpore and Cuttack, including the author, and the rest from the year round observations at weekly or fortnightly intervals by the author. The water bodies involved were mostly fish ponds (nurseries of about 0·04–0·15 ha and 1–1½ metres deep) and rearing and stocking tanks (about 0·2 to 0·5 ha or more and 1½ to 3 meters deep), some multi-purpose public tanks and small reservoirs, shallow fishery bunds (used for fish breeding) a few swamps and moats and two rivers (one of them in a polluted area) under lacustrine conditions. The nursery ponds were invariably kept free of macro-flora while the rest had frequently macro-vegetation at their margins.

2.1a. *Assam* : Sibsagar : (1) Sibsagar Jamuna (8–6–51) ; Joyasagar :—Fish farm (2) N.P. and (3) S.T. (8–6–51) ; (4) S.T. (6–4–55 and 31–5–55) ; (5) N.P. 2 and (6) N.P. 14 and 18 (1/9–6–55) ; (7) N.P. 30 (15–11–65) ; (8) N.P. 38 (1–12–65) ;

(9) N.P. 22 (15/16–3–66) ; (10) N.P. 32 (15–3–66) ; (11) N.P. 25 and (12) N.P. 27 (19–3–66) ; (13) N.P. 38 (21–3–66) ; (14) Gaurisagar tank (10–12–65) ; (15) Beliaghat tank (16–3–66) ; (16) Teok: Rajabari tank (8–1–66) ; Jorhat : (17) Municipal tank (25–10–59) ; Nazira : (18) S.D.O's tank and (19) S.T. 16 (6–4–55) ; Dibrugarh : (20) S.T. 1 (26–5–55).

2.1b. *West Bengal* : 24 Parganas : (21) Museum Pond, Calcutta (March 49–Feb. 50) ; Dum Dum : (22) Mukherjee's Pond (4–10–49) ; (23) Dr. (Mrs.) Ghosh's Pond (5–6–'50) ; Belgharia Fish Farm : (24) S.T. 11 and (25) S.T. 16 (23–5–50) ; (26) S.T. 10 (20–6–50) ; Kamarhati (27) Sagore Dutt Pond–1 (23–5–50) ; Barrackpore : (28) Dhar's Pond (Mar. '49–Feb.'50) ; (29) Central Fisheries Res. Stn. Pond (Mar. '49–Feb.'50) ; (30) Lal Dighi's Pond, Sadar Bazaar (21–1–'50) (31) Nundi's Pond (18–1– '50) ; (32) Palta Exptl. Filter bed (Mar. '49–Feb.'50) ; Hooghly : (33) Choudhry Bagan pond, Serampore (Mar.' 49–Feb.'50) ; Midnapore : Chandrakona Road : (34) Rangamati bund ; (35) Poddar bund and (36) Kachahari bund (16/17–12–52).

2.1c. *Bihar* : Sone : (37) River Dehri at Darihat and Mahadewar Ghat downstream of polluted area (21–5–53) ; Patna : (38) Jail pond (Jul. '49).

2.1d. *Madhya Pradesh* : Bhopal Fish Farm : (39) N.P.1 and N.P. 25 (19–7–54) (40) Nirora tank (20–7–54) ; (41) Tanks, Jehore and (42) Nishatpura (24–7–54) ; Raipur : (43) Turki nursery (21–4–56) ; Jabalpur : (44) Gangasagar (23–4–'56).

2.1e. *Orissa* : Balasore : 20–12–52 (45) B.N.R. Tanks 2 and 11 ; (46) A.B. Mohanty's Pond 1 ; (47) D.M.'s tank ; (48) Hafeezuddin tank (25–11–54) ; Dighi Farm : (49) N.P. 12 (20–12–52) ; (50) N.P. 3,5 and 6 (26–11–54) ; Bhadrak: (51) Purana Bazaar N.P. and (52) S.T. (28–11–54) ; Mayurbhanj : 21–12–52, Tanks (53) Belgodia, (54) Chappal and (55) Himsagar ; Nilgiri (56) Raja's tank (28–11–54) ; Keonjhar ; 19–2–57 : (57) Public tank and (58) N.P. ; Cuttack : Jenapur : (59) N.P.2 and (60) S.T. ; (61) Jobra Fish Farm, Cuttack, N.P. 1–16 (1951–55) ; (62) Cement tank (17–7–51) ; (63) Killa Fish Farm, N.P. 24–41 (1954–56) ; (64) Killa Moat Sec. 5 (26–4–55) ; (65) Moat Extn. (9/11–4–56) ; (66) Office Pond, Central Fisheries, 19, Cant. Rd (1965–66) ; (67) I.G.'s Pond, Cant. Rd (26–6–52) ; (68) Pond, Bengali Sahi (1–3–61) ; (69) Pond, Dolmundi (13–1–66) ; (70) River Mahanadi at Anicut (July '52–May '53) ; (71) Wolffia Pond, Satyabhamapur (26–6–50) ; (72) Nuapara S.T. 5 and 6 (29/30–12–52) ; (73) S.T. 5 and 6 (25–2–57) ; (74) Barai Ponds 1–3 and 9 and (75) Kujang Roadside Pond (30–12–52) ; (76) Birbati pond (25–2–57) ; (77) Narasingpur S.T. 5 (17–12–54) ; (78) Chaudwar Fish Farm S.T. 1 and 3 (2–1–53) ; Dhenkanal : (79) Kamakhyanagar Farm N.P. 1 and (80) N.P. 13 (18–12–54) ; Hindol (81) Farm N.P. 2 (82) Harihat Pukur and (83) Subjail pond (20/21–12–54) (84) Talcher N.P. 6 and 10 (22–12–54) ; (85) Angul Farm N.P. 5, 10 and 16 (22–12–54) ; (86) Golimora Gundu Pukur (25–12–54) ; Sundergarh : (87) Kunseri tank, Bonai Garh (11–12–54) ; Sambalpur : (88) Farm ponds 2,11 and 26 and (89) Gorali tank (12–12–54) ; (90) Padampur, Cota bund (14–12–54) ; Bolangir : (91) Farm pond 10 (29–12–56) ; Puri : Kausalyagang farm : (92) Swamp (10–4–51) ; (93) N.T. 4, S.T. 5, K and N (27–11–52) ; (94) S.T., Harikrishnapur, (95) S.T. 1 and 5, Attaranalla and (96) S.T., Brickfield farms (11–12–52) ; Boudh-Phulbani : (97) Pond, Phulbani (19–12–54) : Ganjam :

(98) S.T., Chatrapur and (99) Berhampur (20–12–54) ; Koraput : (100) Jeypore nursery-1 (19–2–57).

2.1f. *Andhra Pradesh* : Srikakulam : (101) Market Pond and (102) Pala bund and Bokkara bund (3–12–54) ; (103) Jute wetting pond, Vizag Road (3–12–54) ; (104) Yellamanchili market pond (5–12–54) ; Samalkot : (105) Amadalavalaru and (106) Mothapuram ponds (6–12–54) ; Rajamundry : (107) Talkulwa pool, (108) Tobacco Stn. pond and (109) Dwarapudi pond (6–12–54) ; (110) Venkam and (111) Sesham Naidu ponds, Ellore (7–12–54) ; Kakinada : (112) Market pond and (113) Ippur Farm pond-8 (8–12–54) ; (114) Chinnaravoor tank, Tenali and (115) Ayithanagar tank, Tenali West (9–12–54) ; (116) A.R.P. Cement tank, Vijayawada (9–12–54) ; Kurnool : (117) Sunkesala Farm ponds 9, 15 and 16 (10–12–54) ; (118) Kurnool Fish. Office Pond and (119) Mariammakunta (11–12–54) ; Hyderabad : (120) Kunta, Cess pool and (121) Pond 14, Hussainsagar (29–1–53) ; (122) Hayalnagar, (123) Ibrahim Patan and (124) Sadyabag tanks (1–2–53) ; (125) Jafar Charu (1–2–53).

2.1g. *Karnataka* : Bangalore : (126) Lalbagh Hort. Office pond (9–2–53) ; Mysore : (127) Pool, Baratala, (128) Ishadbagh tank, (129) Hasalkot pond, (130) Dhobi Kote and (131) Well–3, Nandi Hills (8–2–53) ; Coorg : (132) Ponnampet ponds 1 and 2 and (133) Beauvoir Farm pond, Mercara (9/10–2–53) ; Mangalore : (134) Fisheries College cement pond-1 (June–Nov. 1974) ; and (135) –do–(Jan.–Feb., 76).

2.1h. *Kerala* : Trichur : (136) Edasserikulam, Cranganore (10/27–2–49) : (136a) Thandankulam. Azhicode (26–2–49) ; (137) Wolffia pond, Azhicode (29–9–51) ; (138) Asarikulam, Madavana (9–10–51) ; (139) Kattukulam, (140) Pandarakulam and (141) Pooppachira and (142) Temple tank, Cranganore (13/15–2–53) ; (143) Vadakechira and (144) Padinjarekulam, Trichur (15–2–53) ; (145) Pond, Chalakudi (13–2–53) ; (146) Pond and (147) Stream among rice fields, Chalakudi (17–2–53) ; (148) Pond, Iringalakuda (13–2–53) ; Quilon : (149) Krishnapuram Res. Stn. pond (24–2–53) ; Trivandrum (150) Aruvikkara reservoir.

2.1i. *Tamilnadu* : (151) Museum Pond, Madras (Dec. '37–Nov. '39) ; (152) Rect. Pond, Chetput fish farm, Madras (11–11–42) ; (153) –do– (31–3–43) ; (154) –do– (27–4–43) and (155) –do– (19–5–43) ; (156) Swamp, Chetput (22–11–44) ; Salem : (157) Mettur Dam, N.P. 5 and 6 (21–12–54) ; (158) N.P. 2 (27–12–54) ; N. Arcot, (159) Moat, Vellore (19–12–54) ; Nilgiris : (160) Muddy N.P., Fish Farm, Ootacamund (11–6–52).

## 3. Estimation of plankton

Plankton were usually collected by straining a constant volume of water through a plankton net made of No. 25 bolting silk (approx. 80 meshes per linear cm) and the strained sample concentrated to a constant volume. Several drops from each sample were examined and from fifty random fields of the mounts the organisms were counted under the low power (about $\times$ 300) of the microscope and their frequencies expressed as symbols, the numerical values for each symbol being adopted from Howland (1931) as follows : $i = 1$–$2$ ; $vr = 3$–$5$ ; $r = 6$–$10$ ; $rc = 11$–$20$ ; $c = 21$–$50$ ; $vc = 51$–$100$ ; $a = 101$–$200$ ; and $va =$ over 200,

When the organisms formed an appreciable scum, their frequencies were estimated roughly as *vc–va* depending on the thickness and spread of the scum.

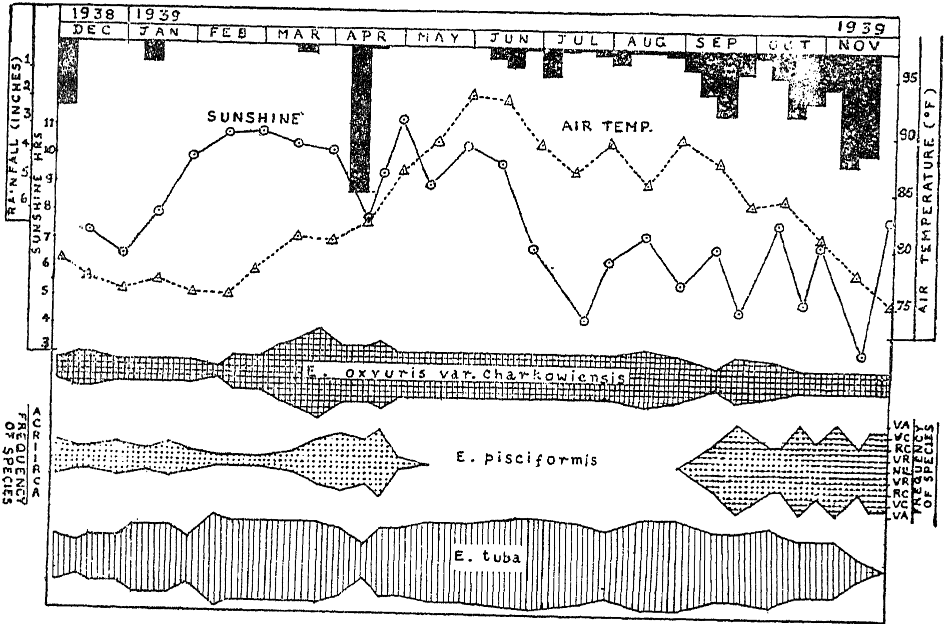
## 4. Ecological notes

Species of *Euglena* are usually found in large numbers in fish ponds, particularly the smaller ones, and to a limited extent in multi-purpose tanks of small size and in other shallow waters with decaying vegetation. Fish ponds are invariably manured with cow dung and other organic manures, especially during May-June, and are rich in organic matter, nitrates and sometimes phosphates. Public tanks are usually polluted and in some of them there is pollution even from sewage.

Some of the species which were observed in large numbers in these water bodies were *E. tuba*, *E. pisciformis*, *E. oxyuris* var. *charkowiensis*, *E. proxima* and sometimes *E. caudata* and its variety *minor*. *E. sanguinea*, *E. viridis*, *E. acus*, *E. granulata* and *E. tuba* var. *pseudotuba f. minima* also occurred in some ponds in large numbers. Of these, *E. oxyuris* var., *E. acus* and *E. granulata* usually occurred in the general plankton while the rest formed fairly thick scums in addition to their presence in the plankton.

*E. tuba* is one of the commonest species found all over north-east and south India (also in west India–see Carter (1869) imparting to the water alternately a brick red coloration during the bright hours of the day and dirty green at other times. During bright periods the red haematochrome pigments are spread all over the cell while in fading light they recede to the hind end. In encysted individuals also the pigments usually remain at the hind end. In var. *pseudotuba* f. *minima* the pigments are restricted to the axial region in fading light or when encysted. *E. tuba* occurs also in the plankton and it has been found (see Philipose 1940) that there is vertical stratification with its concentration gradually increasing towards the surface by about 14 hrs. on bright days and more uniform distribution at all levels at night. The organism usually develops in the bottom silt wherefrom it is recruited to the plankton.

In the filter beds at Barrackpore and ponds at Cuttack and Madras where year round observations were carried out, *E. tuba* occurred abundantly throughout the year except during periods of very heavy rainfall during July-September or October-December. However, even during rainy months sometimes it occurred in abundance during bright spells. *E. pisciformis* occurred abundantly in Museum Pond, Madras, mostly during the heavy monsoon period of September-November and at Cuttack in July. *E. caudata* dominated at Madras in October after heavy rains accompanied by lowering of temperature whereas var. *minor* occurred in two ponds at Barrackpore during the cold rainless month of January. *E. proxima* was generally observed in ponds almost throughout the year with greater development in January-February and December. *E. oxyuris* var. *charkowiensis* usually occurred throughout the year (in Museum Pond, Madras) with maxima during March-April and secondary maxima during June and August. It also occurred in some ponds of Orissa in large numbers in August and December. *E. srinagari* was common in February, June, August and December whereas *E. acus* was more frequent in February, May-June and November-December,




**Text figure.** Correlation between meteorological conditions (av. air temp. av. daily hrs of sunshine and total rainfall) and three species of *Euglena* in Museum Pond, Madras (location 151) during 1938-39. (One year's data redrawn from Philipose 1940).

**Abbreviations** : I—isolated ; VR—very rare ; R—rare ; RC—rather common ; C—common ; VC—very common ; A—abundant ; VA—very abundant.

Text-figure 1 and table 1 give the meteorological and water conditions respectively under which some of the species dominated. *E. tuba* occurred abundantly when there was fairly long sunshine hours, little or no rainfall and fairly high temperature in waters with fairly high total alkalinity, organic matter, albuminoid ammonia, nitrates, chlorides, iron, variable phosphates, and moderate pH. Its abundance in a large number of coastal waters probably suggests that fairly high chlorides have a favourable influence though lower concentrations apparently did not have any inhibiting effect as seen by its abundance in a few interior waters with low chlorides. The fact that it was favourably influenced by bright sunshine and probably high temperature was also borne out by its migration towards the water surface as the day advanced and its dispersal throughout the water late in the afternoon and at night when sunlight was not operative and the temperature was fairly uniform at all levels in small ponds. *E. oxyuris* var. *charkowiensis* also appeared to be favoured by more or less similar conditions but it showed its maximum in Museum Pond when the temperature was moderate, sunshine hours high and there was very little rainfall. It also appeared to be favoured by fairly high nitrates. *E. pisciformis*, on the other hand, dominated when there was lower sunshine hours, fairly good rainfall, and maximum total alkalinity, organic matter, nitrates and iron in Museum Pond during September-October. High temperatures seemed to be unfavourable for its development,

**Table 1.** Showing the ranges and averages of water conditions under which some species of *Euglena* occurred in selected localities.

| Species of *Euglena* | Loc. Nos, and No. of samples | Months and frequencies | Water temp. (° C) | pH value | Total Alkalinity (ppm) | Oxid. Organic matter (ppm) | Free $NH_3$ (ppm) | Alb. ammonia (ppm) | $NO_3$ (ppm) | $PO_4$ (ppm) | Chlorides (ppm) | Total iron (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. tuba* | 151 (7) | 2,5–8 (va) | 26·4–32 (29·7) | 7–7·4 (7·2) | 144–198 (156) | 2·2–6·3 (4·0) | Nil-0·12 (0·04) | 0·48–0·84 (0·56) | 0·44–1·1 (0·51) | 0·01–0·04 (0·019) | 14–44 (26) | 0·3–1·2 (0·49) |
| | 24, 60, 61, 69,72,80 116, 139 (8) | 1–2 11–12 (vc–va) | 22·5–30·5 (26·2) | 7·3–9·2 (8·2) | 46–202 (112) | | | | 0·03–0·30 (0·094) | 0·01–0·48 (0·20) | 9–60 (26·2) | |
| var. *pseudotuba* f. *minima* | 61 (1) | 12 (va) | 27·4 | 8·3 | 132 | | | | 0·30 | 0·02 | | |
| *E. oxyuris* var. *charkowiensis* | 151 (2) | 3,4 (vc–va) | 29·5–31 (30·2) | 7–7·2 (7·1) | 150–162 (156) | 3·6–4·1 (3·8) | Trace | 0·3–0·4 (0·35) | 0·7–0·8 (0·75) | 0·01–0·015 (0·013) | 18–20 (19) | 0·2–0·3 (0·25) |
| | 151 (3) | 6,8 (c) | 28·6–30 (29·2) | 7–7·2 (7·1) | 160–183 (172) | 1·9–6·3 (4·1) | Nil–0·12 (0·04) | 0·62–0·96 (0·80) | 0·44–1·15 (0·71) | 0·01–0·03 (0·020) | 14–36 (24) | 0·5–0·7 (0·57) |
| | 46,63,89 (3) | 8, 12 (c–vc) | 21·9–27·4 (24·0) | 7·3–7·8 (7·6) | 54–170 (98) | | | | 0·01–0·10 (0·053) | 0·05–0·11= (0·80) | | |
| *E. pisciformis* | 151 (5) | 9–11 (a–va) | 25–29·5 (27·6) | 7–7·4 (7·2) | 108–186 (152) | 4–5·2 (4·4) | Nil–0·32 (0·16) | 0·48–0·64 (0·58) | 0·7–1·2 (0·88) | 0·01–0·04 (0·022) | 10–22 (17·5) | 0·7–2·0 (1·3) |
| *E. caudata* | 151(1) | 10 (vc) | 25·0 | 7·0 | 162 | 2·7 | 0·18 | 0·36 | 0·62 | 0·015 | 12 | 0·20 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *E. acus* | 4, 48, 117<br>151<br>(4) | 2, 6, 11,<br>12<br>(c) | 22·2–29<br>(26·4) | 6·8–8·8<br>(8·0) | 26–544<br>(213) | 0·05–0·9<br>(0·30) | 0·17–0·26<br>(1·14) |
| *E. granulata* and<br>*E. vaginicola* | 65<br>(1) * | 4<br>(c) | 32·2 | 9·6 | 128 | 0·12 | 0·24 |
| *E. pseudo*<br>*ehrenbergii* | 112<br>(1) | 12<br>(vr) | 28·0 | 8·5 | 190 | 0·29 | 0·19 |
| *E. helicoideus*,<br>*E. spirogyre* and<br>*E. fusca* | 34, 92<br>(2)<br>* | 4, 12<br>(vr–r) | 22·2–37·4<br>(29·8) | 6·8–7·0<br>(6·9) | 12–13<br>(22) | 0·03–0·09<br>(0·06) | 0·08–0·12<br>(0·10) |

* with decaying vegetation. Abbreviations : vr—Very rare ; r–rare ; c-common ; vc-very common ; a–abundant ; va–very abundant

*E. helicoideus*, *E. spirogyra* and *E. fusca* seemed to be characteristic of shallow waters with fairly low pH and total alkalinity and high organic matter caused by decaying vegetation. *E. granulata* and *E. vaginicola* were quite common in a moat at Cuttack during the bright hot month of April when macroflora were decaying after chemical treatment.

Dodkundi *et al* (1973) observed marked stratification of six species of *Euglena*, *viz*. *E. acus*, *E. viridis*, *E. velata*, *E. gracilis*, *E. elastica* and *E. pisciformis* (in the order given) with increasing densities from bottom to surface after 9 a.m. in a sewage stabilization pond at Dharwar. The zone of maximum density (surface) was characterised by higher values for temperature, pH, dissolved oxygen, carbonates and higher reductions in nutrients. They did not, however, study the variation, if any, in their densities at various levels in relation to sunlight and temperature as the day advanced.

Fritsch and Rich (1913) and Lind (1938) reported maximum development of *Euglena*, especially *E. viridis*, in British ponds when organic matter and sunshine hours were high, with albuminoid ammonia and temperature as possible additional factors. Lund (1943) observed abundance of *E. viridis* in the marginal silt of Clay Pit Pond which had an organic content of 29–54%. In Kenwood Pond which had a fairly low pH, high organic matter (30–50%) and iron compounds in the marginal silt, Philipose (1948) observed abundance of *E. viridis* during February-March and *E. oblonga* during May-August. The latter period corresponded to long hours of sunshine, high temperature and decomposition of organic matter resulting in reducing conditions in the silt accompanied by the release of fairly high concentrations of ferrous compounds into the water. Pringsheim (1956) also stated that most species of *Euglena* preferred water rich in nutritive substances particularly readily available ferrous compounds and ammonium salts.

In Indian waters also a number of workers (Gonzalves and Joshi 1946 ; Zafar 1959 ; Singh 1960 ; Venkateswarlu 1960, 1980 ; Munawar 1970 ; Hosmani and Bharati 1980) correlated high densities of Euglenineae with high oxidisable organic matter and sometimes dissolved iron. Other factors reported as favourable are high carbon dioxide, nitrates and temperature. Zafar (1959) also stated that high nitrites and free ammonia higher than 0.104 ppm had an inhibiting effect on Euglenineae. In Museum Pond, Madras, nitrites ranged from 0.005 to 0.02 ppm when *E. tuba*, *E. oxyuris* var. *charkowiensis* and *E. pisciformis* dominated, which was quite low compared to the figures for the Hyderabad ponds during certain periods. Free ammonia at average concentrations of 0.16-0.18 ppm however, did not have any inhibiting effect on *E. caudata* and *E. pisciformis* in Museum Pond. These two species were also favoured by lowering of temperature after heavy rainfall.

It was also frequently seen by the author in a series of ponds situated side by side at Cuttack that *E. tuba* occurred abundantly in some ponds while it was absent in the others. Apart from slight differences in the nutrient status of these ponds, competition from other bloom forming algae, particularly *Microcystis* and *Anabaena* and some Volvocales and Chlorococcales, appeared to be one of the reasons for this difference. Ponds fertilized with inorganic fertilizers were also usually free of this species at least for some time while organically manured ponds seemed to favour its growth.

## 5. Systematic account

Genus *Euglena* Ehrenberg 1838

Single celled, usually free swimming, crawling or encysted (in resting condition) ; fusiform to elongate-cylindrical with hind end frequently drawn out into a short or long tail ; body fairly rigid, twisted or with varying degrees of " metaboly " (change of shape) ; periplast striate ; vacuolar system a typical cystostome and reservoir ; eye-spot by the side of the reservoir ; flagellum one and of varying length ; chromatophores disc-shaped, band to ribbon like or trough-shaped, of varying number and with or without pyrenoids; paramylum long or short rods or plates, elongated links, small discs or saucer-like sheathing the pyrenoids; haematochrome present in some species ; usually in freshwater, rarely in brackish water.

*Key to the Indian species described:*

I. Body fairly rigid with colourless tip ; chromatophores small, disc–to lens-shaped and without pyrenoids ; paramylum two or more solid rods or plates or elongated links ; nucleus usually ellipsoid and median ; flagellum shorter than body..............................................Group Rigidae

(A) Paramylum in long rods or oblong solid plates, very rarely in links

(a) Paramylum more than two

(i) Body elongate-cylindrical, narrow and ending in a clear point ; paramylum 3–16 rods, rarely links ; cells 65–220 × 4–8–25$\mu$ ..........................................1. *E. acus*

(ii) Body long and flattened with almost parallel sides, twisted spirally ; paramylum 4–10 ; with a prominent tail ; cells 240–530 × 25–40–60$\mu$..............2. *E. helicoideus*

(b) Paramylum usually two

(i) Body more or less spindle-shaped, rarely cylindrical

(1) Usually with a tail spine

\+ Tail spine long ; paramylum one in front and the other behind nucleus ; 52–150 × 6–8$\mu$........... ..............................3. *E. acutissima*

\+ + Tail spine shorter ; paramylum in rods, rarely links, and at same level as nucleus or one in front and the other behind ; 51–82 × 7–11$\mu$...4. *E. limnophila*

Cells smaller and with additional elongate or ovoid paramylum ; 26.5–50 × 6–12$\mu$...var. *minor*

(2) With gradually tapering tail

\+ Body small and not within a gelatinous envelope ; striae longitudinal ; 50–53 × 6–8$\mu$....5. *E. vagans*

++ Body larger and usually within a gelatinous envelope striae spiral ; 53–74 × 15–19.5$\mu$ with sheath ; 41–63.5 × 11–16.7$\mu$ without sheath.......... ..........................6. *E. vaginicola* sp. nov.

(ii) Body flattened or angular in cross-section ; with a prominent tail piece

(1) Body flattened and grooved for part of its length ; usually twisted during locomotion only ; 105–114 (–133) × 13–14 (–15.5)$\mu$..............................7. *E. allorgei*,

(2) Body angular in cross-section ; twisted even at rest 65–210 × 8–25$\mu$.........................8. *E. tripteris*
Smaller form ; 48–52–63 × 9–15$\mu$.........var. *klebsii*

(B) Paramylum in elongated links and usually two, one in front and the other behind the nucleus, rarely more.

(a) Body more or less cylindrical with short colourless tail ; striae smooth ; (65–)85–135 × 7.5–12$\mu$...........9. *E. ignobilis*

(b) Body more or less flattened or cylindrical ; striae with beads.

(i) Cell usually cylindrical ; pellicle yellowish ; striae beaded with hemispherical excrescences ; tail distinct and more or less straight and pointed ; usually 80–130 × 10–15$\mu$...10. *E. spirogyra*

(ii) Cell usually flattened ; pellicle brownish with square, rectangular or L-shaped excrescences ; tail often gradually tapering and oblique ; 153–225 × 117–27.5$\mu$.........11. *E. fusca*

(c) Body flattened in cross-section and twisted or grooved for part of its length even when at rest.

(i) with two or more paramylum links ; body robust, about 10 times as long as broad ; (280–) 375–490(–500) × 30–46 (–61–)$\mu$ ........................................12. *E. oxyuris*

(ii) Paramylum only two

(+) Body about 12 times as long as broad and narrower than in type ; 201–270 (–290) × 16–22.5 (–30)$\mu$.......var. *playfairii*

(++) Body about 6(–8) times as long as broad and smaller than in type (90–) 103–172 (–200) × 17–28 (–30)$\mu$.....var. *charkowiensis*

II. With marked metaboly by bulging, rarely by twisting ; tail when present tapering gradually or short and stumpy ; chromatophores usually larger than in " Rigidae " and lenticular, rarely small and discoid and without pyrenoids ; paramylum usually many and granular to ovoid, rarely in additional long rods ; nucleus ellipsoid to spherical ; flagellum 1/3 to 1½ times the body length.........................................Group Lentiferae

(A) Body flat to cylindrical with both ends rounded ; chromatophores small and discoid ; paramylum small, often in additional long rods ; nucleus ellipsoid ; flagellum shorter than body.

(a) Without any tail ; pellicular striae fine and close ; metaboly by bulging or twisting ; without additional paramylum rods ; 110–200 × 15–40μ..................................13. *E. srinagari*.....

(b) With a short stumpy tail and uniformly prominent double striae ; metaboly by twisting ; with additional paramylum rods ; 228–260 × 19–32–51μ......................14. *E. pseudoehrenbergii* sp. nov.

(B) Body fusiform with gradually tapering tail ; metaboly by bulging ; chromatophores lens-shaped and large ; paramylum large or small and short-cylindrical to ovoid ; nucleus spherical ; flagellum 1–1½ times body length ; cell (39–) 60–93 × (11–) 18–25μ..............15. *E. proxima*

II. Body fusiform with marked metaboly ; chromatophores in oblong, polygonal or laminate plates or elongated bands with a "double pyrenoid" sheathed by saucer-shaped paramylum caps ; with additional paramylum granules in cytoplasm ; nucleus spherical ; flagellum body length or longer.........
..................................................Group Catilliferae

(A) Chromatophores 2 (rarely 3) parietal curved plates ; 16–35 × 5–12μ
..............................................16. *E. pisciformis*

(B) Chromatophores about 5–12 or more circular to angular plates ; without haematochrome

(a) Body fusiform with conical anterior and double drawn out posterior with a nearly cylindrical tail ; chromatophores about 12 angular plates ; 37–115 × 11–27.6 (–30)μ.............17. *E. granulata*

(b) Body fusiform with narrowing posterior and drawn out or nearly cylindrical anterior ; chromatophores circular to saucer-like, usually lobed plates, often more than twelve ; 60–110 × 15–38μ.......
.........................................18. *E. caudata*
Smaller form with 6–16 or more chromatophores ; 30–63 × 10–21μ
..................................................var. *minor*

(c) Body elongate fusiform to cylindrical; chromatophores 7–13 or more circular to ovoid or triangular plates which are curved with the pellicle and irregular due to close proximity ; striae faint ; 31–68 × 6–18–22.5μ....................................19. *E. gracilis*

(C) Chromatophores in elongated spindle-shaped bands ; flagellum usually less than body length ; haematochrome present.

(a) Cysts like round-bottomed flasks with a stalk of varying length ending in a funnel-like base ; chromatophores 5–17 or more ; cell 41–74–96 ×18–30–43μ......................................20. *E. tuba*

(b) Cysts like conical flasks with stalk almost absent and broad rim-like base ; chromatophores numerous ; cell 87–130 × 17–27μ........ ........................................var. *pseudotuba* comb. nov.
Smaller form with 5–10 chromatophores and dimensions 45–82.5 × 17.5–31.5 (–37.5)μ........................f. *minima* f. nov.

(D) Chromatophores numerous and in short or long bands.

(a) Chromatophores peripheral, 16–25, stellate with short bands running parallel to striae as well as radiating from centre ; paramylum two and sheathing pyrenoid ; cell ovoid to ellipsoid ; flagellum 3/4 to 1½ times body length ; without haematochrome ; 50–110 × 16–37 (–40)μ................21. *E. oblonga*

(b) Chromatophores in elongated bands in periphery ; cell ellipsoid to fusiform or elongate cylindrical ; pellicle markedly striate ; flagellum longer than body ; haematochrome present ; 50–150 (–200) × 22–55 (–72)μ.....................................22. *E. sanguinea*

IV. Metaboly less marked than in Groups II and III ; chromatophores ribbon-like often breaking up into short lengths and radiating from 1–3 median " pyrenoid centres ", rarely of two kinds (radial bands and discs) ; paramylum grains usually massed round these centres ; nucleus spherical; flagellum about body length.................................................Group Radiatae

(A) Chromatophores in bands radiating from one centre ; body fusiform sometimes elongate-cylindrical ; 30–65 –73 (–89) × 9–22μ...23. *E. viridis*
Larger form ; 69–94 × 28–40μ...........var. *maxima* var. nov.

(B) Chromatophores in elongated radiating bands as well as irregular discs ; cells 62–86 (–128) × 12–23.5 (–29.5)μ.........24. *E. hemichromata*

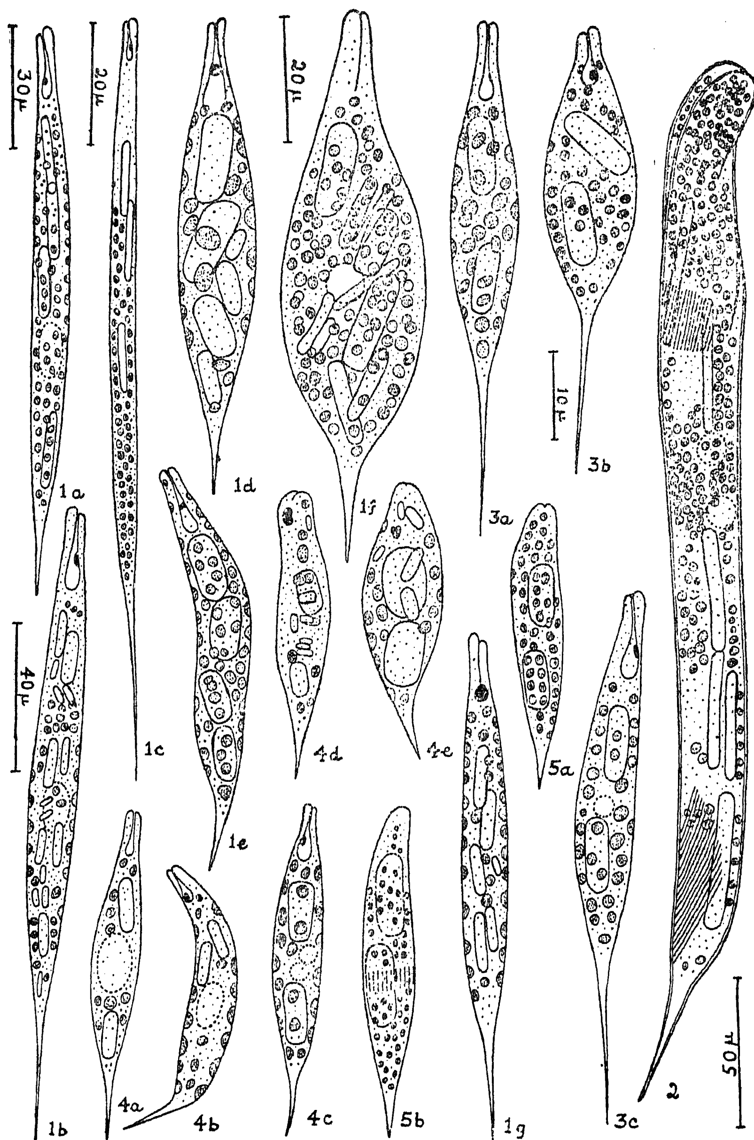
(V) With marked metaboly ; cell cylindrical or with attenuated ends ; chromatophores large, disc–to trough–shaped, without pyrenoids ; flagellum easily shed, locomotion by creeping........................Group Serpente, (No species under this group observed by the author though *E. deses*, *E. guentheri* and *E. intermedia* are reported by other workers).

## I. Group RIGIDAE Pringsheim 1956

### 1. Euglena acus Ehrenberg 1956 1830 (figures 1 a–g)

Lemmermann 1913, p. 129, figure 209 ; Gojdics, 1953, pp. 99–102, Plate 11 figure 1 ; Huber-Pestalozzi 1955, p. 96, figure 15 ; Pringsheim 1956, p. 48 figure 2 ; Hortobágyi 1969 p. 30, figure 50 ; including var. *minor* Hansgirg.

Body more or less rigid, rarely slightly twisted, elongate-cylindrical to spindle-shaped with a slightly drawn-out neck and truncate anterior end ; gullet opening slightly towards one side ; posterior end narrowing into a long or short hyaline




**Figures 1–5. 1 a–g.** *Euglena acus* Ehr. (d and f slightly abnormal specimens,) **2** *E. helicoideus* (Bern.) Lemm. **3 a–c.** *E. acutissima* Lemm. **4 a–c.** *E. limnophila* Lemm. **4 d–e.** var. *minor* Drez. **5 a–b.** *E. vagans* Defl. [Figures of the same magnification bracketed together :—(1a) ; (1b;) (2) ; (1d–f, 4a–d, 5a–b) ; (1g and 1c) ; (3a–c, 4e)].

tail ; chromatophores numerous, small and discoid ; nucleus usually ellipsoid median or slightly behind ; paramylum 3–16 solid rods, rarely oblong plates, of varying lengths ; eye-spot streak-like or nearly round ; flagellum usually up to 1/3 body length only ; striae when observed faint ; cells usually 85–170 × 7–12$\mu$, rarely as narrow as 4$\mu$ or as broad as 23$\mu$, and as short as 64–74$\mu$.

*Habitat* : Planktonic ; common in locations 2, 14, 23, 27, 48, 61 (N.P.9.–May), 73, 82, 118 and 151 (February) ; rare in locations 1, 5, 17–19, 21, 26, 28, 29 (April, May, July, November), 33, 35, 44, 49, 57, 59, 63, 71–72, 75, 78, 80, 82–84, 88–90, 93, 102, 105, 107, 113–18, 127, 134, 141–43, 154 and 156.

Considerable variation in shape and size of cell and in number and size of paramylum bodies was observed. The form from Chetput (location 154, figure 1c) was very narrow in proportion to length (125 × 4μ). An individual from Dum Dum (location 23) was bulged out (figure 1f) towards the posterior end, had a spherical nucleus and measured 88 × 23μ while another from Kamarhati (location 27, figure 1d) was intermediate in size (74 × 11.5μ) with slightly larger chromatophores. A second individual from Kamarhati (figure 1e) measuring 64 × 8μ was slightly twisted. The Dum Dum and Kamarhati material had the paramylum in broad plates or as a mixture of rods and plates. It was also not uncommon to find long and very short rods together in normal narrow individuals.

Van Oye (as cited by Gojdics 1953) gives the range of size of the species as 65–220 × 4–25μ and Chu (1947) as 60–160 × 7–15μ, whereas according to Pringsheim (1956) the normal size comes within 80–150 × 7–12μ, if some of the varieties are excluded. In Indian material Kamat (1961–1964) gives measurements of 100–180 × 6–15μ, Naidu (1962) 98–184 × 7μ and Hortobágyi, *l.c.*, 148 × 15.5μ, as against 64–170 × 4–23μ in the author's material. Skuja's (1949) organism from Burma measured 114–166 × 9–13μ.

The individual from Dum Dum resembled to some extent *E. lata* Swirenko (see Gojdics 1953 p. 178 Plate 36, figure 2) which measured 93–95 × 33–39μ. Though Swirenko treated it as a distinct species, he himself suspected that it could be an abnormal *E. acus*. Gojdics and Pringsheim also consider it as a *E. acus* or a doubtful species. Pringsheim also states that even *Euglena* with a fairly rigid form might become deformed by the overcrowding of paramylum bodies under certain unbalanced nutritional conditions. The individuals from Dum Dum and Kamarhati, therefore, appear to be slightly abnormal.

Annular rings appearing as dark lines along the axes of paramylum rods (see Pringsheim 1956) or as links (see Hortobágyi 1969) were not observed in the author's material.

Distribution in Indian region : Kashmir (Bhatia 1930) ; Gujarat (Kamat 1961–62) ; Maharashtra (Carter 1856, 1858 ; Gonzalves and Joshi 1946 ; Kamat 1963, 1964, 1974, 1976) ; Uttar Pradesh (Hortobàgyi 1969) ; Andhra Pradesh (Naidu 1962 ; Venkateswarlu 1976) ; Karnataka (Dodkundi *et al* 1973) ; Kerala (Suxena *et al* 1973) Assam, W. Bengal, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka, Kerala and Tamilnadu (!) ; Burma (Skvortzov 1937 ; Skuja 1949).

## 2. *Euglena helicoideus* (Bern) .Lemm 1910 (figure 2)

Gojdics 1953, p. 119, Plate 18, figure 1 ; Rino, 1972 p. 149, Plate 5 figure 1 ; = *Phacus helicoideus* Bernard, 1908 p. 206 Plate 16, figure 563 ; = *Euglena oxyuris* var. *helicoidea* (Bern.) Playfair 1921 p. 119, Plate 3, figure 18 ; = *E. gigas* Drezepolski 1925 p. 243, Plate 5 figure 159 ; Huber–Pestalozzi 1955 p. 69, figure 44.

Body long and flattened with more or less parallel sides and twisted spirally ; anterior end rounded ; posterior end with a pointed hyaline tail piece ; pellicle fairly thick and with very fine close spiral striae ; paramylum (4–) 6–8 (–10) long or short hyaline rods or plates ; chromatophores small, discoid and numerous ; eye-spot large ; flagellum not observed ; Body (including tail) 319–330–368 × 25–36–37.5$\mu$ ; tail alone 33.8–52.5$\mu$.

*Habitat* : Rare in plankton at locations 25, 34, 35, 92 and 160.

Though normally the paramylum (only 6–8 observed) were all in narrow rods, in the Belgharia material (location 25) one individual had short (14 × 10.5$\mu$), medium (24.5–28 × 10.5–12.3$\mu$) and long (38.5–54.3 × 8.8–12.3$\mu$) moderately broad solid plates. Nucleus could not be observed clearly in most instances, but in one individual (figure 2) from location 25 there were two small median spherical bodies probably formed by the division of a single nucleus.

This species is distinguished from *E. oxyuris* with more than two paramylum by the paramylum being in solid rods (rarely plates) and not in links. According to Gojdics (1953 p. 120) the striae in *E. oxyuris* also appear as double lines with a distance of 2$\mu$ between the striae. In *E. helicoideus* the striae are fine and close. Pringsheim (1956) does not, however, place much reliance on the absence of paramylum links in *E. helicoideus* and considers it and *E. gigas* as possibly *E. oxyuris.* In material of *E. oxyuris* (360–370 × 40–42$\mu$) with more than two paramylum observed by the author (1948) in a distinctly alkaline London pond (Highgate Pond) the paramylum were in distinct links when viewed along the broad side. In none of the specimens from the five Indian localities this was the case. Because of this and the close fine striae the present species is retained here as *E. helicoideus.*

Huber-Pestalozzi (1955) observed *E. gigas* in highly eutrophic waters of Java. The water bodies from which the present species was collected were also shallow with decaying vegetation and the water was fairly low in pH and total alkalinity. Possibly *E. oxyuris* and *E. helicoideus* are characteristic of two distinct types of waters *viz.* alkaline, and peaty and slightly acidic respectively.

Distribution in Indian region :— W. Bengal, Orissa and Tamilnadu ( ! ).

### 3. *Euglena acutissima* Lemm. 1904 (figure 3 a–c)

Lemmermann, 1910a, p. 496, figure 23 ; 1913, p. 129, figure 210

Body fairly rigid and spindle-shaped but sometimes slightly inflated by contraction along the longitudinal axis. Differs from *E. acus* in having only two paramylum rods, one in front and the other behind a median nucleus, the somewhat spiral arrangement of the chromatophores and a proportionately long hyaline tail ; flagellum not observed ; cells (including tail) 59.5–62 × 7–8$\mu$ ; tail alone 18–19$\mu$.

*Habitat* : Rare in plankton of location 1, 29 (May), 33 (April), 70 (October) 72 and 151 (April, November)

Playfair (1921 pp. 120–21, Plate 4, figures 6–8) gives its dimensions as 110–150 × 7–8$\mu$ with those of his var. *parva* as 54–94 × 6–8$\mu$ and states that the Australian form is really *E. acus*. Gojdics (1953) and Huber-Pestalozzi (1955) merge it with *E. acus* whereas Pringsheim (1956) states that "some species, for

instance *E. acutissima* Lemm. could be considered a variety of *E. acus* in the wider sense". The species is retained here as distinct. The author could not make out the nucleus clearly except in one specimen where it was small and spherical (figure 3c). Johnson's (1944, p. 113, figure 13A–B) *E. acutissima* (120–150 × 9–11μ) with more than two paramylum is obviously a *E. acus*.

Distribution in Indian region : Assam, W. Bengal, Orissa and Tamilnadu (!) ; Burma (Skvortzov 1937).

**4. *Euglena limnophila* Lemm. 1898 (figures 4 a–c)**

Lemmermann 1913, p. 130, figure 205 ; Gojdics 1953 p. 103 Plate 11 figure 6 ; Huber-Pestalozzi 1955 p. 82 figure 59 ; Suxena 1955 p. 430 figure 1 ; Pringsheim 1956 pp. 50–51 figure 3 ; Hortobágyi 1969 p. 30 Plate 4, figure 57

Body spindle-shaped with a slightly drawn out neck, truncate anterior end and with a straight or slightly curved tail at posterior end ; body sometimes slightly curved ; nucleus usually ellipsoid, large and median (rarely two small spherical ones formed by division) ; chromatophores small and not arranged regularly ; paramylum two rods, rarely flattened plates, one in front and the other behind the nucleus or both just in front of nucleus at nearly the same level; eye-spot prominent; flagellum not observed ; cells (including tail) 51–70.5 × 7–7.5μ.

*Habitat* : Rare in plankton of location 33 (April-May).

There is some difference in the dimensions given by various authors. Lemmermann gives it as 82 × 10μ, Skuja (1949) 55–69 × 7–13μ, Pringsheim 68–71 × 9–11μ, Suxena 60 – 80 × 7–10μ, Huber-Pestalozzi, 58–115 × 10μ and Hortobágyi 68 × 9μ. Lemmermann and Suxena noted a tail spine whereas Pringsheim's specimens had a gradually tapering tail. Lemmermann also stated that the paramylum rods were at the same level as the nucleus (also see Suxena 1955). In the present material the tail was either gradually tapering or like a spine and the paramylum just above the nucleus at nearly the same level or one in front and the other behind as in Pringsheim's material. The nucleus is described as spherical by Lemmermann (1913) and Skuja (1948) and ellipsoid by Pringsheim. The longitudinal dark line along the middle of the paramylum rods noted by Pringsheim and Skuja or the link-like paramylum seen by Pringsheim in some specimens were not observed by the author. Nor was there any additional paramylum (see Pringsheim 1956 figure 3A). The eye-spot was also quite small in contrast to the large one mentioned by Huber-Pestalozzi.

Distribution in Indian region : Andhra Pradesh (Suxena *l.c.* ; Zafar 1959) ; Maharashtra (Kamat 1975) ; Uttar Pradesh (Hortobágyi 1969) ; Kerala (Suxena *et al* 1973) West Bengal ( ! ) ; Burma (Skuja 1949).

**var. minor Drezepolski 1925 ( (figure 4 d-e)**

Drezepolski 1925 p. 245 Plate 5, figure 162 ; Gojdics 1953 p. 103, Plate 11, figure 7 ; Huber-Pestalozzi 1955 p. 83 figure 59B ; Hortobágyi 1969 p. 30 Plate 4, figure 55–56.

Differs from the type in its smaller size and in having varying numbers of usually small-sized cylindrical to ovoid paramylum bodies in addition to the normal large ones. Cells 31–46 × 8–8.5$\mu$.

*Habitat* : Rare in plankton of locations 33 (July) and 151 (April).

Hortobágyi's specimens had an irregular outline and there were a number of ring-like and cylindrical to ovoid paramylum bodies. In the author's material there were 7–10 paramylum including two large ones but none of them were ring-like. The two large ones in material of location 151 were also in the form of broad plates.

Distribution in Indian region : Uttar Pradesh (Hortobágyi 1969) ; West Bengal and Tamilnadu ( ! ).

**5. *Euglena vagans* Deflandre 1932 (figure 5 a-b)**

Gojdics 1953 p. 98, Plate 10 figure 8 ; Huber-Pestalozzi 1955 p. 94 figure 173

Body more or less cylindrical for most of its length, slightly tapered towards the anterior end which is obliquely truncate ; posterior end gradually tapering into a short hyaline tail ; chromatophores small, disc-shaped and numerous ; paramylum two and in the form of oblong solid plates, one in front and the other behind; pellicle striated in a longitudinal manner ; nucleus, eye-spot and flagellum not observed ; cells 49–55 × 7–8$\mu$.

Neither Gojdics nor Huber–Pestalozzi refer to the striae

*Habitat* : Rare in plankton of locations 23 and 28 (May).

Distribution in Indian region : West Bengal ( ! ).

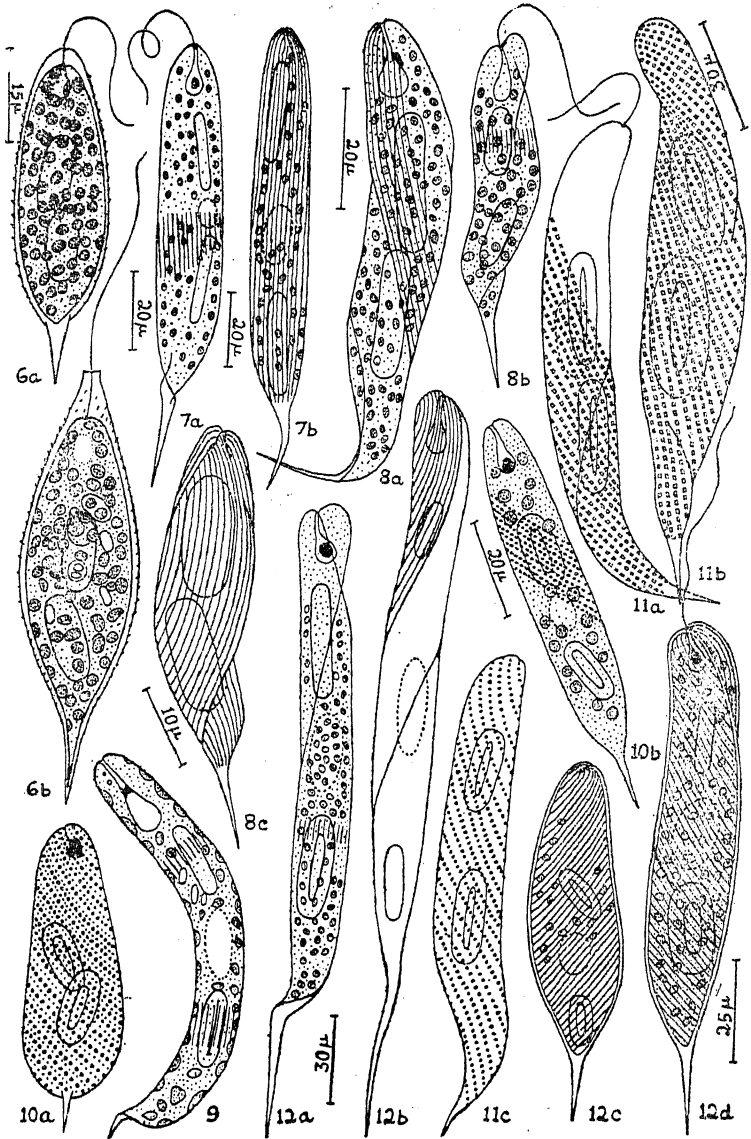
**6. *Euglena vaginicola* sp. nov. (figure 6 a-b)**

Organismi intra cystam late fusiformem irregulariter serrulatam incolentes ; cysta antice late rotundata, aut collo brevi praedita, et aperturam parvam habens ; corpore fusiformi, antice rotundato, postice caudam gradatim attenuatam habente aut in punctum abrupte terminante ; pellicula strias tenues spirales praebens ; chromatophora parva, multa, disciformia, sine pyrenoidibus, aliquantulum spiraliter ordinata ; paramylum ut duae virgulae solidae crassaeque, una anteriore, una posteriore, visum ; grana paramyli additica parva virguliformia ad disciformia interdum praesentia ; fossa et stigma parva ; nucleus non observatus ; flagellum c. 3/4 longitudinis corporis et per aperturam cystae anteriorem emergens ; cellulae 41–63.5 × 11–16.7$\mu$m, vagina (cysta) 53–74 × 15–18.5$\mu$m.

*Habitatio* : Species rara in plancta locorum 23, 61, 63 et 73, frequens in loco 65.

Iconotypus : (figure 6 a-b)

Organism living inside a broadly spindle-shaped serrulate colourless gelatinous envelope (cyst) having a small opening at the anterior end and a gradually tapering smooth posterior end. Anterior end of envelope broadly rounded or with a neck-like portion ; body spindle-shaped with the anterior end broadly rounded and the posterior end with a gradually tapering tail or abruptly ending in a point. Anterior and posterior ends showing some contraction and expansion in living




**Figures 6–12. 6a–b.** *Euglena vaginicola* sp. nov. **7a–b.** *E. allorgei* Defl. **8a.** *E. tripteris* (Duj.) Klebs. **8 b–c.** var, *klebsii* Lemm. **9.** *E. ignobilis* Johnson. **10a–b.** *E. spirogyra* Ehr. **11 a–c.** *E. fusca* (Klebs) Lemm. **12 a–b.** *E. oxyuris* var. *playfairii* Bourr. **12 c–d.** *E. oxyuris* var *charkowiensis* (Swir.) Chu. [Same magnification :—(6a–b, 8b, 9) ; (7a) ; (7b) ; (8a) ; (8c) ; (10a–b) ; (11a, 11c, 12a–c) ; (11b) ; (12d].

material ; pellicle finely striated somewhat spirally with the spiral from left to right ; chromatophores small, numerous, disc-shaped and arranged somewhat spirally ; paramylum two solid stout rods one in front and the other behind ; with or without additional rod-like to discoid paramylum of smaller size ; reservoir and eye-spot small, nucleus not observed ; flagellum about 3/4 body length and

emerging through the anterior opening in the cyst ; cells 41–63.5 × 11–16.7μ ; cysts 53–74 ×15–18.5μ.

*Habitat* : Rare in plankton of locations 23, 61 (N.P.8,9,11–May) ; 63 (N.P.25–January) and 73; common in location 65.

Figure 6 a-b to serve as the type.

The organism differs from all other known species of *Euglena* (except *E. granulata* (Klebs) Schmitz-see Gojdics 1953 p. 138) in having a gelatinous cyst with an aperture at the anterior end through which the flagellum emerges. In species of *Euglena* with cysts, the cyst usually remains closed till the organism is liberated by its breaking up or dissolution, and the flagellum does not develop inside the cyst. In this respect the present organism resembled a *Strombomonas,* but the essential organization of the cell was as in *Euglena* as when seen after removal of the cyst with dilute potassium hydroxide solution. The cyst was also thinner than the lorica of *Strombomonas.*

Though the liberation of the organism from the cyst was not observed, free living individuals resembling in all respects those inside the cyst, except that the cells were more cylindrical and always with a tail, were frequently observed in the collections. In one such individual (57 × 10.6μ) from location 23, distinct spiral rows (12–13) of discoid chromatophores could be observed. There was alsopone short stout paramylum (7–7.7 × 3.5–4.4μ) with a spherical (3.5–4.4μ) paramylum at its inner end in each half of the cell with a distinct gap between the two ests.

Szabados (1950 as cited by Gojdics 1953) observed a bloom of *E. granulata* in Hungary in which the organism was enclosed in flask-shaped cysts resembling the shell of *Trachelomonas similis* with the flagellum emerging through an opening at the end of a short neck, and the organism moved with the cyst. When the cysts were exposed to strong light the organisms squeezed out of the cysts. Once free they assumed the normal shape of *E. granulata* in a short time. The present organism also moved with the cyst. It is quite possible that it was also in a condition of temporary encystment.

The organism inside the cyst showed a certain degree of resemblance to *E. gaumei* Allorge et Lefèvre (1930, pp. 123–124, figures 9–12) in general shape, size (60–70 × 10–12μ) and in the presence of rectangular to oblong paramylum (described as links but shown as solid ones in figures), but differs in having a thin cyst in the encysted condition, in the chromatophores being more or less spirally arranged, in the frequent presence of additional small paramylum bodies, a longer flagellum and in the occasional absence of a tail. The striae in *E. gaumei* also usually runs almost parallel to the long axis, but could be somewhat spiral occasionally, whereas in the present organism they appeared as spiral. An eye-spot and reservoir are not reported in *E. gaumei* ; in the present organism they could be seen faintly in a few specimens.

It also shows some resemblance to *E. vagans* Deflandre in general appearance and the gradually tapering tail, when present, but the anterior end of the present organism is more rounded, the cell is broader and usually within a cyst. As in *E. gaumei*, the striae in *E. vagans* runs almost parallel to the long axis.

The organism under present consideration, is, therefore, treated here tentatively as a new species under the name *E. vaginicola.* However, it has to be observed whether it lives in a free state outside the cyst for any length of time.

Distribution in Indian region : West Bengal, and Orisssa (!).

### 7. *Euglena allorgei* Deflandre 1924 (figures 7 a-b)

Deflandre 1924 pp. 1116–1117, figures 1–2 ; Gojdics 1953, pp. 116–17, Plate 16, figure 2 a-b ; Huber-Pestalozzi, p. 80, figure 56A.

Body nearly flattened in cross-section with the sides nearly parallel ; change of shape only by curving during movement ; anterior end frequently with a slightly curved furrow ; posterior end bent sharply towards one side or nearly straight and ending in a hyaline tail piece ; pellicle striated more or less longitudinally ; chromatophores small, discoid and numerous ; paramylum two long rods one in front and the other behind a median ellipsoid nucleus ; eye-spot small and triangular ; flagellum about one-third body length ; cell (including tail) 100–133 $\times$ 14.5–15.5$\mu$.

*Habitat* : Rare in plankton of locations 26, 61 (December) and 151 (May).

Deflandre's organism is slightly smaller (105–114 $\times$ 13–14$\mu$). He has also shown the nucleus as spherical.

Distribution in Indian region : Maharashtra (Kamat 1975) ; West Bengal, Orissa and Tamilnadu ( ! ).

(Kamat 1975, does not give any description or figures).

### 8. *Euglena tripteris* (Duj.) Klebs 1883 (figure 8a)

Lemmermann 1913 p. 130 figure 201 ; Skuja 1948 p. 198 figure 23, figures 12–13 ; Gojdics 1953, pp. 122–23, Plate 18, figures 3a–d ; Huber-Pestalozzi 1955 pp. 62–63, figure 39 ; Suxena 1955 p. 432 figure 14 ; Pringsheim 1956 pp. 57–59, figure 6.

Body elongate with nearly uniform breadth, rounded anteriorly with the canal opening forming two lips, and ending posteriorly in a long hyaline tail piece ; triangular in cross-section ; twisted markedly so that the body shows 2–3 sections with the sides caved in; pellicle with clear striae which follow the twists ; chromatophores small, discoid and numerous ; paramylum two long rods or oblong plates (28–30 $\times$ 7–9$\mu$), one in front and the other behind an ellipsoid median nucleus ; nucleus frequently not clear when the paramylum are in broad plates almost touching each other ; eye-spot prominent ; flagellum about half body length ; cells (including tail) 94–109 $\times$ 15–17$\mu$.

*Habitat* : In plankton of locations 21 (March), 28 (April-May), 29 (October), 33 (February), 37, 45, 49–51, 56, 61 (N.P. 20-May), 63 (N.P. 32–July), 70 (July), 92, 113, 117, 134, 140 and 151 (November).

Distribution in Indian region : Andhra Pradesh (Suxena ; Zafar 1959 ; Naidu, 1966 ; Venkateswarlu 1976) ; Maharashtra (Kamat 1975) ; West Bengal Bihar, Orissa, Andhra Pradesh, Karnataka, Kerala and Tamilnadu (!) ; Burma, (Skuja 1949).

**Var. *Klebsii* Lemm. 1910 (figure 8 b-c)**

Lemmermann 1910a p. 497 ; 1913, p. 130 ; Johnson 1944, p. 106, figure 4 b-c; Huber-Pestalozzi 1955 p. 63 ; As *E. tripteris*-Kamat 1961–62 p. 16

Smaller than the typical form ; cells 48–63 × 10–14μ (incl. tail) ; tail alone 10.5–14μ ; flagellum 18–20μ ; paramylum 10–15 × 3.5–5.3μ.

*Habitat* : In plankton of locations 13, 14, 23, 28 (December) 33 (April-June), 38, 61 (N.P. 20, 26, 28–29, February, March, May), 63 (N.P. 41–May), 73, 105, 114, 118, 151 (January-February) 152. Common in location 14 and rare in the rest.

Distribution in Indian region : Gujarat (Kamat 1961–62) ; Assam, West Bengal, Bihar, Orissa, Andhra Pradesh and Tamilnadu ( ! ).

Gojdics (1953 pp. 122–23) suppressed all varieties of this species and merged them with the typical form while Huber-Pestalozzi (1955) and Pringsheim (1956 p. 59) retain some of them including var. *klebsii*. Though the size range usually reported for the typical form is 70–80 × 8–14μ (see Lemmermann 1913), Chu (1947) gives it as 70–190 × 11–23μ for his Chinese specimen whereas Skuja (1948) gives it as (75–) 100–205 × 14–22μ with the large paramylum 17–40 × 7–11μ in his Swedish material. Suxena (1955) gives dimensions of 100–210 × 15–23μ and Naidu (1966) 70–80 × 9–11μ. Since the author's smaller form measures only 48–63 × 10–14 μ compared to Hortobágyi's Hungarian material with 58 × 9–10μ and Johnson's American one with 52–63 × 11–15μ, the variety is retained here. Kamat's (1961–62) typical species measuring 25–30 × 5–7μ is much smaller than even var. *klebsii* and could be a juvenile form. Kamat (1975) has not given the dimensions of his typical species from Maharashtra.

Both Gojdics and Pringsheim refer to additional short rod-like, rectangular or ovoid paramylum in the typical species. In the author's material two additional very small rods were observed only in one individual of var. *klebsii*. Johnson (1944) states that var. *klebsii* has less torsion than in the type. In the author's material both well-twisted and individuals with only slight torsion were observed.

**9. *Euglena ignobilis* Johnson 1944 (figure 9)**

Johnson 1944 p. 118 figure 20 A–C

Body more or less cylindrical and curved, with the anterior end slightly narrowed and rounded ; posterior end with a very short hyaline bent tail ; pellicle with prominent continuous striae which are more or less longitudinal ; chromatophores small, discoid to ovoid and numerous ; paramylum two oblong links, one in front and the other behind a median ellipsoid nucleus ; with a few additional rod-like small paramylum ; eye-spot fairly small ; flagellum not observed ; cell 65–69 × 7.5–8μ.

*Habitat* : Rare in plankton of locations 29 (February) 73 and 81.

The organism agreed in most respects with the American one, but was smaller, the American form measuring 85–135 × 8–12μ and with flagellum ¼–½ body length. Also, there was less metaboly and the nucleus was ellipsoid compared to prolate spherical in the American species. According to Pringsheim (1956 p. 57) this species looks more like *E. spirogyra* minus papillae such as commonly occurs in cultures.

Distribution in Indian region : Maharashtra (Kamat 1975 p. 466) ; West Bengal and Orissa (!)

(Kamat 1975 does not give any description or figure.)

**10. *Euglena spirogyra* Ehrenberg 1838 (figure 10 a-c)**

Lemmermann 1910a pp. 488–89 figure 18 (p. 483) ; 1913 p. 131 figure 208 ; Gojdics 1953 pp. 111–112 Plate 14, figure 2a-b ; Huber-Pestalozzi 1955 p. 101, figure 81 ; Suxena 1955 pp. 432–33 figure 5 ; Pringsheim 1956 pp. 53–56 figures 4–5; including var. *marchica* Lemm., 1913 p. 131 ; = *E. fusca* var. *marchica* Lemm., 1910a p. 498.

Body elongated and more or less cylindrical with the sides nearly parallel ; body sometimes bent but not usually twisted ; anterior end slightly tapering with a rounded end, with the canal opening frequently towards one side ; posterior end also somewhat rounded and usually ending abruptly in a distinct hyaline tail piece; pellicle yellowish and with spiral rows of bead-like hemispherical excrescences which are either uniformly developed (as in var. *marchica* Lemm.) or with 1–3 rows of weakly beaded excrescences between two rows of strongly beaded ones ; paramylum two fairly large links one in front and the other behind an ellipsoid median nucleus ; chromatophores small, discoid and numerous ; eye-spot fairly large ; flagellum not observed ; body usually 62.5–86 × 10.5–17.5$\mu$, rarely up to 160 × 16$\mu$.

*Habitat* : Rare in plankton of locations 16, 28, 61 (N.P.5 and 15-December May), 64, 65, 73, 92, 95, 132, 136, 144, 146, 148 and 157.

Bhatia (1930) gives its dimensions as 140 × 18–20$\mu$ and Suxena (1955) as 100–110 × 13–14$\mu$. Dimensions normally accepted for the species (see Pringsheim 1956 p. 55) are 80–130 × 10–15$\mu$. In the author's material they ranged from 62.5–86 × 10.5–17.5$\mu$ with one individual from location 136 measuring (including tail) 160 × 16$\mu$ (see Plate III, figure 10c).

Distribution in Indian region : Kashmir (Bhatia 1930) ; Himachal Pradesh (Kamat 1968a) ; Uttar Pradesh (Singh 1960); Gujarat (Kamat 1961–62); Bombay, Maharashtra (Carter 1856); Andhra Pradesh (Suxena 1955; Zafar 1959); Assam, W. Bengal, Orissa, Karnataka, Kerala and Tamilnadu (!) ; Burma (Skvortzov 1937 ; Skuja 1949).

**11. *Euglena fusca* (*Klebs*) Lemm. 1910 (figure 11 a–c)**

Lemmermann 1910a, p. 496 ; 1913 p. 130 figure 202 ; Gojdics 1953 pp. 112–14 Plate 15 figure 1a–f ; Huber-Pestalozzi 1955 pp. 64–65 figure 41 ; Prowse 1958 pp. 145–46, figure 1 e; = *E. spirogyra* var. *fusca* Klebs, 1883

Differs from *E. spirogyra* in the body being usually flat and ribbon-like with the sides more or less parallel, twisted or slightly bent, posterior end usually gradually tapering and ending in a tail which is sometimes slightly deflected towards one side and the pellicle being beaded with square, rectangular or L-shaped brownish excrescences which are frequently closer and more marked ; paramylum links quite large ; body 150–199 × 20.5–27$\mu$.

*Habitat* : Rare in the plankton of locations 34, 92, 136 and 138.

The most accepted size of the species is 170–225 × 23–27.5$\mu$ though Lemmermann (1910a) gives it as 90–225 × 23–27.5$\mu$, Playfair (1921) as 160–250 × 18–36$\mu$ and

Prowse (1958) as 150–230 × 15–20μ. Kamat (1963, 1964) gives dimensions of 125–200 × 22–30μ and 100–155 × 30–33μ respectively in his Kolhapur and Bombay materials.

Lemmermann also makes a distinction on the basis of length of flagellum, that of *E. fusca* being of body length and that of *E. spirogyra* up to only about one-fourth body length. In the author's material the flagellum could not be observed in *E. spirogyra* and in one instance of *E. fusca* where it was observed (Plate II, figure 11a) it was quite short. Gojdics (1953) states that not much reliance can be placed on length of flagellum to distinguish between the two species. Prowse (1958) and Rino (1972 p. 150) also report a small flagellum in *E. fusca.*

As in *E. spirogyra* the beading on the pellicle may be uniform or with 1–3 rows of weakly beaded rows between two strongly beaded rows or sometimes the beads in the same row may not be uniform (see Gojdics 1953). The author could not find these in his material.

According to Pringsheim (1956 p. 55) "*E. fusca* Lemmermann (1913 p. 130) is so near to *E. spirogyra* that it may be better to return to Klebs' (1883 p. 77) original suggestion of considering it as a variety of the latter".

Distribution in Indian region : Maharashtra (Kamat 1963, 1964, 1976) ; West Bengal, Orissa and Kerala (!).

**12. *Euglena oxyuris* Schmarda 1846**

Lemmermann 1910a p. 497 figure 16 (p. 483) ; 1913, p. 130 figure 207 ; Gojdics 1953 pp. 120–21, Plate 20 figure 1 a-d ; Huber-Pestalozzi 1955, p. 65 figure 42.

Body elongated and more or less flattened with 2–3 twists even when at rest ; anterior end rounded or truncate ; posterior end with a hyaline tail piece ; pellicle fairly thick and striated spirally parallel to the twists, the striae appearing as double lines (see Gojdics 1953) ; chromatophores small, discoid and numerous; nucleus ellipsoid and median ; paramylum usually two elongated links one in front and the other behind the nucleus, rarely more (up to 20) distributed fairly uniformly; eye-spot prominent ; flagellum up to about one-third body length. A fairly robust species which is on the average about ten times as long as broad with the cell (280–) 375–490 (–500)×30–46 (–61) μ.

The typical form was not observed in the author's collections. However, it has been reported from Burma (Skvortzov 1937; Skuja 1949), Maharashtra (Gonzalves and Joshi 1946 ; Kamat 1963, 1975) and Himachal Pradesh (Kamat 1968a). Kamat (1963) gives its dimensions as 280–300 × 30–35μ. From the figure of Gonzalves and Joshi it is about 420 × 38–42μ. On the basis of size, Bhatia's (1930) organism (148× 14–18 μ), Suxena's (1955) measuring 160–200 × 12–18μ and Kamat's *E. estonica* Mölder (Kamat 1967) measuring 120–170 × 15–20μ could be included under *E. oxyuris* var. *charkowiensis* (Swir.) Chu (see below). Skuja's (1949) *E. oxyuris* measuring 152–231 × 20–26 μ could also be a mixture of one or two varieties mentioned below rather than the typical form.

**var. playfairii Bourrelly 1949 (figure 12 a-b)**

Gojdics 1953 p. 122 ; Huber-Pestalozzi 1955 p. 66 ; = *E. oxyuris* var. *gracillima* Playfair 1921 p. 119 plate 3, figure 19

Differs from the typical species in being markedly delicate and narrow in proportion to its length, with the anterior end truncate or with prominent lips, posterior end with a fairly long tail which is straight, curved or slightly deflected to one side ; paramylum two links, one in front and the other behind the nucleus ; eye spot fairly big and more or less rounded; flagellum not observed ; body on the average about twelve times as long as broad with dimensions of 201–270 × 16–22.5μ ; tail alone 47–64μ.

*Habitat* : In plankton of locations 4, 6, 34, 61 (N.P. 27-rather common, August) 63 (N.P. 24, 26–28, 38–July, August, January, March, November), 73, 110, 116 and 132.

The organism in the author's collections was consistently narrow with dimensions of three individuals which could be measured being 200.6 × 16–17.8μ, 206 × 17–20.6μ and 270 × 22.5μ which was in agreement with Playfair's dimensions of 253 × 17μ.

In describing the variety *gracillima* Playfair stressed on the " gracile " (slender) nature of the body. Though the name is probably appropriate, it is not clear why Bourrelly preferred the name fa *playfairii,* the author having not consulted Bourrelly in original.

Distribution in Indian region : Assam, West Bengal, Orissa, Andhra Pradesh and Karnataka (!).

**var *charkowiensis* (Swirenko) Chu 1947 (figure 12 c-d)**

Chu 1947 pp. 125–28, figures 29–35 ; Gojdics 1953 p. 121, Plate 18, figure 2 a-b ; Prowse 1958 p. 146 figure 1 f ; = *Euglena charkowiensis* Swir. 1913 ; Huber-Pestalozzi 1955 p. 61 figure 37 ; Kamat 1961–62 p. 262 ; 1964 p. 9 ; = *E. oxyuris* f. *minor* Defl., 1924, pp. 1117-1118, figure 9 ; Philipose 1940 p. 194 Plate 6 figures 107–108; Huber-Pestalozzi 1955, p. 65; Hortobágyi 1969 p. 30 ; = *E. oxyuris* fa *charkowiensis* Bourrelly, 1949.

Differs from the typical species due to its smaller size, the body being on the average about 6 (–8) times as long as broad ; body 127–144 × 16.5–26μ, rarely 121 × 11.5μ or 135 × 18.5–36μ, the difference in breadth in the last being due to slight posterior inflation (figure 12c) ; tail alone 16–26μ ; paramylum two fairly large links.

*Habitat* : Planktonic ; very common in location 46 ; very common to very abundant (March-April), common (June, August), rather common (May, July September) and stray to rare in other months in location 151 ; rather common to common in locations 63 (N.P. 32–August), 89 and 127 ; stray to rare in locations, 4–6, 13, 18–20, 26, 29 (May-July), 33 (October), 34–35, 37–38, 40, 45, 51, 54–55 58, 61 (May, November) 63 (May, July-September), 72, 75, 81, 83, 86, 88, 93–95 100, 103, 106, 115–17, 119, 121, 127–33, 134 (June), 135, 136, 154 and 157.

Distribution in Indian region : Kashmir (Bhatia 1930 as *E. oxyuris,* 148 × 14-18 μ) ; Rajasthan (Kamat 1967 as *E. estonica* Mölder 120–170 × 15–20 μ) Gujarat (Kamat 1961–62 as *E. charkowiensis* Swir., 130–170 × 24–27 μ) ; Maharashtra (Kamat 1963, 1964, 125–150×22–30μ ; 1974 ; 1975, as *E. charkowiensis*) Andhra Pradesh (Suxena 1955, 160–200×12–18μ; Naidu 1966, 170–180×24–25μ) Uttar Pradesh, (Hortobágyi 1969, as f. *minor* Defl., 143–156 × 20–22μ) ; Assam

West Bengal, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka, Kerala and Tamilnadu (!).

Kamat's *E. oxyuris* (1963) from Maharashtra with dimensions of 75–300 × 15–30 (–50)μ could be a mixture of the typical species and a few other varieties.

There has been considerable confusion regarding the exact distinguishingfeatures, particularly the dimensions, between *E. oxyuris* and its varieties, and in a number of instances there have been overlapping dimensions. In 1949 Bourrelly made a comparative study of the species and its varieties, and on the basis of size created four forms, *viz.* fa *minima* Bourr. with dimensions of 74–86 × 6.5–10μ (see Gojdics 1953 p. 122), fa *charkowiensis* (Swir.) Bourr. (which is synonymous to var. *charkowiensis* (Swir.) Chu. 1947 with dimensions of 125–150 × 20–27μ ; fa *estonica* (Mölder) Bourr. (= *E. estonica* Mölder 1943) with measurements of 160-230 × 16–22μ ; and fa *playfairii* Bourr. with dimensions of 247–290 × 20–30μ.

Gojdics (1953) is of the opinion that since Bourrelly created these forms on the basis of size alone, the last variety is superfluous. Further Gojdics (p. 125) retains *E. estonica* as a separate species on the basis that the paramylum is in the form of two small round bodies.

In the opinion of the author, *E. estonica* is very similar to *E. oxyuris.* In r ich collections of *E. oxyuris* var. *charkowiensis* the author has come across individuals with the paramylum very much reduced in size or sometimes there is only one paramylum. It is just possible that the smaller spherical paramylum in *E. estonica* could be due to improper development. The author is in favour of suppressing this species and merging it partly with var. *charkowiensis* and partly with var. *playfairii* of *E. oxyuris.* Thus, three varieties may be recognised as follows :

(1) var. *minima* Bourrelly 1949 ( = *E. charkowiensis* Swir. f. *minor* Skvortz 1925 ; *E. oxyuris* var. *minor* Prescott 1945 (see Gojdics 1953 p. 121) with size 55.5–86 × 6–10 (15–18)μ, the body being about nine times as long as broad

(2) var. *charkowiensis* (Swir.) Chu. 1947 with cells (90–) 103–172 (–200) × 16.5–28 (–30)μ ,the body being about 6(–8) times as long as broad. Most common size 125–150 × 16.5–27μ ;

(3) var. *playfairii* Bourrelly 1949 with cells 201–270 (–290) × 16–22.5 (–30μ) with a length-breadth ratio of about twelve (rarely up to fifteen). Tail (47–64μ) also relatively longer in this variety.

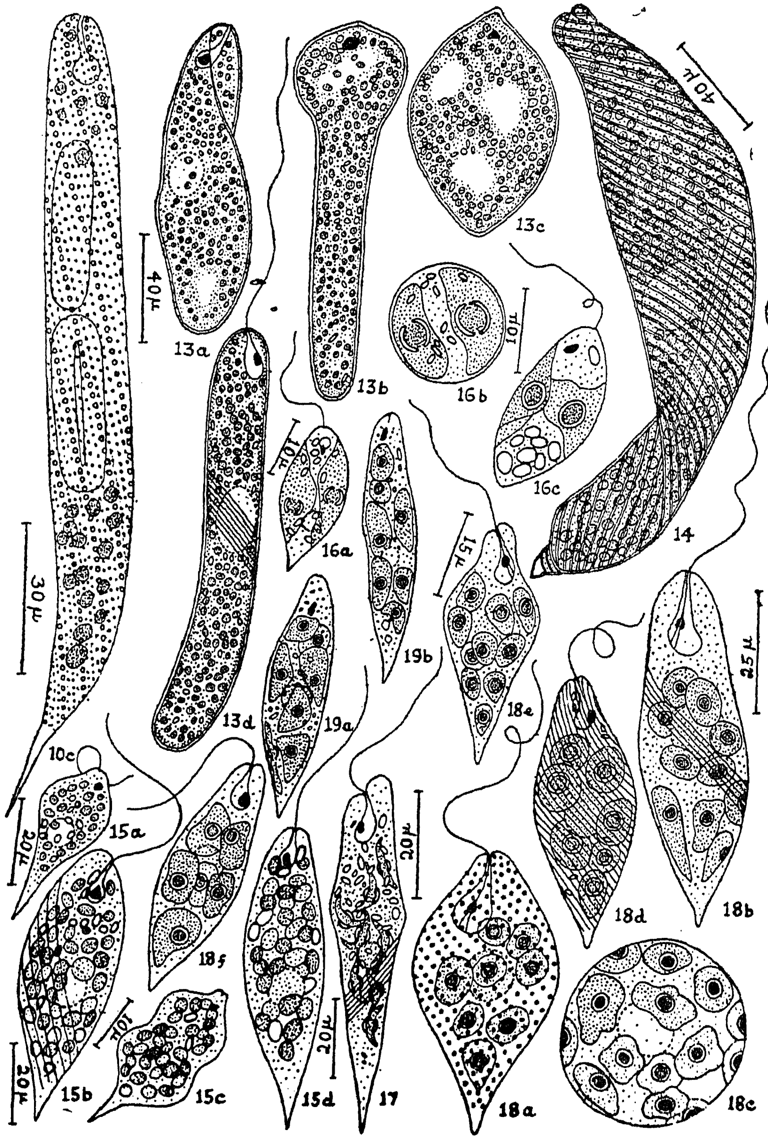
The typical species (with two or more paramylum) will be left with dimensions of (280–) 300–490 (–500) × 30–46 (–61)μ with a length to breadth ratio of about ten. There would still be a little bit of overlapping, but it would be much less.

Kamat (1961–62) gives the dimensions of 80–90–100 × 12–13μ for his var. *minima* Bourr. recorded from Ahmedabad.

## II Group LENTLFERA Pringsheim 1956

### 13. *Euglena Srinagari* (Bhatia) Huber-pest 1955 (figure 13 a—d)

Huber-Pestalozzi 1955 pp. 58–59 figure 32 ; = *Amblyophis srinagari* Bhatiat 1930, p. 363 figure 1 ; *Euglena amblyophis* (Ehr.) Playfair 1921 p. 118 Plate 3) figures 10–11; As *E. ehrenbergii* Klebs–see Philipose 1940 p. 193 Plate 6, figure 124.




**Figures 10 c, 13–19. 10 c.** *Euglena spirogyra* Ehr. **13 a–d.** *E. srinagari* (Bhatia, Huber-Pest. **14.** *E. pseudoehrenbergii* sp. *nov.* **15 a–d.** *E. proxima* Dang. **16 a–c.** *E. pisciformis* Klebs. **17.** *E. granulata* (Klebs) Schmitz. In fignre **17** striae acfully from right to left. Cell accidentally reversed while transferring. **18 a–c.** *E. caudata* Huebner. **18 d–f.** var *minor* Defl. **19 a–b.** *E. gracilis* Klebs. [Same magnification :—[(10c, 15b, 15d) ; (13 a–d, 14) ; (15c, 16a) ; (18 d–f, 19 a–b; (15a) ; (16 b–c) ; (17, 18 a–c)].

Body flat to cylindrical and rounded at both ends with the anterior end frequently narrower ; metaboly by bulging in the middle or rarely at the anterior end, often shortening to about half its normal length and also by twisting once along the longitudinal axis ; chromatophores numerous small and discoid, without pyrenoids ;

nucleus usually ellipsoid, rarely oblong, and median or sub-median ; paramylum many, small and spherical to ovoid or small rod-shaped bodies ; eye-spot fairly small and by the side of the reservoir ; flagellum up to 1/3–3/4 body length, pellicle fairly thick and usually with fine close spiral striae which are sometimes not visible ; body 116–160 × 15–40μ, but in shortened individuals it could be 79 × 52μ.

*Habitat* : Rather common in plankton of locations 61 (N.P. 8 and 13–May and August), 67, 82 and 106 ; stray to rare in locations 17, 34, 35, 53, 57, 61 (N.P. 6,8–May), 63 (N.P. 32–July), 68, 73, 76, 92, 127, 133 and 151 (January-December).

Gojdics (1953 pp. 108–10) considers Bhatia's organism as synonymous to *E. ehrenbergii* Klebs. Playfair's *E. amblyophis*, which is inadequately described has also been considered by most authors (Gojdics 1953 ; Pringsheim 1956 p. 133 as synonymous to *E. ehrenbergii.*) While treating *E. srinagari* as a separate species Huber-Pestalozzi (1955) states that it is either near or the same as *E. ehrenbergii*

However, *E. ehrenbergii* as reported from Europe, America and a number of other areas, has invariably one to ten additional long straight or slightly curved paramylum rods, or, as in Swedish material (see Skuja 1948 p. 192 Taf. 23 figures 7–8) stout solid rods, though occasionally these rods may be absent (see Huber-Pestalozzi 1955 pp. 69–70). In Skuja's material the chromatophores were fairly large and lenticular and the eye-spot fairly large (10–12 × 10μ) and in the form of a curved plate. In material from a London pond observed by the author (1948 Plate 2, figures 41, 48) the chromatophores were small, eye-spot small or large and there was one additional paramylum rod by the side of the nucleus. The range of size given by various authors (including Lemmermann 1913 ; Johnson 1944 ; Gojdics 1953 ; Huber-Pestalozzi 1955) fall within 107–400 × 13–48μ. It would thus appear that *E. ehrenbergii* as conceived by most authors is an extremely variable species. This species (with five paramylum rods) has been reported only once from India (see Gonzalves and Joshi 1946 Plate 5, figure 7) without any accompanying description.

Though the author observed the organism resembling *E. ehrenbergii* in several respects from a number of localities in north-east and south India, surprisingly in none of the individuals he could observe long paramylum rods. Chromatophores and paramylum were always small and discoid, the eye-spot fairly small and the striae when observed were fine and close. With usual dimensions of 116–160 × 15–40μ it came near Bhatia's *Amblyophis srinagari* (114–37μ) and Prowse's (1958 p. 145) *E. ehrenbergii* from Malaysia (also without paramylum rods and 110–200 × 15–20μ). Playfair's (1921) organism though slightly larger (200–300 × 20–25) μ and described as without paramylum resembled *E. srinagari* better than *E. ehrenbergii.* Though Playfair stated there was no flagellum his figure indicated a very short one. Bhatia also figured a very short one. In the author's specimens it was about 1/3 to 3/4 body length. " Metaboly " was also quite marked in the author's material in contrast to limited metaboly in typical *E. ehrenbergii.*

The author is, therefore, of the opinion that organisms resembling *E. ehrenbergii* but without long paramylum rods and some other associated characters are better treated as *E. srinagari* (Bhatia) Huber-Pest.

Distribution in Indian region : Kashmir (Bhatia 1930) ; Assam, West Bengal, Orissa, Andhra Pradesh, Karnataka and Kerala ( ! )

**14. *Euglena pseudoehrenbergii* sp. nov.**

Corpus elongatum applanatumque, latitudine varians ; extremitas anterior manifeste augustata rotundataque, posterior necnon late rotundata, caudam conicam brevem crassam habens ;metabole tortu effecta ; pellicula strias duplices acque crassas praebens ; cauda necnon partim strias habens, estremitate ipsa, autem, levi hyalinaque ; chromatophora parva, multa, disciformia ad lenticularia, sine pyrenoidibus ; nucleus medius, ellipsoideus ad fere sphericum ; paramylum parvum, virguliforme ad discoideum, in cytoplasmate dispersum ; duae additicae virgulae paramyli longae solidaeque iuxta nucleum sitae ; fossa fere spherica, canali brevissimo ; stigma flagellumque non observata ; cellula 228–260 × 19–51 $\mu$m, cauda ipsa 8–10$\mu$m long, striae 8–9 per 40 $\mu$m.

*Habitatio* : Species in plancto loei 112 rara. Iconotypus : Plate III, figure 14.

Body elongate and more or less flattened with varying breadth ; anterior end markedly narrowed and rounded ; posterior end broadly rounded and with a short stout conical tail ; change of shape by twisting ; pellicle with uniformly thick smooth striae which appear as double lines ; tail also partly with striae, the very tip being hyaline and clear of striae; striae spiral from upper left to lower right ; chromatophores numerous, fairly small, disc-shaped to lenticular and without pyrenoids ; nucleus (18.5–27.5$\mu$) ellipsoid to nearly spherical and median ; paramylum small, rod-like to discoid distributed in the cytoplasm ; two additional elongated solid rods (43–48 × 4.5–5.5$\mu$) by the side of the nucleus ; reservoir nearly spherical and with a very short canal ; eye-spot and flagellum not observed ; cell 228–260 × 19–32–51$\mu$ with tail alone 8–10$\mu$, long ; striae 8–9 in 40$\mu$.

*Habitat* : Rare in plankton of location 112 (Market Pond, Kakinada), Plate III. figure 14 to serve as the type.

The organism bears a close resemblance to *E. ehrenbergii* in shape, size and general structure but differs in having a short stout conical tail and its uniformly smooth double striae. It is also somewhat like *E. subehrenbergii* but in the latter the hind end is truncate and hollowed out, the striae are fine and punctate the paramylum is in ovoid-hexagonal bodies and there are no additional paramylum rods. The chromatophores in the present organism are also slightly smaller.

In the presence of striae in double lines the organism resembles three other species, *viz. E. oxyuris* Schmarda, *E. heimii* Lefèvre and *E. mesnili* Defl. *et* Dusi. Though the striae in *E. oxyuris* may be in double lines (see Gojdics 1953, p 120), it is a well defined species with two or more paramylum links and a distinct hyaline tail piece. *E. heimii* Lef. 1934 (see Gojdics 1953 p. 177 Plate 35 figure 13 ; Huber-Pestalozzi 1955 pp. 74–75, figure 52) has thick striae alternating with thin ones, has the general appearance of a *E. ehrenbergii*, has one long straight or curved paramylum rod and is 140–180$\mu$ in length. Though the hind end is described as bluntly or obtusely rounded, figures 52 d and g reproduced by Huber-Pestalozzi suggest a very small conical point or tail ; Both Gojdics (1953) and Pringsheim (1956) consider *E. heimii* as synonymous to *E. ehrenbergii*. The present organism

is essentially different from both these species. *E. mesnili* Defl. *et* Dusi (see Gojdics 1953 p. 88 Plate 7, figure 4) having thick striae alternating with delicate ones or with a delicate striae after several marked ones, has been shown by Pringsheim (1956) to be really a *E. deses* with which the present organism has no resemblance.

The Kakinada organism is, therefore, considered a new species allied to *E. ehrenbergii* under the name *E. pseudoehrenbergii.*

Distribution in Indian region : Andhra Pradesh ( ! ).

Kamat (1963,1974) recorded *E. heimii* from Maharashtra. The dimensions of the Kolhapur organism were 75–110 × 30–45$\mu$.

**15. *Euglena proxima* Dangeard 1901 (figure 15a–d)**

Lemmermann 1910a pp. 498–99 figure 7 (p. 483) ; 1913, p. 129 figure 193; Gojdics 1953 p. 90 Plate 7 figure 7 a–b ; Huber-Pestalozzi 1955 p. 86 figure 64 Pringsheim 1956 p. 62 figure 8 ; Prowse 1958 p. 143 figure 1c; Hortobágyi 1969 p. 30 figure 53.

Body fusiform, somewhat blunt towards the anterior end and tapering gradually to a hyaline point at the posterior end ; changing shape markedly by contraction or bulging without twisting and easily rounding up on irritation ; pellicle with fine spiral striae ; chromatophores lenticular to ovoid and larger than in *E. srinagari* and *E. pseudoehrenbergii* and without pyrenoids ; chromatophores generally absent at the posterior and sometimes at the anterior ends ; paramylum numerous, ovoid to short-cylindrical and almost the size of the chromatophores or sometimes smaller ; nucleus nearly spherical and usually slightly below the median region ; eye-spot elongate, disc-like or nearly triangular; flagellum 1–1½ times body length ; cells 39–83 × 11–22.5$\mu$.

*Habitat* : One of the commonest species found in almost all collections, forming a thin green scum when in abundance, or in the general plankton. Abundant at locations 29 (February 61 (N.P. 9–May)) and 66 (January); Rather common to common in locations 36, 39, 42, 43, 56, 77, 78, 88, 93, 100, 122, 143 and 151 (April and November and stray to rare in other months) ; stray to rare in other localities.

The usual dimensions of this species is 60–93 × 18–25$\mu$. However, Pringsheim (1956) states that there are two size groups with averages of 50 and 60$\mu$ in length, though it is difficult to measure the organism on account of its marked ' metaboly '. Hortobágyi (1969) gives dimensions of 80–90 × 14–16$\mu$, and Prowse (1958) 45–60 × 15–20$\mu$ in his Malaysian material. The smaller paramylum are described by most authors as links. However, the author could not make out links in his material.

Naidu (1962, p. 88, figure 1) describes and figures *E. proxima* (85–90 × 26–30$\mu$) as having two ovoid quite large paramylum bodies, one in front and the other behind a small central spherical nucleus. Obviously this is not a *E. proxima.* The figure (Plate IV, figure 7) given by Gonzalves and Joshi (1946) for this species is also misleading since the striae are shown as punctate and there is only just one paramylum link.

**Distribution in Indian region : Maharashtra (Gonzalves and Joshi 1943 a, 1946 : Kamat 1968, 1974) ; Uttar Pradesh (Hortobágyi 1969); Assam, West Bengal,**

Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka, Kerala and Tamilnadu (!).

**Group III CATILLIFERAE Pringsheim 1956**

**16.** ***Euglena pisciformis*** **Klebs 1883 (figure 16a-c)**

Lemmermann, 1913 p. 125 figure 182 ; Huber-Pestalozzi 1955 p. 41 figure 15 ; Pringsheim 1956 pp. 67–70 figure 12 ; including var. *minor* Hansgirg ; = *E. agilis* Carter 1856 ; Gojdics 1953 pp. 133–36 Plate 1 figure 5 a-e ; Prowse 1952 p. 108 Plate 1, figure h-i ; Naidu 1966 p. 26 figure 7–8.

Body nearly cylindrical with the anterior end rounded and the posterior end rather blunt and pointed ; changing shape by contraction and bulging and rounding up on irritation ; chromatophores two (rarely three) elongated laminate plates, each with a pyrenoid sheathed by paramylum caps ; additional ovoid to short rodlike paramylum in the cytoplasm ; nucleus more or less spherical and in the posterior half ; eye-spot streak-like ; flagellum 1–2 times body length; striae of pellicle not observed ; cells 16–35 $\times$ 7–11$\mu$.

*Habitat* : In plankton or as a green scum when abundant, in locations 28 (April), 29 (common–February ; September–November ; rare–April-May), 33 (April), 62 (common), 125 (rare) and 151 (rather common–December, February, March; nil–May–July or August ; stray to rare-January, August ; common to very common : April ; very abundant-September to November).

Though Carter was the first to describe the species as *E. agilis* his description was considered as inadequate. In 1883 Klebs gave proper description and figures for the species under the name *E. pisciformis* and ever since this name was widely used. Gojdics (1953) revived the name *E. agilis*. However, according to Pringsheim (1956) the long usage of the specific name *pisciformis* and the wrong use of the name *agilis* by Baker in 1926 for another species, *viz E. gracilis* coupled with Carter's inadequate diagnosis warrant the continuation of the name *pisciformis* instead of *agilis*.

Distribution in Indian region : Maharashtra (Carter 1856 ; Hansgirg 1902–as *E. agilis*) ; Andhra Pradesh (Naidu 1966) ; Kerala (Suxena *et al* 1973), Karnataka (Dodkundi *et al* 1973) ; West Bengal, Orissa, Andhra Pradesh and Tamilnadu (!) ; Burma (Skuja 1949).

Naidu (1966) has also recorded var. *piriformis* (Szb.) Gojdics of *E. agilis* from Cuddapah, Andhra Pradesh. Since the name *pisciformis* is more acceptable, this taxon will have to remain in its original name of *E. pisciformis* var. *piriformis* Szabados (measuring 16 $\times$ 12$\mu$ for which Naidu gives dimensions of 25 $\times$ 8$\mu$ compared to 21–28 $\times$ 9–10$\mu$ in his typical species).

**17.** ***Euglena granulata*** **(Klebs) Schmitz 1884 (figure 17)**

Gojdics 1953 pp. 137–39, Plate 24, figure 4 a-g ; Pringsheim 1956 pp. 72–74 figure 14 ; Prowse 1958 p. 148 figure 1m; = *E. granulata* (Klebs) Lemmermann, 1910a p. 501 ; 1913 p. 131 figure 211 ; Huber-Pestalozzi 1955 p. 85 figure 63.

Body fusiform with changes in the ratio of length to breadth caused by bulging slightly in front or behind the middle ; anterior end more or less conical ; posterior

nd double-narrowed and ending in a short nearly cylindrical hyaline tail which s frequently bent to one side ; pellicle with markedly spiral striae ; chromatophores arge, about 6–12, and more or less polygonal with slightly lobed margin and ppearing somewhat spindle-shaped to elongate in side view ; anterior end generally free of chromatophores but at posterior end they may well extend to the audal tip ; each chromatophore with a pyrenoid having double sheaths of saucer-haped paramylum ; additional small ovoid to slightly elongated paramylum odies frequently present in the cytoplasm ; nucleus spherical and median to sub-nedian ; cell contents highly granular ; eye-spot small ; flagellum less than body ength ; cells 50–65 × 11–14$\mu$.

*Habitat* : Rare in plankton of locations 5, 29 (April), 39, 73, 109, 110, 134 and 151 April, August) and 154 ; common in location 65.

According to Pringsheim (1956), though the species is reported very often it is iot certain whether the identification has always been correct. Further, he states hat apart from the shape of the cell with double narrowing at posterior end,the nost reliable feature for identification of the species is the regular spiral rows of ub-cuticular mucus bodies along the striae which do not always show without reatment with vital stains, though Klebs' reason for giving the name "granulata" for his *E. velata* var. *granulata* which is synonymous to *E. granulata*) may have een due to the highly granular nature of the cytoplasm. Johnson (1944) has also ecorded sub-cuticular granules parallel to the striae in this species. Pringsheim ilso states that the flagellum is not more than body length though some authors tate it is longer.

In the author's material the flagellum when observed was shorter than the body. The spiral rows of mucus bodies could not be observed clearly since no vital staining could be done. However, the shape of the cell together with the elongated chromato-phores having sheathed pyrenoids and the granular nature of the cytoplasm pointed to the organism as being an unmistakable *E. granulata*.

Pringsheim (1956) observed two size groups, *viz.* 80–105 × 16–24$\mu$ and 60–83 × 18–25$\mu$ in his strains. Sizes reported by other authors (including Chu 1947) vary from 50–115 × 11–30$\mu$. Pringsheim states that Szabados's organism (37–77 × 20–25$\mu$) is either a smaller variety or it may be an instance of wrong identity. As already stated under *E. vaginicola*, Szabados has reported flask-shaped cysts in this species.

Distribution in Indian region : Assam, West Bengal, Madhya Pradesh, Orissa, Andhra Pradesh and Tamilnadu (!).

### 18. *Euglena caudata* Huebner 1886 (figure 18a–c)

Lemmermann 1913 p. 133 figure 198 ; Chu, 1947, pp. 110–12 figure 21 ; Gojdics 1953 p. 137 Plate 24, figure 2 and Plate 29, figure 3 ; Huber-Pestalozzi 1955 p. 88 figure 67 ; Pringsheim 1956 pp. 74–75 figure 15 ; Prowse 1958 pp. 147–48 figure 1.

Body spindle-shaped with the anterior end drawn out or nearly cylindrical ; posterior end narrowing strongly into a short tail (the name "caudata" being derived from this feature) ; pellicle with fine spiral striae ; regular rows of coloured granules or small spheres seen at wider intervals along the striae (usually along

alternate striae) on vital staining ; chromatophores about 8–10–15 or more, parietal and disc-like with a smooth or slightly lobed margin, the posterior ones being usually elongated, and with a sheathed pyrenoid ; additional small paramylum bodies frequently present ; nucleus nearly spherical and median ; eye-spot prominent ; flagellum $\frac{3}{4}$ to $1\frac{1}{2}$ times body length ; cells 50–73 × 19–26$\mu$. *Habitat* : As a fairly thick scum or in the plankton after overnight heavy rains in location 151 (October 1938).

The organism from Madras agreed fairly well with the typical species (see Pringsheim 1956) but the tail was slightly shorter. Also, in contrast to Pringsheim's material in which the chromatophores were saucer-shaped and lobed, they were disc-like to saucer shaped (the posterior ones being elongated) with smooth or slightly lobed margin in the author's material. Further, Pringsheim stated that the chromatophores extended to the anterior and posterior ends giving the entire organism a green appearance. In the Madras specimens the posterior elongated ones extended to the hind end whereas the front end was usually free of them. The number of chromatophores reported also vary. Huebner (as cited by Pringsheim) gave it as 50, Chu as 6–30 and Pringsheim as more than a dozen whereas in the author's material the maximum number noted was fifteen. Dimensions given by different authors also vary. The usual size is 80–120 × 20–38–50$\mu$ (see Huber-Pestalozzi 1955 and Pringsheim 1956). Chu's organism measured 70–115 × 10–39$\mu$, rarely 60–90 × 10–15–20$\mu$, the latter measurements being more like those of var. *minor* Defl. (63 × 18$\mu$) which is not recognized by some authors. The author's measurements were 50–73 × 19–26$\mu$ compared to 30–49 × 16–21$\mu$ in var. *minor* (see below). Naidu (1966) gave the dimensions of *E. caudata* as 55–77 × 15–17$\mu$.

Reports on flagellar length also vary. Huebner gave it as the body length, Chu twice the body length and Pringsheim less than the body length. As already stated, in the author's material it was $\frac{3}{4}$ to $1\frac{1}{2}$ times body length.

Chu (1947) observed osmophilic granules arranged somewhat spirally under the pellicle in his *E. caudata*, and he stated that *E. granulata* which has such granules is really synonymous to the former. According to Pringsheim (1956), though this feature is more characteristic of *E. granualta*, Chu's organism is really a form of *E. caudata* with such granules well developed, and he retains *E. granulata* and *E. caudata* as distinct species with well defined characteristics. The Madras material also showed the granules in some specimens whereas they were not clear in the majority of specimens. There is no doubt that the Madras form belongs to *E. caudata* because of its characteristic tail, chromatophores and other features as given by Pringsheim.

Distribution in Indian region : Andhra Pradesh (Naidu 1966) ; Maharashtra (Kamat 1975) ; Tamilnadu (!).

**Var. *minor* Deflandre 1924 (figures 18d–f)**

Deflandre 1924 p. 1119 figure 7 ; Huber-Pestalozzi 1955 p. 88 figure 67A.

Differs from the typical species in its smaller size ; posterior part ending in a sharp point or a very short tail ; pellicle striated with fine smooth spiral striae ;

hromatophores 6–16 and discoid to saucer-shaped, and they are larger when maller in number ; flagellum about ⅓ to ½ body length ; cells 30–49 × 16–21μ.
*Iabitat* : As a green scum in locations 29 and 30 (January)

Chu (1947), Gojdics (1953), Pringsheim (1956) and Prowse (1958) do not eparate this variety from the typical species. Since the author's specimens ppeared to be markedly different in size, it is retained under Deflandre's variety.
Distribution in Indian region : West Bengal (!).

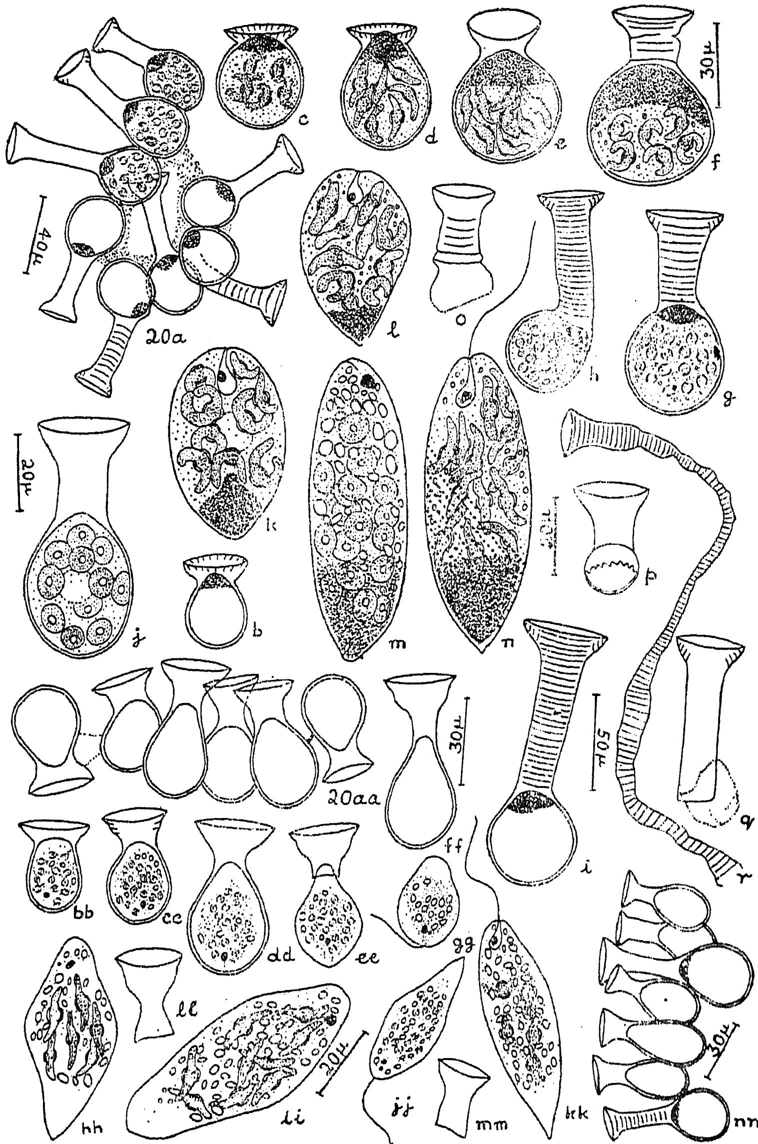
**9. *Euglena gracilis* Klebs 1883 (figure 19a–b)**

Lemmermann 1910a p. 502 figure 17 (p. 483) ; 1913, p. 133, figure 190 ; Gojdics 1953 p. 141 Plate 26 figure 1 ; Huber-Pestalozzi pp. 71–72 figure 48 ; Pringsheim 1956 pp. 81–83 figure 19

3ody elongate fusiform to cylindrical with very little difference between anterior nd posterior ends, the former being somewhat rounded and the latter bluntly ointed ; chromatophores shield-shaped to triangular, varying in number from –10 or 12 and with a sheathed pyrenoid ; with additional small paramylum bodies n the cytoplasm ; nucleus spherical and usually below median region but some-imes nearly median ; eye-spot streak-like ; pellicular striae very faint ; flagellum ot observed (reported to be body length) ; cells 36–47 × 8–13μ.
*Iabitat* : In plankton and scum of locations 30 and 31 (rare).
The dimensions given by various authors (including Prowse 1962) range from 1–68 × 6–18, rarely up to 22.5μ. Pringsheim states that the average length is ot far from 50, with 40 and 70μ as the limits. Kamat (1961–62) gives it as 60–75 × 6–30μ, the maximum breadth being particularly higher than in previous reports. Iowever, in the absence of figures it is not possible to say whether his organism s really a *E. gracilis.*
Distribution in Indian region : Gujarat (Kamat 1961–62) ; Maharashtra (Kamat 975) ; Karnataka (Dodkundi *et al* 1973 ; Hosmani and Bharati 1980, 1980a); Vest Bengal (!).

**0. *Euglena tuba* Carter 1869 *non* Johnson 1944 *emend* Philipose (figure 20a–rand) aa–nn)**

3ody more or less cylindrical or fusiform to ellipsoid rarely nearly spherical with he anterior end broadly rounded and the canal opening slightly towards one side iving it a lipped appearance ; posterior part ending in a sharp blunt point or with very short tail or more often broadly rounded ; changing shape markedly by ulging in the middle or curving at anterior end or by complete rounding up ; ellicle with fine punctate spiral striae ; chromatophores about 5–16 or more and sually in elongated broad bands or somewhat spindle-shaped ; in cysts and reshly liberated individuals often markedly curved with the free ends almost ouching each other ; when inside the cyst or markedly crowded in free living ndividuals, sometimes appearing as spheroid to discoid ; each chromatophore vith a double sheathed pyrenoid, the paramylum being saucer-like ; additional small isc-like paramylum frequently present in the cytoplasm ; nucleus spherical and nedian ; eye-spot fairly small ; flagellum about 1/3 to 1/2 body length, but easily hed ; cell usually with haematochrome pigments spread all over the cell during




**Figures 20a–r and 20aa–nn.** *Euglena tuba* Carter. **20a–r.** individuals with haematochrome. **20aa–mm.** Green individuals without haematochrome. **20nn.** a mixture of green and partly red individuals. **20a, 20aa and 20nn.** Clusters of cysts **20b–f, i–j, 20bb–dd and ff.** Cysts of various size with stalks of varying length and shape **j and m** with discoid chromatophores, the former killed in iodine-formalin; **ee.** liberation of cell from cyst. **20k, l–n, gg–kk.** freshly liberated individualls; **o–q, and ll–mm.** broken remains of cysts. **20r.** an abnormally long stalk found associated with the organism. [same magnification :—(20a and 20nn) ; (20b–i, k–m, o–q, 20aa–gg, jj, ll–mm) ; (20j) ; (20n, hh, ii and rr) ; (20r)].

bright sunshine giving it a brick red appearance or receding to the hind end at other times giving it a dirty green colour ; haematochrome at the hind end in encysted individuals ; cyst in the form of a round-bottomed flask with a tube-like prolongation (stalk) which ends in a funnel-like expansion (base) ; tube frequently striated transversely, short or sometimes quite long, and rarely irregularly jointed ; funnel also some-times with bristle-like hairs at the rim ; a number of cysts may remain together either in a mucilaginous matrix with the tubes projecting all round or attached to each other by mucilaginous pads on either side of the flask, forming an irregular linear row ; encysted organism always with the hind end towards the stalk and the front end towards the bottom of the flask ; very rarely haematochrome absent altogether in encysted and free living state ; flagellum absent in encysted condition ; liberation of the organism by the rupture of the flask near its middle or nearer the stalk or by gelatinization of the flask wall, the flagellum (at first short) appearing shortly after liberation and progressively becoming longer ; locomotion by slow swimming when with flagellum or by slow motion accompanied by change of shape when without flagellum ; cells usually 60–74–96 × 18–30$\mu$ or larger, but in nearly spherical ones 41.5–56 × 36–43$\mu$ ; cysts 34–98$\mu$ long with flasks 31–47 × 26–47$\mu$, stalks up to 34–70$\mu$ or longer and 13–18$\mu$ broad ; base 29–32$\mu$ in diameter.

*Habitat* : As a red or green scum (neuston) or in general plankton and bottom silt, abundant to very abundant throughout the year except during periods of heavy rainfall in locations 24, 32, 66, 151 and in a number of ponds in locations 61 and 63 ; also abundant in locations 7, 51, 60, 69, 72, 73, 80 and 116 ; common in locations 84, 123, 135 and 139 ; also observed in locations 8, 9, 21 (December) 45, 50, 104, 113 and 149. It was frequently associated with *E. sanguinea* (including *E. rubra*) in location 151, and with *E. sanguinea*, *E. viridis* var. and *E. tuba* var. *pseudotuba* f. *minima* (see below), and rarely with *E. proxima* and *E. hemichromata* in location 61.

Though the organism observed in various localities had haematochrome invariably, in two collections from N.P. 12 (location 61) made on 5–12–53 and 14–12–53 it appeared as a pure green scum during the bright hours of the day after fairly heavy rains overnight. In this material, except for very stray individuals with a trace of haematochrome, none of the individuals had the pigment so that the internal contents including the eye-spot could be seen clearly (see figures 20 aa-nn). However, the stalk of the cyst was usually shorter (up to 33$\mu$) and 8.5–14 rarely up to 16$\mu$ broad just above the flask, and with greater bulge below the base. The cysts (33.5–61 × 22.5–34.5$\mu$ with base 18–31$\mu$) and the free living cells. (31–70 × 17–27.5$\mu$) were also on the whole smaller. However, they did not appear to be materially different from normal individuals.

According to Pringsheim (1956 p. 34) the general notion that species like *E. sanguinea* having red pigments may occur in a green state when well supplied with nutritive substances is confirmed by observations in cultures. Green species like *E. gracilis* might also produce red granules in a state of nutritive exhaustion. Probably this might apply to *E. tuba* also.

Even though *E. tuba* is one of the most common species occurring in a number of States in north-east, south and west India, and was first described by Carter in 1869 from Bombay, it is still a controversial species, partly due to Carter's description and figures being inadequate. Apart from the flask-shaped cyst with a tube-like

prolongation ending in a trumpet-like structure characteristic of the species, size of cell about 83 μ, largest cyst 41 μ, shape of cell spindle-shaped to cylindrical flagellum about 1½ times body length (as seen in his figure 13) and presence of haematochrome, he stated that the internal structure of cell was as in *E. viridis* with which it was associated. He could not also establish the real connection between the encysted organism and the free living individual. Further, he thought that the flagellum extended to the tube of the cyst, and with the tubes projecting down into the water, it served for aeration of the encysted organism. Kent (1881 p. 385) had gone to the extent of suspecting that the encysted organism of Carter may not even be connected with the free living one, and the former may not be a *Euglena* at all.

In 1908, Kashyap recorded a red species with flask-like cysts in a pond at Lahore, which he stated was like Carter's *E. tuba*, but his description of the organism as cylindrical to oval ending abruptly in a short tail, chromatophores small and round, paramylum numerous and of various sizes, flagellum body length, eye-spot large, cell 62 × 15μ to 125 × 31μ and pellicle faintly striated, did not help in knowing the exact nature of the organism he observed (though it might to some extent fit in with the structure of *E. tuba*), especially because it was unaccompanied by figures. Walton (1915) gave Kashyap's organism the name of *E. orientalis* based on his description. In all probability it could be a *E. tuba*.

Johnson (1944) described an encysted *Euglena* from Iowa which he wrongly referred to *E. tuba* Carter. Since Johnson's organism is slightly different from that of Carter, and the name cannot stand, Gojdics (1953 p. 159) renamed it as *E. pedunculata* (Johnson) Gojdics. However, the cyst of *E. pedunculata*, as figured in original by Gojdics, appears to be different from that of Johnson, and Johnson's and Gojdics' organisms may not be the same (see below).

Regarding *E. tuba* Carter, Gojdics (1953 p. 190) states that it is a doubtful species and it could very well be a *E. sanguinea*, *E. haematodes* or *E. orientalis* all of which possess haematochrome and may have stalked cysts. Pringsheim (1956 p. 139) is also of the opinion that *E. tuba* Carter is a *E. sanguinea*. Though Huber-Pestalozzi (1955 pp. 43–44, figure 17A a-i) retains Carter's species, his description is based on Johnson's *E. tuba* and mostly his figures. In fact, only figures 17A h-i (cysts) are those of Carter (as reproduced by Kent 1881). Further, he states that Carter's species is doubtful one and that Johnson's species being different, the latter is better named as *E. pseudotuba*.

Hortobágyi (1960) referred an organism occurring in the neuston of fish ponds of Hungary to *E. tuba* Carter. It was olive green, with smooth spiral striae and measured 55–81 × 20·5–32μ. The chromatophores were discoid, 9-numerous, of varying size and without pyrenoid or paramylum. However, he has not shown cysts in his figures.

After studying the organism described as *E. tuba* by Carter (1869) over a number of years and from many localities, and by making undisturbed mounts of the surface scums of the living organism, the author has been in a position to observe details not noted by previous workers, and to study the liberation of the organism from the cyst. Since the organism was invariably mixed with other red forms, *viz.* var. *pseudotuba* f. *minima* (see below) and *E. sanguinea*, observations including measurements were made only from individuals freshly liberated from cysts. As a result, the author is inclined to consider that Carter had actually mixed up *E. tuba*

and *E. viridis*, his figures 13 in plate 17 of an individual in active state having a pointed tail, long flagellum, etc., pointing more to a *E. viridis* than to *E. tuba*. For the reasons stated above, it appears that *E. tuba* Carter is actually a well defined species needing an emended description while the organism of Johnson (1944), which differs mostly in the shape of the cyst, may be considered a variety of *E. tuba* under the name var. *pseudotuba* (Johnson-Huber-Pestalozzi) comb. nov.

Distribution in Indian region : Maharashtra (Carter 1869) ; Andhra Pradesh ? (as *E. orientalis* Walton-see Naidu 1966–60–91 × 18–23μ) ; Assam, West Bengal, Orissa, Andhra Pradesh, Karnataka, Kerala and Tamilnadu (!) ; Pakistan ? (as *Euglena* sp.—see Kashyap 1908 ; = *E. orientalis* Walton 1915).

**var. *pseudotuba* (Johnson-Huber-Pest.) comb. nov.**

= *E. tuba* Johnson 1944 p. 120, figure 22 A–H, *non* Carter 1869 ; = *E. pseudotuba* (Johnson) Huber-Pest., 1955 p. 44

Structure of body and other details more or less as in *E. tuba* Carter with the differences that : (1) the cyst is conical (like a conical flask) to nearly cylindrical to vase-shaped with the stalk almost absent and with a broad rim-like base ; (2) a number of cysts often remain together with their bases almost fused (see Gojdics 1953, p. 139, based on Johnson's personal communication) ; (3) reservoir (spherical) and eye-spot relatively larger ; (4) nucleus below median region ; (5) flagellum nearly body length ; (6) pellicular striae delicate, but not punctate, and (7) cells large, *viz.* 87–130 × 17–27μ, with cysts 34–50μ.

In Johnson's organism also the chromatophores were frequently discoidal (in old laboratory cultures) as in the typical species.

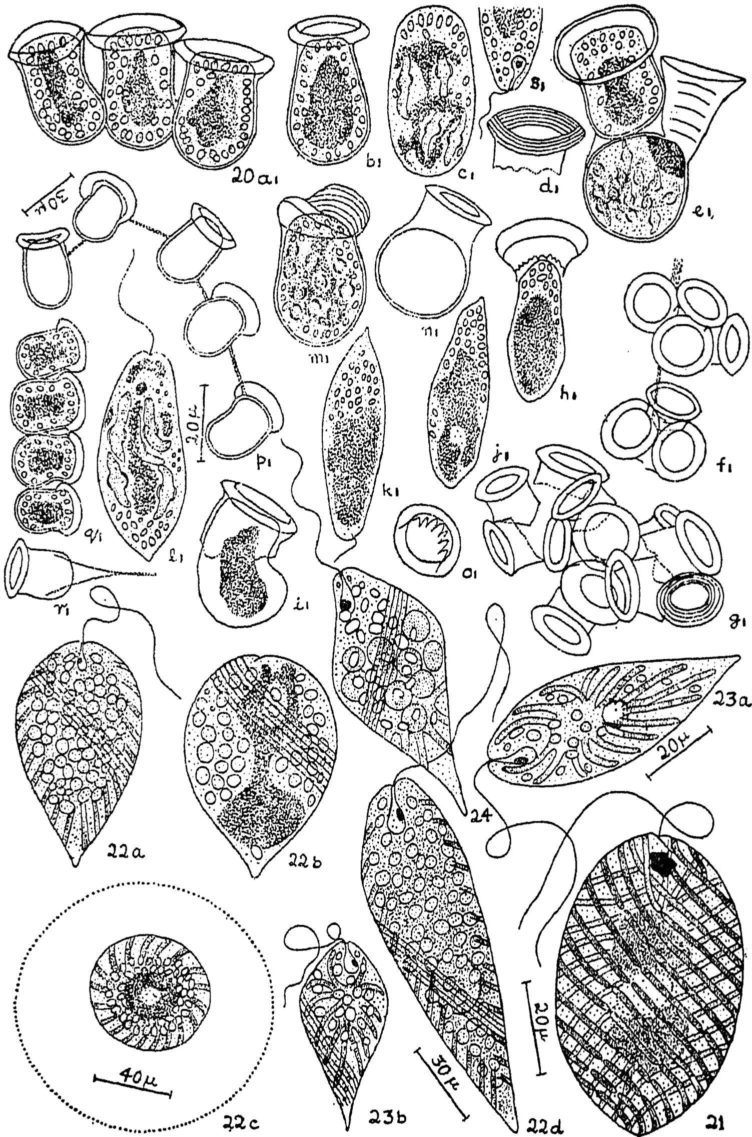
This variety is known only from N. America.

**f. *minima f.* nov. (figure 20a1–s1)**

Forma a var. *pseudotuba* differens ut minor (cellula 45–82·5 × 17·5–37·5μm, atque cysta 35–60 × 24–39μm, basi 29–44μm, diam.) ; extremitas posterior cellulae puncto obtuso aut minuta cauda nodiformi, aut late rotundata terminans ; chromatophora solum c. 5–10 ; paramylum numerosius, maius et in cellula encystata grana paramyli prope extremitatem posteriorem in ordinibus transversis uno, raro doubus, disposita ; flagellum c. dimidium longitudinis corporis ; cystae in corymbis irregularibus aut ordinibus linearibus confertis saepe aggregatae, aut per fila mucosa inter so lateraliter connexae ; basis cystae saepe transverse striata.

*Habitatio* : Plantae ut spuma in stagnis 15 et 16 loci 61.

Differs from var. *pseudotuba* in its smaller dimensions (cell 45–56–82·5 × 17·5–31·5–37·5μ and cysts 35–60 × 34–39μ with base 29–44μ in diameter) ; reservoir and eye-spot correspondingly smaller ; hind end of cell ending in a blunt point or a tiny knob-like tail or sometimes broadly rounded ; paramylum more in number, larger and in encysted cell those near hind end arranged in one (rarely two) transverse rows ; flagellum about half body length ; cysts frequently grouped together in irregular clusters or close linear rows or connected laterally by mucilaginous threads ; base of cysts frequently striated transversely.




**Figures 20–24. 20a1–d1, f1–s1.** *Euglena tuba* var *pseudotuba* (Johnson-Huber-Pest) f. *minima* f. *nov*. **20e1.** *E. tuba* and var *pseudotuba* f. *minima* associated together ; **20a1, q1.** Cysts showing linear arrangement **20p1.** same with lateral attachment by mucilage threads ; **20f1–g1.** Clusters of broken remains of cysts **20r1.** One cyst with bifurcation of mucilage thread before attachment **20h1–i1.** Liberation of organism from cyst ; **j1–k1, l1.** Freshly liberated cells ; **s1.** anterior end of a cell showing reservoir, eye-spot, flagellum, etc. ; **m1–n1.** two abnormal cysts **d1, o1.** broken remains of cysts, **21.** *E. oblonga* Schmitz. **22 a–d.** *E. sanguinea* Ehr. **22 c.** showing cyst. **22 b.** showing haematochrome along axial region (note chromatophores not shown.) **23a–b.** *E. viridis* Ehr. var. *maxima* var. *nov.*; **24.** *E. hemichromata* Skuja. Same magnification —(20a1–b1, d1–r1–k1, m1, s1.22a, b and d, 23b) ; (20f1–g1, o1–r1, n1 ; 22c) ; (20c1, l1 ; 23a, 24, 21.)

*Habitat* : As a scum in ponds 15 and 16, location 61 (October-December and January-February).

In the encysted condition the haematochrome was restricted to the axial region in contrast to the hind end in *E. tuba.* The nucleus and pellicular striae could not be made out due to the haematochrome. Liberation of the organism either by a break near the base (the remains of the cyst showing a toothed appearance just below the base-see figure 20 c1) or in the median region of cyst. Though the cells usually measured 56–82·5 × 17·5–31·5μ, those which were slightly twisted at anterior end and bulged in the middle measured only 45–55 × 33·5–37·5μ. As in *E. tuba,* the flagellum which appeared only after liberation of cell became progressively longer for some time.

The organism was invariably mixed with *E. tuba, E. sanguinea, E. viridis* var. and sometimes with *E. proxima* and *E. hemichromata.* In one or two instances, a cyst of the form was attached to the cyst of *E. tuba* (see figure 20 e1). It could not be ascertained whether this was accidental or otherwise. Gojdics(1953 p.140) also observed one individual of *E. pedunculata* attached to a copepod.

From Johnson's figure 22f (of a broken cyst) and 22 g (of a cyst) there appears to be no doubt that the Indian form is more or less identical to the American organism except for differences in dimensions and other minor details.

Distribution in Indian region : Orissa(!).

It appears to the author that *E. pedunculata* Gojdics (1953 pp. 139–40 plate 25-figure 1 a-e) which she created in the place of Johnson's *E. tuba,* is actually somewhat different from Johnson's organism. It has thin-walled cysts which are like round-bottomed flasks with a fairly long uniformly broad stalk and a broader base (as figured in original by Gojdics) ; cells are liberated by a break on the side of the cyst and not by a break along the median region, and the free living cells are somewhat fusiform with a very gradually tapering posterior in contrast to the nearly cylindrical cell with slight posterior extension or rounded hind end in Johnson's material. Probably, it could be considered as another variety of *E. tuba* under the name var. *pedunculata* (Gojdics) comb. nov. with dimensions 90–120 × 18–22μ.

## 21. *Euglena oblonga* Schmitz 1884 *emend.* Iyengar 1962 (figure 21)

Iyengar 1962 pp. 329–31, figure 9–31 ; = *E. oblonga* Schmitz 1884 ; Lemmermann 1910a, p. 494 ; 1913 pp. 127–28, figure 184 ; Gojdics 1953 pp.64–66 plate 2, figure 2 a-e ; Skuja 1948 pp. 186–88, plate 21, figures 16–19 ; Huber-Pestalozzi 1955 pp. 44–45 figure 18.

Body ovoid to ellipsoid to nearly oblong with the anterior end rounded and lipped ; posterior end rounded or ending in a short point ; chromatophores appearing as numerous short bands arranged peripherally in spiral rows ; pyrenoids and the two sheathing cap-like paramylum could not be made out due to dark cell contents particularly on either side of the nucleus ; nucleus large and median or slightly below ; eye-spot large and irregular in outline ; flagellum body length or slightly longer ; pellicular striae (reported as spiral) not observed ; cells 65–70 × 35–40μ.

*Habitat* : Rare in plankton of locations 6 and 32,

There is difference of opinion regarding the shape of the cell and the nature of the chromatophore in this species. The markedly ovoid shape reported by Schmitz and several others has been considered (see Gojdics 1953) as due to the organism being in the resting stage. Iyengar (1962) gives the shape as fusiform. In the author's material the cell was ellipsoid to nearly oblong. However, in foreign material observed by the author (Philipose 1948) most specimens in the abundant collection were distinctly ovoid with band-like peripheral chromatophores bearing distinct sheathed pyrenoids.

Lemmermann (1910a, 1913) stated that the numerous small chromatophores which lie peripherally continue radially. Later (1913) the same author referred to the chromatophores as star-shaped, probably following Schmitz. By suitable staining techniques, Iyengar (1962) showed that the chromatophores in the species are really stellate and saucer-shaped with 10–12 radiating arms and a central double-sheathed pyrenoid. The chromatophores numbering 16–25 are distributed in the periphery with their arms running radially and spirally along the surface. The organism measures 70–85 × 16–32$\mu$ with nucleus 13·5–15$\mu$ and paramylum 4·5–6 × 3–4.5$\mu$, and has strong spiral pellicular striae. The dimensions of the organism given by various authors range from 50–110 × 16–37$\mu$, the maximum breadth of 57$\mu$ given by Huber-Pestalozzi (1955) being probably a printing error.

Though the author's organism appeared in several respects like a *E. oblonga*, in the absence of all details, it is only tentatively assigned to that species. The organism was also slightly broader in relation to the length.

Distribution in Indian region : Tamilnadu (Iyengar 1962) ; Assam and West Bengal (!).

### 22. *Euglena sanguinea* Ehrenberg 1830 (figure 22a–d)

Lemmermann 1910a p. 494, figure 19 (p. 483) ; 1913 p. 128 figure 185 ; Gojdics 1953 pp. 154–55 plate 31, figure 3 ; Huber-Pestalozzi 1955 pp. 90–93 figure 70 ; Pringsheim 1956 pp. 89-94, figure 22 ; including *E. rubra* Hardy 1911 ; Lemmermann 1913 p. 128 figure 188 ; Philipose 1940 p. 192 plate 6, figure 120.

Body ellipsoid to spindle-shaped with the anterior end broadly rounded and the posterior part tapering gradually to a point or a short knob-like tail ; pellicle with clear spiral striae ; chromatophores band-shaped (ribbon-like) but broken at intervals and lying peripherally as well as radiating towards the inside, but less regularly arranged than in *E. oblonga* ; with sheathed pyrenoids ; chromatophores and pyrenoids frequently obscured by haematochrome, but the former invariably clear at the periphery and hind end ; additional small ovoid to discoid paramylum bodies present in large numbers in the cytoplasm ; eye-spot prominent and usually granulated ; nucleus spherical and median or slightly below, but frequently obscured ; flagellum usually longer than body ; cells 60–91–140 × 24–52, rarely up to 72$\mu$ ; cysts large, spherical and mucilaginous and up to 130$\mu$ in diameter ; encysted cells 48–62$\mu$ in diameter.

*Habitat* : As a brick red scum or in plankton, common in location 15, rare in locations 29 (October), 34, 35, 101, 151 and abundant in location 61 (N.P. 15 and 16, November-December).

Though this species has been reported by many workers from all over the world there is still a lot of confusion regarding its exact structure. This is because the cells are rarely free of haematochrome or the species is sometimes found in association with other red species. According to Pringsheim (1956) the chromatophores are not elongate spindles as described by Gojdics (1953) but actually ribbon-like with gaps here and there, and the ribbons are connected laterally by fine strands. However, the author could not make out these strands. Pringsheim also states that *E. rubra* Hardy with cells 200 × 60μ and *E. haematodes* (Ehr.) Lemm. (1910a p. 495) with cells 75–103 × 28–36μ, are in all probability only *E. sanguinea.* Forms resembling *E. rubra* observed by the author in localities 61 and 151 are also considered here along with *E. sanguinea.* The dimensions given by various authors for this species range from 55–121–150–170 × 22–33–55μ.

Distribution in Indian region : Maharashtra (Kamat 1974) ; Himachal Pradesh (Kamat 1968a) ; Karnataka (Hosmani 1977) ; Assam, West Bengal, Orissa and Tamilnadu (!). According to Biswas (1949 p. 101) the red and green scums of *Euglena* occurring in India and Burma are due to *E. sanguinea* and *E. viridis* respectively. However, he has not taken into consideration *E. tuba* (red or green) and other red and green species.

## IV Group RADIATE Pringsheim 1956

### 23. *Euglena viridis Ehrenberg* 1830

Lemmermann 1910a pp. 491–92, figure 2 (p. 483) ; 1913 p. 127 figure 189 ; Gojdics 1953 pp. 70–71 Plate 4 figure 1 a,b ; Huber-Pestalozzi 1955 pp. 45–47 figure 19; Pringsheim 1956 pp. 102–105 figure 26 ; Hortobágyi 1969 p. 31 figure 54.

Body fusiform with the anterior end broadly rounded and the canal opening slightly towards one side making it lipped ; posterior end more or less conical and ending in a point or in a small narrow cylindrical tail ; pellicle with faint spiral striae ; chromatophores ribbon-shaped and radiating towards the periphery from a pyrenoid centre situated above the nucleus ; nucleus nearly spherical and in the posterior part ; ovoid paramylum bodies found mostly around the central area of the chromatophore group, but some are dispersed in the cytoplasm ; eye-spot small, but bright red ; flagellum usually longer than body ; cells 30–65–73 (-89) × 9–22μ.

The typical species not observed by the author, but reported by Carter (1856, 1858, 1869) from Bombay, by Bhatia (1930) from Kashmir, by Banerji (1936) from lower Bengal, Singh (1960) and Hortobágyi (1969—30-40 × 9.5–11μ) from Uttar Pradesh and Dodkundi *et al* (1973) from Karnataka.

**var. *maxima* var. nov. (figures 23 a–b)**

Varietas a specie typica differens ut maior, cellulis 69–94 × 28–40μm

*Habitatio* : Varietas in spuma planctove locorum 51, 84, 85, 87–88, 101 et 157 rara ; in loco 61 frequens.

Differs from the typical species in its larger size, the cells measuring 69–94 × 28–40μ.

*Habitat* : Rare in the scum or plankton of locations 51, 84, 85, 87–88, 101 and 157 ;

common in location. 61 (N.P. 15–16, Nov.-Dec.) along with *E. tuba*, var. *pseudotuba* f. *minima* and *E. sanguinea*.

Distribution in Indian region : Orissa, Andhra Pradesh and Tamilnadu (!).

**24.** ***Euglena hemichromata Skuja*** **1948 (figure 24)**

Skuja 1948 pp. 185–86 Taf. 21 figures 10–13 ; Gojdics 1953, pp. 130–31, plate 21, figure 6 a–b ; Pringsheim 1956 pp. 107–8, figure 30.

Cell spindle-shaped to cylindric-spindle-shaped or somewhat bulged in the middle due to " metaboly " while in motion ; anterior end truncated obliquely or rarely, more or less rounded ; posterior end tapering to a blunt point or ending in a small hyaline tail ; pellicle with fine faint spiral striae ; chromatophores numerous, parietal, and in the form of spherical to irregular discs as well as elongated ones radiating from the middle of the cell where occasionally a central paramylum group is present ; chromatophores either restricted to the posterior two-thirds of the cell or, sometimes, filling the whole cell and without pyrenoids ; paramylum numerous, short-cylindrical to ovoid or as smaller granules distributed all over or sometimes crowded at the anterior end ; eye-spot bright red ; flagellum 1/3 to 3/4 body length ; nucleus, reservoir and gullet not observed ; cells 63–86 × 17.5–23.5, rarely up to 29.5$\mu$.

*Habitat* : Rare in the scum of location 61 (N.P. 15–February) along with *E. tuba* and var. *pseudotuba* f. *minima*, and *E. proxima*.

Skuja gave the name *hemichromata* on the basis that the chromatophores are mostly restricted to the posterior half or two-thirds of the cell. According to Gojdics (1953) they may be found in the whole cell. Pringsheim (1956) states that the absence of chromatophores at the anterior end in Skuja's material is due to the crowding of paramylum in that region. Both Skuja and Pringsheim place this species near *E. viridis* because of the radial arrangement of the elongated chromatophores from the middle of the cell.

The author's organism agreed in most respects with Skuja's. However, the cells were slightly broader especially when changing shape. Skuja gives the dimensions as 62–128 × 12–22$\mu$, which according to Pringsheim (1956) shows an inhomogeneity in the material. Paramylum in links (as seen in Skuja's drawings) were not observed. Eye-spot was also slightly smaller than in Skuja's organism.

Distribution in Indian region : Orissa (!).

Other species of *Euglena* recorded from the Indian region are as follows :

1. *E. anabaena* Mainz 1926 var. *minima* Mainz 1927
   K V Naidu 1966 p. 26 figure 12. Location Cuddapah, (AP)
2. *E. brevicaudata* Gojdics 1953
   K V Naidu 1962 p. 90, figure 7. Location Cuddapah (AP)
   According to Pringsheim (1956) though a good species, the use of the same name by Schiller has priority
3. *E. choretes* Schiller 1952
   Kamat 1975 p. 466 ; Kamat and Frietas 1976 p. 121. Location Vidarbh and Nagpur respectively

4. *E. deses* Ehrenberg 1833
   H J Carter 1856, E A Gonzalves and D B Joshi 1946 plate 5, figure 9. Location Bombay
5. *E. ehrenbergii* Klebs 1883
   E A Gonzalves and D B Joshi 1946 plate 5, figure 7. Location Bombay
6. *E. elastica* Prescott 1951
   K V Naidu 1962 p. 90 figure 4-6. location Cuddapah (AP) ; Dodkundi *et al* 1973 p. 141 ; S P Hosmani and S G Bharati 1975 p. 151 ; 1980 p. 32, 198; location Dharwar
7. *E. elongata* Schewiakoff 1893
   R K Pandhol and I S Grover 1976; location Ludhiana, Punjab
8. *E. flava* Dangeard 1901
   K V Naidu 1966 p. 26 figure 11. Location Vijayawada (AP)
9. *E. fundoversata* Johnson 1944
   K V Naidu 1966 p. 24 figure 1; Location Vijayawada (AP)
0. *E. gaumei* Allorge *et* Lefèvre 1925
   N D Kamat 1975 p. 466. Location Vidarbh, Maharashtra
1. *E. gibbosa* Schiller 1952
   N D Kamat 1964 p. 9 ; N D Kamat and J F Frietas 1976. Location Bombay and Nagpur respectively
2. *E. guentheri* Gojdics 1953 var. *alpina* (Grandori) Gojdics 1953.
   K V Naidu 1966 p. 26 figure 14. location Vijayawada (AP)
3. *E. heimii* Lefèvre 1934
   N D Kamat 1974 p. 26; Kamat 1963 p. 263. location Marathwada and Kolhapur respectively
   Both Gojdics (1953) and Pringsheim (1956) consider this species as synonymous to *E. ehrenbergii* Klebs ;
4. *E. intermedia* (Klebs) Schmitz 1884
   T Hortobágyi 1969 p. 30 plate 4, figure 52, location UP near Delhi. Pringsheim (1956) considers that this species (= *E. deses* var. *intermedia* Klebs 1883) cannot really be maintained as a species distinct from *E. deses*
5. *E. maharastrensis* Kamat 1963
   N D Kamat 1963 p. 263 plate 13 figure 68–69, location Kolhapur
   A species with brown knobs on the periplast.
6. *E. mucifera* Mainz 1926
   N D Kamat 1975 p. 466. Location Vidarbh, Maharashtra
7. *E. multiformis* Schiller 1952
   N D Kamat 1963 1975. Location Kolhapur and Vidarbh respectively
8. *E. obtusa* Schmitz 1884
   V Venkateswarlu 1976 p. 673 plate 4, figure 51. Location Hyderabad (AP)
9. *E. polymorpha* Dangeard 1901
   Hortobágyi 1969 p. 30 plate 4, figure 51; location UP near Delhi; V Venkateswarlu 1976 p. 673 plate 4, figure 52, location Hyderabad (AP) S P Hosmani and S G Bharati ; 1980a p. 33. location Dharwar
20. *E. pringsheimii* Iyengar 1962
   M O P Iyengar 1962 p. 325 figures 1–8, location Madras

21. *E. pusilla* Playfair 1921 var. *longa* Playfair 1921
    N D Kamat 1964 p. 9, location Bombay. Both the type and var. considered doubtful by most authors due to inadequate descriptions ;
22. *E. rustica* Schiller 1952
    N D Kamat 1963 p. 264, location Kolhapur
23. *E. sacculiformis* Schiller 1952
    N D Kamat 1963 p. 264. location Kolhapur
24. *E. splendens* Dangeard, 1901
    K V Naidu 1966 p. 26, figure 13, location Cuddapah (AP)
25. *E. texta* (Duj.) Huebner 1886
    = *Lepocinclis texta* (Duj.) Lemmermann 1901 ; as *Crumenula texta* Duj. see H J Carter 1856 p. 119, plate 6, figure 53, 56, 60 and 60a. Location Bombay
26. *E. tuberculata* Drezepolski 1925
    N D Kamat 1961–62, 1963. Location Ahmedabad, Gujarat and Kolhapur, Maharashtra respectively
27. *E. spathirhyncha* Skuja 1948
    K V Naidu 1966 p. 24, figure 3, location Cuddapah and Hyderabad (AP)
28. *E. subehrenbergii* Skuja 1948
    T Hortobágyi 1969 p. 31 plate 4, figure 49, location UP near Delhi
29. *E. velata* Klebs 1883
    Dodkundi *et al* 1973 p. 141, location Dharwar

Quite a number of these species are only just listed, sometimes with measurements, or when described and illustrated, complete details are lacking or the illustrations are not satisfactory. However, Iyengar (1962) and Hortobágyi (1969) have given good descriptions and illustrations for the taxa recorded by them. *E. texta* (Duj.) Huebner is also a well-known species. A good number of the remaining records may have to be re-investigated.

As already stated elsewhere, *E. estonica* and *E. charkowiensis* have been considered in this account under *E. oxyuris*, *E. orientalis* under *E. tuba* and *E. agilis* under *E. pisciformis*.

*E. alata* Thompson 1938 (see Kamat and Frietas 1976 p. 121) has been considered by Pringsheim (1956) as synonymous to *E. tripteris*, the species differing from the latter only in its paramylum being in links. Pringsheim also treats *E. archaeo-plastidiata* Chadefaud 1937 (see Naidu 1966 p. 25 figure 2) as synonymous to *E. pisciformis* while *E. minima* Francé 1894 and *E. minuta* Prescott (1944) are treated by him as probably a variety of/or related to *E. pisciformis* respectively. Skuja (1949) and Kamat and Frietas (1976) have reported *E. minima* and *E. minuta* respectively from Burma and Maharashtra.

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