THE ROLE OF ENVIRONMENT DURING SEED DEVELOPMENT ON SUBSEQUENT SEED QUALITY OF COWPEA (*Vigna unguiculata*)

By

AN-CHING CHENG TANG

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1982

To my parents.

ACKNOWLEDGEMENTS

The author wishes to express her gratitude to Dr. Daniel J. Cantliffe, chairman of the supervisory committee, for his support, guidance and patience throughout the course of this research.

She extends appreciation to Dr. C.B. Hall, Dr. T.E. Humphreys, Dr. T.A. Nell and Dr. I.K. Vasil for serving as her committee.

Special thanks go to Dr. T.A. Nell for his assistance in the greenhouse experiments. Appreciation is extended to Frank Woods and Jeanne Fischer for their technical assistance and to Miss Rena Herb for her typing of this manuscript.

The author would also like to thank those in the Vegetable Crop Deparment for their friendship and help during her study.

Most of all, thanks go to the author's family. Without their support and encouragement this study would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

Table		Page

LIST OF FIGURES

Figure		page

Abstract of Dissertation Presented to the Graduate Council of
the University of Florida in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

THE ROLE OF ENVIRONMENT DURING SEED DEVELOPMENT ON
SUBSEQUENT SEED QUALITY OF COWPEA (*Vigna unguiculata*)

By

An-Ching Cheng Tang

May, 1982

Chairman: Daniel J. Cantliffe
Major Department: Vegetable Crops

Seed yields of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were significantly reduced when seeds were produced under high temperature, drought stress, low light intensity, or deficient levels of N or P in the growth media, but were unaffected by variation in photoperiod.

Seed vigor of either cultivar was unaffected by photoperiod or light intensity. Hardseededness developed in 'Pinkeye Purple Hull' under shortdays.

Drought stress of -4 or -8 bars during seed development reduced subsequent seedling axis growth of 'Texas Cream 40' compared to a nonstressed treatment. At seed maturity smaller embryo size and less nutrient accumulation, especially starch, in the cotyledons accounted for this reduction in seed vigor. Under similar conditions, 'Pinkeye Purple Hull' produced seeds as vigorous as the unstressed control.

At day/night temperatures of $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$, seeds of both cultivars had higher vigor characteristics than seeds which developed at $38^{o}/30^{o}C$ or $23^{o}/15^{o}C$. In 'Texas Cream 40', heat stress at $38^{o}/30^{o}C$

appeared to reduce the ability of seedlings to utilize food reserves effectively during germination. Reduced seed vigor of 'Pinkeye Purple Hull' at $38^{o}/30^{o}C$ was related to a shortage of reserve food, especially starch, at seed maturity. Vigor of seeds of both cultivars produced at $23^{o}/15^{o}C$ was less than seeds produced at $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$ but was not as reduced as those produced at $38^{o}/30^{o}C$.

In nutritional experiments, seed vigor of 'Texas Cream 40' was unaffected by various nutritional treatments imposed from sowing to seed maturity. 'Pinkeye Purple Hull' plants grown on a deficient level of N, P or S had reduced seed vigor compared with those grown in complete Hoagland's solution. Seed vigor of this cultivar was also reduced by doubling the N level in the nutrient media. When imposed after anthesis, increased N and P levels in the nutrient media of the parent plant did not alter seed vigor of either cultivar. However, reducing N or P levels to one-quarter of those in a complete Hoagland's solution enhanced axis growth of the progenies of both cultivars.

INTRODUCTION

There is a great demand for increased research emphasis on high protein species to meet the food needs of the ever increasing world population. Although cowpea is nutritionally poor in S-containing amino acids, it is a main source of dietary protein in many tropical countries (Summerfield _et al_., 1974). This is because cowpea normally contains 20 to 25% protein in the mature seed and is more widely adapted compared to other legumes.

Low and inconsistent yields of cowpea have led to the initiation of more research to gain a better understanding of yield components for this species. Photoperiod, light intensity, temperature, soil moisture, and soil fertility have been shown to affect plant development and the final seed yield of cowpea. Rapid loss of viability during storage and extremely poor seedling establishment in the field constantly plague cowpea growers. Because cowpea is usually grown as a rainfed crop, the environment can greatly alter yields through a reduction in seed vigor. Thus, the importance of optimizing seed vigor in order to achieve higher yields is indisputable.

Although genetically determined, seed vigor is often altered by external factors. This includes the environment during seed production, seed maturity, field weathering, pathogen and insect attack, processing, and storage conditions. Altering any of these factors could reduce seed vigor. Compared to other factors, environment during seed development is beyond human control in the field. Variation in seed vigor due to different growing seasons or locations has been shown in several species including cowpea.

For many species there is abundant literature on the influence of altered environmental conditions during seed development on subsequent seed quality. However, most of the literature for cowpea is restricted to studies on plant development, N fixation, and seed yields. Little is known about the influence of environment on subsequent seed vigor. Therefore, research concerning the role of environment during seed development on seed vigor of the progeny of cowpea was conducted. The objectives of the present experiments are (1) to investigate the influence of different photoperiods, light intensities, water stresses, temperatures, and nutritional media during cowpea seed development on subsequent seed vigor, and (2) to examine closely the portion of the seed in which vigor is affected. The results should be of benefit for the improvement of cowpea seed production practices to ultimately produce higher quality seed.

CHAPTER I
LITERATURE REVIEW

Research on seed vigor has drawn great attention in the past several decades. An understanding of what seed vigor is and the cause of low vigor may help to improve the yields of high quality crops which may have a significant impact on economical returns to farmers. Seed vigor is defined, according to the Association of Official Seed Analysts (A.O.S.A.), as "the sum total of all those properties in seeds which, upon planting, result in rapid and uniform production of healthy seedlings under a wide range of environment including both favorable and stress conditions" (Woodstock, 1976, p. 4). Seedlings from high vigor seeds develop more rapidly and yield more than those from low vigor seeds.

An accurate assessment of seed vigor cannot be made before a clear understanding of the cause of low vigor is understood. Several vigor tests have been established for certain species in the A.O.S.A. seed vigor testing handbook (Woodstock, 1976). They are the accelerated aging test, cold test, conductivity test, cool germination test, seedling growth rate test, seedling vigor classification test, and tetrazolium test. Other methods based on biochemical metabolism during germination, such as respiration and enzyme activity, have been correlated with seedling growth, but are not recommended as routine procedures for vigor testing because of variability in the tests (Woodstock, 1976).

Although genetically determined, seed vigor is often modified by external factors. Several of these can affect seed vigor during seed development. These include the environment and nutritional status under which the mother plant grows, the stage of seed maturity at harvest, field deterioration, and pathogen attack in the field (Austin, 1972; Delouche, 1980). Environmental and nutritional stress can affect seed vigor early in the life of the seeds. If seeds which develop under adverse environments are low in vigor, they will be more susceptible to field deterioration, pathogen attack and improper handling during harvest and storage than seeds which develop under optimum conditions. Therefore, understanding how seed vigor will be affected by the environment and nutritional status with which the seed develops is highly important. Yet, research on this area is rather limited compared with the other external factors which affect vigor.

Influence of Light Stress on Seed Vigor of the Progeny

Effect of photoperiod on seed vigor. Although the inductive effect of photoperiod on flower initiation has been intensively studied for many years, the influence of daylength during seed maturation on seed quality was not reported until the 1960's (Koller, 1962). The photoperiodic response of flower induction and seed quality is not always the same. For example, prickly lettuce (*Lactuca scariola*) which flowered only under 16 hours daylength produced high vigor seeds when the seeds developed under 8 hours daylength compared with seeds developed under 16 hours daylength (Gutterman *et al*., 1975).

When seeds developed under 8 hours daylength, the seed weights of soybean (*Glycine max*) and restharrow (*Ononis sicula*) decreased, while those of lettuce (Lactuca sativa) and lamb's quarter (Chenopodium album) increased, compared with their counterparts which developed under longdays (16 to 20 hours daylength) (Koller, 1962; Wentland, 1965; Evenari et al., 1966; Patterson et al., 1977). A change in daylength during the last 12 days of seed ripening affected the germinability of 'Grand Rapids' lettuce (Gutterman, 1973). Seeds which developed under continuous light had increased tolerance to germination at high temperature both in continuous darkness and after a short light break, compared with seeds developed under 8 hours daylength. Conversely, pigweed (Amaranthus retroflexus) seeds which developed under 8 hours daylength had higher percentage of germination in the dark and at 30^{o}C after a short illumination or low temperature (5 to 10^{o}C) pretreatment than seeds which developed under 16 hours daylength (Kigel et al., 1977).

Seed dormancy can also be affected by photoperiod during seed development. Both restharrow grown under 20 hours daylength and lamb's quarter grown under 16 hours daylength produced a higher proportion of dormant seeds than those under 8 hours daylength (Wentland, 1965; Evenari et al., 1966; Karssen, 1970; Gutterman and Evenari, 1972). The dormant seeds, which gave rise to scattered stands upon sowing, possessed water impermeable seed coats. Seedling growth following germination was not reported in the species discussed above.

Purslane (Portulaca oleracea) seeds which matured under 8 hours daylength reached 50% germination 4 days earlier than those matured under 11 or 13 hours daylengths (Gutterman, 1974). The reduction in daylength from 16 to 8 hours during the last 8 days of seed maturation increased germination rate. Continuous light during seed development and maturation of red beet (Beta vulgaris) increased the proportion of empty seed balls and reduced the germinability of normally developed seeds by 12% (Heide et al., 1976). The progeny yield of roots was decreased by 10% compared with those grown under 8 hours daylength. Similarly, seeds of prickly lettuce which developed under 8 hours daylength were larger in size and had higher germination percentage and rate than those developed under 16 hours daylength (Gutterman et al., 1975). Seedling growth, measured as hypocotyl length and cotyledon size, in seeds which developed under 8 hours daylength was 25% greater than in seeds which developed under 16 hours daylength. Progeny plants flowered earlier in the former situation. The high seed vigor of prickly lettuce which developed under 8 hours daylength was related to an increase in gibberellic acid content of the seeds.

Effect of light intensity and quality on seed vigor. Little is known about the relationship of light intensity and quality during seed development to subsequent seed vigor. A reduction in light intensity to 27% of incident radiation during pigweed seed development affected its germination. Seed germination in the dark or after a low temperature pretreatment was less in longday shaded seeds than a longday control, but was higher in shortday shaded seeds than the shortday control (Kigel et al., 1977).

In mouse-ear cress (Arabidopsis thaliana), germination in the dark from seeds grown under cool white fluorescent lamps was 45%, under cool white plus incandescent lamps, 12%, and under incandescent lamps, 0% (McCullough and Shropshire, 1970).

The phytochrome mediated system was thought to control this germination response because the incandescent lamps provided a larger proportion of light energy at wavelengths greater than 700 μm than cool white fluorescent lamps. This mechanism was not apparent in purslane because continuous red illumination during seed maturation did not improve germination in the dark (Gutterman, 1974). However, red-far red interruption during maturation under shortday affected germinability of purslane. Red interruption increased seed germination at 40^{o}C, while far-red interruption increased germination at 25^{o} to 30^{o}C.

Influence of Water Stress on Seed Vigor of the Progeny

Rainfall during seed maturation of cotton (*Gossypium hirsutum*) accounted for 27% of the variability in seed vigor (Peacock and Hawkins, 1970). Higher rainfall led to progeny with better seedling growth than seeds produced in drier weather. The percentage of abnormal seedlings increased from 7% to 16% in peanut (*Arachis hypogaea*) when seeds developed without irrigation (Cox *et al*., 1976). Excessive irrigation of bean (*Phaseolus vulgaris*) plant bearing mature seeds reduced seed vigor (Siddique and Goodwin, 1980b). The number of normal seedlings was reduced by 10% from plants watered with overhead irrigation every day, compared with those with surface watering every other day.

Seed vigor of 'Moapa' alfalafa (*Medicago sativa*) was unaffected by water stress of up to -10 bars imposed during the reproductive stage (Walter and Jensen, 1970). Another cultivar 'Ranger' emerged earlier and had greater seedling weight and volume when seeds developed at soil moistures of -1 to -10 bars compared with -1/6 to -1/3 bars. Varietal differences in seed vigor due to drought stress during seed development were also reported for peanut (Pallas et al., 1977). The large-seeded Virginia type 'Florigiant' was the most sensitive cultivar, the Spanish type 'Tifspan' the least and the runner type 'Florunner' intermediate. The number of mature seeds of 'Florigiant' was decreased by 34%, seed weight reduced by 20%, and germination of mature seeds was 20% less when the mother plant was grown at -15 bars soil water tension compared with those grown above -2 bars. 'Tifspan' grown at -15 bars had similar seed size and germinability as those grown above -2 bars.

Lettuce seed germination was high (97-100%) regardless of water stress during seed development (Izzeldin et al., 1980b). Seed wieght, however, was 40% higher when seeds developed at -5 bars than seeds which developed at -0.3 or -0.8 bars. The percentage of abnormal seedlings doubled and radicle length was 20% less in seeds which developed at -0.3 bars compared with -5 bars. A similar change in seed vigor by drought stress was reported for Indian ricegrass (Oryzopsis miliacea) (Whalley et al., 1966). Seed weight and subsequent seedling growth of the progeny increased by 10% when the mother plant was grown in severe water stress (-15 bars) compared with an adequate water supply (-1 bar).

'Luther Hill' sweet corn (Zea mays) tolerated drought stress up to -5 bars during seed development without influencing seed size distribution or viability (El-Forgany and Makus, 1979). A seed vigor index, the combination of a cold test, germination rate and the accelerated aging test, in the progeny was the same regardless of water stress, but decreased significantly when -3 or -5 bars water stress were imposed during silking. Those plants exposed to water stress 3 to 6 weeks after silking produced seeds as vigorous as the control.

Influence of Temperature Stress on Seed Vigor of the Progeny

The influence of temperature during seed development on subsequent seedling growth was observed in spring wheat (Triticum aestivum) in the 1930's (Kostjucenko and Zarubailo, 1937). Exposure of the plants to low temperatures (<14^{o}C) during the milk-ripe stage of seed development significantly reduced the requirement for vernalization. Progeny plants grew and developed more rapidly when seeds matured at 15.5^{o}C instead of 26.5^{o}C (Riddell and Gries, 1958).

Day/night temperature treatments (25^{o}/20^{o}C, 20^{o}/15^{o}C and 15^{o}/10^{o}C) during seed development of Italian ryegrass (Lolium multiflorum), perennial ryegrass (Lolium perenne) and meadow fescue (Festuca pratensis) had a significant influence on subsequent seed germination and vigor (Akpan and Bean, 1977). The germination percentage was the highest in seeds which developed at 25^{o}/20^{o}C, especially at germination temperatures of 13^{o} to 20^{o}C. Seeds from the 15^{o}/10^{o}C treatment germinated 2 to 3 days slower, but produced seedling with 20 to 24% more dry weight than those which developed at higher temperatures (25^{o}/20^{o}C and 20^{o}/15^{o}C). In pearl millet (Pennisetum americanum), seed viability and field emergence were unaffected by the temperatures (33^{o}/28^{o}C, 30^{o}/25^{o}C, 27^{o}/22^{o}C and 21^{o}/16^{o}C) at which the seeds developed (Fussell and Pearson, 1980). However, grains which developed at 21^{o}/16^{o}C produced seedlings with 40 to 60% more hieght and dry weight than those at 33^{o}/28^{o}C.

Similar improvements of seed vigor after maturation under low temperatures were reported for several legumes (Green et al., 1965;

Harris *et al.*, 1965; Walter and Jensen, 1970; Harrison and Perry, 1973; Perry and Harrison, 1973). Soybean plants which matured in hot weather (>32°C) produced seeds with a lower percentage of germination and field emergence than those produced in cool weather (Green *et al.*, 1965). Seedling growth and seed yields in the progeny of soybean were reduced by high temperature (27°C) during the last 45 days of seed maturation compared with those produced under low temperature (21°C) (Harris *et al.*, 1965). Seed germination was delayed and seedling growth was reduced in pea (*Pisum sativum*) when the mother plant encountered high temperatures (>35°C) during the 10 day period after the pods had started to wrinkle compared with those which developed at 30°C (Harrison and Perry, 1973; Perry and Harrison, 1973). Seed yields in the progeny were reduced when the seeds were originally matured at high temperature. In alfalfa, seedling weights and leaf numbers were greater in 'Ranger' when the seeds matured at 13°C than at 24°C (Walter and Jensen, 1970). Seed vigor of another cultivar 'Moapa' was unaffected by the temperatures at which seeds had matured. When 10 genotypes of bean were subjected to six temperature regimes (33°/28°C to 18°/13°C) from anthesis to seed maturity, all produced seeds of higher vigor at the lower temperatures (21°/16°C and 18°/13°C) than at the higher temperatures (33°/28°C and 30°/25°C) (Siddique and Goodwin, 1980a). Resistance to mechanical injury increased in seeds which developed under low temperatures. Seed size and vigor of 'Apollo' bean decreased linearly as temperature increased from 21°/16°C to 33°/28°C during seed development and maturation (Siddique and Goodwin, 1980b). This adverse effect of high temperature on seed vigor was observed even on plants with well-developed seeds at the yellow fleshy pod stage.

Germination of tobacco (Nicotiana tabacum) was similar regardless of the temperatures ($22^o/18^oC$, $26^o/22^oC$ and $30^o/26^oC$) during seed maturation (Thomas and Raper, 1975a). Rate of emergence, however, was 1 to 2 days slower and seedling growth, determined as dry weight, fresh weight and leaf size, was reduced by 17% when seeds matured at $33^o/26^oC$ compared with $22^o/18^oC$ or $26^o/22^oC$. When a low temperature ($18^o/14^oC$) was imposed on tobacco plants during vegetative growth, seed germination of the progeny was 14% less than those grown at the higher temperatures ($22^o/18^oC$ to $33^o/26^oC$) (Thomas and Raper, 1975b). Seedling growth following germination was not reported.

Contrary to the above, seeds of bracted plantain (Plantago aristata) which matured at warm temperature (27^oC) germinated 1 to 2 days earlier and produced seedlings of 25% greater growth than seeds matured at cool temperature (16^oC) (Stearns, 1960). The difference in seedling growth persisted for 120 days. Similarly, seed germination of red beet decreased by 50% and the plants yielded 13% less when the mother plant was exposed to low temperature (12^oC) during seed development compared with those exposed to higher temperatures (18^o and 24^oC) (Heide et al., 1976). Controversial results, due to different experimental conditions, were reported for cotton (Peacock and Hawkins, 1970; Quisenberry and Gipson, 1974). Longer seedlings and higher yields were obtained when the mother plant matured at a cool minimum temperature (15^oC) in the field compared with a warm temperature (21^oC) (Peacock and Hawkins, 1970). When plants matured at night temperatures of 11^o, 15^o, 21^o and 27^oC

in the growth chamber, 20 to 70% less seedlings emerged and the resultant plants yielded 20% less cotton in the field from seeds which matured at 11^{o} and $15^{o}C$ than at 21^{o} and $27^{o}C$ (Quisenberry and Gipson, 1974).

Influence of Nutritional Status of the Mother Plants on Seed Vigor of the Progeny

Seed viability of pepper (*Capsicum annuum*), carrot (*Daucus carota*) and lettuce were similar regardless of the N levels (ranging from 23mM to 0.23 mM) in which the mother plants were grown (Harrington, 1960). Seedling growth, however, was not measured in these studies. A 10 fold increase in the level of N in the culture media of the mother plant did not affect seed germination and seedling growth of pea (Austin, 1966b), nor did N application at 141 Kg/ha to the mother plant influence subsequent progeny seed germination and root yield of carrot (Austin and Longden, 1965, 1966). In tobacco, an increase of N application from 50 to 94 Kg/ha to the mother plant reduced the time to germination and enhanced seedling uniformity of the progeny (Thomas and Raper, 1979). Seedling dry weights in the progeny of wheat and Indian ricegrass were also increased by the previous N supplement (Whalley *et al.*, 1966; Ries and Everson, 1973).

A high percentage of seed germination was reported in pepper, carrot and lettuce regardless of the P levels (1mM to 0.0078mM) in the culture media of the mother plant (Harrington, 1960). Red cotyledon, a physiological disorder in lettuce, increased by 50% when the mother plant was grown under P deficiency conditions. These seeds germinated 2 to 3 days slower and plant development, as demonstrated by the formation of new leaves, accumulation of leaf area, and vegetative maturity, was delayed 1 to 2 weeks compared with those grown in complete Hoagland's solution. Formation of a firm commercial lettuce head in the progeny was significantly reduced by P deficiency in the mother plant (Izzeldin et al., 1980a). A reduction in seed vigor due to P deficiency in the mother plant was observed in pea, watercress (Rorippa nasturtium aquaticum) and carrot (Austin and Longden, 1965, 1966; Austin, 1966a, 1966b). Seed germination and emergence of these species were 95 to 100% regardless of the P levels imposed on the mother plants. Seedling weight and pea yields were 25% less from seeds grown in 0.4mM P than in 4.0mM P (Austin, 1966b). In watercress, early seedling dry weight and seed yields were 20 to 30% less in seeds produced at low P levels than those produced at high P levels (Austin, 1966a). A 10% increase in carrot root yield was related to an increase in seed P content which resulted from the P supplement (133 Kg/ha) to the mother plants (Austin and Longden, 1966). Phosphorus application to the mother plant of Indian ricegrass also improved subsequent seedling growth (Whalley et al., 1966).

When K from 6mM to 0.094mM or Ca from 5mM to 0.078mM was applied to the mother plants, the germination percentages of pepper, carrot and lettuce seeds were not altered (Harrington, 1960). Seed longevity, however, was significantly reduced when seeds developed on K or Ca deficient plants. A high proportion of red cotyledon developed in lettuce and the subsequent seedling growth was adversely affected when deficient levels of Ca were imposed on the mother plant (Harrington, 1960; Izzeldin et al., 1980a). Calcium content of the peanut seeds was reduced from 420ppm to 200ppm when the mother plant was grown without Ca supplement (Cox et al., 1976). Seeds low in Ca germinated 40% less and produced seedlings with a higher incidence of abnormality, such as dark plumule and watery hypocotyl, than seeds high in Ca. Another abnormality, hollow heart, developed in peanuts when the mother plant was grown in low B soil (Harris and Brolmann, 1966). Similarly, soybeans grown in Mo deficient soil were low in seed Mo content which led to a reduction in seedling growth and seed yield (Harris et al., 1965).

In brief, the nutritional status of the mother plant had little effect on seed germinability, but seed vigor was either reduced by nutrient deficiencies or improved by additional applications of nutrients to the mother plant. Some of the 'carry-over' effects were via changes in chemical composition (P or Ca) of the seeds, as observed in carrot, watercress and peanut (Austin, 1966a; Austin and Longden, 1966; Cox et al., 1976).

An interesting relationship between seed protein content and seed vigor was reported in bean, wheat and oat (Avena sativa) (Schweizer and Ries, 1969; Ries, 1971; Ries and Everson, 1973). Supplemental N (50 to 100 Kg/ha) to the mother plant increased seed protein content in bean which was highly correlated with subsequent seedling size (r=0.95***) and yield (r=0.61**) (Ries, 1971). Protein content was increased in wheat seeds by the application of herbicide or N to the mother plant (Ries et al., 1970; Ries and Everson, 1973). Progeny plants grown from high protein seeds were more advanced in morphological development and yielded more seeds than those from low protein seeds. A 21 to 42% increase in oat grain yield was related to an increase in seed protein content by herbicide applications to the mother plant (Schweizer and Ries, 1969).

In conclusion, seed vigor was altered by environmental and nutritional stress imposed on the mother plant. In order to understand how the environment alters seed development and subsequent seed vigor, the present research was undertaken.

CHAPTER II
SEED YIELD AND VIGOR IN COWPEA SEEDS WHICH DEVELOPED UNDER DIFFERENT PHOTOPERIODS AND LIGHT INTENSITIES

Variation in light duration, intensity or spectral quality can affect plant development. Light duration (photoperiod) is well-known for its influence on flower initiation, while light intensity controls photosynthesis, and light quality affects phytochrome mediated processes (Leopold and Kriedemann, 1975). Thus, changes in light during seed development might affect seed viability and vigor. A shortday (SD) treatment during seed development of red beet increased yield in the progeny compared with a longday (LD) treatment (Heide *et al.*, 1976). Improvements in seedling growth due to SD treatments during seed development of the mother plant were reported for purslane (Gutterman, 1974) and prickyly lettuce (Gutterman *et al.*, 1975).

Diverse responses in seed yield of cowpeas to SD (11 hours 40 minutes) and LD (13 hours 20 minutes) treatment were reported for 61 cultivars examined (Huxley and Summerfield, 1974). A 50% reduction in light intensity decreased seed yield of 'Prime' cowpea, but increased seed weights (Summerfield *et al.*, 1976). The effect of light on seed germinability and vigor in the progeny was not reported. The objective of the present experiments was to investigate the influence of different photoperiods and light intensities during seed development on seed vigor of cowpea.

Materials and Methods

Biological materials

Ten cowpea cultivars were provided by Dr. R. L. Fery, USDA, Charleston, South Carolina (Table A-1). All seeds were propagated at the University of Florida, Horticulture Unit in Gainesville in the spring of 1979. The maturity date and seed weight were recorded at the time of harvest (Table A-1). After preliminary tests which included a standard germination test and an accelerated aging test (Tables A-2, A-3), 'Texas Cream 40' and 'Pinkeye Purple Hull' were chosen for the experiments which follow.

Experiment I. Effect of photoperiod during seed development on seed vigor

A greenhouse experiment was conducted in the winter of 1980 at a temperature of $24^{o} \pm 3C$ day and $19^{o} \pm 3C$ night under natural irradiation. 'Texas Cream 40' and 'Pinkeye Purple Hull' were grown in 8 liter pots filled with 3:1 peat:perlite mixture. All pots were watered daily to field capacity and fertilized once a week with N:P:K (2:1:2). Insecticides and fungicides were applied as needed. At anthesis, a SD treatment was initiated by covering the plants with black plastic to reduce daylength to 8 hours. One-half of these plants were subjected to a 1 hour light break (50 $\mu E/m^2/s$) in the middle of the 16 hours dark period. This constituted the LD treatment. Daylength treatments were continued until all seeds were harvested. The experimental design was a split plot with four replications.

Experiment II. Effect of light intensity during seed development on seed vigor

'Texas Cream 40' and 'Pinkeye Purple Hull' were grown in the greenhouse in the summer of 1980, using the same cultural procedures as Experiment I. At anthesis, plants were transferred to growth chambers (Conviron E-15) where light intensities varied accordingly: 275, 475, or 775 $\mu E/m^2/s$. Photoperiod and temperature were maintained constant in all chambers: 12 hours day and 12 hours night at a temperature of 25°C day and 20°C night. The experimental design was a split plot with four replications within each cultivar in the growth chamber.

Evaluation of seed vigor

Seed yield. In both experiments flowers were tagged at anthesis and the maturity date of individual pods was recorded. Seeds were harvested as the pods dried. The pod development period (Pod Dev. Per.), pod number per plant, seed number per pod, seed yield, and seed weight were recorded. After cleaning by hand, seeds were stored at 10°C and 50% RH.

Standard germination test. Twenty-five or 50 seeds (dependent on the quantity of seeds available) were germinated on moist paper towels at 25°C (A.O.S.A., 1970). Germination, defined as radicle protrusion, was counted daily. At the end of the fifth day, the total number of germinated and abnormal seedlings were counted, hypocotyl and radicle lengths were measured, and fresh and dry weights of the axis and cotyledons were taken. An abnormal seedling was classified according to the A.O.S.A. rules for testing seeds (A.O.S.A., 1970). A formula $\Sigma G_i T_i / \Sigma G_i$ was used to calculate the average days to germination (ADG), where G_i was the number of germinated seeds at day T_i, and T_i was the i-th day of germination (Quisenberry and Gipson, 1974).

Accelerated aging test. A preliminary test was conducted with field grown cowpeas to determine the appropriate aging period

(Table 1). Fifty seeds were aged at 41^{0}C and 100% RH for 0, 4 and 5 days, then germinated as in the standard germination test (Woodstock, 1976). Measurements of seedling growth were taken as above.

Cold test. A cold test was verified for cowpea as recommended in the A.O.S.A. seed vigor testing handbook (Woodstock, 1976). Fifty seeds were sown in unsterilized soil, then imbibed at 10^{0}C for seven days, and finally placed at 25^{0}C for seven days after which germination percentage was determined.

Determination of seed coat thickness. Seed coat thickness was measured in 'Pinkeye Purple Hull' seeds which developed under SD treatment. The hard and nonhard seeds were separated and dried in a 30^{0}C oven for three days after imbibing in water for 24 hours. A cross-section was made through the hilum portion with a single edged razor. Sections of the seeds were then mounted on aluminum stubs with tube coat (G.C. Electronics Co., Rockford, Illinois), and coated with 50nm of gold-paladium using a Hammers V sputter coater. Samples were viewed in a Hitachi scanning electron microscope, Model-450, with an accelerated voltage of 20 Kv and seed coat thickness was measured.

Statistical analysis. Analysis of variance for the experimental data was performed with the aid of the Northeast Regional Data Center in Gainesville. Analysis of variance was used to determine whether the differences between mean values of the treatments were significant.

Results and Discussion

'Texas Cream 40' and 'Pinkeye Purple Hull' were used because both had similar maturity dates (2½ months) and seed weights (Table A-1). Seed germination and seedling growth were similar in both cultivars with the standard germination test and after aging at $41^{\circ}C$ and 100% RH for four days (Table 1). Seed vigor of 'Pinkeye Purple Hull' was unaffected by five days of accelerated aging, but that of 'Texas Cream 40' was dramatically reduced. Only 32% of the seedlings were classed as normal in 'Texas Cream 40' after this period. Germination rate and radicle lengths of these seedlings from the five day aging treatment were significantly less than those from unaged seeds. Because of the severity of the five day treatment on 'Texas Cream 40', an aging period of 4 days was used to compare seed vigor of both cultivars after the various environmental treatments. The cold test was excluded as a vigor index for cowpea because no seed of either cultivar was viable after exposure to $10^{\circ}C$ for seven days (data not presented).

Experiment I. Effect of photoperiod during seed development on seed vigor

A greater number of heavier seeds of 'Pinkeye Purple Hull' led to a 50% increase in seed yield compared with 'Texas Cream 40' (Table 2). Although the period of seed development in 'Texas Cream 40' was five days longer than 'Pinkeye Purple Hull', seed weight of the former was 40% less than that of the latter. Differences in photoperiod had no effect on seed yield, weight or the total time of pod development in these two cultivars of cowpea. Robertson *et al*. (1962) reported that peas which developed under 16 hours daylength after flowering produced heavier seeds than those under eight hours daylength. In this case, seed weight differences might have reflected plant growth differences due to the longer light period during seed development. Seed numbers per pod were similar in 'Pinkeye Purple Hull' under both daylength treatments, but fewer seeds per pod were produced

Table 1

Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas after Accelerated Aging

Cultivar	Aging period	Germination		ADG	Seedling length		Seedling wt	
		total	abnormal		hypocotyl	radicle	fresh	dry
	day	%		day	cm		mg/plant	
Texas Cream 40	0	100a[z]	3b	1.8b	10.0a	17.4a	1050a	92a
	4	95a	2b	1.8b	10.8a	15.7a	940a	85a
	5	63b	31a	2.2a	9.5a	11.7b	950a	91a
Pinkeye Purple Hull	0	99a	0a	1.9a	9.2a	16.1a	1110a	92a
	4	100a	0a	1.6a	9.1a	15.5a	930a	89a
	5	91a	7a	1.7a	9.1a	16.5a	1090a	98a

[z]Values followed by the same letter within cultivar columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

Table 2

Effect of Photoperiod during Seed Development on Seed Yields of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpea

Variable	Seed yield	Pods/plant	Seeds/pod		Wt/100 seeds	Pod Dev. Per.
	g/plant	no.	no.		g	day
Cultivar						
Texas Cream 40	18.6b[z]	13.0b	9.1b		15.9b	27.4a
Pinkeye Purple Hull	27.7a	16.3a	10.7a		16.6a	22.6b
Photoperiod			Texas Cream 40	Pinkeye Purple Hull		
SD	24.6a	15.3a	8.6b	10.9a	16.1a	25.0a
LD	21.7a	13.9a	9.5a	10.6a	16.3a	25.0a
Interaction						
Cv x Photo	NS	NS	*		NS	NS

[z]Values followed by the same letter within variable columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*Significant interaction at the 5% level in 'Texas Cream 40' when plants developed under SD than under LD (Table 2). Huxley and Summerfield (1976) reported an increase in seed number per pod of 'K2809' cowpea under a LD (13 hours 20 minutes) compared with a SD (11 hours 40 minutes) treatment.

Germinability of 'Texas Cream 40' was the same regardless of the photoperiod treatment during seed development (Table 3). However, germination was 20% less in 'Pinkeye Purple Hull' seeds which developed under SD than under LD. This difference was due to hardseededness, since germination was 100% after scarification of the seed coat. Seed coat thickness of both hard and nonhard types of 'Pinkeye Purple Hull' developed under SD were similar, 60 to 70μm (Table A-4). Hardseededness might be caused by the change in seed coat structure and/or composition. Gutterman and Heydecker (1973) found that the cuticle layer in the thinner part of restharrow seed coat was well-developed and thickened in hard seeds which matured under 20 hours daylength. Werker et al. (1979) observed that hardseeded pea species had a continuous and very hard pectinaceous layer on caps of the palisade cells. The presence of quinone in a continuous layer around the seed coat, either in the palisade cells or osteosclereids, correlated with water impermeability.

Hard seeds of 'Pinkeye Purple Hull' grew as well as nonhard seeds; thus, seed vigor was not reduced. Hardseededness in cowpea which developed under SD might serve as a survival mechanism for this tropical species. Oropeza (1976) observed that rainfall combined with high temperature during seed maturation reduced germinability of 'Mognolia' cowpea by 20% within several days. Potts et al. (1978) reported that hardseededness in soybean was beneficial in preventing

Table 3

Effect of Photoperiod during Seed Development on Subsequent Seed Germination of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas with the Standard Germination Test and the Accelerated Aging Test

		Standard Germination Test			Accelerated Aging Test		
		Germination			Germination		
Cultivar	Photoperiod	total	abnormal	ADG	total	abnormal	ADG
		%		day	%		day
Texas Cream 40	SD	100a[z]	7a	1.5a	93a	9a	1.6a
	LD	100a	4a	1.4a	92a	12a	1.7a
Pinkeye Purple Hull	SD	81b	3a	1.6a	93b	7a	1.6a
	LD	100a	4a	1.4a	100a	8a	1.4a

[z]Values followed by the same letter within cultivar columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

viability loss during field deterioration. The hardseeded line 'D67-5677-1' was able to maintain viability for up to 9 weeks in the field when seeds were exposed to warm and humid weather, while a normal nonhard line 'Dare' lost viability by 50% during the same period.

Seedling size of 'Texas Cream 40' from both photoperiod treatments was generally larger than that of 'Pinkeye Purple Hull' after the standard germination test (Table 4), but these differences, except hypocotyl length, were not significant after accelerated aging (Table 5). Seedling growth of both cowpea cultivars was unaffected by photoperiod. Heide et al. (1976) observed that root yield in the progeny increased in red beet when seeds developed under SD (8 hours) than under LD (24 hours). Gutterman et al. (1975) reported that larger seedlings developed in prickly lettuce from seeds which matured under SD (8 hours) than those under LD (16 hours). In this case, the improvement of seed vigor in seeds which matured under SD was related to an increase in gibberellic acid content of the seeds.

Experiment II. Effect of light intensity during seed development on seed vigor

Average seed yield of 'Texas Cream 40' was 41% greater than that of 'Pinkeye Purple Hull' (Table 6). More pods per plant were produced in 'Texas Cream 40' compared with 'Pinkeye Purple Hull', while the number of seeds per pod and seed weight were similar for both cultivars. 'Texas Cream 40' had a slightly longer seed development period than 'Pinkeye Purple Hull'.

Yields of seeds which developed under 275 $\mu E/m^2/s$ were 66% of those which developed under the higher light intensities (Table 6). This appeared to be due to a reduction in the number of pods produced at this light intensity. A 50% decrease in full daylight at the

Table 4

Effect of Photoperiod during Seed Development on Subsequent Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas with the Standard Germination Test

Variable	Seedling length		Fresh wt			Dry wt		
	hypocotyl	radicle	axis	cotyledon	seedling	axis	cotyledon	seedling
	cm		mg/parts			mg/parts		
Cultivar								
Texas Cream 40	10.8a[z]	17.5a	1001a	178a	1179a	65.3a	53.7a	119.0a
Pinkeye Purple Hull	7.6b	16.3b	810b	192a	1002b	59.6b	57.3a	116.9a
Photoperiod								
SD	9.1a	17.0a	892a	190a	1081a	61.5a	56.9a	118.4a
LD	9.3a	16.8a	919a	180a	1099a	63.4a	54.1a	117.5a
Interaction								
Cv x Photo	NS	NS	NS	NS	NS	NS	NS	NS

[z]Values followed by the same letter within variable columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table 5

Effect of Photoperiod during Seed Development on Subsequent Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas after Accelerated Aging

Variable	Seedling length		Fresh wt			Dry wt		
	hypocotyl	radicle	axis	cotyledon	seedling	axis	cotyledon	seedling
	cm		mg/parts			mg/parts		
Cultivar								
Texas Cream 40	10.9a[z]	16.8a	971a	163a	1134a	70.1a	44.5a	114.6a
Pinkeye Purple Hull	8.0b	15.8a	861a	182a	1043a	64.2a	49.7a	113.9a
Photoperiod								
SD	9.5a	16.6a	915a	169a	1084a	67.0a	46.3a	113.3a
LD	9.3a	16.0a	917a	176a	1093a	67.3a	47.9a	115.2a
Interaction								
Cv x Photo	NS	NS	NS	NS	NS	NS	NS	NS

[z]Values followed by the same letter within variable columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

Table 6

Effect of Light Intensity during Seed Development on Seed Yield Measurement of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Variable	Seed yield	Pods/plant	Seeds/pod	Wt/100 seeds	Pod Dev Per.
	g/plant	no.	no.	g	day
Cultivar					
Texas Cream 40	19.5a[z]	12.5a	8.7a	17.7a	21.6a
Pinkeye Purple Hull	13.8b	9.3b	8.3a	17.7a	20.4b
Light Intensity					
275 $\mu E/m^2/s$	12.4b	8.3b	8.4a	17.8a	22.9a
475 $\mu E/m^2/s$	18.4a	12.8a	8.2a	17.0a	19.5b
775 $\mu E/m^2/s$	19.7a	12.2a	8.9a	18.1a	20.6b
Interaction					
Cv x Light	NS	NS	NS	NS	NS

[z] Values followed by the same letter within variable columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

reproductive stage of 'Prima' cowpea led to a 20% reduction in seed yield (Summerfield et al., 1976). In this case, rapid leaf senescence and possibly a reduction in photosynthetic rate under low light intensity decreased available nutrients for seed development, which in turn reduced seed yield. Pod maturity of 'Texas Cream 40' and 'Pinkeye Purple Hull' was delayed two to three days when seeds developed under the lowest light intensity compared with seeds which developed under the higher two light intensities. Sofield et al. (1977) observed that the seed development period in wheat was unaffected by light intensities ranging from 8.1 to 48.4 Klux after anthesis. However, seed size decreased under shading. In 'Prima' cowpea, Summerfield et al. (1976) reported that seed weight increased 14% when plants developed under a 50% reduction of normal sunlight. Robertson et al. (1962) found that seed weight decreased when peas developed under 1000 f.c. compared with 1500 f.c. The capacity of nutrient redistribution and sink size (seed number per plant) probably contributed to the variation of seed weight in the species discussed above.

Seeds of 'Texas Cream 40' germinated faster and generally developed into larger seedlings than 'Pinkeye Purple Hull' in both the standard germination test (Table 7) and the accelerated aging test (Table 8). Seed germinability and vigor of both cultivars before and after aging were unaffected by the intensity of light imposed after flowering. A decrease in radicle length and axis weights from seeds which developed under 475 $\mu E/m^2/s$ with the standard germination test was not thought to be of practical significance (Table 7). This was possibly related to a slight but

Table 7

Effect of Light Intensity during Seed Development on Subsequent Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas with the Standard Germination Test

Variable	Germination		ADG	Seedling length		Fresh wt			Dry wt		
	total	abnormal		hypocotyl	radicle	axis	cotyledon	seedling	axis	cotyledon	seedling
	%		day	cm		mg/parts			mg/parts		
Cultivar											
Texas Cream 40	100a[z]	0a	1.11b	9.7a	16.3a	1023a	198a	1221a	70.3a	58.8a	129.1a
Pinkeye Purple Hull	100a	0a	1.20a	7.4b	14.7b	814b	209a	1023b	60.0b	64.1a	124.1a
Light Intensity											
275 $\mu E/m^2/s$	100a	0a	1.14a	8.5a	15.9a	919ab	211a	1130a	64.9ab	65.0a	129.9a
475 $\mu E/m^2/s$	100a	1a	1.18a	8.4a	15.1b	867b	193a	1060b	61.6b	57.4a	119.0a
775 $\mu E/m^2/s$	100a	0a	1.14a	8.8a	15.6a	970a	206a	1176a	68.9a	62.1a	131.0a
Interaction											
Cv x Light	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Values followed by the same letter within variable columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table 8

Effect of Light Intensity during Seed Development on Subsequent Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas after Accelerated Aging

Variable	Germination		ADG	Seedling length		Fresh wt			Dry wt		
	total	abnormal		hypocotyl	radicle	axis	cotyledon	seedling	axis	cotyledon	seedling
	%		day	cm		mg/parts			mg/parts		
Cultivar											
Texas Cream 40	95a[z]	9a	1.15b	8.9a	15.5a	993a	205a	1098a	68.5a	57.4a	125.9a
Pinkeye Purple Hull	93a	7a	1.40a	6.5b	11.6b	769b	221a	990b	58.1b	63.4a	121.5a
Light Intensity											
275 $\mu E/m^2/s$	94a	6a	1.22a	7.6a	14.1a	874a	212a	1086a	63.0a	60.6a	123.6a
475 $\mu E/m^2/s$	93a	5a	1.34a	7.8a	13.6a	875a	207a	1082a	62.1a	58.3a	120.4a
775 $\mu E/m^2/s$	94a	11a	1.26a	7.7a	13.0a	893a	220a	1113a	64.7a	62.2a	126.9a
Interaction											
Cv x Light	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Values followed by the same letter within variable columns are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

non significant decrease in individual seed weight of seeds which developed under this light intensity.

From these results, it appeared that seed vigor in two cultivars of cowpea was unaffected when the mother plants encountered a change in daylength or a reduction in light intensity during seed development. Seeds produced under different light conditions should be of high vigor although seed yields might be somewhat reduced by the lower light intensities.

Summary

'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were subjected to different photoperiods and light intensities during seed development to investigate the influence of these environmental factors on seed yield and vigor.

Photoperiods, shortday (8 hours day/16 hours night) versus longday (8 hours day/16 hours night with a 1 hour light break in the middle), did not influence seed yield, size or vigor of either cultivar. Hardseededness was observed in 'Pinkeye Purple Hull' when seeds developed under shortdays. Once the coat of such seeds was ruptured, germination and seedling growth were normal. Differences in seed coat thickness were not observed between hard and nonhard seeds; thus, it appeared that seed coat composition was altered.

Seed yields of both cultivars decreased significantly when light intensity was reduced from 775 to 275 $\mu E/m^2/s$. However, neither seed weight nor seed vigor was affected by light intensity differences imposed during seed development.

CHAPTER III
REDUCTION IN SEED YIELD AND VIGOR OF COWPEA DUE TO WATER STRESS DURING SEED DEVELOPMENT

Water stress during seed development can affect seedling growth in the progenies of lettuce (Izzeldin _et al_., 1980b), Indian ricegrass (Whalley _et al_., 1966), peanut (Cox _et al_., 1976), alfalfa (Walter and Jensen, 1970), and sweet corn (El-Forgany and Makus, 1979). Seed vigor was reduced in peanut and alfalfa, improved in lettuce and Indian ricegrass, and unaffected in sweet corn by drought stress. An increase in seed weight under drought conditions was related to an improvement in seed vigor of lettuce (Izzeldin _et al_., 1980b) and Indian ricegrass (Whalley _et al_., 1966). Decreased seed vigor of Spanish peanuts was associated with reduced ethylene production during early seed germination from plants grown under low soil moisture stress (Ketring _et al_., 1978). When 'Florigiant' peanuts matured under a drought condition, seeds low in Ca were produced which gave rise to seedlings with a physiological disorder (Cox _et al_., 1976). Seed chemical composition was altered when water stress was imposed during seed development of sorghum (Eck and Musick, 1979), wheat (Terman et al., 1969; Campbell and Davidson, 1979) and oat (Chinnici and Peterson, 1979); however, the relation of this change in composition to seed vigor was not reported.

Water stress, either a deficit or excess, adversely affected nodule growth in cowpea (Doku, 1970; Hong _et al_., 1977). Seed yield varied with water stress levels and the growth stage at which the water stress was imposed. Treatments that decreased the number of mature pods reduced cowpea seed yields (Hiler et al., 1972; Kamara, 1976; Minchin et al., 1978; Turk et al., 1980). The influence of water stress on progeny seed vigor was not observed. The present research was conducted to investigate the influence of water stress during seed development on subsequent seed vigor of cowpea. Since a decrease in vegetative growth by water stress might severely affect seed development and vigor, plants were grown with similar quantities of available water until flowering. Active embryo metabolism and nutrient supply from the cotyledons determine seedling growth at the early stages of plant development. Thus, the chemical composition of the seeds was analyzed to determine changes induced by water stress and its possible relationship with changes in seed vigor. Also, in order to determine if there was drought injury to the embryos themselves, embryos were cultured to eliminate the influence of cotyledons during early seedling development.

Materials and Methods

Experimental water stress treatments during seed development

A greenhouse experiment was conducted in the summer of 1980 at a temperature of $33^{o} \pm 3$ C day and $25^{o} \pm 3$ C night under natural light intensity and photoperiod. 'Texas Cream 40' and 'Pinkeye Purple Hull' were germinated in flat trays filled with a 3:1 peat:perlite mixture. Seedlings with fully-expanded primary leaves were rinsed under tap water to remove debris on the root surfaces, then were transferred to 1-liter jars filled with complete Hoagland's solution (Hoagland and Arnon, 1938). The pH of the solution was adjusted to 5.5-6.0. The jars were wrapped with aluminum foil to eliminate algae growth and the solutions were aerated with an air pump.

At anthesis, polyethylene glycol-6000 (PEG-6000) was added at the rate of 160 or 250 g/Kg water to the culture solutions to reduce water potential to -4 or -8 bars, respectively (Michel and Kaufmann, 1973). A control, Hoagland's solution, was maintained for comparison. The amount of PEG-6000 was increased slowly so that water potential dropped 1 to 2 bars every 3 to 4 days. The stress conditions were maintained until seed harvest. The experiment was a completely randomized design with four replications.

Evaluation of seed vigor

Influence of water stress on seed yield and vigor. Seed yield measurements and seed vigor tests were conducted as previously reported (Chapter II).

Influence of water stress on seed chemical composition. Mature seeds were ground in a Wiley mill to pass through a 40 mesh sieve. Total seed nitrogen (N) was determined by the micro-Kjeldahl method (A.O.A.C., 1975). Total inorganic phosphorus (P) was determined with a dry-ashed sample by the Fiske-Subbarow method (Fiske and Subbarow, 1925). Total protein was extracted with 0.5N NaOH in a homogenizer (Sorvall Omni mixer) at high speed for 3 minutes. After trichloroacetic acid precipitation, the pellet was resuspended in 0.1N NaOH and an aliquot was taken to determine protein content by Lowry's method (Lowry et al., 1951). Bovine albumin was used as a standard.
Starch was analyzed by a modified method of Dekker and Richards (1971). Starch was extracted with 0.5N NaOH over a boiling water bath

for 15 minutes, then neutralized with 0.5N acetic acid. An aliquot was taken and hydrolyzed with amyloglucosidase (Sigma, Rhizopus genus) at 55^{o}C for 30 minutes. The starch concentration in glucose equivalents was measured in a glucose analyzer (YSI Model 27).

Embryo culture. A preliminary test was conducted to determine the optimum culture medium. Sterilization of the seeds was found to be unnecessary. Embryos were excised from the dry mature seeds, transferred to sterilized media containing 1.5% agar, 2% sucrose, and 0.1% casein hydrolysate. Petri dishes with embryos were set upright at an 80^{o} angle. The embryos were grown at 25^{o}C for 5 days, then the axis fresh weight, hypocotyl and radicle lengths were measured. Dry weights were determined after drying at 70^{o}C for 24 hours.

Statistical analysis. Analysis of variance was performed as previously described (Chapter II). Regression analysis was followed, if significant differences were observed, to determine the trend of changes in vigor characteristics due to water stress treatments. Lines shown in the figures were drawn from the regression equation for each measurement.

Results and Discussion

Influence of water stress on seed yield and vigor

Seed yields of 'Pinkeye Purple Hull' decreased linearly as water stress intensified during seed development, while that of 'Texas Cream 40' decreased to half of the control (-0.8 bars) under both moderate (-4 bars) and severe (-8 bars) stress (Fig. 1A). Reduction in seed yield was directly correlated with a decrease in pod number per plant (r=0.90***) (Fig. 1B). Seed number per pod

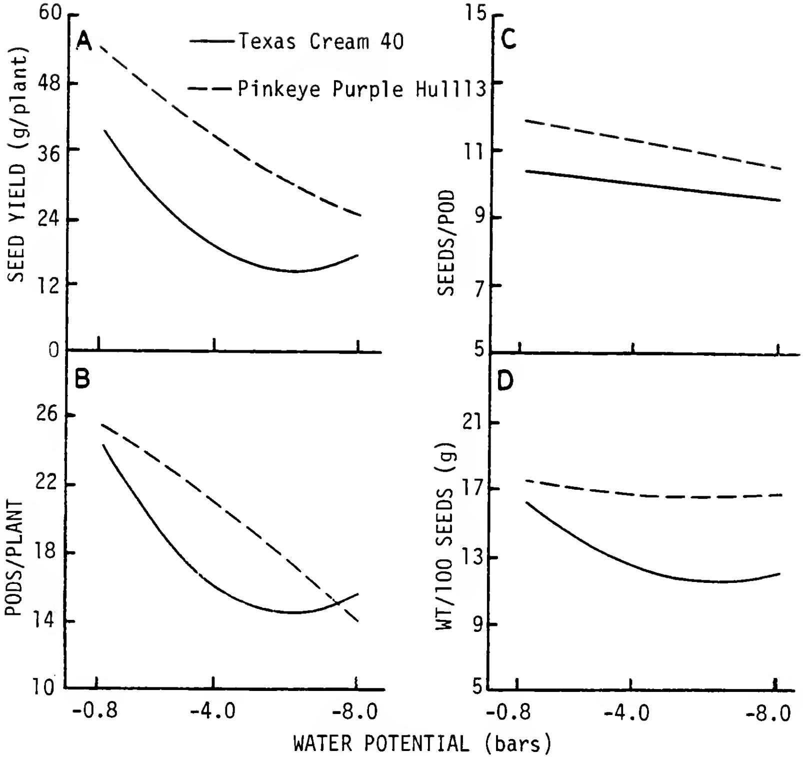

Fig. 1 Effect of water stress during seed development on seed yield measurements of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.

A. seed yield, B. pod number per plant, C. seed number per pod, D. weight of 100 seeds.

decreased in both cultivars under drought stress (Fig. 1C). A similar reduction in seed yields by drought stress during seed development was reported for 'Bungundy' (Hiler et al., 1972), 'Temne' (Kamara, 1976), 'California No. 5', and 'Chino 3' (Turk et al., 1980) cowpeas. Decreases in pod number per plant and/or seed number per pod also accounted for the reduction in seed yields of these other cowpea cultivars. When imposed after anthesis, drought stress did not influence the length of time to pod maturation of 'Texas Cream 40' and 'Pinkeye Purple Hull' (19 days under all water stress levels) or seed weight of 'Pinkeye Purple Hull' (Fig. 1D). A 23% reduction in seed weight was observed in 'Texas Cream 40' when plants developed under both moderate and severe stress compared with the control (Fig. 1D). Kamara (1976) reported that seed weight of 'Temne' cowpea decreased by 70% after withdrawing irrigation at the podding stage compared with a control watered to field capacity. The average seed yield of 'Pinkeye Purple Hull' was 54% more than that of 'Texas Cream 40', due to a greater number of heavier seeds produced by 'Pinkeye Purple Hull'.

The percentage and rate of seed germination under optimum conditions were unaffected in 'Texas Cream 40' by drought stress imposed on the mother plant (Figs. 2A, B). Hypocotyl length was also unaffected by water stress, but radicle length was slightly reduced in seeds which developed under severe water stress (Figs. 2C, D). The fresh and dry weights of the axis and whole seedling declined linearly as water stress intensified (Figs. 3A, B, D, E). A decrease in cotyledon weights (25 to 30%) from seeds which developed under -4 and -8 bars water stress was highly correlated (r=0.91***) with weight decrease in the mature seeds (23%) (Figs. 3C, F).

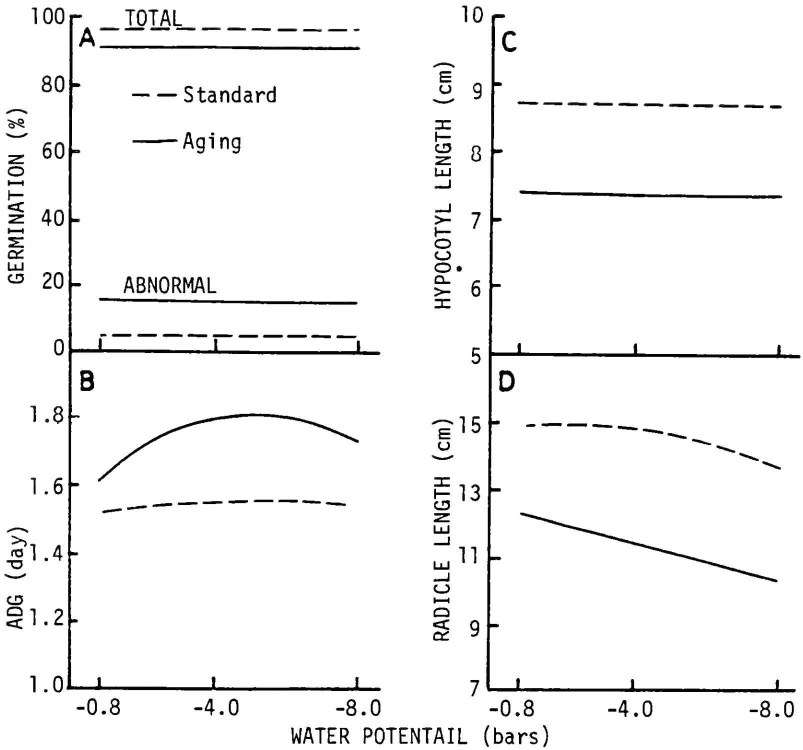

Fig. 2 Effect of water stress during seed development on seed germination, germination rate and seedling lengths of the progeny of 'Texas Cream 40' with the standard germination test and after accelerated aging.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

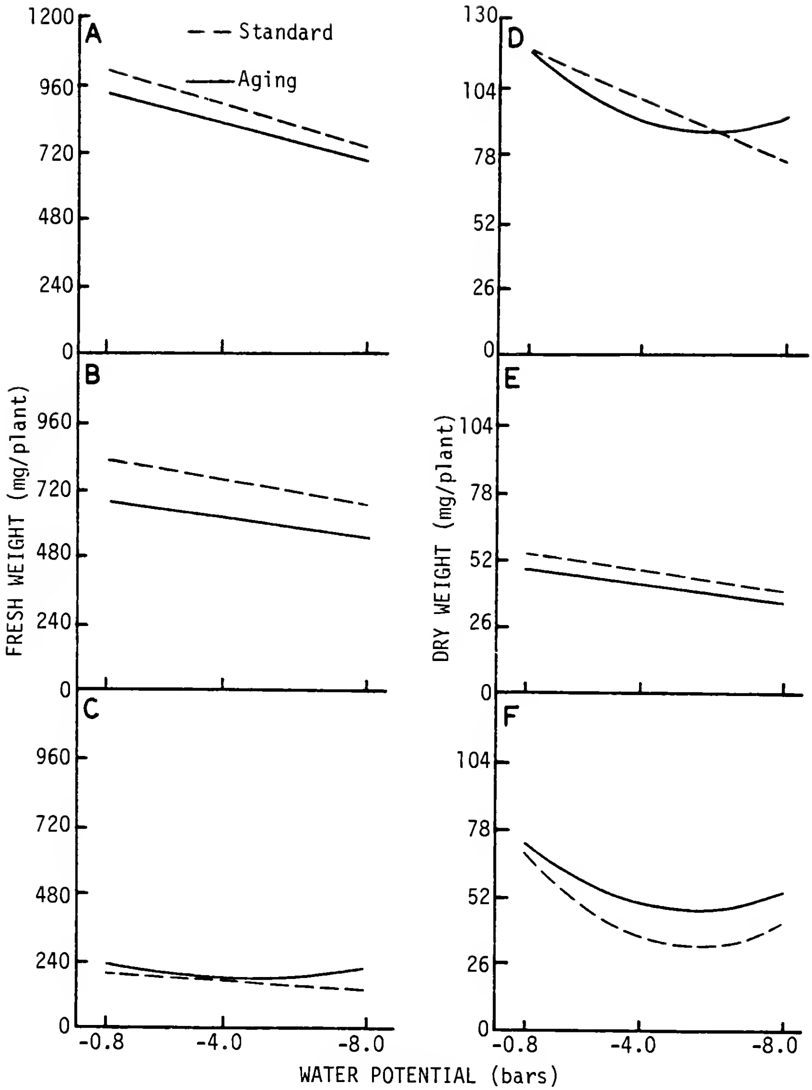

Fig. 3 Effect of water stress during seed development on seedling weight of the progeny of 'Texas Cream 40' with the standard germination test and after accelerated aging.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, E. axis, F. cotyledon.

The percentage of germinated and abnormal seedlings, and hypocotyl lengths of 'Texas Cream 40' after accelerated aging were unaffected by water stress (Figs. 2A, C). Germination was delayed in seeds which developed under -4 and -8 bars water stress, however (Fig. 2B). Radicle length (Fig. 2D), axis weights and seedling fresh weight decreased linearly as water stress that was imposed on the mother plant intensified (Figs. 3A, B, E). Cotyledon weight and seedling dry weight were correlated with the original seed weight (r=0.96***) (Figs. 3C, D, F).

The influence of water stress on seed germination and seedling growth in the progeny of 'Texas Cream 40' were similar in both the standard germination test and the accelerated aging test (Figs. 2, 3). More abnormal seedlings arose after aging. Compared with the unstressed control, seeds which developed under -4 and -8 bars water stress germinated slower and had shorter radicle lengths after aging. This did not occur after the standard germination test, however. The overall radicle growth of aged seeds was 21% less than that of unaged seeds, while hypocotyl length was reduced by 1.3 cm after aging. Aging also led to a 10 to 15% decrease in axis weight and the corresponding increase in cotyledon weight, when compared with the standard germination test.

Seed germination and seedling growth of 'Pinkeye Purple Hull' in the standard germination test were unaffected by water stress up to -8 bars (Figs. 4, 5). After accelerated aging, germination percentage and axis lengths of 'Pinkeye Purple Hull' were also unaffected by water stress (Fig. 4). Seeds which developed under the most severe water stress, however, germinated slower and had

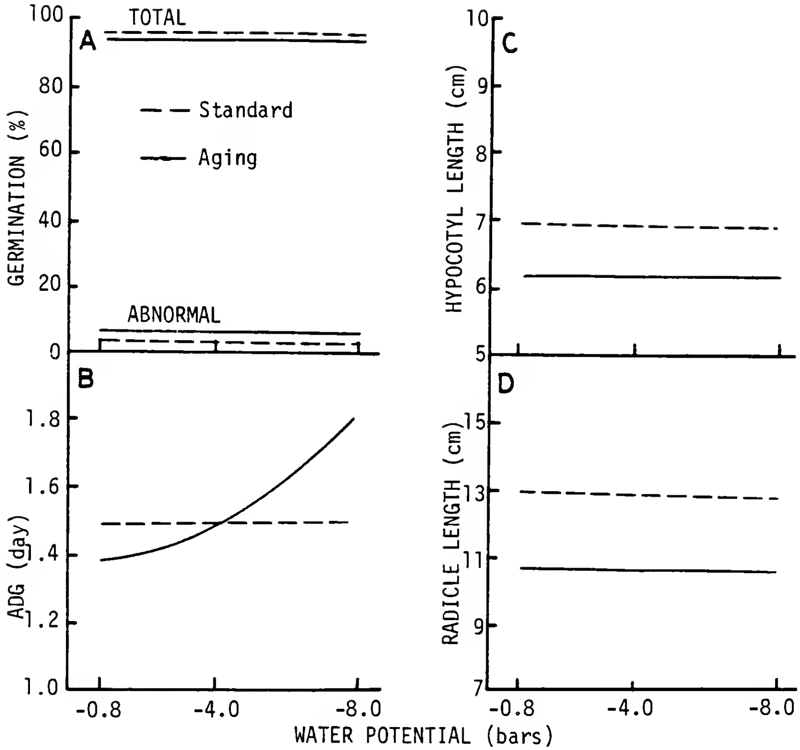

Fig. 4 Effect of water stress during seed development on seed germination, germination rate and seedling lengths of the progeny of 'Pinkeye Purple Hull' with the standard germination test and after accelerated aging.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

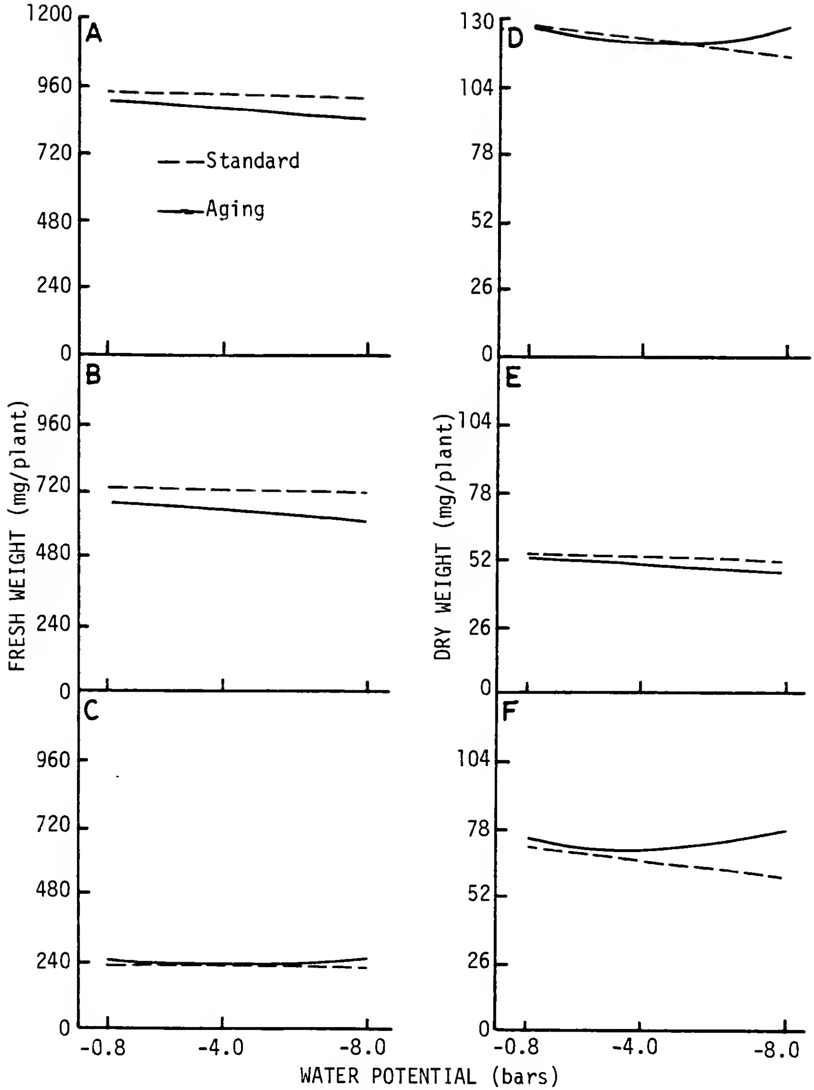

Fig. 5 Effect of water stress during seed development on seedling weight of the progeny of 'Pinkeye Purple Hull' with the standard germination test and after accelerated aging.

Fresh weight: A. seedling, B. axis, C. cotyledon
Dry weight: D. seedling, E. axis, F. cotyledon

lower axis weights after aging when compared with the control (Figs. 5B, E). Therefore, severe water stress reduced seed vigor slightly but significantly. Compared with the standard germination test, aging did not alter seed germinability or the development of abnormal seedlings, but reduced hypocotyl lengths by 11%, radicle lengths by 17.5%, and axis dry weights by 8.4% in 'Pinkeye Purple Hull'.

Overall, axis growth of 'Pinkeye Purple Hull' after aging decreased linearly as water stress levels increased; but the rate was much slower in 'Pinkeye Purple Hull' (-0.64 mg per plant per bar) than in 'Texas Cream 40' (-1.28 mg per plant per bar) (Figs. A-2, A-4). Average differences in axis dry weights between aged and unaged seeds were smaller in 'Pinkeye Purple Hull' (5%) than in 'Texas Cream 40' (12%). Thus, seed vigor of 'Texas Cream 40' was significantly reduced by moderate to severe water stress. Vigor was further reduced if seeds germinated under aging stress. Seed vigor of 'Pinkeye Purple Hull' was only reduced in seeds which developed under the most severe water stress and then germinated in an adverse condition. Compared with the control, decrease in axis dry weight of seeds which developed under -8 bars water stress was less in 'Pinkeye Purple Hull' (9%) than in 'Texas Cream 40' (19%). Thus, 'Pinkeye Purple Hull' appeared to be more tolerant to drought stress than 'Texas Cream 40'.

Drought sensitive 'Texas Cream 40' produced small and low vigorous seeds under -4 and -8 bars water stress without any apparent influence on seed germinability and germination rate. Pallas <u>et al</u>., (1977) reported similar decreases in seed size and normal seedling development in Virginia type peanuts by drought stress up to -15 bars during seed development. The close relationship between seedling weights and seed weight in 'Texas Cream 40' (r=0.96) was also reported in lettuce by Izzeldin et al., (1980b) and in Indian ricegrass by Whalley et al., (1966). Heavy seeds produced seedlings with longer radicles in lettuce and seedlings of greater length and weight in Indian ricegrass than light seeds. However, the change in seed weight by drought stress in 'Texas Cream 40' cowpea was opposite to those in lettuce and Indian ricegrass. Stresses up to -5 bars in lettuce and -15 bars in Indian ricegrass reduced seed yields of both crops, but compensatorily increased seed weight by 40% in lettuce and 9% in Indian ricegrass. In 'Texas Cream 40' cowpea, seed weight was reduced 23% under water stress conditions. Hence, drought stress during seed development did not appear to affect vigor in lettuce and Indian ricegrass to the extent it did with 'Texas Cream 40' cowpea.

'Pinkeye Purple Hull' was drought tolerant and able to produce seeds with comparable size and vigor as the control under -4 bars water stress. A similar observation was made in 'Luther Hill' sweet corn by El-Forgany and Makus (1979). Seed size and vigor index from a cold test, an accelerated aging test, and germination rate were unaffected by soil moisture depletion up to -5 bars during corn seed development. Severe drought stress (-8 bars), however, reduced seed vigor of 'Pinkeye Purple Hull' slightly after aging.

Because seedling weight of cowpea was well-correlated with seed weight (r=0.96), an attempt to separate the effect of drought from the effect of seed size was made. Little variation was found in size distribution of 'Pinkeye Purple Hull' with or without water stress, while more small seeds were produced in 'Texas Cream 40' under water stress (Table 9). Most seeds of 'Texas Cream 40' from drought stress treatments appeared less-filled and most had a wrinkled seed coat (Fig. 6). A further separation on seed fullness was performed with a slot sieve. The proportion of less-filled seeds increased from 1% to 13% as drought stress intensified. Since weight differences still existed after final sieving, water stress apparently reduced nutrient deposit in the developing seeds of 'Texas Cream 40'. Comparison of seed vigor with the same seed weight between water stress treatments was not feasible in this experiment.

Influence of water stress on seed chemical composition

Water stress during seed development significantly altered the chemical composition of mature seeds (Fig. 7). Compared with unstressed seeds, N concentration increased 13% in 'Texas Cream 40' and 7% in 'Pinkeye Purple Hull' seeds under both moderate and severe water stress (Fig. 7A). The protein concentration of 'Texas Cream 40' increased from 17.5% to 20.5% as drought levels intensified, while that of 'Pinkeye Purple Hull' increased significantly over the control only under the -8 bars water stress (Fig. 7B). Similar increases in seed N and protein concentrations due to water stress during seed development were reported for wheat by Terman et al. (1969), for oat by Chinnici and Peterson (1979), and for sorghum by Eck and Musick (1979). Ries (1971) observed that seed vigor in bean was associated with seed protein concentration. Larger seedlings and higher bean yield were obtained from high protein seeds compared with low protein seeds of the same size. This relationship was also reported in wheat by Lowe and Ries (1972). However, in the present experiment with 'Texas Cream 40' cowpea drought stress led to the

Table 9

Effect of Water Stress during Seed Development on Seed Size Distribution of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Cultivar	Water stress	Seed size category: large	Seed size category: medium	Seed size category: small
	bars	% or (mg)	% or (mg)	% or (mg)
		>0.71mm	0.54-0.71mm	< 0.54mm
Pinkeye Purple Hull	-0.8	3	96(176)[z]	1
	-4.0	1	97(169)	2
	-8.0	3	96(170)	1
		>0.63mm	0.51-0.63mm	< 0.51mm
Texas Cream 40	-0.8	9	84(162)	7
	-4.0	1	90(125)	9
	-8.0	3	84(124)	13
			>0.40mm[y] / < 0.40mm[y]	
	-0.8		83(163) / 1	
	-4.0		83(127) / 7	
	-8.0		71(125) / 13	

[z]Figures in parenthesis are per seed weight in mg.

[y]Seed fullness screening on medium size 'Texas Cream 40'.

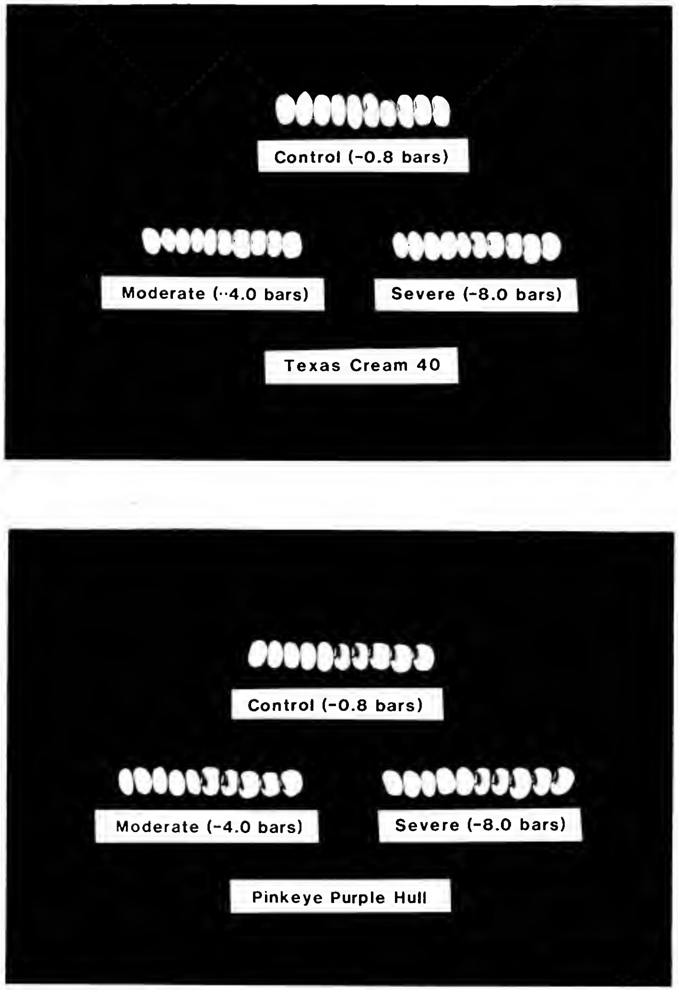

Fig. 6 Final size and appearance of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas as affected by water stress during seed development

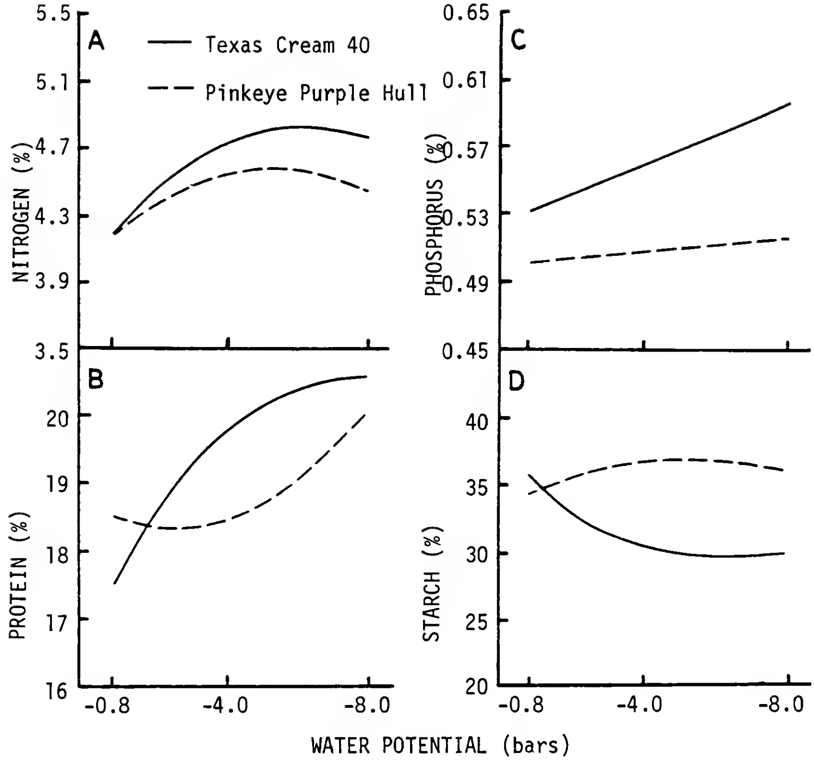

Fig. 7 Effect of water stress during seed development on seed composition (% dry weight basis) of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.
A. nitrogen, B. protein, C. phosphorus, D. starch.

high protein but low vigor seeds regardless of seed size. Similar responses were observed in seeds of 'Pinkeye Purple Hull' developed under severe water stress, but the reduction in vigor was less in 'Pinkeye Purple Hull' than in 'Texas Cream 40'.

Seed P concentration increased linearly in 'Texas Cream 40' but remained unchanged in 'Pinkeye Purple Hull' as water stress intensified (Fig. 7C). Eck and Musick (1979) reported that the concentration of P in sorghum seeds was unaffected by drought stress up to a leaf water potential of -24 bars during seed development. Austin (1966a, 1966b) and Austin and Longen (1966) reported that seedling growth and crop yields of carrot, watercress and pea were related to seed P content. The higher the P concentration of the seed, the higher the seed vigor of the progeny. A similar relationship was not observed in the present work with 'Texas Cream 40' cowpea. In this case, seeds with higher P concentrations after water stress had reduced seedling vigor.

The starch concentration of cowpea seeds increased 9% in 'Pinkeye Purple Hull', but decreased 14% in 'Texas Cream 40', under drought stress compared with unstressed seeds (Fig. 7D). Unlike seed N, protein and P concentrations, which were negatively correlated with the axis growth in both cultivars (r=-0.61**, -0.53** and -0.75** for N, protein and P, respectively), starch concentration was positively correlated with axis growth in 'Texas Cream 40' (r=0.61**).

Vegetative growth of the cowpea plants was similar before treatment in the present experiment. After imposing drough stress with PEG-6000, leaf senescence occurred and new pods were no longer produced. The increases in seed N, protein and P concentrations in seeds of both cultivars under water stress might simply be the result of the decrease in the sink (seed number and weight) compared with the available nutrient source (current metabolism and vegetation) for seed development. The decrease in starch concentration of 'Texas Cream 40' seeds under water stress indicated that photosynthesis or carbohydrate metabolism might be reduced in this more drought sensitive cultivar compared with the N and P metabolism.

Total nutrient quantities on a per seed basis were directly correlated with the seed weight in both cultivars (r=0.95***, 0.91***, 0.96*** and 0.97*** for N, protein, P and starch, respectively). Therefore, water stressed seeds of 'Texas Cream 40' contained less reserve food than unstressed seeds (Fig. 8). The total quantity of N decreased from 13 to 14%, protein 10 to 12%, P 15 to 18% and starch 33%, in 'Texas Cream 40' seeds which developed under water stress compared with the control. In 'Pinkeye Purple Hull', however, total quantities of N, protein, P and starch in water stressed seeds varied to within $\pm$5% of unstressed seeds (Fig. 8). The composition of N, protein, P and starch accounted for 30 to 50% variation in seed vigor of both cultivars. This indicated that factors other than nutrient supply from the cotyledons might possibly be involved in vigor determination.

Embryo culture

Selection of a medium for embryo culture was conducted with media containing different sucrose concentrations (Table 10). Hypocotyl length of both cultivars decreased as the sucrose concentration increased from 2% to 6%. Radicle length and axis

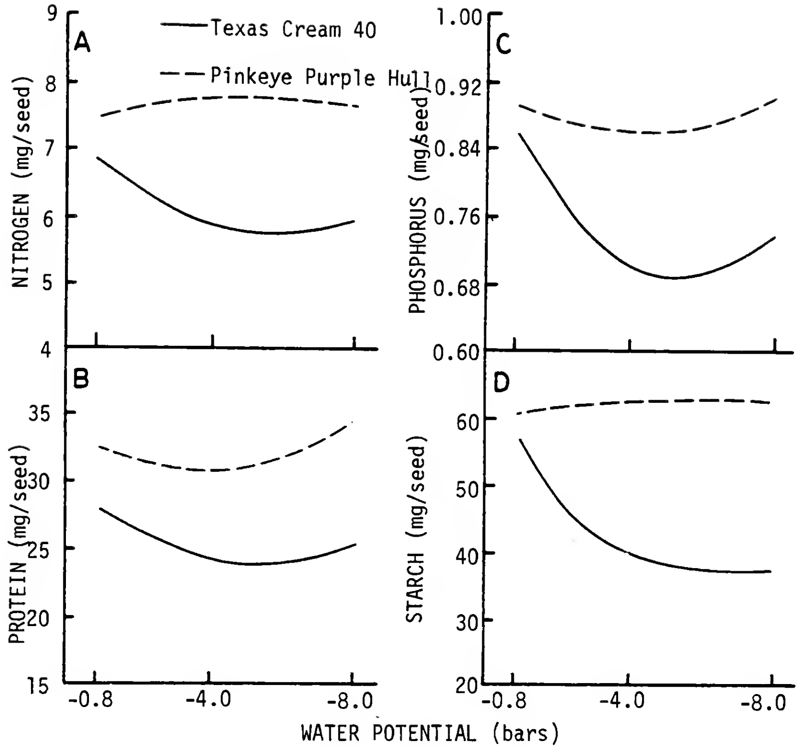

Fig. 8 Effect of water stress during seed development on seed composition (mg per seed) of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.

A. nitrogen, B. protein, C. phosphorus, D. starch.

Table 10

Embryo Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas in Media Containing Different Sucrose Concentrations.

Cultivar	Sucrose[z]	Axis length		Axis wt	
		hypocotyl	radicle	fresh	dry
	%	cm		mg/axis	
Texas Cream 40	0	0.9b[y]	1.8b	28.9c	2.0d
	2	1.7a	3.1a	44.3b	3.4c
	4	1.2a	3.6a	49.5a	4.7b
	6	1.0b	3.4a	42.9b	5.5a
Pinkeye Purple Hull	0	1.0a	1.6b	36.0c	2.3d
	2	1.9a	3.8a	53.0b	3.8c
	4	1.8a	4.3a	57.7a	5.0b
	6	1.3b	3.9a	54.0b	6.3a

[z]All media contained 0.1% casein hydrolysate and 1.5% agar.

[y]Values followed by the same letter within cultivar column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

fresh weight reached maximum in 4% sucrose, then declined as sucrose concentration increased to 6%. Embryonic axis dry weight increased linearly as the sucrose concentration in the media increased. Because of a possible osmotic effect leading to a reduction in axis length, a medium containing 2% sucrose was used. Combinations of sucrose and casein hydrolysate in 1.5% agar media were also examined (Table 11). Embryos of both cultivars grew better, shown by increases in axis length and weight, with sucrose in the media than without. Casein hydrolysate in the presence of sucrose increased radicle length of 'Texas Cream 40' and axis weight of 'Pinkeye Purple Hull'. Therefore, both sucrose and casein hydrolysate were used in the medium for the cowpea embryo culture experiment.

Embryos from 'Texas Cream 40' seeds which developed under -4 and -8 bars water stress were smaller in size and had shorter radicle lengths and reduced axis weights after 5 days culture at 25°C compared with the unstressed control (Table 12). Hypocotyl length of 'Texas Cream 40' was unaffected by water stress treatments to the mother plant. Although embryo size of 'Pinkeye Purple Hull' was unaffected by water stress treatments, and axis dry weight and hypocotyl length were similar after five days of culture, radicle lengths and axis fresh weights declined as the water stress treatment to the mother plant intensified.

Anatomically, embryos of drought stressed seeds of both cultivars were the same as unstressed seeds, regardless of embryo size (data not presented). Seed development of 'Pinkeye Purple Hull' seemed unaffected by drought stress. A decrease in embryonic axis fresh weight and radicle lengths of culture embryos of this cultivar

Table 11

Embryo Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas in Different Culture Media

Cultivar	Media[z]		Axis length		Axis wt	
	SU	CH	hypocotyl	radicle	fresh	dry
	%		cm		mg/axis	
Texas Cream 40	0	0	0.8a[y]	2.0c	13.9b	0.96b
	2	0	1.0a	2.9b	16.3a	1.33a
	0	0.1	0.8a	2.0c	12.7b	0.96b
	2	0.1	1.1a	4.0a	15.7a	1.31a
Pinkeye Purple Hull	0	0	0.9a	2.3b	15.6b	1.11b
	2	0	1.0a	4.1a	15.4b	1.32ab
	0	0.1	1.0a	2.5b	16.3b	1.17b
	2	0.1	1.1a	4.3a	18.1a	1.56a

[z]SU: sucrose, CH: Casein hydrolysate.

[y]Values followed by the same letter within cultivar column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table 12

Effect of Water Stress during Seed Development on Embryo Size at Seed Maturity and Its Growth in Culture of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Cultivar	Water stress	Embryo wt	Axis length		Axis wt	
			hypocotyl	radicle	fresh	dry
	bars	mg	cm		mg/embryo	
Texas Cream 40	-0.8	3.6a[z]	1.2a	3.6a	42a	3.4a
	-4.0	3.2b	1.1a	3.3b	38ab	3.2ab
	-8.0	3.0b	1.2a	3.2b	36b	2.9b
Pinkeye Purple Hull	-0.8	4.4a	1.7a	4.5a	58a	4.5a
	-4.0	4.3a	1.7a	4.3ab	56a	4.4a
	-8.0	4.4a	1.6a	3.9b	53b	4.4a

[z]Values followed by the same letter within cultivar column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

indicated that severe drought stress during seed development might exert damage on the growth of the embryo. Since embryo morphology and size of 'Pinkeye Purple Hull' were unaffected, internal injury in ultrastructure or metabolism was possible. Thus, drought stress imposed after anthesis might have adversely affected cowpea embryo development. The embryo size and growth of 'Texas Cream 40' and embryo growth of 'Pinkeye Purple Hull' were significantly reduced by water stress. Ketring et al. (1978) reported that low soil moisture during maturation of Spanish type peanuts reduced ethylene production during early germination which resulted in reduced seedling growth. The embryo, being the major site of ethylene production (Ketring and Morgan, 1969), was possibly impaired by drought stress although it was not investigated in Ketring's work.

The influence of water stress on embryo growth of the progeny of 'Texas Cream 40' in culture (Table 12) was simliar to that in the whole seed vigor test (Figs. 2, 3). In both, radicle lengths and axis weights decreased linearly, while hypocotyl lengths remained unchanged, as drought stress treatments intensified during seeds development. Apparently, axis growth of 'Texas Cream 40' was a reflection of embryo size at seed maturity and thus its potential to grow. Compared with unstressed seeds, axis growth of 'Texas Cream 40' from seeds which developed under moderate and severe stress was 6% and 14% less, respectively, in embryo culture. A further decrease in axis growth was observed in the whole seed vigor test: 14% and 19% less under moderate and severe stress, respectively, compared with the control. The decrease in seed nutrient content in 'Texas Cream 40' under water stress (Fig. 8) might have led to the further reduction in axis growth in the whole seed. Since axis dry weight increase in culture was proportional to available sucrose (Table 10), the 33% decrease in available starch of 'Texas Cream 40' seeds which developed under water stress might be a major contributing factor that led to the reduction of axis growth.

In conclusion, water stress during seed development reduced cowpea seed yield and vigor. The influence was more detrimental to 'Texas Cream 40' than to 'Pinkeye Purple Hull'. When developed under drought stress during seed development, 'Pinkeye Purple Hull' produced vigorous seeds although a reduction in seed yield resulted. In the same situation, adequate water supply was necessary for 'Texas Cream 40' to produce more vigorous seeds.

Summary

'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were subjected to water stresses of -4 and -8 bars during seed development to investigate the influence of drought stress on subsequent seed vigor in the progeny. Seed yields of both cultivars were significantly reduced by water stress imposed after anthesis. When developed under water stress, drought sensitive 'Texas Cream 40' produced seeds which were smaller in size than the unstressed control. Decreases in embryo size and available nutrient reserves, especially starch, in the water stressed seeds accounted for the reduction in seed vigor of 'Texas Cream 40'. Drought tolerant 'Pinkeye Purple Hull' was able to produce seeds of the same size and vigor under water stress as the unstressed control. Embryo size and available nutrients in 'Pinkeye Purple Hull' seeds which developed under drought conditions were similar to the unstressed seeds.

CHAPTER IV

REDUCTION IN SEED YIELD AND VIGOR OF COWPEA DUE TO TEMPERATURE STRESS DURING SEED DEVELOPMENT

Temperature stress during seed development can affect subsequent seed germinability, stand uniformity and early seedling growth. Seed viability of alfalfa (Walter and Jensen, 1970), lettuce (Koller, 1962), tobacco (Thomas and Raper, 1975a, 1975b), barley (Khan and Laude, 1969) and ryegrass (Wiesner and Grabe, 1972) increased when seeds matured at high temperature. Improvement of seedling growth due to low temperature during seed development was reported in pearl millet (Fussell and Pearson, 1980), pea (Perry and Harrison, 1973), soybean (Harris et al., 1965), bean (Siddique and Goodwin, 1980a, 1980b) and alfalfa (Walter and Jensen, 1970). However, little is known regarding how temperature affects seed vigor in the progeny. An increase in seedling weight of pearl millet was related to an increase in size of seeds which developed at low temperature (Fussell and Pearson, 1980). A physiological disorder, hollow heart, developed in pea seeds which matured at high temperature (Perry and Harrison, 1973). This abnormality led to poor seedling growth (Harrison and Perry, 1973).

Night temperature can have a profound influence on cowpea reproductive ontogeny (Huxley and Summerfield, 1974, 1976). Plants flowered only at night temperatures above $19^{o}C$; the higher the temperature, the earlier the plant flowered. Seed vigor of cowpea has been reported to be similar at temperatures of $33^{o}/24^{o}C$ and $27^{o}/19^{o}C$ during seed development (Ndunguru et al., 1978). In the present experiments temperatures were varied during seed development to determine their effect on seed vigor of cowpea. The composition of mature seeds was analyzed to determine changes induced by temperature stress during seed development and the possible relationship of this to seed vigor. Embryos were cultured in vitro to determine possible injury to the axis by temperature stress during seed development. Seedling growth and changes in the major storage components of starch and protein in the axis and cotyledons during germination were studied to evaluate the relationship between food utilization and seedling vigor.

Materials and Methods

Experimental treatments during seed development

'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were grown in the greenhouse in the spring of 1980 using cultural practices as previously described (Chapter II). At anthesis, plants were transferred to growth chambers (Coviron E-15) in which the day/night temperatures were set at $38^{o}/30^{o}C$, $33^{o}/25^{o}C$, $28^{o}/20^{o}C$ and $23^{o}/15^{o}C$. Light intensity, 775 $\mu E/m^2/s$, and photoperiod, 12 hours day and 12 hours night, were the same in all chambers. The experiment was a split plot design with four replications within each cultivar in the growth chamber.

Evaluation of seed vigor

Influence of temperature stress on seed yield and vigor. Cowpea yield measurements and vigor tests were evaluated as previously described (Chapter II).

Influence of temperature stress on seed composition. Total seed N, protein, P and starch contents were analyzed as previously described (Chapter III).

Embryo culture. Embryos were excised from mature seeds and cultured as previously described (Chapter III).

Partitioning of seedling weight and nutrient composition during germination. Ten to 15 axes were excised from cotyledons after 0, 1, 3 and 5 days of germination. Both axis and cotyledons were weighed, then dried in a VirTis freeze-drier for two days. After dry weights were taken, each plant part was ground and assayed for amino acid and soluble carbohydrate contents. The tissues were extracted three times with 80% alcohol over a water bath at 82^{o}C for 20 minutes. The alcohol extract was evaporated to near dryness, then diluted with distilled water. Total amino acid content was determined by the ninhydrin method (Moore and Stein, 1954; Moore, 1968) and total soluble carbohydrate by the phenol-sulfuric acid method (Dubois et al., 1956). Both starch and protein were dissolved in 0.5N NaOH by heating the residue of the alcohol extraction over a boiling water bath (Cruz et al., 1970). After centrifugation, total protein and starch contents in the supernatant were determined as previously described (Chapter III).

Statistical analysis

Analyses of variance and regression were analyzed as previously described (Chapter II, III).

Results and Discussion

Influence of temperature stress on seed yield and vigor

Seed yield of 'Texas Cream 40' decreased from 49g to 14g per plant as temperature increased from $23^0/15^0C$ to $33^0/25^0C$ (Fig. 9). In 'Pinkeye Purple Hull', seed yield was comparable at temperatures of $23^0/15^0C$ and $28^0/20^0C$, then declined from 23g to 5g per plant as temperature increased from $28^0/20^0C$ to $38^0/30^0C$. Seed yields of both cultivars were directly correlated (r=0.91***) with pod number per plant (Fig. 9B). Stewart et al. (1980) reported that seed yield of 'K2809' cowpea was reduced by 27% when plants were grown at $33^0/24^0C$ compared with $27^0/19^0C$, and lower pod numbers also led to the lower seed yield. The delay of pod maturation at low temperature appeared to be related to an increase in seed weight of both 'Texas Cream 40' and 'Pinkeye Purple Hull' (Fig. 10), since these factors were highly correlated (r=0.81***) (Figs. 9C, D). Seed weights at $23^0/15^0C$ were double those at $38^0/30^0C$.

Wien and Ackah (1978) reported a similar relationship between pod development period, maturation temperature and seed weight of several cowpea cultivars. Roberts et al. (1978) reported that 'K2809' cowpea had higher seed growth rate and heavier seeds when plants were grown at $27^0/19^0C$ than at $33^0/24^0C$. Huxley and Summerfield (1976) observed that day-night temperatures affected seed development of 'K2809' cowpea differently. Seed weight was 19% less at a night temperature of 24^0C compared with 19^0C, but was 18% more at a day temperature of 33^0C compared with 27^0C. Siddique and Goodwin (1980b) reported that seed maturity in bean was delayed 11 days but that seed size increased 2.5 times when developmental temperatures decreased from $33^0/28^0C$ to $18^0/13^0C$. A similar delay in seed development and increase in seed weight at low maturation temperature was reported in pea (Robertson et al., 1962). Egli and Wardlaw (1980) reported

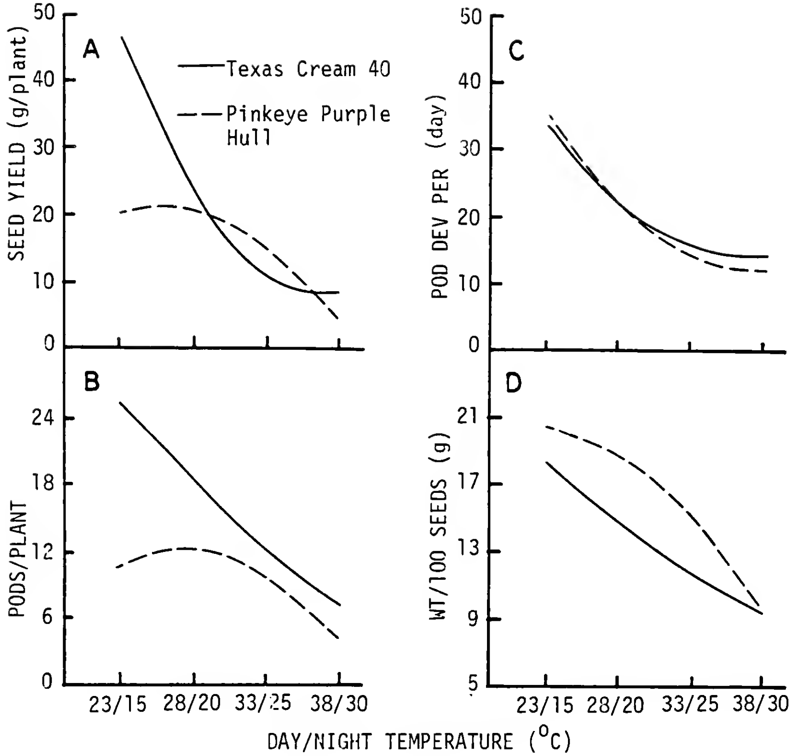

Fig. 9 Effect of different temperatures during seed development on seed yield measurements of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.

A. seed yield, B. pod number per plant, C. pod development period, D. weight of 100 seeds.

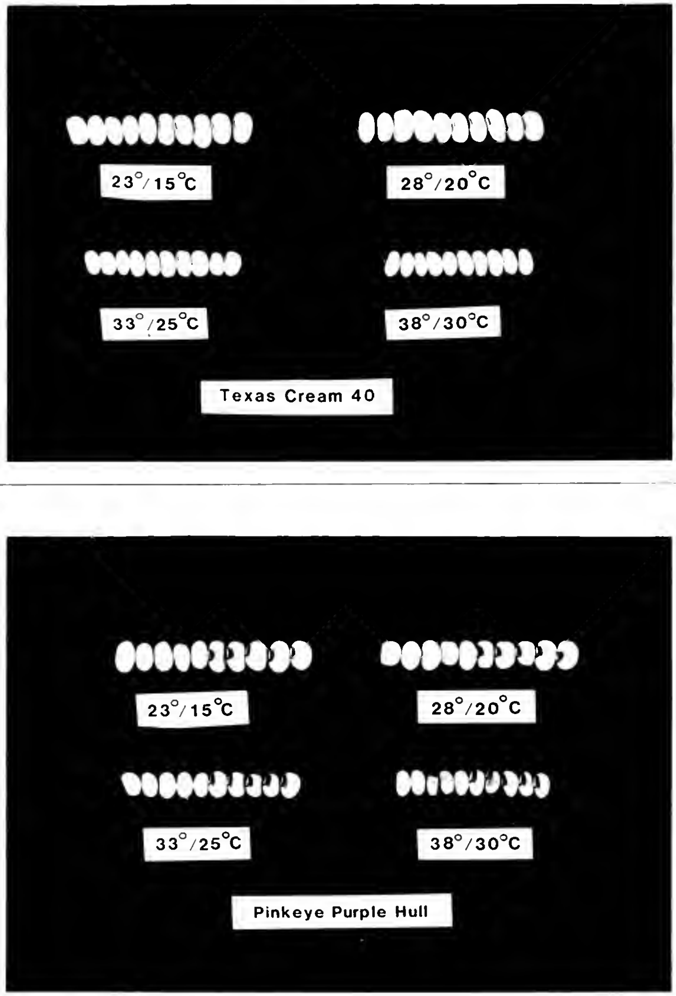

Fig. 10 Final size and appearance of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas as affected by temperature during seed development.

that the duration of soybean seed growth was unaffected by temperatures of $24^{0}/19^{0}C$ to $30^{0}/25^{0}C$, but was 3 days less at $33^{0}/28^{0}C$. Seed weight of mature soybean was 25% less at both $33^{0}/28^{0}C$ and $18^{0}/13^{0}C$ than at temperatures between this range.

Seed germinability, abnormal seedling development, and radicle length of 'Texas Cream 40' with the standard germination test were unaffected by temperature during seed development (Fig. 11). Germination rate in the progeny was slower in seeds from the $23^{0}/15^{0}C$ treatment and hypocotyl length was shorter in seeds from the $38^{0}/30^{0}C$ treatment compared with those from $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$. Although seed weight increased at low temperature, seedling fresh weight and axis weight in the progeny of 'Texas Cream 40' were the greatest in seeds which developed at $28^{0}/20^{0}C$, followed by $23^{0}/15^{0}C$ and $33^{0}/25^{0}C$, and the least at $38^{0}/30^{0}C$ (Figs. 12 A, B, E). Seedling dry weight and cotyledon weights were related to the original seed weight (r=0.97***) (Figs. 12 C, D, F).

After accelerated aging, germinability of 'Texas Cream 40' was reduced in seeds which developed at $23^{0}/15^{0}C$ compared with seeds which developed at the higher temperatures (Fig. 11). Germination rate, normal seedling development and axis growth in the progeny were the greatest at developmental temperatures of $33^{0}/25^{0}C$ and $28^{0}/20^{0}C$, and the least at $38^{0}/30^{0}C$ (Figs. 11, 12). Hypocotyl length was 30% less, radicle length 25% less, and axis weight 42% less in seeds which developed at $38^{0}/30^{0}C$ compared with $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$. Low temperature ($23^{0}/15^{0}C$) incurred during seed development led to a reduction in hypocotyl length by 20% and axis weight by 11% in the progeny compared with optimum temperatures. Seedling dry weight and cotyledon weight after aging were related to seed weight at maturity (r=0.97***).

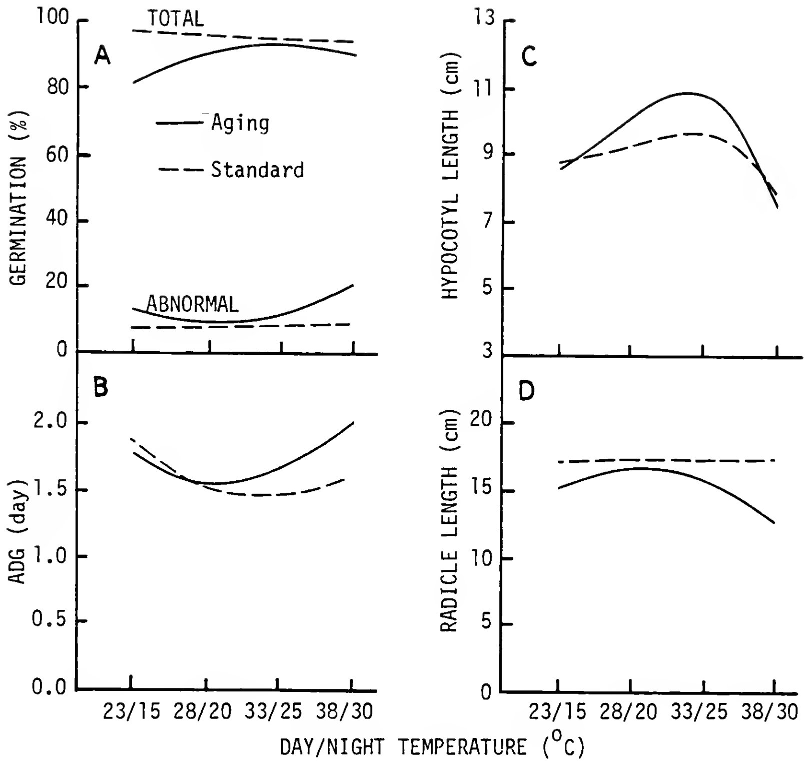

Fig. 11 Effect of different temperatures during seed development on seed germination, germination rate and seedling lengths of the progeny of 'Texas Cream 40' cowpea with the standard germination test and after accelerated aging.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

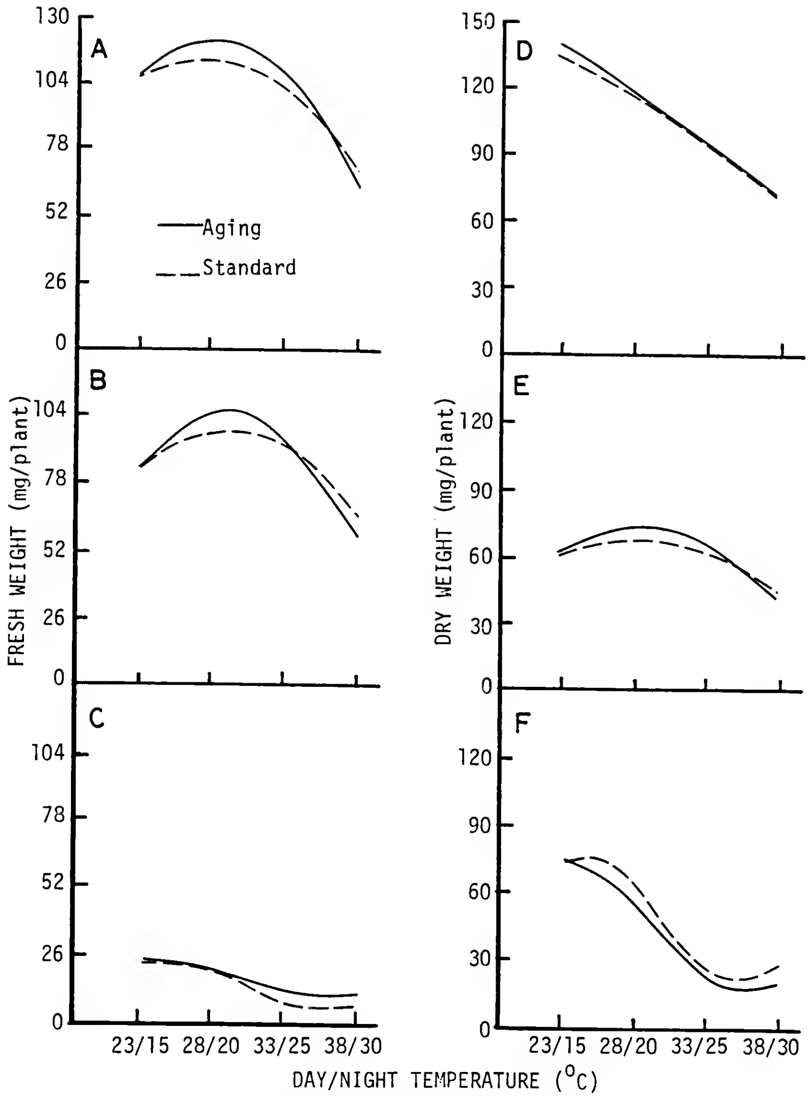

Fig. 12 Effect of different temperatures during seed development on seedling weight of the progeny of 'Texas Cream 40' cowpea with the standard germination test and after accelerated aging.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, E. axis, F. cotyledon.

'Texas Cream 40' seeds which developed at $23^{o}/15^{o}C$ were larger in size but produced smaller seedlings before or after aging than seeds which developed at $28^{o}/20^{o}C$. Axis growth from seeds which developed at $38^{o}/30^{o}C$ were 42% less after aging and 31% less before aging, compared with seeds which developed at temperatures of $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$. Heat stress ($38^{o}/30^{o}C$) during seed development of 'Texas Cream 40' led to the production of seeds with the lowest vigor. Cool temperature ($23^{o}/15^{o}C$) led to seeds of intermediate vigor, and temperatures in between led to seeds with the highest vigor.

In 'Pinkeye Purple Hull', seed viability, abnormal seedling development and radicle length with the standard germination test were unaffected by temperature treatments imposed during seed development (Fig. 13). Seed germination in the progeny was delayed at developmental temperatures of $23^{o}/15^{o}C$ and $38^{o}/30^{o}C$ compared with $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$. Hypocotyl length was 20% less from seeds which developed at $23^{o}/15^{o}C$ than at the higher temperatures. The optimum temperatures for subsequent high seedling fresh weight and axis weights of 'Pinkeye Purple Hull' were $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$ (Fig. 14). Temperatures below or above these values reduced axis growth in the progeny by 20%. Seedling dry weight and cotyledon weights were correlated with the initial seed weight (r=0.97***).

After accelerated aging, germination of 'Pinkeye Purple Hull' was reduced by 20% and more abnormal seedlings were produced when seeds developed at $23^{o}/15^{o}C$ compared with $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$ (Fig. 13). Seeds from $28^{o}/20^{o}C$ and $33^{o}/25^{o}C$ treatments during seed development germinated faster and had longer hypocotyls than seeds from $23^{o}/15^{o}C$ and $38^{o}/30^{o}C$ treatments. After aging, radicle

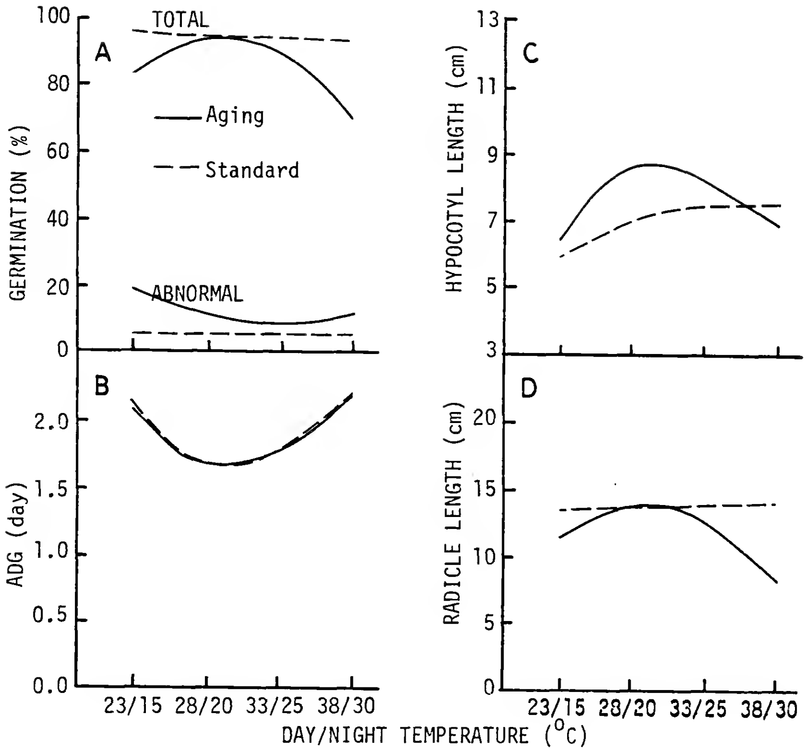

Fig. 13 Effect of different temperatures during seed development on seed germination, germination rate and seedling lengths of the progeny of 'Pinkeye Purple Hull' cowpea with the standard germination test and after accelerated aging.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

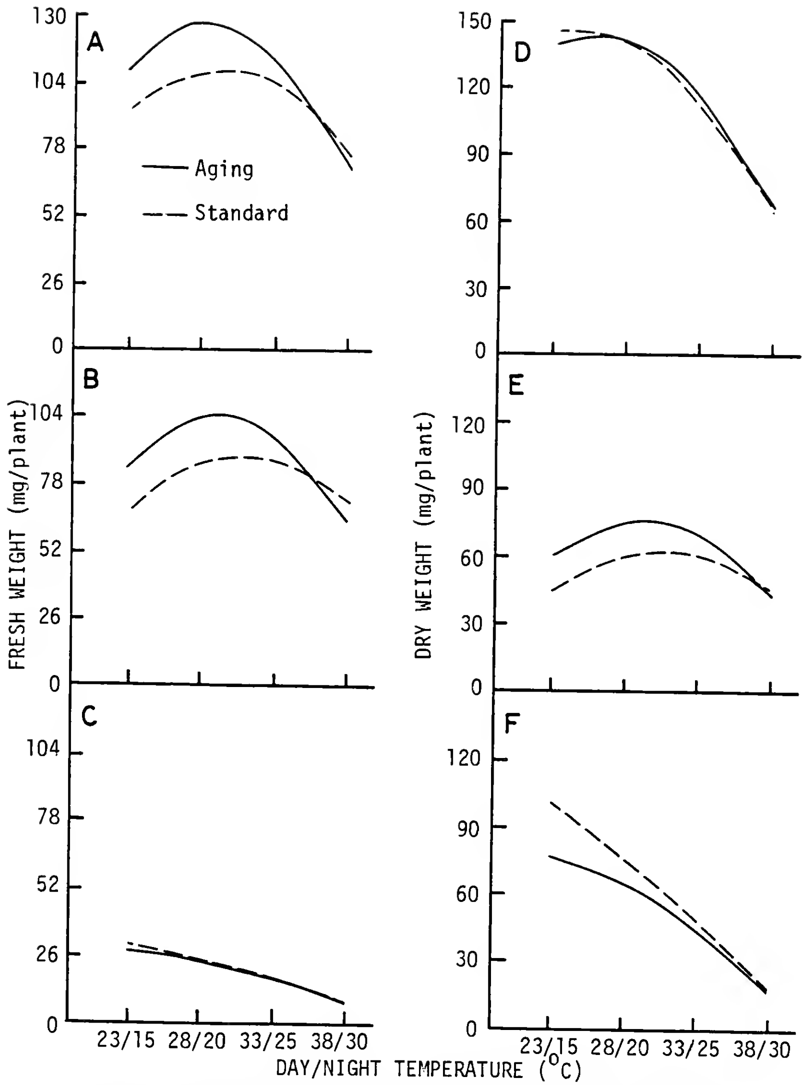

Fig. 14 Effect of different temperatures during seed development on seedling weight of the progeny of 'Pinkeye Purple Hull' cowpea with the standard germination test and after accelerated aging.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, B. axis, C. cotyledon.

length, axis weight and seedling fresh weight were greater from seeds which developed at $28^{0}/20^{0}C$ than at $33^{0}/25^{0}C$ or $23^{0}/15^{0}C$ (Figs. 13, 14). These values were the least from seeds which developed at $38^{0}/30^{0}C$. Seedling dry weight and cotyledon weight followed the same trend as initial seed weight, namely, weights increased as temperatures decreased.

Axis growth of 'Pinkeye Purple Hull' seeds which developed at $23^{0}/15^{0}C$, $33^{0}/25^{0}C$ and $38^{0}/30^{0}C$ were 80%, 91% and 60%, respectively, of those which developed at $28^{0}/20^{0}C$ after aging, compared with 80%, 100% and 80% before aging. Thus, seedling growth after aging was reduced from seeds which developed at $33^{0}/25^{0}C$ and $38^{0}/30^{0}C$. Overall, temperatures of $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$ led to the highest quality seeds of 'Pinkeye Purple Hull', while temperature of $38^{0}/30^{0}C$ led to the lowest quality seeds.

The influence of temperature treatments during seed development on seed vigor was similar in both cowpea cultivars. Both produced small and low vigor seedlings when seeds developed at $38^{0}/30^{0}C$. The heaviest seeds were produced in both cultivars at $23^{0}/15^{0}C$, but axis growth in the progeny from these seeds was reduced compared with those which developed at $28^{0}/20^{0}C$. The reduction in axis growth of both cultivars which developed at $23^{0}/15^{0}C$ was less than that from seeds which developed at $38^{0}/30^{0}C$. Although axis growth in seeds which developed at the lower temperatures was greater in 'Texas Cream 40' than in 'Pinkeye Purple Hull' with the standard germination test (Fig. A-6), this difference was not significant after aging (Fig. A-8). This indicated that 'Texas Cream 40' seeds which developed at low temperature appeared to be more sensitive to stress during germination than 'Pinkeye Purple Hull'.

Seeds with the highest vigor were produced in 'Texas Cream 40' and 'Pinkeye Purple Hull' when developed at $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$. A similar result was reported by Ndunguru et al. (1978) for 'K2809' cowpea. Plant development, final seed yield and weight were unaffected by developmental temperatures of $33^{0}/24^{0}C$ and $27^{0}/19^{0}C$ imposed on the mother plant. Large seeds produced larger seedlings at the early stages of development than small seeds from both temperature treatments.

The adverse effect that high temperature (>$32^{0}C$) during seed development had on seed vigor was also observed by Green et al. (1965) in soybean. Compared with low temperature ($21^{0}C$), high temperature ($27^{0}C$) during the last 45 days of seed maturation in soybean reduced subsequent seedling growth and seed yield (Harris et al., 1965). Siddique and Goodwin (1980a, 1980b) reported that normal seedling development in bean decreased linearly as temperature during seed development and maturation increased from $21^{0}/16^{0}C$ to $33^{0}/28^{0}C$. Walter and Jensen (1970) reported that seeds with similar vigor were produced in 'Moapa' alfalfa at both $13^{0}C$ and $24^{0}C$, while seedling weight and leaf number in the progeny were greater in 'Ranger' when seeds developed at $13^{0}C$ instead of $24^{0}C$. If seedling weight of cowpea was taken as a vigor index, the influence of temperature during seed development on vigor would be similar to those of bean and alfalfa; for example, the lower the developmental temperature, the higher the seed vigor. However, axis weight from seeds which developed at $23^{0}/15^{0}C$ was less than that from $28^{0}/20^{0}C$ (Figs. 12, 14). Because larger seeds had a less axis weight, the use of seedling weight as a vigor index in the above case was concealed by the influence of temperature on seed size.

Influence of temperature stress on seed composition

Temperature variation during seed development had a profound influence on the chemical composition of the seeds produced (Fig. 15). Seed N and protein concentrations of 'Texas Cream 40' were similar at $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$, 6% higher than those values at $38^{0}/30^{0}C$, and 13.5% lower at $23^{0}/15^{0}C$. In 'Pinkeye Purple Hull', seed N and protein concentrations decreased as temperature increased from $23^{0}/15^{0}C$ to $33^{0}/25^{0}C$, then increased to 5.6% total N and 22% protein as temperature increased to $38^{0}/30^{0}C$. Seed P concentration increased linearly in 'Texas Cream 40' and quadratically in 'Pinkeye Purple Hull' as developmental temperature increased from $23^{0}/15^{0}C$ to $38^{0}/30^{0}C$. Starch concentrations, however, decreased in both cultivars as temperature increased. On a per seed basis, all these nutrients decreased as temperature increased (Fig. 16). Thus, seed size determined the total available nutrients.

Robertson et al. (1962) reported that protein and starch synthesis in pea seeds was delayed by low maturation temperature; however, starch content on a fresh weight basis increased at low temperature due to prolonged duration for accumulation. Sofield et al. (1977) observed in wheat that although seed N and P concentrations increased at high developmental temperature ($30^{0}/25^{0}C$) the seeds produced were smaller in size than those which developed at the lower temperatures. This led to the reduced total N and P quantities in the seed (mg per seed). Similar results of low N and P contents in seeds produced at high temperature were reported by Chowdhury and Wardlaw (1978) in wheat, oat and sorghum. High production temperatures led to reduction in seed size, starch, and protein content in wheat

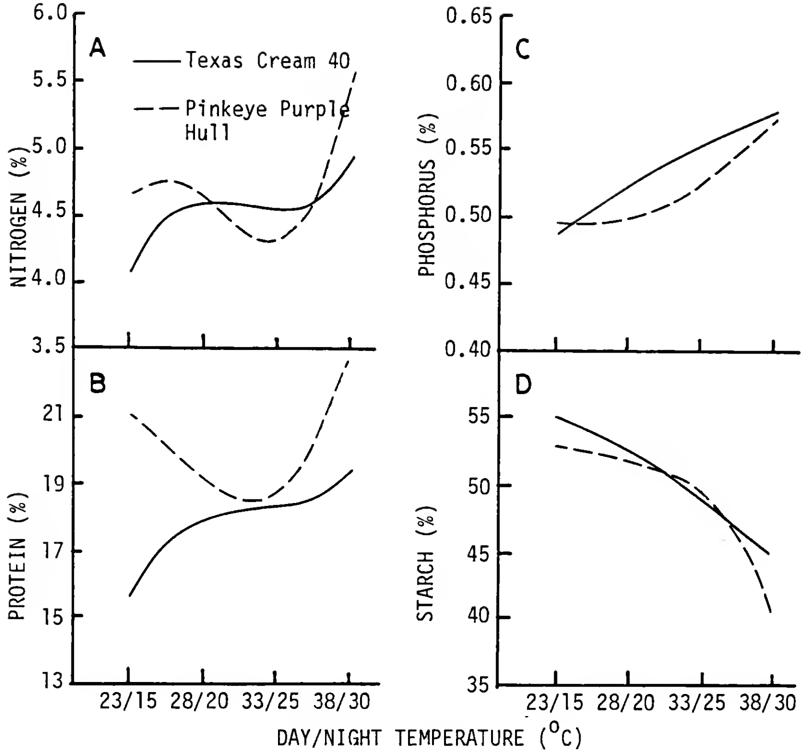

Fig. 15 Effect of different temperatures during seed development on seed composition (% dry weight basis) of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.

A. nitrogen, B. protein, C. phosphorus, D. starch.

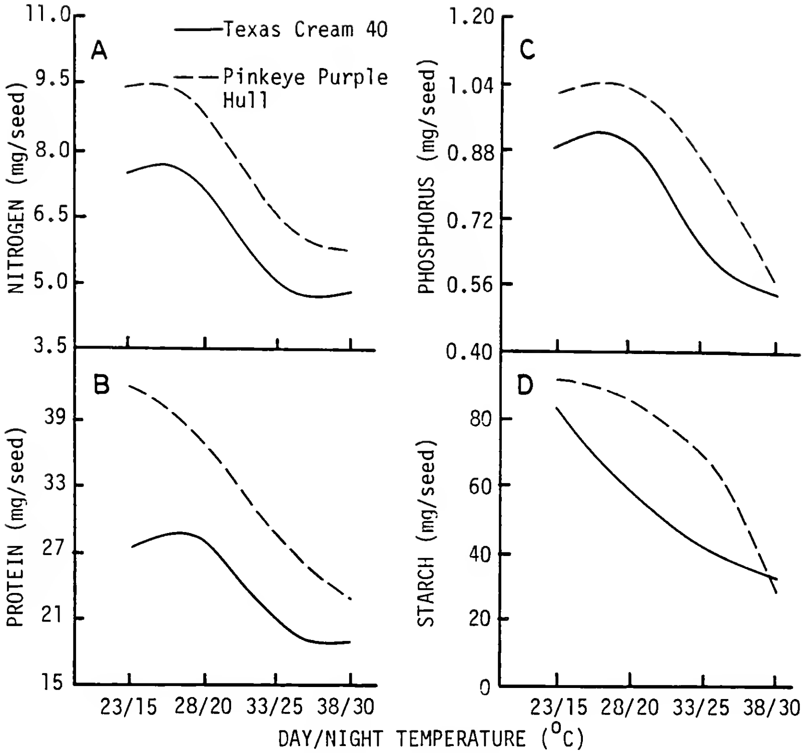

Fig. 16 Effect of different temperatures during seed development on seed composition (mg per seed) of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.

A. nitrogen, B. protein, C. phosphorus, D. starch.

(Spiertz, 1977) and in pearl millet (Fussell et al., 1980). The influence of temperature on seed vigor of the progeny was not reported in these studies, however.

Ries (1971) reported that larger seedlings and higher bean yields were obtained from seeds high in protein compared to those low in protein of the same size. Austin and Longden (1965, 1966) observed that higher P contents in seeds of carrot, watercress and pea led to better seed vigor. These relationships were not found in the present experiments with cowpea after temperature stress during seed development. The greatest axis growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' was from seeds which developed at $28^{0}/20^{0}$C and $33^{0}/25^{0}$C, yet neither had excessively high N and P concentrations. Seed development at $38^{0}/30^{0}$C led to the highest concentrations of N, protein, and P in the seeds but the poorest seedling axis growth in the progeny. After five days of germination, lack of nutrient supply probably accounted for the reduction in axis weight from seeds which developed at $38^{0}/30^{0}$C. However, this would not explain why axis growth of seeds which developed at $23^{0}/15^{0}$C was less than seeds which developed at $28^{0}/20^{0}$C and $33^{0}/25^{0}$C because seeds produced at $23^{0}/15^{0}$C were the largest in size. Efficiency in the utilization of storage nutrients during seed germination might be impaired by low developmental temperature or embryo damage might have occurred at low temperature.

Embryo culture

Although seed weights of both cultivars increased as developmental temperatures decreased, embryos excised from these seeds were, for the most part, similar in size (Table 13). Radicle length and axis weight of both cultivars after five days culture at 25^{0}C were related to the embryo size, although only the axis dry weight was significantly different among the various temperature treatments.

Table 13

Effect of Different Temperatures During Seed Development on Embryo Size at Seed Maturity and Its Growth in Culture of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Cultivar	Day/Night temperature	Wt/embryo	Axis length		Axis wt	
			hypocotyl	radicle	fresh	dry
	°C	mg	cm		mg/embryo	
Texas Cream 40	23/15	3.4a[z]	0.99a	3.0a	32a	3.2a
	28/20	3.3a	0.96a	3.2a	35a	3.0a
	33/25	2.9b	0.97a	2.8a	28a	2.5b
	38/30	3.1ab	0.95a	2.8a	34a	3.0a
Pinkeye Purple Hull	23/15	4.2a	1.11a	3.1a	36a	3.5a
	28/20	3.5b	0.94a	2.8a	30a	3.0b
	33/25	4.3a	1.02a	3.0a	36a	3.6a
	38/30	3.9ab	1.27a	2.7a	38a	3.4a

[z]Values followed by the same letter within cultivar column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Chang and Struckmeyer (1976) reported that onion (Allium cepa) produced many aborted seeds with retarded endosperm growth when seeds developed at $43^{o}C$. Similar seed abortion was observed in 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas at $38^{o}/30^{o}C$. These data were not included in seed yield. Embryos surviving heat stress were able to develop normally, although the shorter duration of the seed growth period and more rapid leaf senescence appeared to lead to a reduction in nutrient accumulation in the cotyledons. Compared axis growth of embryos in culture (Table 13) with that of whole seeds (Figs. 12, 14), embryo size and its relative growth obviously did not lead to the reduction in seed vigor at stress tempeatures of $38^{o}/30^{o}C$ and $23^{o}/15^{o}C$ during seed development. Therefore, utilization of food reserves during germination was probably affected by adverse seed developmental temperatures.

Partitioning of seedling weight and nutrient composition during germination

Generally, changes in seedling growth and utilization of storage reserves during germination were similar in 'Texas Cream 40' and 'Pinkeye Purple Hull' seeds which developed at different temperatures (Tables A-5, A-6). Seedling fresh weight increased almost linearly in both cultivars, but dry weight decreased slightly over the five day germination period. Protein content of the whole seedling decreased during germination, accompanied by an increase in amino acid content. Loss of starch in the whole seedling during germination was not accompanied by an immediate increase in soluble carbohydrate. In fact, soluble carbohydrate decreased during the first three days of germination from seeds which developed at $28^{o}/20^{o}C$ or higher. This loss of carbohydrate was assumed to be transformed to structural components and/or used for respiration.

The hydrolysis of storage protein and starch, and the loss of soluble carbohydrate during germination coincided with rapid radicle growth and hypocotyl elongation (Tables A-5, A-6). From this time on, axis weights increased dramatically, accompanied by a decrease in cotyledon dry weight. The protein, amino acid, and soluble carbohydrate in the axis during the first day of germination was apparently used for respiration. No starch was found in the axis throughout germination. A constantly low amino acid content and a decrease of soluble carbohydrate in the cotyledons during germination indicated that protein and starch were hydrolyzed and moved to the axis without significant accumulation of products in the cotyledons.

Although cotyledon weight and nutrient reserves in 'Texas Cream 40' seeds which developed at $23^{o}/15^{o}C$ and $28^{o}/20^{o}C$ decreased at similar rates during germination, axis weight increase was slower at the third day of germination in seeds which developed at $23^{o}/15^{o}C$ than seeds which developed at $28^{o}/20^{o}C$ (Table 14). The decrease in amino acid and soluble carbohydrate in the cotyledons and the corresponding influx of these materials in the axis were also slower in the fomer compared to the latter at the third day of germination. Decreases in axis growth and movement of amino acid and soluble carbohydrate from cotyledons to the axis, as shown in the partitioning experiment with the standard germination test (Table 14), might have been further slowed down after aging. This possibly led to the low seed vigor in seeds which developed at $23^{o}/15^{o}C$ (Fig. 12).

After five days of germination axis growth rates were similar in 'Texas Cream 40' seeds which developed at $33^{o}/25^{o}C$ or less, regardless of seed size at maturity (Table 14). Cotyledon weight and nutrient reserves decreased more rapidly in seeds which developed at $33^{o}/25^{o}C$

Table 14

Effect of Different Temperatures during Seed Development on the Percentage Changes in Weight and Nutrient Composition in the Axis and Cotyledons of 'Texas Cream 40' Cowpea during Germination

Measurement	Day/Night temperature	Axis 1	Axis 3	Axis 5	Cotyledon 1	Cotyledon 3	Cotyledon 5
	°C	% change			% change		
Fresh wt	23/15	452a[z]	6780b	22500a	87a	92a	44a
	28/20	531a	8250a	26100a	109a	105a	41a
	33/25	501a	7850a	25500a	107a	97a	19b
	38/30	374a	6600b	18100b	94a	84a	4c
Dry wt	23/15	25a	530b	1750a	-8a	-20a	-49a
	28/20	32a	660a	2000a	-3a	-20a	-53a
	33/25	31a	630a	1850a	-2a	-26ab	-65b
	38/30	14a	540b	1310b	-8a	-32b	-69b
Protein	23/15	16a	243a	760a	1a	-15a	-48a
	28/20	-2a	225a	680a	3a	-16a	-53a
	33/25	-2a	218a	670a	-6ab	-28b	-70b
	38/30	9a	228a	570a	-9b	-33b	-71b
Amino acid	23/15	87a	1660b	6750b	51a	39a	-17a
	28/20	113a	1990a	7520ab	49a	19b	-37b
	33/25	86a	1980a	7670a	19b	-9c	-61d
	38/30	61a	1630b	5170c	29b	15b	-48c
Soluble carboydrate	23/15	-37a	600b	2030a	-1a	-35a	-76a
	28/20	-35a	750a	2220a	-1a	-66b	-84b
	33/25	-35a	730a	2050a	-17b	-77c	-89c
	38/30	-47a	540b	1160b	-4a	-73c	-90c
Starch	23/15				-11a	-22a	-50a
	28/20				-8a	-17a	-57ab
	33/25				-6a	-21a	-67bc
	38/30				1a	-28a	-71c

[z]Values followed by the same letter within measurement column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

compared with the lower temperatures. This appeared to be related to the initial seed size (Table A-5), because changes in total quantities of weight and nutrients over the five days germination period were less in seeds which developed at $33^{0}/25^{0}C$ than at $28^{0}/20^{0}C$ (Table 18). A decrease in axis weight in seeds which developed at $33^{0}/25^{0}C$ over those which developed at $28^{0}/20^{0}C$ during germination was probably due to a smaller embryo size in the former at seed maturity (Table A-5).

Similar seed and embryo sizes were obtained in 'Texas Cream 40' when seeds developed at $38^{0}/30^{0}C$ and $33^{0}/25^{0}C$ (Table A-5). However, axis growth rate decreased in seeds which developed at $38^{0}/30^{0}C$ after three days of germination (Table 14). Loss of protein and starch from the cotyledons during germination was similar in seeds which developed at both of the above temperatures (Table 14), but movement of amino acid and soluble carbohydrate to the axis was slower in seeds which developed at $38^{0}/30^{0}C$ than at $33^{0}/25^{0}C$ (Tables 14, 15). Therefore, the former seemed unable to utilize reserve food as efficiently as the latter. A lower starch concentration in the cotyledons, at seed maturity and during germination, was found in seeds which developed at $38^{0}/30^{0}C$ compared with seeds which developed at $33^{0}/25^{0}C$ (Table 15). This might partially explain the large decrease in soluble carbohydrate accumulation in the axis of heat stressed seeds after five days of germination.

'Pinkeye Purple Hull' seeds which developed at $23^{0}/15^{0}C$ and $28^{0}/20^{0}C$ had similar seed weights at maturity (Table A-6). However, changes in weight and nutrient composition were generally delayed in seeds which developed at $23^{0}/15^{0}C$ compared with seeds which developed at $28^{0}/20^{0}C$ or $33^{0}/25^{0}C$ (Table 16). The actual increases in weight

Table 15

Effect of Different Tempeatures during Seed Development on Nutrient Concentrations in the Axis and Cotyledons of 'Texas Cream 40' Cowpea after, 0, 1, 3 and 5 days of Germination

Nutrient	Day/Night temperature	Axis				Cotyledon			
		0	1	3	5	0	1	3	5
	°C	—%[y]—				—%—			
Protein	23/15	20.0b[z]	18.6b	10.9a	9.3b	14.6b	16.0b	15.4c	14.9b
	28/20	24.9a	18.4b	10.7a	9.3b	16.5a	17.4a	17.2ab	16.6a
	33/25	23.9a	17.9b	10.4a	9.3b	16.7a	16.1b	16.4b	14.3b
	38/30	21.0b	19.9a	10.8a	10.0a	18.1a	17.8a	17.7a	16.9a
Amino acid	23/15	3.0b	4.5c	8.3c	11.1b	1.1b	1.9b	2.0c	1.8b
	28/20	3.7a	5.9a	10.1a	13.3a	1.5a	2.3a	2.2b	2.0b
	33/25	3.3ab	4.7bc	9.4b	13.1a	1.6a	2.0b	2.0c	1.9b
	38/30	3.5ab	4.9b	9.5b	13.2a	1.7a	2.3a	2.8a	2.8a
Soluble carbohydrate	23/15	26.1a	13.2a	28.9a	30.3a	9.8b	10.5bc	7.8a	4.6a
	28/20	24.2a	11.7a	27.2a	26.8b	9.8b	10.0c	4.2c	3.5c
	33/25	25.0a	12.5a	28.7a	27.5b	12.8a	10.9b	4.0c	4.1ab
	38/30	27.3a	12.7a	27.3a	24.5c	12.3a	12.9a	4.8b	3.8bc
Starch	23/15					54.5a	53.7a	54.1a	54.5a
	28/20					52.4a	49.5b	54.6a	48.7b
	33/25					49.0b	50.3b	52.4a	46.1bc
	38/30					44.4c	45.4c	47.0b	41.7c

[z] Values followed by the same letter within nutrient column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

[y] % dry weight basis

Table 16

Effect of Different Temperatures during Seed Development on the Percentage Changes in Weight and Nutrient Composition in the Axis and Cotyledons of 'Pinkeye Purple Hull' Cowpea during Germination

Measurement	Day/Night temperature	Axis			Cotyledon		
		1	3	5	1	3	5
	°C	% change			% change		
Fresh wt	23/15	259b[z]	5000b	17000bc	101ab	105a	76a
	28/20	480a	7340a	22700a	85b	83a	35b
	33/25	510a	6260ab	19000b	82b	84a	22bc
	38/30	530a	5030b	14500c	118a	94a	9c
Dry wt	23/15	2b	400b	1340bc	1a	-11a	-37a
	28/20	14ab	560a	1710a	-7a	-22b	-51b
	33/25	25a	520a	1520ab	-7a	-21b	-58b
	38/30	20ab	450ab	1110c	4a	-29b	-69c
Protein	23/15	5a	270a	700b	-5a	-30a	-52a
	28/20	17a	300a	830ab	-10a	-29a	-63b
	33/25	30a	320a	890a	-12a	-31a	-75c
	38/30	-1a	160b	440c	-7a	-35a	-84d
Amino acid	23/15	40c	960b	3970b	38b	50a	5a
	28/20	94b	1550a	5870a	73a	52a	-28b
	33/25	139a	1520a	5380a	52ab	9b	-47c
	38/30	81b	1020b	3510b	34b	14b	-54c
Soluble carbohydrate	23/15	-53a	410b	1490b	3a	-25a	-68a
	28/20	-58a	640a	2100a	-13a	-60b	-82b
	33/25	-45a	560ab	1680ab	-14a	-68b	-85bc
	38/30	-45a	450ab	910c	-14a	-80c	-90c
Starch	23/15				1a	-25a	-46a
	28/20				1a	-26a	-56b
	33/25				4a	-25a	-63b
	38/30				0a	-48b	-79c

[z]Values followed by the same letter within measurement column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

and nutrient composition from day three to five in the axis of the former were close to those changes in the latter (Table A-7). Therefore, the reduction in axis growth of seeds from the $23^{0}/15^{0}C$ treatment was probably due to a lag in active growth in the first day of germination (Table A-7).

'Pinkeye Purple Hull' seeds which developed at $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$ had similar growth rates in the axis during germination (Table 16), regardless of seed and embryo sizes at maturity (Table A-6). Hydrolysis of food reserves in the cotyledons and utilization of the breakdown products in the axis were, for the most part, at similar rates in both seeds.

Reduction in growth and nutrient accumulation in the axis of 'Pinkeye Purple Hull' seeds which developed at $38^{0}/30^{0}C$, compared with seeds which developed at $28^{0}/20^{0}C$ and $33^{0}/25^{0}C$, occurred after three days of germination (Table 16). As germination progressed, the highest rates (Table 16), but the least actual quantities (Table 18), of weight and storage reserve loss in the cotyledons were observed in seeds which developed at $38^{0}/30^{0}C$. The same seeds were the smallest in size (Table A-6) and contained the least concentration of starch (Table 17). This most likely led to a reduction in the amount of carbohydrate available for axis growth. After five days of germination most of the starch was utilized in these heat stressed seeds (Table A-5), which, in turn, probably led to the low concentration of soluble carbohydrate in the axis (Table 17). Because changes in weight and nutrient composition in the axis and cotyledons during early germination were unaffected by developmental temperatures of $38^{0}/30^{0}C$ and $33^{0}/25^{0}C$, low seed vigor at $38^{0}/30^{0}C$ was probably due to an insufficient reserve food at seed maturity.

Table 17

Effect of Different Temperatures during Seed Development on Nutrient Concentrations in the Axis and Cotyledons of 'Pinkeye Purple Hull' Cowpea after 0, 1, 3 and 5 days of Germination

Nutrient	Day/Night temperature	Axis 0	Axis 1	Axis 3	Axis 5	Cotyledon 0	Cotyledon 1	Cotyledon 3	Cotyledon 5
	°C	—%[y]—				—%—			
Protein	23/15	18.8b[z]	19.4a	13.7a	10.4a	18.0ab	16.9ab	14.3bc	13.5a
	28/20	19.6b	19.9a	11.8a	10.0a	16.9bc	16.3bc	15.7b	12.9a
	33/25	16.6c	17.2a	11.2a	10.2a	15.9c	15.1c	13.9c	10.5b
	38/30	23.9a	19.8a	11.6a	10.8a	20.2a	18.2a	18.5a	9.7b
Amino acid	23/15	3.3b	4.5b	7.0c	9.3c	1.1bc	1.5c	1.9b	1.9b
	28/20	3.2b	5.5a	7.9ab	10.5b	1.0c	1.7c	1.8bc	1.7b
	33/25	3.1b	5.5a	7.5bc	9.8bc	1.2b	2.0b	1.7c	1.5c
	38/30	4.2a	6.3a	8.5a	12.5a	2.1a	2.7a	3.3a	3.1a
Soluble carbohydrate	23/15	24.7a	11.4a	25.1a	27.4a	8.9c	9.1a	7.5a	4.5a
	28/20	23.8a	8.8a	26.7a	28.7a	8.5c	7.9b	4.4b	3.3c
	33/25	25.1a	10.9a	26.7a	27.6a	10.0b	9.2a	4.1bc	3.6bc
	38/30	23.1a	10.6a	23.1a	19.4b	12.1a	10.0a	3.4c	3.9b
Starch	23/15					53.3a	53.1a	45.1a	45.6a
	28/20					51.7a	55.6a	49.2a	47.2a
	33/25					50.3a	56.5a	47.6a	44.3a
	38/30					40.8b	39.4b	29.8b	27.7b

[z]Values followed by the same letter within nutrient column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

[y]% dry weight basis

Table 18

Effect of Different Temperatures during Seed Development on Changes in Weight and Nutrient Composition of Axis and Cotyledons of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpea during a 5 Day Germination Period

Measurement	Day/Night temperature	Texas Cream 40		Pinkeye Purpule Hull	
		Axis	Cotyledon	Axis	Cotyledon
	°C	mg		mg	
Fresh wt	23/15	776	74	709	137
	28/20	821	56	815	64
	33/25	722	19	788	32
	38/30	602	4	597	7
Dry wt	23/15	55.5	-74	52.9	-62
	28/20	57.0	-66	58.3	-86
	33/25	48.2	-59	58.8	-78
	38/30	40.0	-58	41.9	-51
Protein	23/15	4.8	-10.7	5.2	-15.8
	28/20	4.8	-10.7	5.5	-17.7
	33/25	4.1	-10.8	5.7	-15.7
	38/30	3.7	-10.6	4.0	-12.6
Amino acid	23/15	6.4	-0.3	5.2	-0.1
	28/20	7.9	-0.7	6.4	-0.3
	33/25	6.6	-0.9	6.0	-0.8
	38/30	5.5	-0.7	5.6	-0.8
Soluble carbohydrate	23/15	17.0	-11.3	14.6	-10.2
	28/20	15.4	-10.1	17.0	-11.6
	33/25	13.5	-10.2	16.3	-11.4
	38/30	9.8	-9.4	8.0	-8.0
Starch	23/15		-40.5		-40.9
	28/20		-36.9		-48.2
	33/25		-29.5		-42.6
	38/30		-26.4		-23.8

Lowe and Ries (1973) reported an influence of storage tissue on seed vigor of wheat. A greater dry weight in wheat seedlings derived from high protein seeds than from low protein seeds was attributed to the endosperm, not the embryo. Reduction in seed vigor due to high temperature during seed development was reported to be related to a decrease in seed size of pearl millet (Fussell and Pearson, 1980). However, information regarding which part of the seed contributed to the low seed vigor due to temperature stress was not reported.

In the present study, low seed vigor of 'Pinkeye Purple Hull' cowpea which developed at high temperature was due to the insufficient food reserve in the cotyledons, not due to an injury to the embryo.

Although cowpea is tropical in origin, high temperature stress during seed development had a detrimental effect on seed yield and vigor. Seed vigor was also reduced by low temperature during cowpea seed development, even though large seeds were produced. The adverse effect of high or low temperature on seed vigor was probably related to a reduction in nutrient build up on the seeds or to a reduction in the ability to utilize food reserves efficiently during germination. No apparent injury to the embryo was found in the seeds harvested from the different temperature treatments in this experiment.

In order to produce seeds of high vigor in cowpea, low or high temperature during seed development should be avoided by altering the planting date and/or by using short season cultivars.

Summary

'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were grown at day/night temperatures of $38^{o}/30^{o}C$, $33^{o}/25^{o}C$, $28^{o}/20^{o}C$, and $23^{o}/15^{o}C$

during seed development to investigate the influence of temperature stress on seed vigor.

Seed yields and seed weights of both cultivars decreased as temperature increased. The greatest axis growth was observed in seeds of both cultivars which developed at $33^{0}/25^{0}C$ and $28^{0}/20^{0}C$. Both could utilize storage nutrients efficiently during germination, regardless of seed size. Seeds of both cultivars which developed at $38^{0}/30^{0}C$ had the least axis growth. Seeds of 'Texas Cream 40' which developed at $38^{0}/30^{0}C$ appeared unable to utilize storage nutrients as efficiently as seeds which developed at $28^{0}/20^{0}C$ or $33^{0}/25^{0}C$. In 'Pinkeye Purple Hull', low seed vigor at $38^{0}/30^{0}C$ was related to a shortage of reserve food, especially starch, at seed maturity. Exposure to low temperature ($23^{0}/15^{0}C$) during seed development reduced seed vigor of both cultivars to a less extent than the high temperature ($38^{0}/30^{0}C$). In 'Texas Cream 40', $23^{0}/15^{0}C$ exposure probably caused a slower rate of utilization of storage nutrients during germination. In 'Pinkeye Purple Hull', a lag in active seedling growth and utilization of reserve food during early germination probably led to reduced vigor of seeds produced at this temperature. No apparent injury to the embryo was found by low or high temperature stress in this study.

CHAPTER V

CHANGES IN SEED VIGOR OF COWPEA DUE TO NUTRITIONAL TREATMENTS IMPOSED AT DIFFERENT GROWTH STAGES

Seed vigor of Indian ricegrass (Whalley _et al_., 1966), wheat (Ries _et al_., 1970) and snapbean (Ries, 1971) was improved by N application to the parent plant, while that of carrot (Austin and Longden, 1966) and pea (Austin, 1966b) was unaffected by N deficiency in the parent plant. Reduction in seedling growth and yield of the progenies were observed in pea (Austin, 1966b), carrot (Austin and Longden, 1966), lettuce (Harrington, 1960) and watercress (Austin, 1966a) after maturing on parent plants which suffered from P deficiency. Calcium deficiency led to the development of abnormal seedlings in the progenies of lettuce (Harrington, 1960) and peanut (Cox _et al_., 1976). Changes in seed composition due to an alteration in the nutritional status of the parent plant were reported to affect seed vigor of wheat (Lowe and Ries, 1972), snapbean (Ries, 1971), pea (Austin, 1966b), carrot (Austin and Longden, 1966) and watercress (Austin, 1966a).

Cowpea yields were increased by the application of N, P or S (Ezedinma, 1965; Nangju, 1976); however, the influence of these nutritional treatments on seed vigor of the progeny was not reported. Sulfur amino acids, which are quantitatively low in cowpea seeds, and N contents of cowpea seeds were increased by additional N, P or S fertilizer (Evans _et al_., 1977; Nangju, 1976). Increases in seed S-amino acids and N contents improved the protein quality and quantity in cowpea, yet their relationship to seed vigor was, again, not reported. The following experiments were conducted to determine the influence of various nutritional treatments on seed vigor of the progeny of cowpea. The nutritional treatments were initiated at the time of sowing or after anthesis of the first flowers in order to compare the influence of nutritional treatments imposed at different growth stages on subsequent seed vigor. A possible relationship between chemical composition of the seed and seed vigor due to nutritional treatment was examined.

Materials and Methods

Effect of nutritional treatments imposed at the seedling stage of the parent plant on seed vigor of the progeny

A sand culture experiment was conducted in the greenhouse in the fall of 1979 at a temperature of 27^{o} $\pm$3C day and 22^{o} $\pm$3C night under natural irradiation. 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were seeded in 8 liter pots filled with sterilized #2 grade sand. Nutritional treatments (Table 19) were initiated immediately after sowing and were continued for the duration of the experiment. Pesticides were applied as needed. The experiment was a completely randomized design with four replications.

Effect of nutritional treatments imposed at anthesis in the parent plant on seed vigor of the progeny

A similar experiment was conducted in the greenhouse in the fall of 1980 at a temperature of 30^{o} $\pm$3C day and 24^{o} $\pm$3C night under natural irradiation except that the plants received a complete Hoagland's solution until flowering. At that time each pot was flushed twice with 10 liters of deioinized water to remove residual nutrients from the sand. Three levels of N (420ppm, 210ppm and 52.5ppm) in combination with three levels of P (62ppm, 31ppm and 7.8ppm) were

Table 19

Nutritional Treatments Imposed at the Seedling Stage of the Parental Plant

Nutrient solution variable[z]	Elemental concentration		
	N	P	S
	ppm		
control[y]	210.0	31.0	64.0
420ppm N	420.0	31.0	64.0
52.5ppm N	52.5	31.0	64.0
62ppm P	210.0	62.0	64.0
7.8ppm P	210.0	7.8	64.0
128ppm S	210.0	31.0	128.0
16ppm S	210.0	31.0	16.0

[z]The following elements were in all treatments: K 234ppm, Ca 160ppm, Mg 48ppm, Fe 0.6ppm, B 0.5ppm, Mn 0.5ppm, Zn 0.05 ppm, Cu 0.02ppm, Mo 0.01ppm.

[y]Complete Hoagland's solution (Hoagland and Arnon, 1938).

applied throughout the seed development period. The experiment was a 2x3x3 factorial in a completely randomized design with four replications.

Evaluation of seed vigor

Effect of nutritional treatments on seed yield and vigor. Seed yield measurements and vigor tests were conducted as previously described (Chapter II).

Effect of nutritional treatments on seed composition. Total seed N, protein, P and starch were analyzed as previously described (Chapter III). The seed S-amino acids, cysteine and methionine, were determined by a microbiological method, using *Leuconostoc mesenteroides* (Difco, ATCC 8042) growth as an indicator of S-amino acid content (Evans *et al*., 1976; Hannah *et al*., 1977).

Statistical analysis. Analysis of variance was performed as previously described (Chapter II).

Results and Discussion

Effect of nutritional treatments imposed at the seedling stage of the parent plant on seed vigor of the progeny

Effect of nutritional treatments on seed yield and vigor. Average seed yield and weight were similar for both cultivars (Table 20). However, the influence of various nutritional treatments on seed yield and weight varied with the cultivar. Seed yield of 'Texas Cream 40' decreased by 41% at 52.5ppm N compared with the higher two N levels, while seed weight increased by 10% at 420ppm N compared with the lower two N levels (Table 20). The 7.8ppm P treatment led to a 38% decrease in seed yield of 'Texas Cream 40' compared with the higher two P levels, however, seed weight was unaffected by P treatment. Neither seed yield nor seed weight was affected by different S levels.

Table 20

Effect of Nutritional Treatments Imposed at the Seedling Stage of the Parent Plant on Seed Yields and Weights of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Variable	Seed yield		Wt/100 seeds	
	g/plant		g	
Cultivar				
Texas Cream 40	17.1a[z]		16.8a	
Pinkeye Purple Hull	17.5a		17.7a	
Treatment	Texas Cream 40	Pinkeye Purple Hull	Texas Cream 40	Pinkeye Purple Hull
N(ppm)				
52.5	10.6b	9.8d	15.6c	14.1c
210.0[y]	18.1a	22.3a	16.5bc	17.0b
420.0	19.7a	18.4b	18.1a	19.0a
P(ppm)				
7.8	13.1b	13.5c	15.7c	18.0ab
31.0[y]	18.1a	22.3a	16.5bc	17.0b
62.0	18.5a	24.1a	16.5bc	18.3ab
S(ppm)				
16.0	19.9a	16.4bc	17.8ab	19.3a
64.0[y]	18.1a	22.3a	16.5bc	17.0b
128.0	19.6a	17.8b	17.5ab	17.8b
Interaction				
Cv x Treatment	**		*	

[z]Values followed by the same letter within each variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*,**Significant interaction at 5%, 1% level, respectively.

[y]Control: complete Hoagland's solution.

Seed yield of 'Pinkeye Purple Hull' decreased by 17% at 420ppm N and 56% at 52.5ppm N, compared with the 210ppm N treatment (Table 20). Seed weight increased as the supply of N increased. 'Pinkeye Purple Hull' yielded 40% less at 7.8ppm P than the higher two P levels, but seed weight was unaffected by P treatment. Deviation from 64ppm S in the nutrient solution reduced seed yields by 20 to 26%. Seed weight, however, increased by 10% at 16ppm S compared with the higher two S levels.

After the standard germination test, seed germination and seedling axis growth of 'Texas Cream 40' were generally less than those of 'Pinkeye Purple Hull' (Table 21). The former germinated more rapidly than the latter, however.

In 'Texas Cream 40', seed germinability and abnormal seedling development after the standard germination test were unaffected by various nutritional treatments imposed on the parent plant (Table 21). Germination rate was similar regardless of N treatment. Axis lengths and weights were less in seeds which were produced with 420ppm N compared with the lower two N levels, although the former had a heavier seed weight at seed maturity than the latter. Compared with the 31ppm P treatment, seeds from the 7.8ppm P treatment germinated more rapidly, while seeds from the 62ppm P treatment had shorter hypocotyl lengths. Radicle length and axis weight were similar regardless of P treatment. Generally different S levels in the growth media had no influence on germination rate or seedling growth of the progeny of 'Texas Cream 40'.

Seed germinability and abnormal seedling development of 'Pinkeye Purple Hull' seeds after the standard germination test were unaffected by altering nutrient supply to the parent plant (Table 21). Germination rate was slower in seeds from the 52.5 or 420ppm N treatments compared with the 210ppm N treatment. Seeds which were produced at 420ppm N

Table 21

Effect of Nutritional Treatments Imposed at the Seedling Stage of the Parent Plant on Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas with the Standard Germination Test

Variable	Germination: total	Germination: abnormal	ADG		Seedling length: hypocotyl		Seedling length: radicle	Seedling fresh wt: axis		Seedling fresh wt: cotyledon	Seedling fresh wt: seedling	Seeding dry wt: axis		Seeding dry wt: cotyledon	Seeding dry wt: seedling
	%	%	day		cm		cm	mg/part		mg/part	mg/part	mg/part		mg/part	mg/part
Cultivar															
Texas Cream 40	91b[z]	4a	1.7b		5.6b		13.4a	518b		264a	782b	39.0b		88.0a	127.0a
Pinkeye Purple Hull	98a	4a	1.9a		6.0a		12.9a	609a		248a	857a	45.7a		79.6a	125.3a
Treatment			Texas Cream 40	Pinkeye Purple Hull	Texas Cream 40	Pinkeye Purple Hull		Texas Cream 40	Pinkeye Purple Hull			Texas Cream 40	Pinkeye Purple Hull		
N(ppm)															
52.5	92a	3a	1.9a	1.8a	5.6ab	7.1a	13.8ab	458b	634abc	203d	749c	39.2ab	46.7abc	63.5d	106.4d
210.0	97a	6a	1.9a	1.5b	6.2a	6.2bc	13.4ab	579a	656ab	235cd	852ab	41.6a	49.6ab	74.6cd	120.2cd
420.0	95a	7a	1.6a	2.1a	4.7c	5.3c	11.9c	450b	380bc	304a	818ab	34.0b	43.6c	103.5a	142.3a
P(ppm)															
7.8	96a	3a	1.4b	1.9a	6.2a	5.5c	13.3ab	548ab	545c	257bc	804abc	41.1a	41.6c	82.3bc	123.6bc
31.0	97a	6a	1.9a	1.5b	6.2a	6.2bc	13.4ab	579a	656ab	235cd	852ab	41.6a	49.6ab	74.6cd	120.2cd
62.0	93a	3a	1.7a	1.9a	5.3bc	5.9bc	12.9bc	497ab	581bc	257bc	796bc	36.8ab	42.1c	83.9bc	123.3bc
S(ppm)															
16.0	96a	8a	1.6a	1.9a	5.9ab	5.4c	13.0ab	579a	563bc	279ab	851ab	42.0a	44.8c	94.6ab	138.0ab
64.0	97a	6a	1.9a	1.5b	6.2a	6.2bc	13.4ab	579a	656ab	235cd	852ab	41.6a	49.6ab	74.6cd	120.2cd
128.0	95a	0a	1.6a	1.9a	5.3bc	6.6ab	14.0a	516ab	703a	257bc	866a	38.2ab	51.5a	84.2bc	129.0abc
Interaction															
Cv x Treatment	NS	NS	**		**		NS	**		NS	NS	*		NS	NS

[z] Values followed by the same letter within variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*, ** Significant interaction at 1% and 5% levels, respectively.

had smaller axis lengths and weights compared with those which developed at the lower two N levels. Cotyledon and seedling weights were correlated with original seed weight. Phosphorus levels other than 31ppm led to slower germination rates and less axis dry weights in the progeny. Axis length and fresh weight were unaffected by P treatment. Seeds of 'Pinkeye Purple Hull' which were produced with 64ppm S germinated more rapidly than the other S levels. In spite of a higher initial seed weight, seeds from the 16ppm S treatment had less axis growth than that which occurred at the higher two S levels.

After accelerated aging, germination of 'Texas Cream 40' seeds was less than 'Pinkeye Purple Hull' (Table 22). Although 'Texas Cream 40' germinated more rapidly and had longer axis lengths than 'Pinkeye Purple Hull', axis weights were similar for both cultivars.

The number of viable and abnormal seedlings from aged 'Texas Cream 40' seeds were unaffected by altering various nutrients to the parent plant (Table 22). Seeds germinated at similar rates regardless of N treatment. Seedlings with shorter axis lengths but similar axis weight were produced from seeds grown with 52.5 or 420ppm N compared with 210ppm N. Seeds produced on 7.8ppm P had more rapid germination rates compared with those from the higher two P levels. Axis growth was similar regardless of P treatment. Different S levels in the nutrient solution had no influence on germination rates in the progeny. Subsequent axis weight, however, increased as the supply of S increased.

In 'Pinkeye Purple Hull', germinability, abnormal seedling development and germination rate after aging were unaffected by various nutritional treatments imposed on the parent plant (Table 22). Seeds which were produced with 52.5 or 420ppm N developed into smaller seedlings than seeds which were produced with 210ppm N. Seedling growth

Table 22

Effect of Nutritional Treatments Imposed at the Seedling Stage of Parent Plant on Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas after Accelerated Aging

Variable	Germination total	Germination abnormal	AOG	AOG	Seedling length hypocotyl	Seedling length radicle	Seedling fresh wt axis	Seedling fresh wt axis	Seedling fresh wt cotyledon	Seedling fresh wt seedling	Seedling dry wt axis	Seedling dry wt axis	Seedling dry wt cotyledon	Seedling dry wt seedling
	%	%	day		cm	cm	mg/part		mg/part	mg/part	mg/part		mg/part	mg/part
Cultivar														
Texas Cream 40	62b[z]	8a	1.9b		5.1a	8.6a	480a		269a	749a	37.6a		87.3a	374.9a
Pinkeye Purple Hull	87a	7a	2.1a		4.6b	8.7b	489a		276a	765a	39.2a		87.3a	126.5a
Treatment			Texas Cream 40	Pinkeye Purple Hull			Texas Cream 40	Pinkeye Purple Hull			Texas Cream 40	Pinkeye Purple Hull		
N(ppm)														
52.5	73a	6a	2.0a	2.0a	4.7abc	8.7bc	430bc	472c	248c	674b	35.0bc	34.1bc	77.4b	106.9d
210.0	74a	10a	1.8a	2.1a	5.4a	10.1a	507a	613a	245c	803a	38.2ab	47.2a	75.9b	118.6cd
420.0	72a	5a	1.8a	2.0a	4.3c	8.0c	469ab	436c	313a	765ab	36.7bc	36.6bc	103.8a	140.1a
P(ppm)														
7.8	72a	9a	1.7b	2.7a	4.8abc	8.7abc	474ab	433c	279abc	733ab	38.3ab	34.9bc	89.8ab	176.5abc
31.0	74a	10a	1.8a	2.1a	5.4a	10.1a	507a	613a	245c	803a	38.2ab	47.2a	75.9b	118.6cd
62.0	80a	7a	2.2a	2.0a	5.5a	9.9ab	509a	554ab	256bc	787a	40.1a	43.3a	80.6b	122.3bc
S(ppm)														
16.0	78a	9a	2.1a	2.0a	4.5bc	9.0abc	463ab	465bc	306ab	770ab	34.6bc	37.6bc	101.3a	137.4ab
64.0	74a	10a	1.8a	2.1a	5.4a	10.1a	507a	613a	245c	803a	38.2ab	47.2a	75.9b	118.6cd
128.0	72a	5a	1.8a	2.1a	5.2ab	9.9ab	516a	499bc	260bc	768ab	41.1a	41.0ab	87.4ab	128.4abc
Interaction														
Cv x Treatment	NS	NS	***		NS	NS	**		NS	NS	**		NS	NS

[z]Values followed by the same letter within variable column are not significantly different at 5% level, Duncan's Multiple Range Test.

*** Significant interaction at 0.1% level.

in the progeny was less from the 7.8ppm P treatment compared with the higher two P treatments. Axis weights, but not axis lengths, of seeds which were produced with 64ppm S was greater compared with seeds produced at higher or lower S levels.

Effect of nutritional treatments on seed composition. In general, 'Pinkeye Purple Hull' seeds contained more N, protein and P, but less starch and S-amino acids than 'Texas Cream 40' seeds (Table 23). Various nutrient treatments altered seed composition of each cowpea cultivar differentially, however.

Seed N and protein concentrations of 'Texas Cream 40' increased as the supply of N increased (Table 23). Seeds with a higher N but lower protein concentration were produced with 7.8ppm P compared with the higher P levels. Varying S levels in the nutrient media had no effect on N or protein concentration in the seeds.

In 'Pinkeye Purple Hull', seed N and protein concentrations increased as N supply in the culture media increased (Table 23). Sasseville and Mills (1979) reported that seed N concentration of 'Pinkeye Purple Hull' decreased from 4.6% to 3.5% when N level in the culture solution was lowered from 150ppm to 75ppm. In the present experiment, seed N concentration of 'Pinkeye Purple Hull' increased as P supply decreased, while protein concentration was higher with 7.8ppm P than the higher two P levels. Sulfur treatments did not alter seed N or protein concentrations.

Ries (1971) reported that seed vigor of bean was improved when seed protein content increased after the addition of supplemental N. In the present study with 'Pinkeye Purple Hull' cowpea, there was a 23 to 27% decrease in total quantities of N and protein in seeds which were produced with 52.5ppm N. This appeared to be related to a 28% reduction

Table 23

Effect of Nutritional Treatments Imposed at the Seedling Stage of the Parent Plant on Seed Composition of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Variable	N		Protein		P		Starch	Methionine	Cysteine	
	%[y]		%		%		%	%	%	
Cultivar										
Texas Cream 40	3.99b[z]		17.0b		0.425b		47.9a	0.310a	0.216a	
Pinkeye Purple Hull	4.10a		18.5a		0.545a		45.8b	0.264b	0.150b	
Treatment	Texas Cream 40	Pinkeye Purple Hull	Texas Cream 40	Pinkeye Purple Hull	Texas Cream 40	Pinkeye Purple Hull			Texas Cream 40	Pinkeye Purple Hull
N(ppm)										
52.5	3.57c	3.64d	15.9c	16.9c	0.431ab	0.568b	46.5ab	0.266c	0.190b	0.139b
210.0	3.98b	4.18b	17.3b	18.2b	0.421b	0.527c	46.2ab	0.284b	0.196b	0.153a
420.0	4.18a	4.12bc	18.6a	19.6a	0.430ab	0.563b	48.4a	0.303a	0.223a	0.152a
P(ppm)										
7.8	4.19a	4.48a	16.1c	19.6a	0.360c	0.363d	44.8b	0.293ab	0.230a	0.149a
31.0	3.98b	4.18b	17.3b	18.2b	0.421b	0.527c	46.2ab	0.284b	0.196b	0.153a
62.0	3.96b	3.97c	17.3b	18.1b	0.456a	0.613a	48.0a	0.286b	0.222a	0.153a
S(ppm)										
16.0	3.99b	4.19b	17.3b	18.7ab	0.450a	0.594ab	47.8a	0.287b	0.231a	0.156a
64.0	3.98b	4.18b	17.3b	18.2b	0.421b	0.527c	46.2ab	0.284b	0.196b	0.153a
128.0	4.04ab	4.14b	16.9bc	18.0b	0.430ab	0.590ab	46.5ab	0.290b	0.223a	0.151a
Interaction										
Cv x Treatment	*		**		***		NS	NS	*	

[z]Values followed by the same letter within each variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*, **, *** Significant interaction at 0.1%, 1% and 5%, respectively.

[y]% dry weight basis

in subsequent axis growth compared with seeds which were produced with 210ppm N. A 10 to 20% increase in seed N and protein from the 420ppm N treatment, however, reduced axis growth of 'Pinkeye Purple Hull' by 22% compared with seeds produced at 210ppm N. Therefore, a decrease in seed N and protein content below some threshold level appeared to affect axis growth of 'Pinkeye Purple Hull'. The reduction in vigor characteristics of 'Pinkeye Purple Hull' seeds produced with 420ppm N cannot be explained at present. Changes in total quantities of N and protein in 'Texas Cream 40' seeds did not affect seed vigor. An increase in N concentration of carrot seeds from a 141 Kg/ha N application to the parent plant did not affect seedling growth or root yield in the progeny.

The concentration of P in 'Texas Cream 40' seeds was unaffected by N treatment, but increased as the level of P in the nutrient solution increased (Table 22). The 16ppm S treatment led to the highest, 128ppm S intermediate, and 64ppm S the lowest, P concentration in 'Texas Cream 40' seeds.

'Pinkeye Purple Hull' seeds which were produced from 210ppm N contained less P than seeds from a lower or higher N level (Table 23). As P supply increased in the growing media, seed P concentration increased. Sulfur levels other than 64ppm led to the production of seeds which contained a higher P concentration.

Austin (1966a, 1966b) and Austin and Longden (1966) reported that P deficiency led to seed with a low P content in pea, carrot and watercress. Upon germination these seeds developed into smaller seedlings and yielded less than seeds produced at a normal P level. In the present study with cowpea, the total quantity of P in 'Pinkeye Purple Hull' seeds decreased by 27% when grown with 7.8ppm P. During germination a possible reduction in energy supply in these low P seeds might partially attribute to the 26% reduction in its axis growth. An increase in the total quantity of P in seeds produced with 62ppm P did not improve seed vigor of 'Pinkeye Purple Hull'. Thus, a threshold level of P might exist in 'Pinkeye Purple Hull' seeds, below which seed vigor could be reduced. Seed vigor of 'Texas Cream 40', however, was the same regardless of seed P concentration resulting from different P supplies in the growth media.

Starch concentration of either cultivar was unaffected by N or S treatment, but increased as P level in the nutrient solution decreased (Table 23). The change in seed starch concentration due to various P levels appeared to be too small (±5%) to play an important role in changing growth patterns during early seedling development.

Seed methionine concentration increased in both cultivars as the N level in the nutrient solution increased, but was unaffected by P or S treatment (Table 23). Cysteine concentration of 'Texas Cream 40' was 14% more when seeds were produced with 420ppm N compared with the lower two N levels (Table 23). 'Texas Cream 40' seeds produced with 7.8ppm P had a lower cysteine concentration than seeds from the other P levels. A low cysteine concentration was observed in 'Texas Cream 40' seeds when they were produced with an intermediate level of S.
In 'Pinkeye Purple Hull' seeds, the concentration of cysteine decreased 9% at 52.5ppm N compared with the higher two N levels, but was unaffected by P or S treatment.

Dessauer and Hannah (1978) reported a 10% increase in free methionine, total methionine and total protein contents of cowpea seeds when methionine was added to the growth media. Evans et al. (1977) reported that methionine and cysteine contents in three cowpea cultivars increased as the level of sulfate-S in the growing media increased to 5ppm. Above 5ppm sulfate-S cysteine concentration remained unchanged.

Failure to increase methionine and cysteine concentrations in cowpea seeds by an application of 20 Kg/ha S was also observed by Nangju (1976). In the present study, the content of S-amino acids in seeds of 'Texas Cream 40' and 'Pinkeye Purple Hull' were unaffected by the S level given to the parent plant. This indicated that the lowest S level (16ppm) was too high to induce S deficiency in cowpea seeds. There was no apparent influence of the S-amino acid content of the seeds on subsequent seedling growth.

Effect of nutritional treatments imposed at anthesis in the parent plant on seed vigor of the progeny

Effect of nutritional treatments on seed yield and vigor. Average seed yields of 'Pinkeye Purple Hull' were higher than 'Texas Cream 40' (Table 24). This was probably due to more seeds per pod produced by 'Pinkeye Purple Hull'. Pod number per plant and seed weight were similar in both cultivars, while the time to pod maturation was three days longer in 'Texas Cream 40' than in 'Pinkeye Purple Hull'. This cultivar difference in seed yield was not observed in the previous experiment.

Seed yield, pod number per plant and seed number per pod of either cultivar were unaffected by N or P treatment imposed during seed development (Table 24). Pod maturation was one to two days earlier at the 210ppm N-31ppm P and 420ppm N-31ppm P treatments compared with the other nutritional treatments (Table 25). Seeds which developed under these two nutritional treatments, however, were heavier than those which developed under the 52.5ppm N-31ppm P treatment (Table 25). An increase in seed weight of both cultivars at the high N level (420ppm) was observed at the high P level (62ppm). Summerfield et al. (1976) reported that compared with a continuous N supply, withdrawal of N after flowering reduced seed yield of 'Prima' cowpea by 33%. Seed size, however, was not affected.

All seeds germinated normally in both cultivars after the standard germination test (Table 26). 'Texas Cream 40' germinated more rapidly

Table 24

Effect of Nutritional Treatments Imposed at Anthesis of the Parent Plant on Seed Yields of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Variable	Seed yield	Pod/plant	Seed/pod	Wt/100 seeds	Pod Dev Per
	g/plant	No.	No.	g	day
Cultivar					
Texas Cream 40	14.3b[z]	9.8a	9.4b	15.6a	23.1a
Pinkeye Purple Hull	16.4a	10.1a	10.2a	16.2a	20.0b
N(ppm)					
52.5	15.8a	10.5a	9.9a	15.3b	21.9a
210.0	14.7a	9.6a	10.0a	15.6b	21.4a
420.0	15.6a	9.9a	9.5a	16.8a	21.6a
P(ppm)					
7.8	14.5a	9.5b	9.7a	15.9a	21.8a
31.0	15.2a	9.7b	10.1a	15.8a	21.1b
62.0	16.3a	10.7a	9.7a	15.9a	21.9a
Interaction					
Cv x N	NS	NS	NS	NS	NS
Cv x P	NS	NS	NS	NS	NS
N x P	NS	NS	NS	*	*
Cv x N x P	NS	NS	NS	NS	NS

[z]Values followed by the same letter within variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

* Significant interaction at 5% level.

Table 25

Effect of Nutritional Treatments Imposed at Anthesis on the Pod Development Period and Seed Weights of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Variable	N (ppm)	P (ppm) 7.8	31	62
Pod Dev. Per. (days)	52.5	21.7a[z]	21.9a	22.0a
	210.0	21.9a	20.3b	21.9a
	420.0	21.9a	21.0b	21.7a
Wt/100 seed (mg)	52.5	15.7a	15.2b	15.0b
	210.0	15.6a	16.4ab	14.8b
	420.0	16.5a	17.5a	16.5a

[z]Values followed by the same letter within variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table 26

Effect of Nutritional Treatments Imposed at Anthesis of the Parent Plant on Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas with the Standard Germination Test

Variable	Germination		ADG	Seedling length			Seedling fresh wt					Seedling dry wt			
	total	abnormal		hypocotyl		radicle	axis		cotyledon	seedling		axis		cotyledon	seedling
	%		day	cm			mg/part					mg/part			
Cultivar															
Texas Cream 40	100a[z]	1a	1.11b	7.9a		14.6a	830a		183a	1013a		57.5a		57.5b	112.0a
Pinkeye Purple Hull	99a	0a	1.37a	6.3b		12.1b	629b		195a	824b		48.8b		66.1a	111.9a
N(ppm)				Texas Cream 40	Pinkeye Purple Hull		Texas Cream 40	Pinkeye Purple Hull		Texas Cream 40	Pinkeye Purple Hull	Texas Cream 40	Pinkeye Purple Hull		
52.5	98a	0a	1.23a	8.5a	5.9b	13.5a	866a	586b	184c	1037a	772b	59.2a	45.2b	57.9b	110.1b
210.0	100a	0a	1.28a	7.9b	6.5a	13.3a	814ab	642a	211b	989a	833a	56.3a	49.8a	59.7b	112.7b
420.0	100a	0a	1.23a	7.4c	6.5a	13.3a	808b	660a	229a	1012a	868a	56.9a	51.4a	67.8a	122.0a
P(ppm)															
7.8	99a	1a	1.27a	7.1a		13.4b	734a		185a	919ab		53.3a		60.3a	113.6a
31.0	100a	0a	1.30a	6.8b		12.9c	691b		193a	884b		50.5b		64.0a	114.5a
62.0	100a	0a	1.16b	7.4a		13.8a	753a		188a	951a		55.6a		61.0a	116.6a
Interaction															
Cv x N	NS	NS	NS	***		NS	**		NS	**		**		NS	NS
Cv x P	NS	NS	*	NS		NS	NS		NS	NS		NS		NS	NS
N x P	NS	NS	NS	NS		NS	NS		NS	NS		NS		NS	NS
Cv x N x P	NS	NS	**	NS		NS	NS		NS	NS		NS		NS	NS

[z]Values followed by the same letter within variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*, **, *** Significant interaction at 0.1%, 1% and 5% levels, respectively.

and had a greater axis growth than 'Pinkeye Purple Hull'.

Changes in N or P level in the nutrient solution during seed development had no influence on seed germinability or abnormal seedling development of either cultivar when they were germinated under optimum conditions (Table 26). Germination rate of 'Texas Cream 40' was unaffected, while that of 'Pinkeye Purple Hull' varied among nutritional treatments (Table 27). Alteration in germination rate of 'Pinkeye Purple Hull' was not related to subsequent seedling growth, however.

As the level of N increased in the nutrient media during seed development of 'Texas Cream 40', hypocotyl length and axis fresh weight in the progeny decreased after the standard germination test (Table 26). Radicle length, axis dry weight and seedling fresh weight remained unchanged. In 'Pinkeye Purple Hull', seed development at the lowest N level (52.5ppm) led to the production of smaller seedlings in the progeny compared with seeds from the higher two N levels. Cotyledon weight and seedling dry weight were related to the initial seed weight of both cultivars.

Varying the P supply throughout the seed development period affected seedling growth in the progeny similarly in both cultivars after the standard germination test (Table 26). Seeds which developed with 31ppm P gave rise to smaller seedlings than seeds from the other two P levels. Cotyledon weight and seedling dry weight were the same regardless of P treatment.

After accelerated aging, over 80% of the seeds germinated normally in both cultivars (Table 28). 'Texas Cream 40' germinated more rapidly and had a greater axis growth than 'Pinkeye Purple Hull'. This was similar to that found with the standard germination test.

Table 27

Effect of Nutritional Treatments Imposed at Anthesis of the Parent Plant on the Average Days to Germination of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas with the Standard Germination Test

Cultivar	N (ppm)	P (ppm) 7.8	31	62
		ADG (day)		
Texas Cream 40	52.5	1.1a[z]	1.1a	1.1a
	210.0	1.1a	1.2a	1.1a
	420.0	1.2a	1.1a	1.1a
Pinkeye Purple Hull	52.5	1.6a	1.3b	1.2b
	210.0	1.3b	1.6a	1.4a
	420.0	1.3b	1.5ab	1.2b

[z]Values followed by the same letter within cultivar column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table 28

Effect of Nutritional Treatments Imposed at Anthesis of the Parent Plant on Seed Germination and Seedling Growth of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas after Accelerated Aging

Variable	Germination total	Germination abnormal	ADG	Seedling length hypocotyl	Seedling length radicle	Seedling fresh wt axis	Seedling fresh wt cotyledon	Seedling fresh wt seedling	Seedling dry wt axis	Seedling dry wt cotyledon	Seedling dry wt seedling
	%	%	day	cm	cm	mg/part	mg/part	mg/part	mg/part	mg/part	mg/part
Cultivar											
Texas Cream 40	91a[z]	9a	1.20b	7.6a	13.0a	774a	199b	973a	55.1a	57.8b	112.9a
Pinkeye Purple Hull	92a	4a	1.34a	5.6b	10.2b	618b	217a	835b	47.8b	65.2a	113.0a
N(ppm)											
52.5	90a	7a	1.22a	7.2a	12.2a	742a	184c	926a	54.0a	53.5c	107.5b
210.0	92a	6a	1.32a	6.3b	11.3a	658b	211b	869b	49.1b	62.4b	111.5b
420.0	93a	7a	1.27a	6.3b	11.3a	688b	229a	917a	51.3b	68.6a	119.9a
P(ppm)				Texas Cream 40 / Pinkeye Purple Hull		Texas Cream 40 / Pinkeye Purple Hull		Texas Cream 40 / Pinkeye Purple Hull	Texas Cream 40 / Pinkeye Purple Hull		
7.8	93a	6a	1.23a	8.8a / 5.8a	12.4a	833a / 650a	196b	1061a / 864a	60.8a / 49.7a	56.9b	112.2a
31.0	92a	10a	1.28a	7.2b / 5.4a	11.0b	722b / 585b	213ab	924b / 809a	51.6b / 45.8b	63.4a	112.1a
62.0	90a	4a	1.30a	6.9b / 5.6a	11.4b	716b / 619ab	216a	933b / 833a	52.9b / 47.9ab	64.2a	114.6a
Interaction											
Cv x N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cv x P	NS	NS	NS	***	NS	**	NS	*	*	NS	NS
N x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x N x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Values followed by the same letter within variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*, **, *** Significant interaction at 0.1%, 1% and 5% levels, respectively.

Alteration of the N level given to the parent plant during seed development affected subsequent seed germinability and seedling growth of both cultivars similarly after aging (Table 28). Seed viability, abnormal seedling development and germination rate of either cultivar were unaffected by N treatment. Both, however, had longer hypocotyl lengths and greater axis weights from seeds which developed with 52.5ppm N compared to seeds produced with the higher two N levels.

Cultivar differences due to P treatment during seed development were significant after accelerated aging (Table 28). In 'Texas Cream 40', seed germination, abnormal seedling development and germination rate were similar regardless of P treatment. Seeds which developed with 7.8ppm P had longer axis lengths and heavier axis weights compared with seeds from the higher P levels. In 'Pinkeye Purple Hull', seed viability, abnormal seedling development, germination rate and hypocotyl length in the progeny were unaffected by the P level supplied to the parent plant. Radicle lengths decreased by 13% after aging from seeds which developed with 7.8ppm P compared with those from the higher two P levels. Seed development with 7.8ppm P led to the greatest, 62ppm intermediate, and 31ppm the least, axis weight in the progeny.

Different responses of axis growth before or after aging were apparent when seeds were produced in deficient levels of N or P. Since the aging test evaluated seed vigor after stress, both 'Texas Cream 40' and 'Pinkeye Purple Hull' produced seeds of higher vigor with a reduced supply of N or P after anthesis.

Effect of nutritional treatments on seed composition. 'Pinkeye Purple Hull' seeds contained more N than 'Texas Cream 40' (Table 29). The concentration of protein and P in the seeds were similar in both cultivars. Compared with the previous experiment, seed N content of

Table 29

Effect of Nutritional Treatments Imposed at Anthesis of the Parent Plant on Seed Composition of 'Texas Cream 40' and 'Pinkeye Purple Hull' Cowpeas

Variable	N	Protein	P
	%[y]	%	%
Cultivar			
Texas Cream 40	4.01b	17.3a	0.492a
Pinkeye Purple Hull	4.11a	17.3a	0.494a
N(ppm)			
52.5	4.00c	16.5b	0.495a
210.0	4.06b	17.7a	0.495a
420.0	4.12a	17.7a	0.489a
P(ppm)			
7.8	4.11a	17.8a	0.481c
31.0	4.02b	17.2b	0.494b
62.0	4.05b	16.9b	0.502a
Interaction			
Cv x N	**	**	***
Cv x P	**	**	*
N x P	NS	*	*
Cv x N x P	NS	NS	NS

[z]Values followed by the same letter within variable column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

*, **, *** Significant interaction at 0.1%, 1% and 5% levels, respectively.

[y]% dry weight basis

cowpea was more consistent than the protein and P contents. Larger variation in the latter implied that seed protein and P contents were probably more susceptible to environmental changes under which the plants had grown and developed.

The concentration of N in 'Texas Cream 40' seeds increased as N supply to the parent plant increased during seed development (Fig. 17A). The N concentration was higher at 7.8 or 62ppm P than at 31ppm (Fig. 17D). Seed protein concentration of 'Texas Cream 40' decreased at the lowest level of N compared with the higher two N levels, but increased at the lowest P level when compared with the higher two P levels (Figs. 17B, E). The concentration of P in 'Texas Cream 40' seeds was unaffected by N treatment, but increased with a higher level of P (Figs. 17C, F).

In 'Pinkeye Purple Hull', seed N concentration was unaffected by N treatment during seed development, but increased at the lowest level of P when compared with the higher two P levels (Figs. 17A, D). Protein concentration in 'Pinkeye Purple Hull' seeds increased as N supply increased in the nutrient media during seed development, but decreased as the level of P increased (Figs. 17B, E). The concentration of P in 'Pinkeye Purple Hull' seeds decreased either at the highest level of N when compared with the lower two N levels, or at the lowest level of P when compared with the higher two P levels (Figs. 17C, F).

Comparing these two nutritional experiments, chemical composition of the seeds and seed vigor were altered by the nutritional treatments whether they were initiated at the time of sowing or at anthesis. Changes in seed composition due to nutritional treatment were similar in both experiments. The differences of seed chemical composition among nutritional treatments were more significant when they were imposed

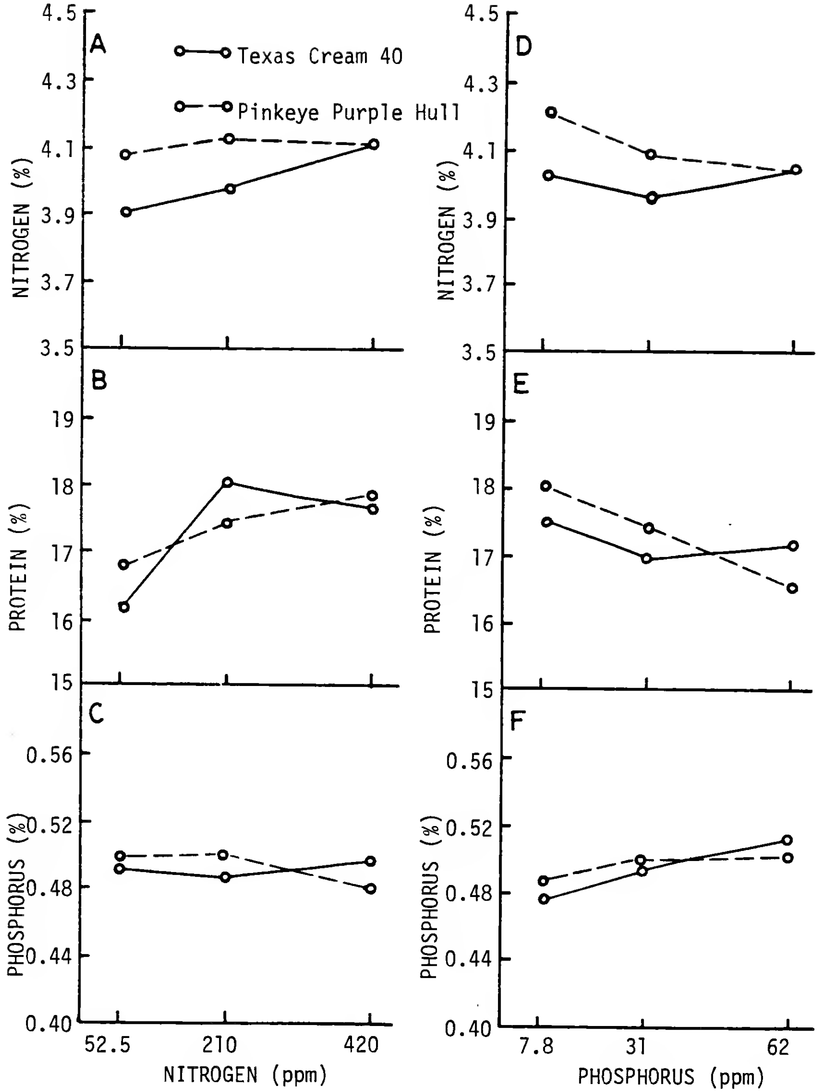

Fig. 17 Effect of nutritional treatments imposed at anthesis of the parent plant on seed composition (% dry weight basis) of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas.

A. nitrogen, B. protein, C. phosphorus, D. nitrogen, E. protein, F. phosphorus.

at the seedling stage. The influence of nutrient treatment on seed vigor, however, varied in these two experiments. When imposed during seed development of the parent plant, deficient levels of N or P led to seeds of high vigor. Seed vigor of 'Pinkeye Purple Hull' was reduced when the same nutritional treatments were imposed from sowing to seed maturity. In both experiments, 'Texas Cream 40' and 'Pinkeye Purple Hull' produced seeds which were larger in size at the highest level of N compared with the lower two N levels. Axis growth in these seeds was reduced when the highest N level was imposed at the seedling stage, but was not altered when imposed at anthesis. Therefore, a change in nutrient supply at different growth stages during cowpea seed production affected seed vigor differentially. From these results, an adequate supply of N and P during vegetative growth of cowpea at least to flowering was required for maximum seed quality.

Summary

'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas were subjected to various nutritional treatments beginning at the seedling stage or after anthesis to determine their influence on seed vigor of the progeny.

Seeds of high vigor were produced in 'Texas Cream 40' when various N, P or S rates in the growth media were imposed from sowing to seed maturity. Seed vigor of 'Pinkeye Purple Hull' was reduced by a deficient level of N, P or S given to the parent plant. When grown with a high N level (420ppm) in the nutrient solution, the same cultivar produced seeds with a lower vigor compared to those from the 210ppm N treatment. A reduction in seed vigor of 'Pinkeye Purple Hull' grown with 52.5ppmN appeared to be attributed to decreases in total quantities of N and

protein in the seeds produced. A similar reduction in seed vigor due to a decrease in the total quantity of P in the seeds was apparent in 'Pinkeye Purple Hull' when grown with 7.8ppm P.

Decreasing N and P supply during seed development improved subsequent seed vigor of both cultivars. No apparent relationship between chemical composition of the seeds and seed vigor was found when nutritional treatments were imposed at anthesis.

SUMMARY AND CONCLUSIONS

A comprehensive review article on cowpea was written by Summerfield et al. (1974). It included the general characteristics of cowpea, its origin and utilization, physiology and breeding, yield variation, weed and pest control, some specific problems in African farming, and dietetic attributes of the species. More recently research efforts with cowpea have been initiated in the areas of symbiotic N fixation and the influence of environment on seed yield. Little has been reported on the influence of environmental stress during seed development on subsequent seed vigor. Therefore, the present work was conducted to investigate the influence of varying environmental conditions during seed development on seed vigor of the progeny. Seed vigor of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas was significantly affected by altering water supply, temperature or nutrient media, but not photoperiod or light intensity, of the parent plant during seed development.

Alteration of temperature during seed development had the greatest influence on seed quality of the progeny. The optimum day/night temperature range during seed development for the highest seed quality of both cultivars appeared to be between $33^{\circ}/25^{\circ}C$ and $28^{\circ}/20^{\circ}C$. Increasing the day/night temperatures to $38^{\circ}/30^{\circ}C$ dramatically reduced seed yields and vigor of both cultivars. Lowering the temperatures to $23^{\circ}/15^{\circ}C$ increased seed yield of 'Texas Cream 40', but had no effect on that of 'Pinkeye Purple Hull'. Seed vigor, however, was reduced in both cultivars when seeds developed at this temperature compared to the optimum temperatures. The reduction in vigor was to a lesser extent than that which occurred under heat stress.

Reduction in vigor of seeds produced at high temperature during seed development was attributed to reduced nutrient accumulation and/or damage of the ability of seedling to utilize food reserves effectively upon germination. Embryo size and subsequent growth in vitro were unaffected by the various temperature treatments. Thus, reduction in utilization of food reserves probably was caused by reduced hydrolysis of storage nutrients or translocation of these products to the axis.

Although cowpea is usually grown in the tropics and subtropics, periods of high temperatures for two weeks or longer could conceivably have an adverse influence on seed yield and vigor. High temperatures during seed development have been reported to lead to high respiration and low photosynthesis rates in the leaves of wheat (Spiertz ,1977), grains of pearl millet (Fussell and Pearson, 1980), leaves and ears of rice and sorghum (Chowdhury and Wardlaw, 1978). This might have occurred in the present study, as demonstrated by the decrease in starch concentration of seeds which developed at $38^{o}/30^{o}C$. If cowpea is grown in a temperate region, the first frost might come before seed development is complete due to a prolonged growth period at the lower temperatures. This might result in seed immaturity, which would further reduce seed vigor. When searching for high yield traits in cowpea, breeders should observe any adverse influences of high or low temperature during seed development on subsequent seed vigor and separate it from real genetic characteristics.

Under water stress 'Pinkeye Purple Hull' produced only a few seeds, but of high quality. 'Texas Cream 40' was drought sensitive; both seed yield and vigor were reduced. Decreases in embryo size and nutrient accumulation in the cotyledons contributed to the reduced seed vigor of this cultivar.

Many immature pods abscised and no flowers set new pods after exposure to drought stress. This implied that drought had a direct influence on fruit set, thus, seed development. Decreases in starch concentration in seeds of 'Texas Cream 40' probably resulted from a reduction in photosynthesis during water stress, as observed by the rapid leaf senescence which occurred. Because cowpea is usually grown on residual soil moisture instead of with irrigation, there can be great risks of encountering drought during later plant developmental stages, thus resulting in reduced seed quality.

The mechanisms by which drought and heat stress affected cowpea seed vigor appeared to be different. Under water stress 'Pinkeye Purple Hull' produced less seeds, but of high quality. High developmental temperature led to reduced nutrient reserves in 'Pinkeye Purple Hull' seeds, which contributed to poor seedling growth. In 'Texas Cream 40', reduced seed vigor under water stress was attributed to both low nutrient accumulation and small embryo size at seed maturity. Although nutrient accumulation also decreased in this cultivar with high temperatures during seed development, an inability to utilize reserve food effectively during seed germination played a major role in the reduced seedling growth.

Changes in the supply of N or P were found to affect seed vigor of cowpea differentially when the treatments were imposed either after anthesis or from the time of sowing to seed maturity. Seed vigor of

'Texas Cream 40' was unaffected by various nutritional treatments which were imposed at the seedling stage of the parent plant. 'Pinkeye Purple Hull', however, produced seeds of low vigor at deficient levels of N or P when the treatments were imposed during the same period of time. An excessive supply of N also reduced seed vigor of this cultivar. When imposed at anthesis, reduced N or P levels in the nutrient media enhanced subsequent axis growth of both cultivars, while increased N or P levels did not alter seed vigor of either cultivar.

Decreases in seed N, protein or P concentrations were correlated with reduced seed vigor of 'Pinkeye Purple Hull' when grown on deficient levels of N or P. The influence of reduced nutrient accumulation in the seeds on subsequent seedling growth was similar to that which occurred under temperature stress or water stress during seed development. Starch was the limiting factor in vigor determination in the latter two cases, however. This was probably related to the fact that most N and P in the seeds was retranslocated from vegetative tissue after anthesis, while carbohydrate came from photosynthesis in the leaves during seed development.

Seed vigor of both cultivars was the same regardless of light intensity during seed development, but seed yield was reduced under the lowest light intensity (275 $\mu E/m^2/s$). Many immature pods (3 to 4 mm long) were aborted, indicating that continued seed growth, not fruit set, was severely affected by this low light intensity. Therefore, reduction in seed yield under extremely low light intensity was the result of competition among fruiting structures for the limited nutrient source, probably due to reduced photosynthesis. Instead of producing many seeds of poor vigor, cowpea appeared to have the ability to maintain seed quality under shading by reducing the total number of seeds produced. This can be an important trait for breeders to consider; and they should include seed vigor as a genetic trait in their studies. The low yield of cowpea when intercropped with sorghum or millet (Summerfield et al., 1974) might be partly attributed to shading. By adjusting sowing dates so that cereals are harvested before cowpea seed development begins, cowpea yield and ultimately seed quality might be improved.

Although seed yield and seed vigor were unaffected by different photoperiods during seed development, hardseededness developed in 'Pinkeye Purple Hull' under shortdays. This indicated that dormancy, a trait that could ultimately prolong seed viability under adverse storage conditions, could be induced by alteration of seed production conditions. Under prolonged field weathering or adverse storage conditions, hardseededness would be of benefit in maintaining both seed viability and vigor because moisture levels within the seed could be controlled by the seed coat. Growers would have to scarify the seed before planting in order to get emergence and uniform stands in the field, however.

In general, many crops have little capacity to adjust seed number to the available resources once seeds begin to develop. If stress is imposed at an early stage of plant development, vegetative growth would be altered. This usually reduces the number of flower buds formed, thus, the number of seeds produced. The influence of environment on seed vigor might interact with the quantity of seeds at the onset of seed growth. Differences in physiological responses resulting from reduced vegetative growth might add still another variable to the response of seed vigor to environmental stress during seed development.

Therefore in the present study various environmental conditions, except nutritional treatments, were imposed after anthesis to eliminate the possible effect of vegetative differences on subsequent seed development and vigor.

Individual environmental factors were studied in the present experiments. However, the overall components of the environment under field conditions are very complex. For example, drought often develops in hot weather. Both would reduce total seed number produced to adjust to the limited carbohydrate source under stress. Seed vigor, however, would not benefit from a reduction in total seed number because in this study both high temperature and drought conditions reduced seed size and seed vigor in addition to the low seed yield. Additional variations would result in more complicated responses and might further reduce vigor from that which was found in the present study. Additional investigations on combinations of environmental stresses, especially temperature, soil moisture and fertility during seed development, would provide information about their influence, and the possible interaction between these factors on seed vigor of the progeny.

LITERATURE CITED

Akpan, E.E.J., and E.W. Bean. 1977. The effects of temperature upon seed development in three species of forage grasses. Ann. Bot. 41: 689-95.

Association of Official Agricultural Chemists. 1975. Methods of analysis. A.O.A.C. Washington pp:927-28.

Association of Official Seed Analyst.. 1970. Rules for testing seeds. Proc. Assoc. Offic. Seed Anal. 60:1-116.

Austin, R.B. 1966a. The growth of watercress (Rorippa nasturtium aquaticum L. Hayek) from seed as affected by the phosphorus nutrition of the parent plant. Plant Soil 24:113-20.

Austin, R.B. 1966b. The influence of the phosphorus and nitrogen nutrition of pea plants on the growth of their progeny. Plant Soil 24:359-68.

Austin, R.B. 1972. Effects of environment before harvesting on viability. In Viability of seed (E.H. Roberts, Ed). Syracuse Univ. Press, Syracuse, N.Y. pp:114-49.

Austin, R.B., and P.C. Longden. 1965. Effects of nutritional treatments of seed-bearing plants on the performance of their progeny. Nature 205:819-20.

Austin, R.B., and P.C. Longden. 1966. The effects of manurial treatments on the yield and quality of carrot seed. J. Hort. Sci. 41:361-70.

Campbell, C.A., and H.R. Davidson. 1979. Effect of temperature, nitrogen fertilization and moisture stress on yield, yield components, protein content and moisture use efficiency of Manitou spring wheat. Can. J. Plant Sci. 59:963-74.

Chang, W.N., and B.E. Struckmeyer. 1976. Influence of temperature on seed development of Allium cepa L. J. Amer. Soc. Hort. Sci. 101:296-98.

Chinnici, M.F., and D.M. Peterson. 1979. Temperature and drought effects on blast and other characteristics in developing oats. Crop Sci. 19:893-97.

Chowdhury, S.I., and I.F. Wardlaw. 1978. The effect of temperature on kernel development in cereals. Aust. J. Agric. Res. 29:205-23.

Cox, R.F., G.A. Sullivan, and C.K. Martin. 1976. Effect of calcium and irrigation treatments on peanut yield, grade and seed quality. Peanut Sci. 3:81-85.

Cruz, L.J., G.B. Cagampang, and B.O. Juliano. 1970. Biochemical factors affecting protein accumulation in the rice grain. Plant Physiol. 46:743-47.

Dekker, R.F.H., and G.N. Richards. 1971. Determination of starch in plant material. J. Sci. Fd Agric. 22:441-44.

Delouche, J.C. 1980. Environmental effects on seed development and seed quality. HortSci. 15:775-80.

Dessauer, D.W., and L.C. Hannah. 1978. Inhibition of cowpea seedling growth by methionine analogs. Crop Sci. 18:593-97.

Doku, E.V. 1970. Effect of daylength and water on nodulation of cowpea (Vigna unguiculata L. Walp) in Ghana. Expl. Agric. 6:13-18.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-56.

Eck, H.V., and J.T. Musick. 1979. Plant water stress effects on irrigated grain sorghum. II. Effects of nutrients on plant tissues. Crop Sci. 19:592-98.

Egli, D.B., and I.F. Wardlaw. 1980. Temperature response of seed growth characteristics of soybeans. Agron. J. 72:560-64.

El-Forgany, M., and D.J. Makus. 1979. Effect of water stress on seed yield and quality of the sweet corn inbred 'Luther Hill'. J. Amer. Soc. Hort. Sci. 104:102-04.

Evans, I.M., D. Boulter, R.T. Fox, and B.T. Kang. 1977. The effects of sulphur fertilisers on the content of sulpho-amino acids in seeds of cowpeas (Vigna unguiculata). J. Sci. Fd. Agric. 28:161-66.

Evans, I.M., J.E. Ford, L.C. Hannah, and D. Boulter. 1976. Comparison of chemical and microbiological methods in the estimation of methionine in cowpea (Vigna unguiculata) seeds. Br. J. Nutr. 36:289-93.

Evenari, M., D. Koller, and Y. Gutterman. 1966. Effects of the environment of the mother plant on germination by control of the seed coat permeability to water in Ononis sicula Guss. Aust. J. Biol. Sci. 19:1007-16.

Ezedinma, F.O.C. 1965. The influence of seed size and fertilizer on the development and yield of cowpea (Vigna sinesis Endl.). Nigerian Agric. J. 2:75-79.

Fiske, C.H., and Y.P. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Fussell, L.K., and C.J. Pearson. 1980. Effects of grain development and thermal history on grain maturation and seed vigor of Pennisetum americanum. J. Exp. Bot. 31:635-43.

Fussell, L.K., C.J. Pearson, and M.J.T. Norman. 1980. Effect of temperature during various growth stages on grain development and yield of Pennisetum americanum. J. Exp. Bot. 31:621-33.

Green, D.E., E.L. Pinnell, L.E. Cavanah, and L.F. Williams. 1965. Effect of planting date and maturity date on soybean seed quality. Agron. J. 57:165-68.

Gutterman, Y. 1973. Differences in the progeny due to daylength and hormone treatment of the mother plant. in Seed ecology (W. Heydecker, ed) Butterworths, London. pp:59-80.

Gutterman, Y. 1974. The influence of the photoperiodic regime and red-far red light treatments of Portulca oleracea L. plants on the germinability of their seeds. Oecologia 17:27-38.

Gutterman, Y., and M. Evenari. 1972. The influence of day length on seed coat color, an index of water permeability, of the desert annual Ononis sicula Guss. J. Ecol. 60:713-19.

Gutterman, Y., and W. Heydecker. 1973. Studies of the surfaces of desert plant seeds. I. Effect of day length upon maturation of the seed coat of Ononis sicula Guss. Ann. Bot. 37:1049-50.

Gutterman, Y., T.H. Thomas, and W. Heydecker. 1975. Effect on the progeny of applying different day length and hormone treatments to parent plants of Lactuca scariola. Physiol. Plant. 34:30-38.

Hannah, L.C., B.B. Rhodes, and I.M. Evans. 1977. Examination and modification of the use of Leuconostoc mesenteroides for measurements of the sulfur-containing amino acids from Vigna unguiculata. J. Agric. Fd. Chem. 25:620-23.

Harrington, J.F. 1960. Germination of seeds from carrot, lettuce and pepper plants grown under severe nutrient deficiencies. Hilgardia 30:219-35.

Harris, H.C., and J.B. Brolmann. 1966. Comparison of calcium and boron deficiencies of the peanut. II. Seed quality in relation to histology and viability. Agron. J. 58:578-82.

Harris, H.B., M.B. Parker, and B.J. Johnson. 1965. Influence of molybdenum content of soybean seed and other factors associated with seed source on progeny response to applied molybdenum. Agron. J. 57:397-99.

Harrison, J.G., and D.A. Perry. 1973. Effects of hollow heart on growth of peas. Ann. Appl. Biol. 73:103-09.

Heide, O.M., O. Junttila, and R.T. Samuelsen. 1976. Seed germination and bolting in red beet as affected by parent plant environment. Physiol. Plant. 36:343-49.

Hiler, E.A., C.H.M. Vanbavel, M.M. Hossain, and W.R. Jordan. 1972. Sensitivity of southern peas to plant water deficit at three growth stages. Agron. J. 64:60-64.

Hoagland, D.R., and D.I. Arnon. 1938. The water culture method for growing plants without soil. Circ. Calif. Agric. Exp. Stat. No. 347. Berkeley.

Hong, T.D., F.R. Minchin, and R.J. Summerfield. 1977. Recovery of nodulated cowpea plants (Vigna unguiculata L. Walp) from waterlogging during vegetative growth. Plant Soil 48:661-72.

Huxley, P.A., and R.J. Summerfield. 1974. Effects of night temperature and photoperiod on the reproductive ontogeny of cultivars of cowpea and of soybean selected for the wet tropics. Plant Sci. Letters 3:11-17.

Huxley, P.A., and R.J. Summerfield. 1976. Effects of daylength and day/night temperatures on growth and seed yield of cowpea cv. K2809 grown in controlled environments. Ann. Appl. Biol. 83:259-71.

Izzeldin, H., L.F. Lippert, and F.H. Takatori. 1980a. Performance of lettuce plants originating from seedlings affected with physiological necrosis (red cotyledon). J. Amer. Soc. Hort. Sci. 105:54-57.

Izzeldin, H., L.F. Lippert, and F.H. Takatori. 1980b. An influence of water stress at different growth stages on yield and quality of lettuce seed. J. Amer. Soc. Hort. Sci. 105:68-71.

Kamara, C.S. 1976. The effects of excess and deficient soil moisture on the growth and yield of cowpea. Trop. Grain Legume Bull. 6:4-8.

Karssen, C.M. 1970. The light promoted germination of the seeds of Chenopodium album L. III. Effect of the photoperiod during growth and development of the plants on the dormancy of the produced seeds. Acta. Bot. Neerl. 19:81-94.

Ketring, D.L., and P.W. Morgan. 1969. Ethylene as a component of the emanations from germinating peanut seeds and its effect on dormant Virginia-type seeds. Plant Physiol. 44:326-30.

Ketring, D.L., C.E. Simpson, and O.D. Smith. 1978. Physiology of oil seeds. VII. Growing season and location effects on seedling vigor and ethylene production by seeds of three peanut cultivars. Crop Sci. 18:409-13.

Khan, R.A., and H.M. Laude. 1969. Influence of heat stress during seed maturation on germinability of barley seed at harvest. Crop Sci. 9:55-58.

Kigel, J., M. Ofir, and D. Koller. 1977. Control of the germination responses of Amaranthus retroflexus L. seeds by their parental photothermal environment. J. Exp. Bot. 28:1125-36.

Koller, D. 1962. Preconditioning of germination in lettuce at time of fruit ripening. Amer. J. Bot. 49:841-44.

Kostjucenko, I.A., and T.J. Zarubailo. 1937. Vernalization of seed during ripening and its significance in practice. Herbage Rev. 5:146-57.

Leopold, A.C., and P.E. Kriedemann. 1975. Plant growth and development. Second edition. McGraw-Hill, Inc. New York pp:349-74.

Lowe, L.B., and S.K. Ries. 1972. Effects of environment on the relation between seed protein and seedling vigor in wheat. Can. J. Plant Sci. 52:157-64.

Lowe, L.B., and S.K. Ries. 1973. Endosperm protein of wheat seed as a determinant of seedling growth. Plant Physiol. 51:57-60.

Lowry, O.H., N.J. Rosebrough, A. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-75.

McCullough, J.M., and W. Shropshire, Jr. 1970. Physiological predetermination of germination responses in Arabidopsis thaliana L. Heynh. Plant Cell Physiol. 11:139-48.

Michel, B.E., and M.R. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51:914-6.

Minchin, F.R., R.J. Summerfield, A.R.J. Eaglesham, and K.A. Stewart. 1978. Effects of short-term waterlogging on growth and yield of cowpea (Vigna unguiculata). J. Agric. Sci. 93:355-66.

Moore, S. 1968. Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. J. Biol. Chem. 243:6281-83.

Moore, S., and W.H. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211:907-13.

Nangju, D. 1976. Effect of fertilizer management on seed sulfur content of cowpea (Vigna unguiculata L. Walp). Trop. Grain Legume Bull. 4:6-8.

Ndunguru, B.J., R.J. Summerfield, R.F. Minchin, and K.A. Stewart. 1978. Effects of environment during seed maturation, seed size and seed protein N content on growth and yield of cowpea plants in the subsequent generation. Reading Univ.-IITA Int. Comm. No. 19 pp:1-8.

Oropeza, F. 1976. Seed maturation in cowpea (Vigna unguiculata). MS Thesis, Mississippi State Univ., Mississippi State.

Pallas, J.E. Jr., J.R. Stansell, and R.R. Bruce. 1977. Peanut seed germination as related to soil water regime during pod development. Agron. J. 69:381-83.

Patterson, D.T., M.M. Peet, and J.A. Brunce. 1977. Effect of photoperiod and size at flowering on vegetative growth and seed yield of soybean. Agron. J. 69:631-35.

Peacock, H.A., and B.S. Hawkins. 1970. Effect of seed source on seedling vigor, yield and lint characteristics of upland cotton, Gossypium hirsutum L. Crop Sci. 10:667-69.

Perry, D.A., and J.G. Harrison. 1973. Causes and development of hollow heart in pea seed. Ann. Appl. Biol. 73:95-101.

Potts, H.C., J. Duangpatia, W.G. Hairston, and J.C. Delouche. 1978. Some influences of hardseededness on soybean seed quality. Crop Sci. 18:221-24.

Quisenberry, J.E., and J.R. Gipson. 1974. Growth and productivity of cotton grown from seed produced under four night temperatures. Crop Sci. 14:300-02.

Riddell, J.A., and G.A. Gries. 1958. Development of spring wheat: III. Temperature of maturation and age of seeds as factors influencing their response to vernalization. Agron. J. 50:743-46.

Ries, S.K. 1971. The relationship of protein content and size of bean seed with growth and yield. J. Amer. Soc. Hort. Sci. 96:557-60.

Ries, S.K., and E.H. Everson. 1973. Protein and seed size relationships with seedling vigor of wheat cultivars. Agron. J. 65:884-86.

Ries, S.K., O. Moreno, W.F. Meggitt, C.J. Schweizer, and S.A. Ashkar. 1970. Wheat seed protein: Chemical influence on and relationship to subsequent growth and yield in Michigan and Mexico. Agron. J. 62:746-48.

Roberts, E.H., R.J. Summerfield, F.R. Minchin, K.A. Stewart, and B.J. Ndunguru. 1978. Effects of air temperature on seed growth and maturation in cowpea (Vigna unguiculata). Ann. Appl. Biol. 90:437-46.

Robertson, R.N., H.R. Highkin, J. Smydzuk, and F.W. Went. 1962. The effect of environmental conditions on the development of pea seeds. Aust. J. Biol. Sci. 15:1-15.

Sasseville, D.N., and H.A. Mills. 1979. N form and concentration: Effects on N absorption, growth and total N accumulation with southernpeas. J. Amer. Soc. Hort. Sci. 104:586-91.

Schweizer, C.J., and S.K. Ries. 1969. Protein content of seed: Increase improves growth and yield. Science 165:73-75.

Siddique, M.A., and P.B. Goodwin. 1980a. Maturation temperature influences on seed quality and resistance to mechanical injury of some snap bean genotypes. J. Amer. Soc. Hort. Sci. 105:235-38.

Siddique, M.A., and P.B. Goodwin. 1980b. Seed vigor in bean (Phaseolus vulgaris L. cv. Apollo) as influenced by temperature and water regime during development and maturation. J. Exp. Bot. 31:313-23.

Sofield, I., L.T. Evans, M.G. Cook, and I.F. Wardlaw. 1977. Factors influencing the rate and duration of grain filling in wheat. Aust. J. Plant Physiol. 4:785-97.

Sofield, I., I.F. Wardlaw, L.T. Evans, and S.Y. Zee. 1977. Nitrogen, phosphorus and water contents during grain development and maturation in wheat. Aust. J. Plant Physiol. 4:799-810.

Spiertz, J.H. 1977. The influence of temperature and light intensity on grain growth in relation to the carbohydrate and nitrogen economy of the wheat plant. Neth. J. Agric. Sci. 25:182-97.

Stearns, F. 1960. Effects of seed environment during maturation on seedling growth. Ecol. 41:221-22.

Stewart, K.A., R.J. Summerfield, F.R. Minchin, and B.J. Ndunguru. 1980. Effects of contrasting aerial environments on yield potential in cowpea (Vigna unguiculata L. Walp). Trop. Agric. 57:43-52.

Summerfield, F.J., P.A. Huxley, P.J. Dart, and A.P. Hughes. 1976. Some effects of environmental stress on seed yield of cowpea (Vigna unguiculata L. Walp) cv. Prima. Plant Soil. 44:527-46.

Summerfield, R.J., P.A. Huxley, and W. Steele, 1974. Cowpeas (Vigna unguiculata(L.) Walp). Field Crop Abstracts 27:301-12.

Terman, G.L., R.E. Ramig, A.F. Dreier, and R.A. Olson. 1969. Yield-protein relationship in wheat grain, as affected by nitrogen and water. Agron. J. 61:755-59.

Thomas, J.F., and C.D. Raper, Jr. 1975a. Differences in progeny of tobacco due to temperature treatment of the mother plant. Tobacco Sci. 19:37-41.

Thomas, J.F., and C.D. Raper, Jr. 1975b. Seed germinability as affected by the environmental temperature of the mother plant. Tobacco Sci. 19:104-06.

Thomas, J.F., and C.D. Raper, Jr. 1979. Germinability of tobacco seed as affected by culture of the mother plant. Agron. J. 71:694-95.

Turk, K.J., A.E. Hall, and C.W. Asbell. 1980. Drought adaptation of cowpea. I. Influence of drought on seed yield. Agron. J. 72:413-20.

Walter, L.E., and E.H. Jensen. 1970. Effect of environment during seed production on seedling vigor of two alfalfa varieties. Crop Sci. 10:635-38.

Wentland, M.J. 1965. The feect of photoperiod on the seed dormancy of Chenopodium album. Dissertation Abst. Int. B 26:4998. The University of Wisconsin, Wisconsin State.

Werker, E., I. Marbach, and A.M. Mayer. 1979. Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus Pisum. Ann. Bot. 43:765-71.

Whalley, R.D.B., C.M. McKell, and L.R. Green. 1966. Effect of environmental conditions during the parent generation on seedling vigor of the subsequent seedlings of Oryzopsis miliacea L. Benth and Hook. Crop Sci. 6:510-12.

Wien, H.C., and E.E. Ackah. 1978. Pod development period in cowpeas: Varietal differences as related to seed characters and environmentnal effects. Crop Sci. 18:791-94.

Wiesner, L.E., and D.F. Grabe. 1972. Effect of temperature preconditioning and cultivar on ryegrass (Lolium sp) seed dormancy. Crop Sci. 12:760-64.

Woodstock, L.W. 1976. Progress report on the seed vigor testing handbook. Assoc. Offic. Seed Anal. Newsletter 50:1-78.

APPENDIX

Table A-1

Seed Coat Color, Seed Weight and Relative Maturity of Ten Cowpea Cultivars

Seed color	Cultivar	Wt/100 seeds	Maturity^z
		g	
White/Cream	White Acre	8.7	L
	Snapea	13.2	I
	Texas Cream 40	13.9	E
	Zipper Cream	20.3	L
Colored	Iron	11.0	I
	Pinkeye Purple Hull	14.4	E
	Mississippi Purple	15.4	E
	Mississippi Silver	15.4	E
	Knuckle Purple Hull	19.9	I
	California Blackeye #5	21.4	E

^{z}E: early, 70 days
I: intermediate, 90 days
L: late, 130 days

Table A-2

Seed Germination and Seedling Growth of Ten Cowpea Cultivars with the Standard Germination Test

Seed Color	Cultivar	Germination		ADG	Seedling length		Seedling wt	
		total	abnormal		hypocotyl	radicle	fresh	dry
		%		day	cm		mg/plant	
White/ Cream	Snapea	96a[z]	4a	2.2bc	10.2a	14.5d	1030c	88c
	Texas Cream 40	97a	3a	1.8de	10.0a	17.4ab	1050c	92bc
	White Acre	98a	2a	1.7e	9.2ab	17.8a	640d	49e
	Zipper Cream	96a	4a	2.4b	7.5c	15.6cd	1250b	123a
Colored	California Blackeye #5	96a	4a	2.0cd	10.2a	16.4abc	1580a	136a
	Pinkeye Purple Hull	99a	1a	1.9d	9.2ab	16.1bc	1110c	92bc
	Mississippi Purple	99a	1a	2.2bc	8.3bc	16.6abc	1120c	103b
	Knuckle Purple Hull	99a	1a	2.4b	8.5bc	16.4abc	1140bc	127a
	Mississippi Silver	100a	0a	2.0cd	9.2ab	15.7cd	1100c	99bc
	Iron[y]	48b	0a	3.7a	5.9d	12.3e	560d	71d

[z]Values followed by the same letter within each column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test

[y]Hardseededness of Iron led to the low germination percentage

Table A-3

Seed Germination and Seedling Growth of Ten Cowpea Cultivars after Accelerated Aging at 41°C and 100% RH for 5 days

Seed color	Cultivar	Germination total	Germination abnormal	ADG	Seedling lenth hypocotyl	Seedling lenth radicle	Seedling wt fresh	Seedling wt dry
		%	%	day	cm	cm	mg/plant	mg/plant
White/ Cream	Snapea	87a[z]	24abcd	2.5b	10.7a	12.8c	970de	83cd
	Texas Cream 40	63b	31ab	2.2bc	9.5abc	11.7c	950e	91cd
	White Acre	86a	20bcde	2.5b	10.2ab	15.8ab	700f	58e
	Zipper Cream	52c	36a	2.5b	7.8de	13.6bc	1200b	130a
Colored	California Blackeye #5	67b	30abc	2.1c	9.9abc	16.9a	1480a	145a
	Pinkeye Purple Hull	91a	7e	1.7d	9.1bcde	16.5a	1090bcd	98bc
	Mississippi Purple	70b	14de	2.3bc	9.2bcd	15.3ab	1150bc	111b
	Knuckle Purple Hull	59bc	17bcde	2.2bc	7.7e	17.4a	1110bcd	134a
	Mississippi Silver	89a	9e	2.1cd	9.1bcde	16.6a	1020cde	100bc
	Iron	94a	16cde	3.1a	8.6cde	16.0ab	710f	77d

[z] Values followed by the same letter within each column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table A-4

Seed Coat Thickness of Hard and Nonhard 'Pinkeye Purple Hull' Seeds Which Developed under SD

Seed coat layer	Hard	Nonhard
	µm	
Cuticle	1.6-2.3	1.7-2.0
Palisade	40-47	40-50
Basal	14-25	16-20
Total thickness	55.6-74.3	57.7-72.0

Table A-5

Partitioning of Seedling Weight and Nutrient Composition during Germination of 'Texas Cream 40' Cowpea as affected by Temperatures during Seed Development

Measurement	Day/Night temperature	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5
	°C	mg/seedling				mg/axis				mg/cotyledon			
Fresh wt	23/15	171a[z]	332a	558a	1020a	3.4a	19.0a	237b	779a	168a	313a	321a	242a
	28/20	140b	306a	543a	1017a	3.2ab	19.9a	263a	824a	137b	286a	280b	193b
	33/25	101c	219b	417b	841b	2.8b	17.0a	225b	725a	98c	202b	193c	117c
	38/30	96c	197b	393b	702c	3.3a	15.8a	222b	605b	93c	181b	171c	97d
Dry wt	23/15	156a	144a	143a	136a	3.2a	4.0a	20.1ab	58.7a	152a	140a	123a	78a
	28/20	127b	124b	120b	118b	2.9a	3.8a	21.7a	59.9a	124b	121b	99b	58b
	33/25	92c	91c	85c	82c	2.6b	3.4b	18.9b	50.8b	90c	88c	66c	31c
	38/30	87c	81c	77c	69d	3.1a	3.5b	19.5b	43.1c	84c	78c	58c	26c
Protein	23/15	22.9a	23.1a	21.1a	17.0a	0.64b	0.74a	2.19ab	5.43a	22.3a	22.4a	18.9a	11.6a
	28/20	21.0a	21.6a	19.3a	15.1b	0.71a	0.70a	2.31a	5.54a	20.3b	20.9a	17.0a	9.6b
	33/25	15.6b	14.7b	12.8b	9.2c	0.62b	0.61b	1.96b	4.74b	15.2c	14.1b	10.9b	4.4c
	38/30	15.9b	14.5b	12.3b	8.7c	0.64b	0.70a	2.10ab	4.29b	15.0c	13.8b	10.2b	4.4c
Amino acid	23/15	1.8a	2.8a	4.1b	8.0b	0.10ab	0.18b	1.68b	6.54b	1.72a	2.60a	2.38a	1.42a
	28/20	2.0a	3.0a	4.4a	9.1a	0.11a	0.22a	2.19a	7.97a	1.85a	2.76a	2.20a	1.17b
	33/25	1.5b	1.9b	3.5c	7.2b	0.09b	0.16b	1.78b	6.66b	1.46b	1.74b	1.32c	0.58c
	38/30	1.5b	2.0b	3.1d	6.4c	0.11a	0.17b	1.85b	5.65c	1.39b	1.78b	1.60b	0.72d
Soluble carbohydrate	23/15	15.7a	15.3a	15.4a	21.4a	0.83a	0.53a	5.81a	17.8a	14.9a	14.8a	9.6a	3.6a
	28/20	12.8b	12.5b	10.0b	18.0b	0.69ab	0.45a	5.89a	16.1ab	12.1b	12.0b	4.1b	2.0b
	33/25	12.1bc	9.9c	8.1c	15.3b	0.65b	0.43a	5.43a	14.1b	11.5c	9.5c	2.7c	1.3c
	38/30	11.2c	10.5c	8.1c	11.6c	0.84a	0.45a	5.32a	10.6c	10.0c	10.0c	2.8c	1.0c
Starch	23/15	83a	75a	66a	43a					83.0a	75.5a	66.4a	42.5a
	28/20	65b	60b	54b	28b					65.0b	59.8b	53.9b	28.1b
	33/25	44c	44c	35c	15c					44.0c	44.2c	34.9c	14.5c
	38/30	37c	35c	27c	11c					37.4c	35.4c	27.1c	11.0c

[z]Values followed by the same letter within measurement column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table A-6

Paritioning of Seedling Weight and Nutrient Composition during Germination of 'Pinkeye Purple Hull' Cowpea as Affected by Temperatures during Seed Development

Measurement	Day/Night temperature	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5	Day 0	Day 1	Day 3	Day 5
	°C	mg/seedling				mg/axis				mg/cotyledon			
Fresh wt	23/15	189a[z]	386a	591b	1035a	4.2a	15.0c	213b	713ab	185a	371a	378a	322a
	28/20	188a	362a	605a	1068a	3.6b	20.9b	268a	819a	185a	341a	337b	249b
	33/25	151b	292b	533b	970a	4.2a	25.3a	264a	792a	147b	267b	270c	179c
	38/30	86c	205c	371c	690b	4.1a	26.0a	211b	601b	82c	179c	160d	89d
Dry wt	23/15	172a	173a	169a	163a	4.0a	4.9b	19.7b	56.9a	168a	169a	149a	106a
	28/20	171a	161a	153b	143b	3.4b	3.9b	22.4ab	61.7a	168a	157a	131b	82b
	33/25	138b	129b	129c	119c	3.9a	4.9a	24.1a	62.7a	134b	124b	105c	56c
	38/30	78c	81c	73d	69d	3.8a	4.5ab	20.7ab	45.7b	74c	77c	53d	23d
Protein	23/15	30.9a	29.3a	24.0a	20.3a	0.74ab	0.78a	2.71a	5.94ab	30.2a	28.6a	21.3a	14.4a
	28/20	29.0a	26.4b	23.2a	16.7b	0.66b	0.78a	2.62a	6.15a	28.3a	25.6b	20.5a	10.6b
	33/25	21.8b	19.5c	17.3b	11.7c	0.65b	0.84a	2.69a	6.37a	21.1b	18.7c	14.6b	5.4c
	38/30	15.9c	14.8d	12.1c	7.4d	0.91a	0.90a	2.36a	4.93b	15.0c	13.9d	9.7c	2.4d
Amino acid	23/15	2.0a	2.8a	4.2a	7.3ab	0.13a	0.18b	1.39a	5.33a	1.88a	2.58a	2.81a	1.96a
	28/20	1.8a	2.8a	4.1a	7.9a	0.11a	0.21b	1.79a	6.46a	1.72ab	2.58a	2.33b	1.39b
	33/25	1.7a	2.8a	3.6b	7.0ab	0.12a	0.27a	1.81a	6.14a	1.61b	2.45a	1.75c	0.86c
	38/30	1.7a	2.3a	3.5b	6.4b	0.16a	0.29a	1.77a	5.71a	1.52b	2.04b	1.74c	0.71c
Soluble carbohydrate	23/15	16.0a	15.8a	16.1a	20.4a	0.98a	0.46ab	4.94a	15.6a	15.0a	15.4a	11.2a	4.8a
	28/20	15.1a	12.8b	11.8b	20.5a	0.81a	0.34b	6.03a	17.8a	14.3a	12.4b	5.7b	2.7b
	33/25	14.3a	12.0b	10.8b	19.3a	0.97a	0.54a	6.42a	17.3a	13.4a	11.5b	4.3c	2.0b
	38/30	9.8b	8.2c	6.6c	9.7b	0.88a	0.48ab	4.80a	8.9b	8.9b	7.7c	1.8d	0.9c
Starch	23/15	90a	90a	67a	49a					89.5a	90.0a	67.2a	48.6a
	28/20	87a	87a	64a	39b					86.7a	87.3a	64.3a	38.5b
	33/25	67b	70b	50b	25c					67.3b	69.8b	50.4b	24.7c
	38/30	30c	30c	16c	6d					30.2c	30.1c	15.6c	6.4d

[z]Values followed by the same letter within measurement column are not significantly different at 5% level of probability according to Duncan's Multiple Range Test.

Table A-7

Effect of Developmental Temperature on Changes in Weight and Nutrient Composition in the Axis of 'Pinkeye Purple Hull' Seeds during Germination

Measurement	Day/Night temperature	Germination period (days) 0 to 1	3 to 5
	°C	—mg—	—mg—
Dry wt	23/15	0	37.2
	28/20	0.5	39.3
	33/25	1.0	38.6
	38/30	0.7	25.0
Protein	23/15	0.04	3.23
	28/20	0.12	3.53
	33/25	0.19	3.68
	38/30	0.09	2.57
Amino acid	23/15	0.05	3.94
	28/20	0.10	4.67
	33/25	0.15	4.33
	38/30	0.13	3.94
Soluble carbohydrate	23/15	-0.52	10.7
	28/20	-0.47	11.8
	33/25	-0.43	11.0
	38/30	-0.40	4.1

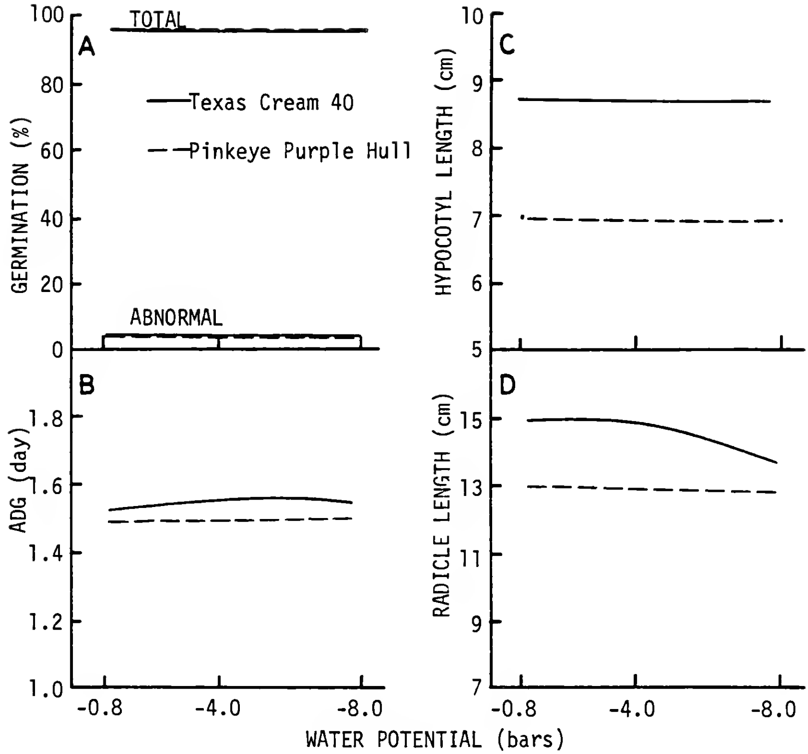

Fig. A-1 Effect of water stress during seed development on seed germination, germination rate and seedling lengths of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas with the standard germination test.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

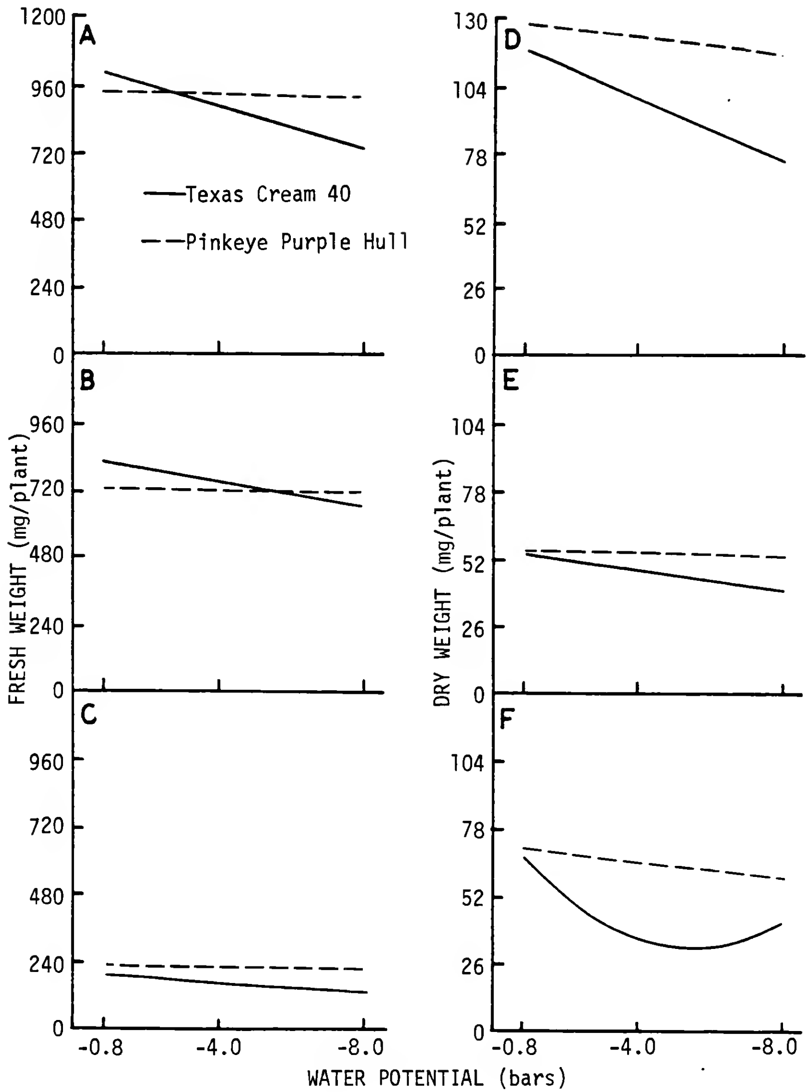

Fig. A-2 Effect of water stress during seed development on seedling weights of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas with the standard germination test.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, E. axis, F. cotyledon.

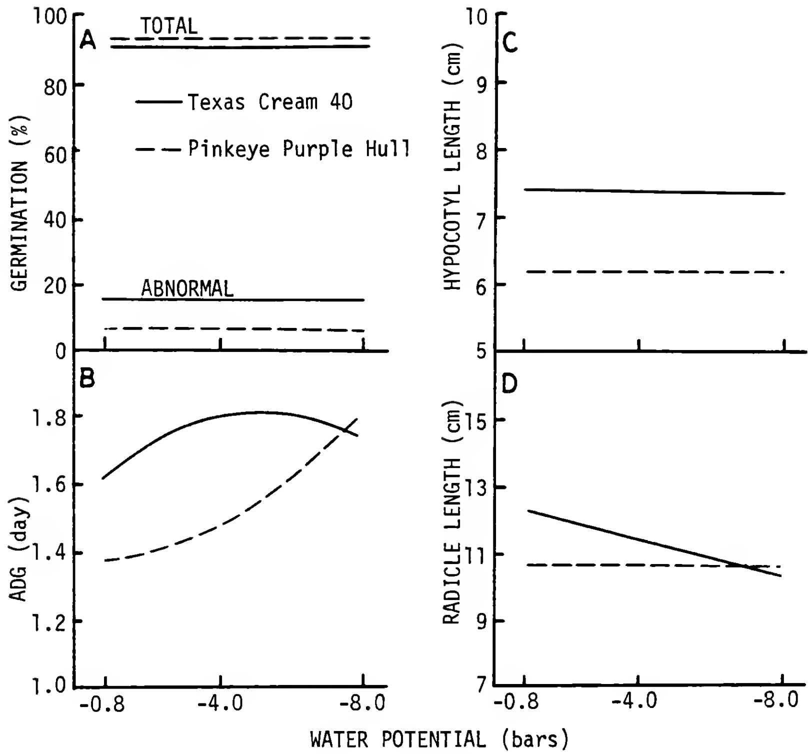

Fig. A-3 Effect of water stress during seed development on seed germination, germination rate and seedling lengths of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas after accelerated aging.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

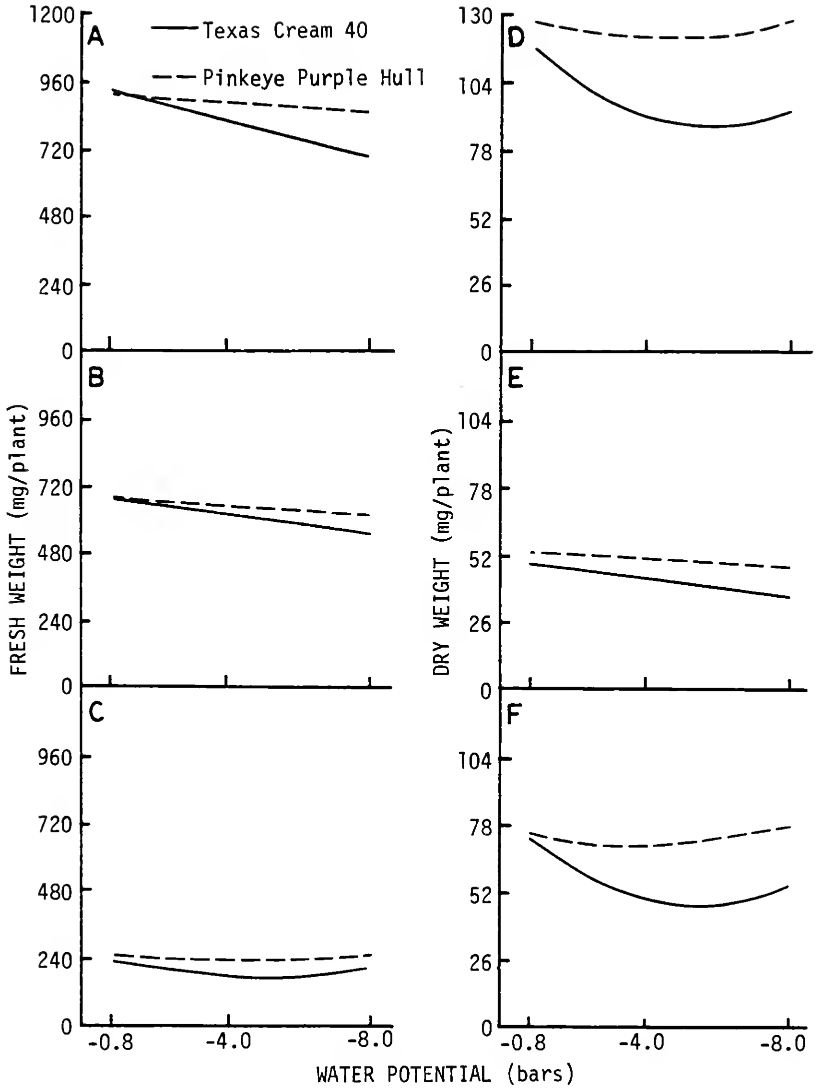

Fig. A-4 Effect of water stress during seed development on seedling weights of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas after accelerated aging.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, E. axis, F. cotyledon.

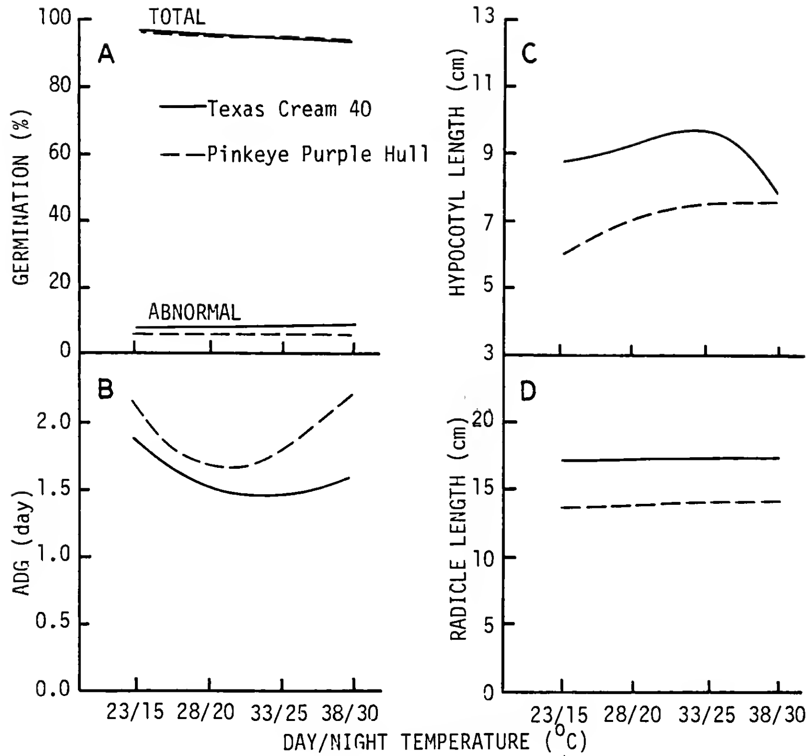

Fig. A-5 Effect of different temperatures during seed development on seed germination, germination rate and seedling lengths of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas with the standard germination test.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

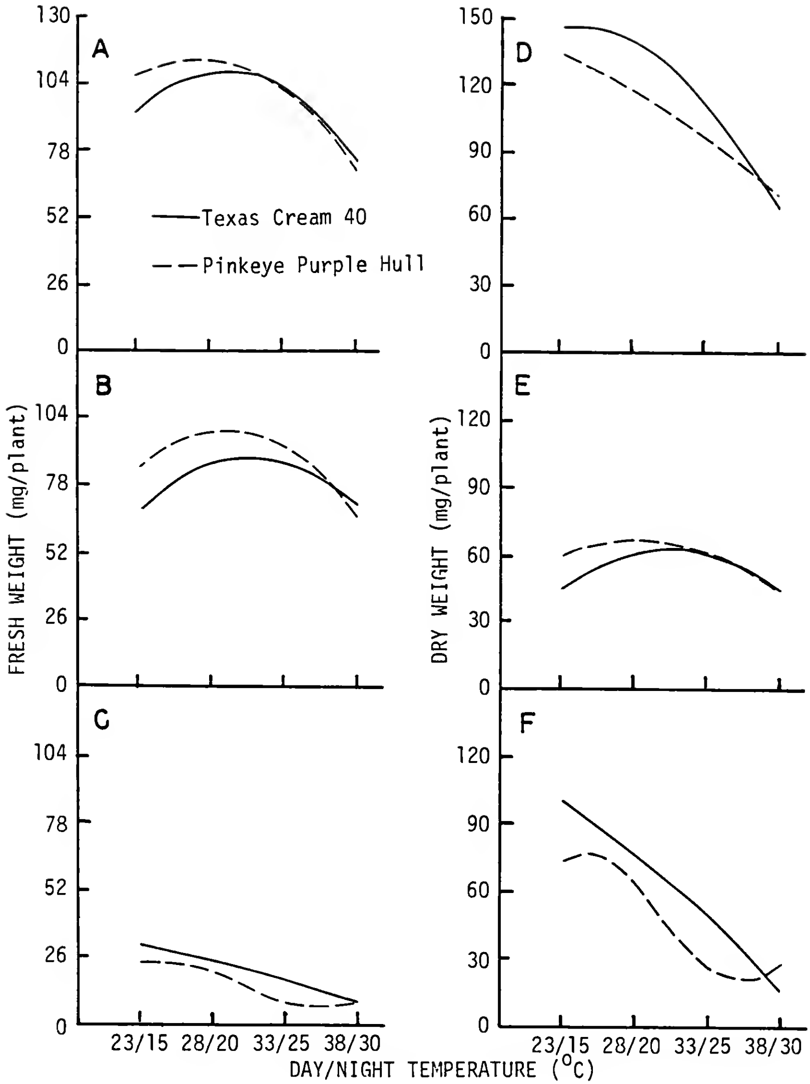

Fig. A-6 Effect of different temperatures during seed development on seedling weights of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas with the standard germination test.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, E. axis, F. cotyledon.

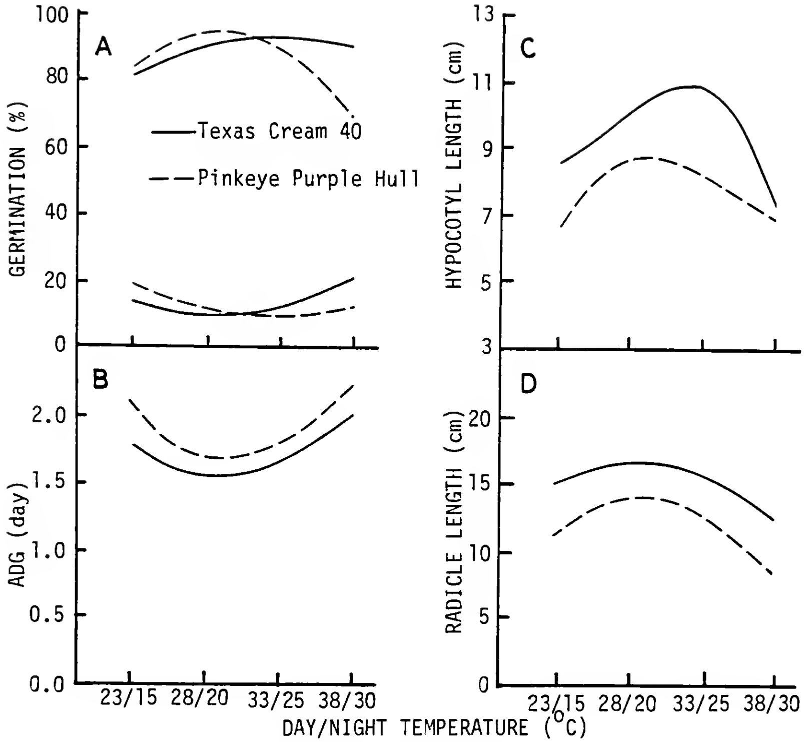

Fig. A-7 Effect of different temperatures during seed development on seed germination, germination rate and seedling lengths of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas after accelerated aging.

A. germination percentage, B. average days to germination, C. hypocotyl length, D. radicle length.

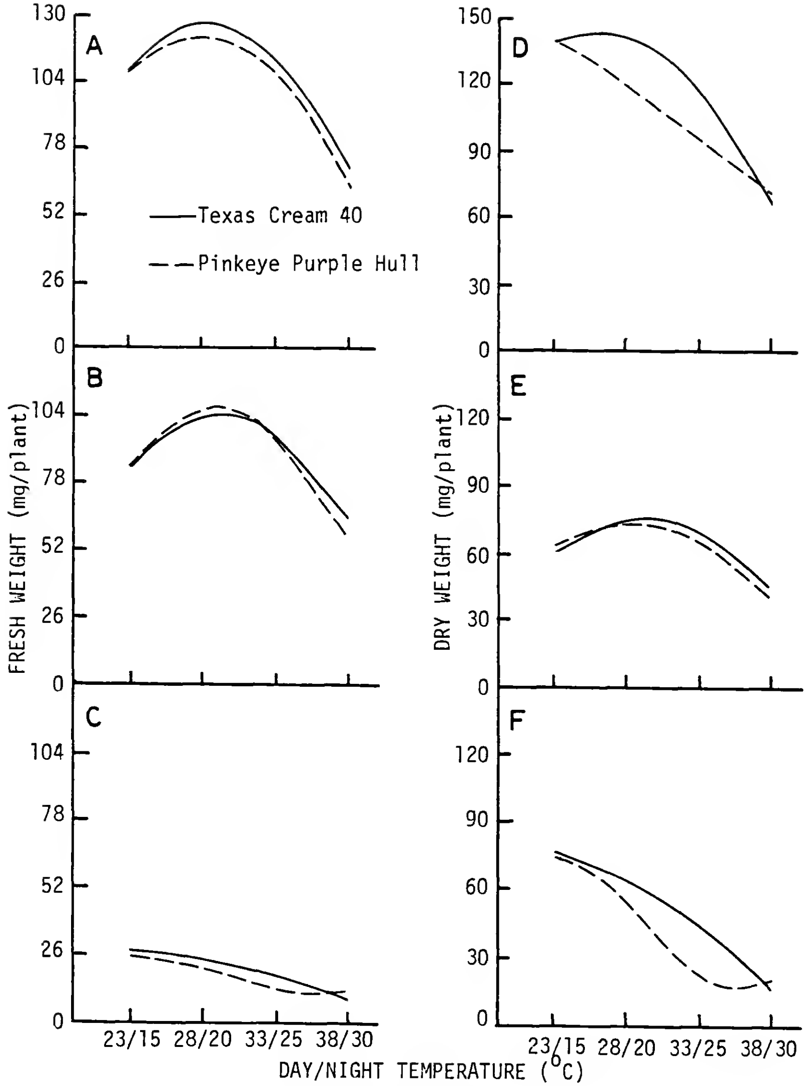

Fig. A-8 Effect of different temperatures during seed development on seedling weights of the progenies of 'Texas Cream 40' and 'Pinkeye Purple Hull' cowpeas after accelerated aging.

Fresh weight: A. seedling, B. axis, C. cotyledon.
Dry weight: D. seedling, E. axis, F. cotyledon.

BIOGRAPHICAL SKETCH

The author was born in Nantou, Taiwan, on June 23, 1952. She enrolled at National Taiwan University, Taipei, Taiwan, in September, 1970, from which she received her Bachelor of Science degree with a major in agronomy in June, 1974. After receiving a Master of Science degree in agronomy from National Taiwan University in June, 1976, she worked in the Plant Physiology Department of the Asian Vegetable Research and Development Center in Shanhua, Taiwan, until November, 1977. In March, 1978, she entered the Vegetable Crops Department at the University of Florida to pursue her Doctor of Philosophy degree. She is married to Shih-Tsan Tang and has a son, Chiaming.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Daniel J. Cantliffe, Chairman
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Chesley B. Hall
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas E. Humphreys
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Terril A. Nell
Assistant Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Indra K. Vasil
Graduate Research Professor of Botany

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1982

Dean, College of Agriculture

Dean for Graduate Studies and Research

UNIVERSITY OF FLORIDA
3 1262 08553 3643