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RESEARCH ARTICLE

VARIETAL DIFFERENCES AMONG *CAJANUS CAJAN* (L.) GENOTYPES IN GERMINATION, ELECTROPHORESIS AND MINERAL ANALYSIS

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ABSTRACT

In the present investigation twelve genotypes of pigeonpea (*Cajanus cajan* (L.) Millspaugh) which were divided into three groups based on the duration for flower initiation i.e. Short duration (ICPL151, ICPL87, ICPL1, ICPL6), Medium duration (T21, HY2 mutant, Pusa agheti, C11) and Long duration (ICPL270, ST1, PDM1, LRG30) were selected and was raised at the Experimental Farm of the Department of Botany, Andhra University, Waltair, Visakhapatnam, A.P., India. In the present study fresh weight, dry weight, seed water content and percent moisture, electrophoresis, germination percentage and mineral analysis were studied in all the twelve genotypes of pigeonpea. There was a gradual increase in pods fresh and dry weights were recorded in all the genotypes. Water content in the seeds increased up to 30 days after flowering followed by a decline till maturity and decrease in the per cent moisture content of the seed throughout the developing period. The polyacrylamide gel electrophoresis of different genotypes of pigeonpea seed soluble proteins were exhibited with high intensity, however short duration genotypes showed more intensive protein band staining than some medium duration and all long duration genotypes. The macronutrients phosphorous, calcium and magnesium were comparatively low in the short duration and among the micronutrients iron recorded greater values than the Zinc, copper and manganese in all the genotypes. Among all the genotypes studied the T21 of medium duration with its small seed size exhibited greatest germination percentage.

INTRODUCTION

Seed germination is an important phase of crop life which forms the basis for the seedling growth and its establishment. The process of seed germination is complex and needs more attention. The physiology and biochemistry of seed germination has been reviewed by several workers (Mayer and Poljakoff Mayber, 1975; Bewley and Black, 1978). Pigeonpea exhibits hypogeal germination. Studies in controlled environments have shown a broad optimum temperature range of 19 ºC to 45 ºC requirement for germination, with an optimum between 29 ºC and 36 ºC (deJabrun *et al*., 1981). Seed yield is the product of metabolic events that are dictated by numerous physiological and biochemical processes (Sherrard *et al*., 1984). It is possible to enhance yields by genetic manipulation of these processes. However, identification of a particular process which promotes or limit yield is difficult. Adequate dry matter production and its appropriate partitioning towards seeds favours high yields. Enhanced dry matter production per unit area is also important for good yields.

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Dry matter production by a crop depends upon leaf area development, leaf area index, the rate of photosynthesis, the rate of respiration and photorespiration, nutrient uptake and assimilation and water use. The utilization of these traits to boost yields is dependent on the genetic variability present in the species for the trait or traits, the heritability of the trait and the positive association of the trait with the yield. The important phases of growth which determine and contribute to crop productivity are seed germination and seedling establishment, vegetative growth, flowering, seed development and maturity. These are under genetic control and thus can be manipulated, to provide a mechanism for complementation between vegetative and reproductive structures. Thus, the information gained on the physiological components of different genotypes is likely to help in the breeding programmes for higher seed yields. From a physiological stand point, the yielding, ability depends on photosynthesis, translocation of the photosynthates as well as on the potentiality of the seeds to accumulate storage substances. The source of nutrients for developing seeds and pods of legumes is not well understood. Allen and Morgan (1972) reported that assimilates from leaves and green pods themselves contributed to the increase in pod size and weight. Littleton *et al*. (1979b) found a strong relationship between final number of pods and total plant dry weight. In pigeonpea the number of pods per plant is strongly related to assimilate supply during first two

weeks after flowering, and on pod retention for the next two weeks (Thirathon *et al*., 1987a). Several workers have reported that a considerable proportion of carbon assimilated during pod growth was diverted to stems and other storage organs (Rawson and Constable, 1981; Deshpande and Nimbalkar, 1982; Setter *et al*., 1984). Thus the pigeonpea plants should be able to supply carbon assimilates to more pods than they do (Sheldrake and Narayanan, 1979). Kaplan and Koller (1974) investigated the accumulation rate of seed dry mater during the liner phase of seed growth in soybean genotypes and found that the seed number did not differ significantly among the genotypes. Pattern of dry matter accumulation in developing fruit parts of early and late maturing genotypes of pigeonpea was reported by Khatra *et al*. (1986). They compared dry matter accumulation in the pod wall, seed coat and seed throughout the reproductive development and found significant water loss and dry matter accumulation in fruit parts which begin much earlier in early maturing genotypes than in late maturing genotypes.

There is a large increase in seed size of pigeonpea between 14 to 28 days after flowering. Fresh weight per seed reached a maximum at 28 days after flowering, whereas dry weight per seed increased up to 35 days after flowering in all the pigeonpea genotypes studied (Singh *et al*., 1980). These studies revealed that the pod wall has lost significant amount of dry matter when the seeds reach maximum dry weight. Fruits of short duration genotypes accumulated more photosynthates than long duration genotypes at all comparable stages. The pattern of pod and seed development indicated that the optimum harvest date for pigeonpea was 42 days after anthesis (Balakrishnan *et al*., 1984). Seeds from brown pods are more superior to those of green pods (Kumari and Ram, 1987) and large dark tan colour seeds were superior to small and off-coloured seeds (Karivaratharaju *et al*., 1982). Pigeonpea seeds are smooth coated, with a small white hilum and are usually round or oval. Seed colour varies from dark tan to cream white among the genotypes. Hundred seed weight of different genotypes of pigeonpea usually range from 4 to 24 g (Narayanan *et al*., 1981b). The increase in seed size was accompanied by linear increase in 100-seed weight, germination percentage, soluble proteins, free amino acids, DNA and RNA contents among the genotypes (Karivaratharaju *et al*.,1982; Vanangamudi *et al*., 1988).

Electrophoretic analysis of seed proteins may provide information on the variability of breeding material. This may also provide a method for genotype identification. Differences between genotypes of the same species have been observed by electrophoretic separation of proteins of *Lupinus angustifolins* (Blagrove and Gillespie, 1978), *Pisum sativum* (Hynes, 1968; Thomson and Schroeder, 1978; Thomson *et al*., 1978), *Arachis hypogea* (Dawson and Mclutosh, 1973), *Glycine max* (Larsen, 1967; Lowry *et al*., 1974), *Phaseolus vulgaris* L. (Yuma and Bliss, 1978), *Vicia faba* (Barratt, 1980) and cotton (Goyal, 1992). Singh *et al*. (1981) compared seed protein fractions of wild relatives and cultivated species of pigeonpea. All these investigations indicated considerable differences in the number and concentrations of the major protein fractions of the genotypes. Grain legumes are also rich source of vitamins, especially the B-complex. They are also rich in minerals such as calcium and iron (Meiners *et al*., 1976; Gopalan *et al*., 1978). Some of the minerals particularly phosphorous, calcium and magnesium have been reported to play an important role in influencing the quality of pigeonpea seeds (Sharma *et al*., 1977). Kadwe *et al*. (1974) reported large varietal differences in the mineral composition within several crop plants. No much information on the variations in the mineral composition of the seeds of pigeonpea genotypes are available and therefore needs further investigation. Recently, Mehrota *et al*. (1987) reported that susceptible cultivars contain more phosphorous values when compared to resistant pigeonpea cultivars. Keeping this in view an attempt was made to find out the genotypic variation in the dry matter production, electrophoresis, germination percentage and mineral analysis and its partitioning in pigeonpea. This is based on the extent to which variations in total biomass production and harvest index are responsible for pigeonpea seed yields.

MATERIALS AND METHODS

Twelve genotypes of pigeonpea (*Cajanus cajan* (L.) Millspaugh) were selected for the investigation which were divided into three groups based on the duration for flower initiation and is presented in the following table:

Group	Genotypes
Short duration	ICPL151, ICPL87, ICPL1, ICPL6
Medium duration	T21, HY2 mutant, Pusa agheti, C11
Long duration	ICPL270, ST1, PDM1, LRG30

The seeds were obtained from International Crops Research Institute for the Semi-Arid Tropics, Patancheru, All India Co-ordinated Pulse Improvement Programme, Hyderabad and other places of Andhra Pradesh. The pigeonpea crop was raised at the Experimental Farm of the Department of Botany, Andhra University, Waltair, Visakhapatnam, A.P., India. The Experimental Farm is situated in a congenial place on latitude 17^0 35' north and longitude 83^0 17' 8" east and at 100 feet high above mean sea level. The crop was grown for three seasons. Seeds of pigeonpea were inoculated with Rhizobium and were sown 4 cm deep in the plots of 10X10 m with a spacing of 75 cm between the rows and 50 cm between the plants within the rows, every growth season of the years. The pigeonpea crop was grown as sole crop. In addition to rainfed conditions, the crop was subjected to monthly irrigation whenever required. The farm yard manure and fertilizers were supplied at the rates shown in the following table:

Manure/Fertilizer	Kgs/ha	No.of doses	Stages
Farm yard manure	5000	1	Soil incorporation
Nitrogen	25	1	Before sowing
Phosphorus	50	1	Before sowing

For recording the data on each parameter, ten plants were collected from each plot and the mean values were presented at monthly intervals. Finally, the mean value of all the three growth season data was given. The data collected and analysed include both field observations and laboratory experiments.

Fresh weight: Samples of ten plants per pot were taken for estimation of fresh weight at monthly intervals. Roots, stems and leaves and pods were separated and their fresh weights were determined.

Dry weight: Immediately after taking fresh weights of the of roots, stems and leaves and pods they were placed in paper

bags kept in a hot air oven maintained at 80ºC for 48 h, by which time the dry weights were recorded. Fallen leaf material was also considered in order to obtain the total dry weight of the whole plant.

Seed water content and percent moisture: The amount of water content in different plant parts was obtained by subtracting the values of dry weights from the respective fresh weights. The difference will be the water content present in the plant parts. Per cent moisture was also calculated by using water content and fresh weight values in all the twelve genotypes.

Electrophoresis: Electrophoretic separation of soluble proteins on acrylamide gel.

Extraction of proteins: The seed coats of the mature seeds of pigeonpea genotypes were removed and the protein was extracted following the procedure of Larsen, 1967. Five grams of seed samples of each genotype were ground finely with 20 ml of 0.1 M acetate buffer (pH 4.8) after an overnight extraction at 4 ºC. The gruel was centrifuged at 30,000 x g at 0 ºC for 30 minutes. The pellet was discarded and the supernatant was adjusted to pH 8.0. The solution was kept at 0 ºC for one hour and then centrifuged at 15,000 x g and then kept it again at 0 ºC for 15 mins. The supernatant was collected and 0.5 ml of this solution was used for separation on polyacrylamide gel electrophoresis (PAGE).

Working solutions

Running gel: There were made fresh each time and the stock solutions were brought to room temperature before mixing to avoid formation of bubbles during polymerization. One part of solution A (10 ml) and one part of solution B (10 ml) were mixed and to this, two parts (20 ml) of solution C was added and mixed gently.

Stacking Gel: These were made fresh each time, and brought to room temperature before mixing to avoid formation of bubbles during polymerization. One part of solution D (2 ml) one part of solution E (2 ml) two parts of solution F (4 ml) and 4 parts of solution G (8 ml) were mixed gently.

Electrophoresis: Polyacrylamide gel electrophoresis was performed according to the method of Davis (1964) in 10% polyacrylamide 2 mm thick slab gels. When the gels were polymerized, the water from the top of the gels were shaken and carefully removed. The slab was fixed, in the electrophoresis apparatus. Protein extract (0.05 ml) in 0.5 M sucrose of each genotype was applied in each slot. The electrophoretic tank was filled carefully with 0.1 M Tris glycine buffer (pH 8.3). A Sharp interference between the gel and the protein and between the protein and the buffer was maintained. A drop of bromophenol blue solution was applied to each slot on the gel as a marker. The tris-glycine buffer was filled in the reservoir to cover the slab gel until the top terminal. The electrophoretic run was conducted with a current of 30 mA at room temperature for about three to four hours, or until the dye marker was approximately 0.5 cm from the bottom of the gel.

Staining: At the end of the electrophoretic run, the slab gel was removed carefully without breaking with a fine stream of water. Immediately the gel was immersed in 0.6% coomassie brilliant blue R 250 in 7.0% acetic acid and stained for about 1 to 5 hours at room temperature. The gel was then destained by placing them in a mixture of acetic acid, methanol and water in a ratio of 2:3:15 and by changing the destaining solution several times until the washing solution was no longer blue.

Mineral analysis: For the estimation of different minerals, the seed (cotyledons and embryonic axis without seed coat or testa) powders were digested by the wet oxidation method (Jackson, 1973). To 1 gram of dry seed powder in a 100 ml conical flask, 10 ml of concentrated nitric acid was added and then placed on a hot plate. The suspension was boiled until nearly dry. This predigestion with nitric acid requires about 45 minutes and was cooled slightly. Then 10 ml of ternary mixture of acids HNO_3:H_2SO_4:$HClO_4$ in the ratio of 10:1:4 was added. Digestion was carried out until dense white fumes of H_2SO_4 and $HClO_4$ were evolved. Digestion was continued until the acid liquid was largely volatalised. The digestion was stopped and the residue in the flask was dissolved in the double distilled water to a volume of 25 ml and can be used for mineral analysis.

Phosphorus: Phosphorus was estimated colorimetrically by the method of Fiske and Subba Row (1925) as modified by Bartlett (1957). A volume of 4.2 ml of the extract was taken into a thick walled heat resistant test tube to which 0.5 ml of 10 N H_2SO_4 was added. After thorough shaking 0.4 ml of ammonium molybdate solution and 0.2 ml of Fiske and Subba Row's reagent were added. It was then heated in a water bath for 7 minutes and cooled with tap water. The colour developed was measured at 660 nm in a ECIL'S junior Spectrophotometer GS 866C. The standard curve was prepared using disodium hydrogen phosphate (Na_2HPO_4).

Preparation of reagents

- Molybdate solution: It was prepared by dissolving 25 g of ammonium Molybdate in 20 ml of distilled water and 300 ml of 10 N H_2SO_4. The solution was made up to a litre and stored.
- Fiske and Subba Row's reagent: To a 100 ml solution of 15% sodium bisulphite, 500 mg of sodium sulphite and 250 mg of 1,2-amino naphthol-4-sulphonic acid were added, mixed and filtered. The filtrate was stored in a dark bottle and was used up to 4 weeks.

Potassium: Potassium in the acid extract was estimated by using 121, Digital FPM 125, systronics Flame photometer and the results were expressed as mg potassium per 100 g dry weight. The standard solution of potassium was prepared by using potassium chloride (KCl).

Calcium, magnesium, zinc, copper, iron and manganese: calcium, magnesium, zinc, copper, iron and manganese in the seed extracts of different genotypes were analysed using atomic absorption spectrophotometer (Varian 275, Australia). The data were expressed as mg per 100 g dry weight of the mature seed material. The standard solutions for calcium, magnesium, zinc, copper, iron and manganese were prepared using calcium carbonate, magnesium carbonate, metallic zinc, metallic copper, metallic iron and manganese chloride respectively.

Germination percentage: The seeds of all the 12 genotypes were surface sterilized with 0.01% mercuric chloride for 3 minutes, washed thoroughly with distilled water and 25 seeds of each lot were placed in petri dishes lined with moist filter paper and allowed to germinate at 30±1 ºC in the dark. Counts for germination were taken up to 48 hours at 24 hours intervals. Seeds with 0.5 cm radicle emergence were considered as germinated. The germination data recorded at the end of 48 hours were expressed as germination percentage.

RESULTS

Fresh and dry weights: The fresh and dry weights of developing pods of the 12 genotypes showed marked differences. There was a gradual increase in pods fresh weight in all the genotypes from the 10th to the 30th day after flowering followed by a decline until harvest. However, the dry weights of pods recorded a gradual increase till harvest in all the genotypes studied. The ICPL151 of short duration, the Pusa agheti of medium duration and the ICPL270 of long duration genotypes exhibited greatest dry weight values at the time of harvest in their respective groups (Fig-1a, b,c,d,e,f).

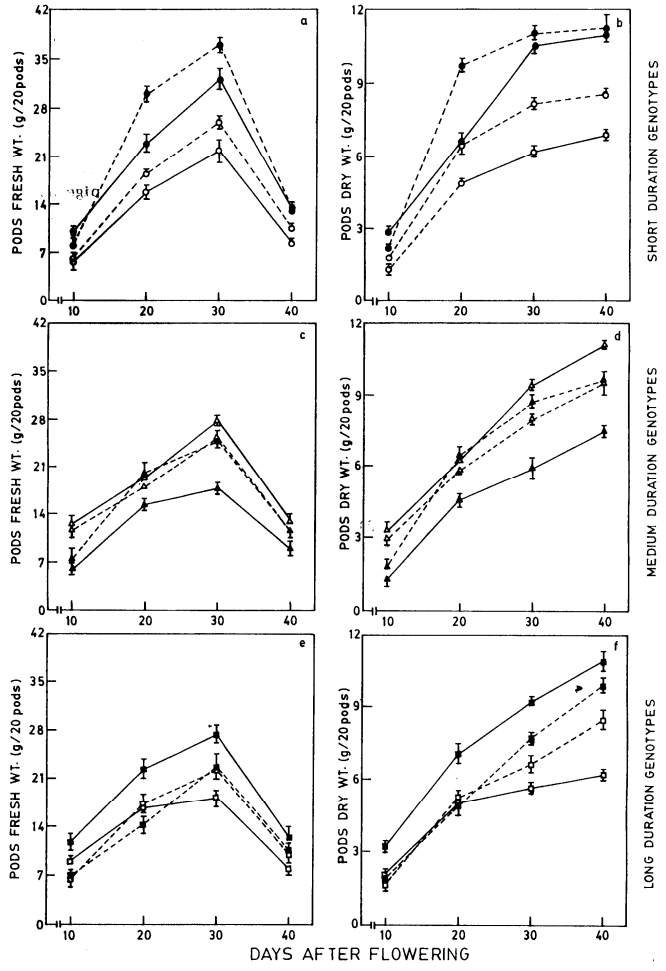

Fig. 1. Fresh (a,c,e) and dry weights (b,d,f) of the developing pods of pigeonpea genotypes. (vertical bars represent S.E.) ●—● ICPL151;●---● ICPL87;o—o ICPL1;o---o ICPL6;▲—▲ T21;▲---▲ HY2 mutant;Δ---Δ Pusa agheti;Δ—Δ C11;■—■ ICPL270;■---■ ST1;□—□ PDM1;□---□LRG30

The 100-seed fresh weight showed an active increase from the 10th to the 30th day after flowering followed by a sharp decrease. The dry weights of developing seeds of all the genotypes exhibited a steady increase till the harvest. The ICPL151 of short duration, Pusa agehti of medium duration and ICPL270 of long duration genotypes recorded greater 100-seed dry weights at the end of the seed maturation period (Fig-2a, b, c, d, e,f).

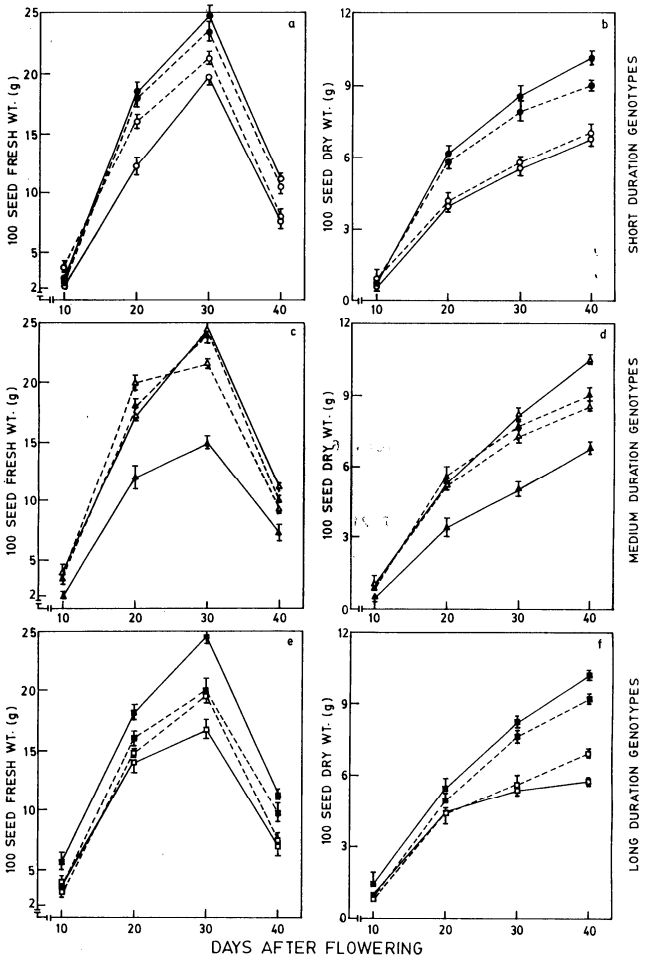

Fig-2:Hundred seed fresh (a,c,e) and dry weights (b,d,f) of the developing seeds of pigeonpea genotypes. (vertical bars represent S.E.) ●—● ICPL151;●---● ICPL87;o—o ICPL1;o---o ICPL6;▲—▲ T21;▲---▲ HY2 mutant;Δ---Δ Pusa agheti;Δ—Δ C11;■—■ ICPL270;■---■ ST1;□—□ PDM1;□---□LRG30

Seed water content and per cent moisture: The water content of seed increased from 10 to 30 days after flowering and then decreased at 40 days in all the pigeonpea genotypes. The ICPL270 of long duration recorded greatest value of water content and the T21 of long duration recorded lowest value at 30 days of seed development (Fig-3a, b, c, d, e,f). The per cent moisture content of seed showed a gradual decrease throughout the developing period until seed maturation. However, at maturity the ICPL87 of short duration, HY2 mutant of medium duration and ICPL270 of long duration expressed higher values of per cent moisture in their respective groups. The developing pattern of pods and seeds of all the genotypes at the 10th, 20th, 30th and 40th day after flowering are presented in the Plates-1,2,3,4. The pod wall develops more rapidly at the initial 20 days after flowering than the young seeds and thereafter exhibited slow growth. The pods are oblong, straight or sickle-shaped depending on the genotype. Pods are green in colour until 20 days after flowering, appear straw-coloured around 30 days after flowering and often streaked to various degrees with purple colour when ripe or at harvest. The pod length in general varied from 4 to 8 cm and width ranged between 0.6 to 1.2 cm.

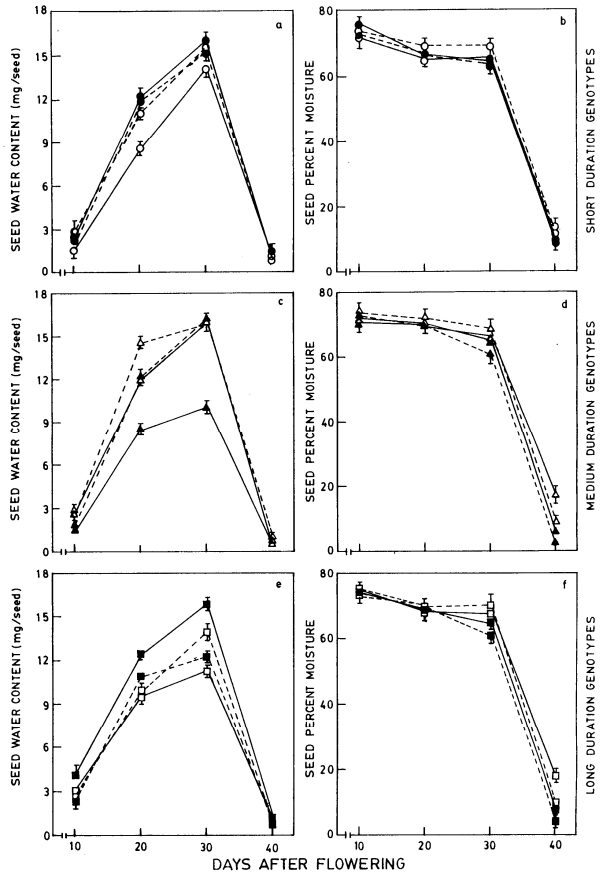

Fig. 3. Water content (a,c,e) and percent moisture (b,d,f) of the developing seeds of pigeonpea genotypes. (vertical bars represent S.E.). ●—● ICPL151;●---● ICPL87;o—o ICPL1;o---o ICPL6;▲—▲ T21;▲---▲ HY2 mutant;Δ---Δ Pusa agheti;Δ—Δ C11;■—■ ICPL270;■---■ ST1;□—□ PDM1;□---□LRG30

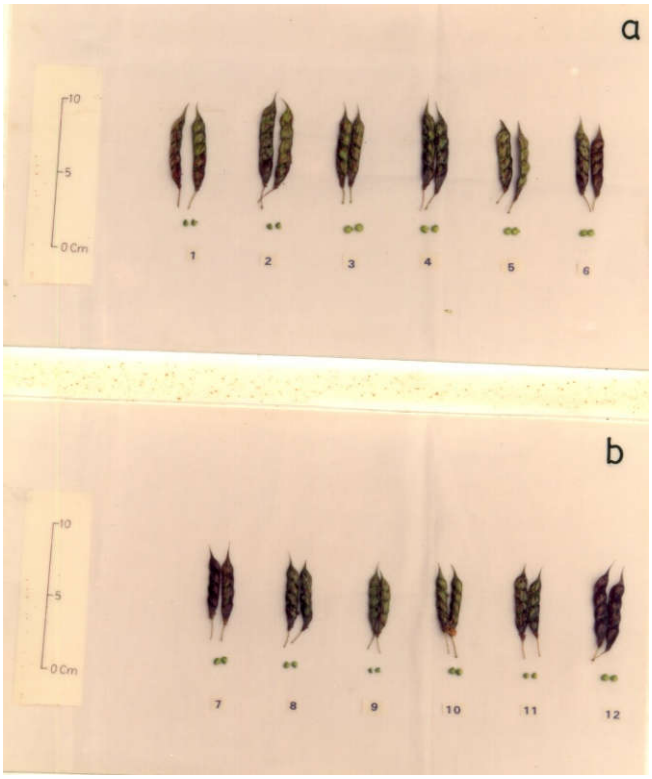

Plate 1. Ten day old pods and seeds of pigeonpea genotypes
1. ICPL151; 2. ICPL87; 3. ICPL1; 4. ICPL6; 5. HY2 mutant; 6. T21
b) 7. C11; 8. ST1; 9. ICPL270; 10. Pusa agheti; 11. PDM1; 12. LRG30

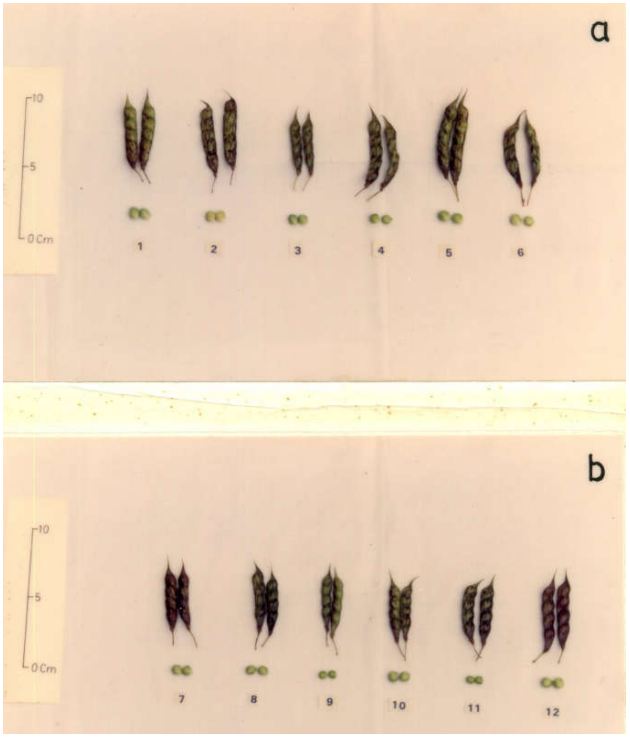

Plate-2: Twenty day old pods and seeds of pigeonpea genotypes.
a) 1. ICPL151; 2. ICPL87; 3. ICPL1; 4. ICPL6; 5. HY2 mutant; 6. T21
b) 7. C11; 8. ST1; 9. ICPL270; 10. Pusa agheti; 11. PDM1; 12. LRG30

The pod length attained its maximum on 30th day after flowering in all the genotypes and showed a slight reduction due to dehydration thereafter at seed maturity. Maximum increase was between 10 to 20 days after flowering. The maximum pod length was observed in the genotype HY2 mutant and the minimum in the genotype PDM1 at the harvest. Usually pods are flattened with diagonal depressions between seeds and are beaked. Pods generally contain 3-4 seeds. However, the genotypes ICPL151, ICPL87 and HY2 mutant produced 5-6 seeds per pod. Seeds are green when young followed by pale yellow and ultimately show a range from white to brown to red. All the seeds reached maximum size at 30 days after flowering. At maturity, seeds showed a slight reduction in size due to dehydration. The seeds are usually round or oval and smooth coated. Specifically the seed coat colour of the genotypes was creamish white in HY2 mutant and Pusa agheti, greyish brown in the ICPL151, ICPL87 and ICPL6, orange red in the ICPL1, T21 and PDM1 and dark red in the C11, ICPL270, ST1 and LRG30. Relatively the genotypes, ICPL151, ICPL87, Pusa agheti, HY2 mutant and ICPL270 exhibited large sized seeds. The polyacrylamide gel electrophoresis of soluble proteins of pigeonpea seeds of different genotypes were presented in the Plate-5. Each genotype exhibited 12 protein bands some with high intensity and the others with low intensity staining. The short duration genotypes showed more intensive protein band staining than some medium duration and all long duration genotypes. The mineral composition of the mature seeds of all the genotypes were presented in Table-1. The values were expressed as mg/100 g dry wt. The phosphorous content of the genotypes varied from 224 to 342 mg/100 g dry wt.

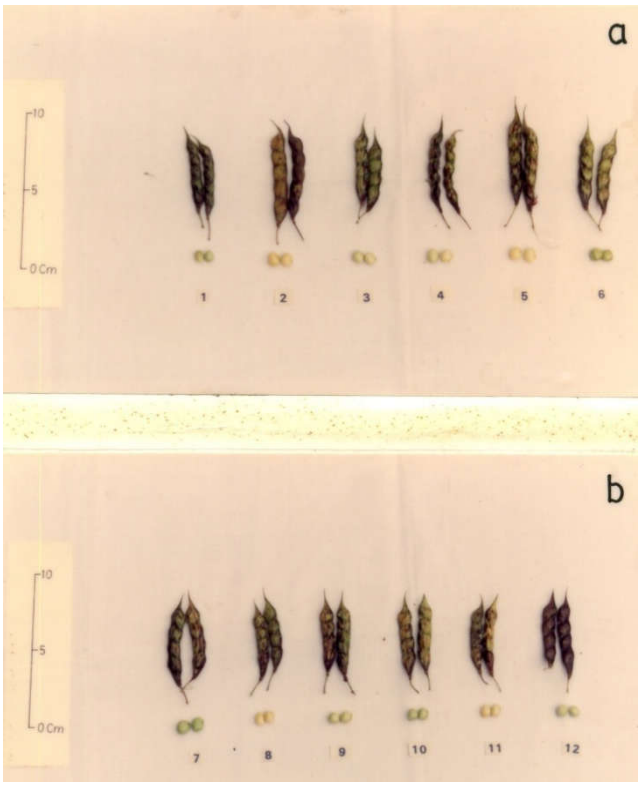

Plate 3. Thirty day old pods and seeds of pigeonpea genotypes
a) 1. ICPL151; 2. ICPL87; 3. ICPL1; 4. ICPL6; 5. HY2 mutant; 6. T21
b) 7. C11; 8. ST1; 9. ICPL270; 10. Pusa agheti; 11. PDM1; 12. LRG30

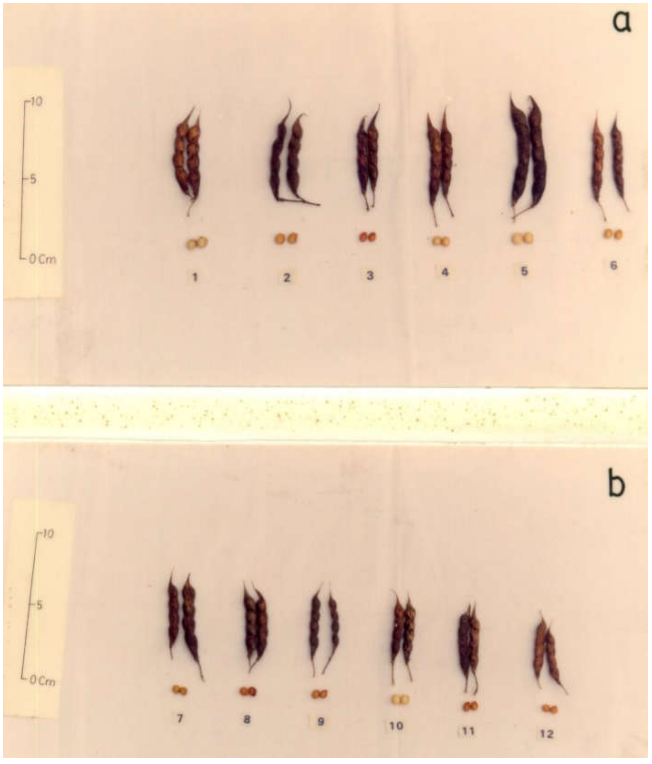

Plate-4: Forty day old pods and seeds of pigeonpea genotypes
a) 1. ICPL151; 2. ICPL87; 3. ICPL1; 4. ICPL6; 5. HY2 mutant; 6. T21
b) 7. C11; 8. ST1; 9. ICPL270; 10. Pusa agheti; 11. PDM1; 12. LRG30

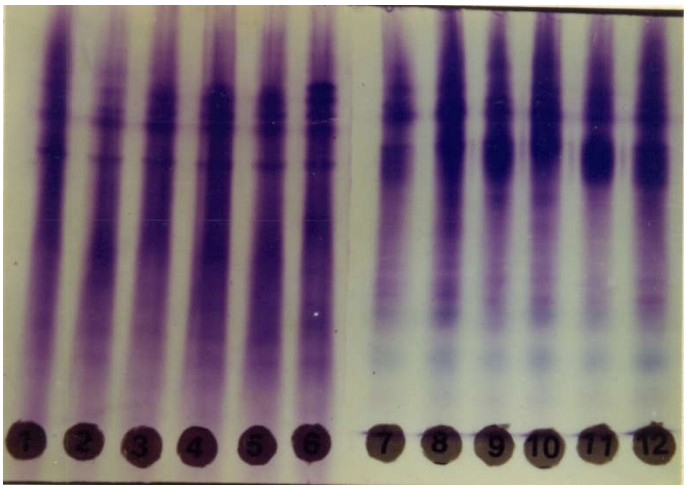

Plate 5. Electrophoretic separation of the soluble proteins of mature seeds of pigeonpea genotypes.
a)1. ICPL151; 2. ICPL87; 3. ICPL1; 4. ICPL6; 5. HY2 mutant; 6. T21
b) 7. C11; 8. ST1; 9. ICPL270; 10. Pusa agheti; 11. PDM1; 12. LRG30

The PDM1 of the long duration genotypes and the ICPL87 of the short duration genotypes exhibited the highest and the lowest values respectively among all the genotypes studied. The short duration genotypes always exhibited lower phosphorous content when compared to medium and long duration genotypes. The potassium content of the genotypes studied varied between 1282 to 1448 mg/100 g dry wt. The T21 of the medium duration and the ST1 of long duration genotypes recorded the highest and lowest quantities respectively. The genotypic differences in relation to potassium content were not conspicuous. Calcium content of the genotypes studied varied between 85.10 and 127 mg/100 g dry wt. The PDM1 of the long duration and the ICPL151 of the short duration genotypes expressed the maximum and minimum values respectively. The long duration genotypes exhibited higher quantities of calcium followed by medium duration and short duration genotypes. The magnesium concentration of mature seeds of the genotypes varied between 128 and 168 mg/100 g dry wt. The PDM1 long duration and the ICPL151 of short duration genotypes recorded the highest and lowest quantities of magnesium. Trace elements were also expressed as mg/100 g dry wt. The zinc content exhibited a maximum value of 2.52 mg/100 g dry wt in ICPL87 and a minimum value of 2.12 mg/100 g dry wt in ST1 among the genotypes analysed. The copper content of different genotypes varied between 1.17 to 1.96 mg/100 g dry wt. The genotypes ICPL87 and C11 recorded the maximum and minimum values of copper content respectively. Of all the microelements studied, iron content recorded greater values. Its values varied between 3.39 and 4.55 mg/100g dry wt. Among the genotypes analysed ICPL87 recorded the maximum and ICPL1 the minimum quantities of iron respectively. The manganese content of the different genotypes varied between 1.05 and 1.44 mg/100 g dry wt. The manganese content recorded the maximum in the T21 and the minimum in the PDM1. The cobalt, nickel and lead contents were found in trace quantities only. An inspection of the mineral analysis data indicated that the ICPL87 of short duration, the T21 of medium duration and the PDM1 of long duration genotypes exhibited greater values of mineral elements within the genotype groups studied.

Table 1. Seed mineral composition of pigeonpea genotypes (mg/100 g dry wt) (mean of 3 replications ± S.E.)

	Macro elements				Trace elements			
Genotypes	Phosphorous	Potassium	Calcium	Magnesium	Zinc	Copper	Iron	Manganese
Short duration								
ICPL151	241.00±4.20	1375.00±13.45	85.10±1.99	128.00±0.99	2.21±0.03	1.37 ±0.10	4.19±0.06	1.10±0.03
ICPL87	264.00±4.45	1418.00±11.36	97.40 ±2.31	145.00±1.02	2.52±0.05	1.96 ±0.15	4.55±0.09	1.17±0.04
ICPL1	258.00±5.70	1332.00±12.75	91.61±2.02	138.00±0.88	2.30±0.03	1.39±0.11	3.39±0.05	1.14±0.03
ICPL6	267.00 ±5.34	1347.00±15.00	96.80±2.15	157.00±1.01	2.49±0.06	1.38±0.09	3.53±0.06	1.13 ±0.02
Medium duration								
T21	317.10±6.12	1448.00±11.85	105.21±2.30	160.00±1.06	2.70±0.05	1.51±0.12	4.67±0.07	1.44±0.22
HY2 mutant	289.00 ±5.79	1408.00±12.34	109.32±2.64	167.00±1.11	2.30±0.04	1.27±0.13	4.18±0.03	1.30±0.03
Pusa agheti	308.00±5.60	1385.00±12.28	103.60±1.89	165.00±1.15	2.36±0.03	1.46±0.17	4.30±0.06	1.16±0.04
C11	255.00 ±5.33	1298.00±11.89	111.25±2.25	152.00±1.14	2.19±0.02	1.17 ±0.16	4.53±0.07	1.19±0.05
Long duration								
ICPL270	275.00±5.20	1285.00±11.65	112.50±2.19	155.00±1.16	2.39±0.03	1.41±0.15	4.28 ±0.08	1.17±0.03
ST1	250.50±4.65	1282.00±12.50	115.60±2.00	146.00±1.08	2.12±0.02	1.45±0.14	3.48 ±0.06	1.13±0.02
PDM1	342.00±5.31	1398.00±12.37	127.00 ±2.34	168.00±1.19	2.42 ±0.04	1.38±0.13	4.51±0.09	1.05±0.02
LRG30	233.75 ±4.69	1355.00±12.44	119.80±1.98	162.00±1.03	2.25±0.05	1.26±0.11	4.40±0.07	1.23±0.04

Table 2. Per cent Seed Germination of Pigeonpea Genotypes (Mean Of 10 Replications ± S.E.)

Genotype	Per cent
Short duration	
ICPL151	93.18 ± 1.50
ICPL87	94.33 ± 2.25
ICPL1	94.66 ± 1.45
ICPL6	92.49 ± 1.02
Medium duration	
T21	98.10 ± 0.90
HY2 mutant	81.56 ± 3.00
Pusa agheti	88.40 ± 2.64
C11	83.36 ± 2.10
Long duration	
ICPL270	84.00 ± 2.10
ST1	87.00 ± 2.95
PDM1	96.66 ± 1.46
LRG30	90.16 ± 2.31

Genotypic variation of per cent germination of pigeonpea seeds were presented in Table-2. Within the given group the ICPL1 (94.66%) of short duration, the T21 (98.10%) of medium duration and the PDM1 (96.66%) of long duration genotypes recorded greater values of per cent germination. The HY2 mutant (81.56%) expressed lowest per cent germination among all the genotypes studied.

DISCUSSION

The knowledge of physiological and biochemical changes that occur during the development of pods and seeds may have great significance in evaluating the genotypes of the given crop. The fresh weight of pods and seeds attained a maximum on the 30th day after flowering followed by a decline on the 40th day in all the pigeonpea genotypes studied (Fig-1a, c, e; Fig-2a, c, e). However, there was a continuous increase in the dry weight of pods and seeds throughout their development. The ICPL151 of short duration, the Pusa agheti of medium duration and the ICPL270 of long duration genotypes exhibited higher values in their respective groups (Fig- 1b, d, f; Fig- 2b, d, f). The seed size and 100-seed weight of these genotypes were also greatest in their respective groups (Plates- 1, 2, 3 and 4). Thus, in pigeonpea, the dry matter accumulation in seeds is directly correlated with genetic differences in final seed size. The fresh and dry weight of developing seeds exhibited sigmoid growth pattern. The rapid growth phase was observed between 10 and 30 days of seed development. During seed development 70 per cent of the dry matter was accumulated during this period. Thus, this period appears to be the most active phase for accumulation of reserve material.

Water content in the seeds increased up to 30 days after flowering followed by a decline till maturity (Fig-3a, c, e). In response, a slight decrease in the fresh weight was observed between 30 and 40 days although the dry weight continued to increase. There was a continuous decrease in the per cent moisture content of the seed throughout the developing period (Fig-3b, d, f). The increase in dry weight was coincided with the decreasing moisture content (Harrington, 1973; Mayer, 1973). In all the pigeonpea genotypes, both the pod length and seed size attained maximum values on the 30th day after flowering followed by a retraction thereafter (Plates- 1, 2, 3 and 4). The retraction in pod length and seed size may be in response to the loss of water content with advancing pod and seed maturity. This loss of water from the seeds was associated with the cessation of seed growth activities. Many other seeds such as peas (Manohar and Sachan, 1974) and mung bean (Savitri *et al*., 1978) were also exhibited reduced size with maturation. At maturity the seed colour varied greatly among the genotypes. They exhibited cream (cv.HY2 mutant), orange red (cvs. ICPL151 and ICPL87) or reddish brown (cvs. ICPL1, ICPL6, T21, Pusa agheti, C11, ICPL270, ST1, PDM1 and LRG30) in colour. The seed shape also varied from oval to pea shaped (Plates- 1, 2, 3 and 4). The dark coloured seeds exhibited high germination percentage when compared to light coloured ones (Powell and Mathews, 1979; Karivaratharaju *et al*., 1982; Olivera *et al*., 1984; Alison *et al*., 1986). The polyacrylamide gel electrophoresis of soluble proteins of pigeonpea seeds revealed that the short duration genotypes showed intensive protein band staining than some of the medium duration and all the long duration genotypes (Plate-5). Perhaps, it may be due to their greater protein content when

compared to the medium and long duration genotypes. The mineral composition of different genotypes of pigeonpea revealed that, the macronutrients phosphorous, calcium and magnesium were comparatively low in the short duration and some medium duration genotypes than the long duration genotypes (Table-1). It was reported that the lower values of phosphorous induces susceptibility for the seed to storage and the resulting loss of seed viability (Mehrota *et al*., 1987). Perhaps, this may be one of the reasons for the lower physiological stamina in the short duration and HY2 mutant of medium duration genotypes (Kalpana, 1992). Long duration pigeonpea genotypes contained more calcium and therefore presumably may be more resistant to storage.

The genotypic variation in relation to potassium content were not that much conspicuous. Among the micronutrients iron recorded greater values than the zinc, copper and manganese in all the genotypes. The study revealed that ICPL87, T21 and PDM1 belonging to the short, medium and long duration genotypes exhibited greater values of mineral elements in their respective groups. The emergence of 0.5 cm radical from the seed after 24 hours of imbibitions was considered as seed germination. Among all the genotypes studied the T21 of medium duration with its small seed size exhibited greatest germination percentage. The HY2 mutant with its large seed size exhibited lowest seed germination (Table-2). The physical observation of the seeds revealed that the testa of the white and light coloured seeds loosely adhered to the cotyledons and this feature allowed water to enter the seed more freely in the gap between the testa and cotyledons resulting in a rapid rate of water uptake (Powell and Mathews, 1979; Oliveira *et al*., 1984; Alison *et al*., 1986). It may be presumed that no yield advantage was associated with using high vigour seed. The seeds of ICPL87 and ICPL151 with low vigour and low percent germination, exhibited higher seed yields. The high per cent germination is only assured of adequate field emergence and stand establishment.

Conclusion

There was a continuous increase in pod and seed weights throughout the period of seed development. The ICPL151 of short duration, the pusa agheti of medium duration and the ICPL 270 of long duration genotypes expressed greater dry weight values in their respective groups. The seed size and 100-seed weight of these genotypes were also greatest in their respective groups. The seed coat colour varied greatly from cream to reddish brown among the genotypes. The dark coloured seeds exhibited higher germination percentage than the light coloured ones. Interestingly, the protein content showed conspicuous genotypic difference ranging from 19.96 to 24.18 per cent. The highest value was recorded in the ICPL87 of short duration and the lowest value in the ST1 of long duration pigeonpea genotypes. The macronutrients, phosphorus, calcium and magnesium were comparatively low in the short and some medium duration genotypes than the long duration genotypes.

REFERENCES

Alison A, De A, Powell M, Oliveira A and Mathews S., 1986. Seed vigour in cultivars of dwarf french beans (*Phaseolus vulgaris*) in relation to the colour of the testa. *J. Agric. Sci*., (Camb) 106: 419-425.

Allen EJ and Morgan DG., 1972. A quantitative analysis of the effects of nitrogen on the growth, development and yield of oil seed rape. *J. Agric. Sci*. (Camb), 78: 315-324.

Balakrishnan K, Vanangamudi K, Vanangamudi M and Natarajaratnam N., 1984. Seed development and maturation studies in pigeonpea. *Madras Agricultural Journal*, 71(6): 387-390.

Barratt DHP., 1980. Cultivar identification of *Vicia faba* (L.) by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of seed globulins. *J. Sci. Food Agric*., 31: 813-819.

Bartlett GR., 1957. Phosphorus assay in column chromatography. *J. Bio. Chem*., 234:466-468.

Bewley JD and Black M., 1978. Physiology and biochemistry of seeds in relation to germination I. Development, germination and growth. Springer-Verlag, Berlin.

Blagrove RJ and Gillespie JM., 1978. Comparative studies on the proteins from seeds of *Lupinus angustifolius* L. *Aust. J. Plant Physiol*., 5: 651-663.

Davis BJ., 1964. Disc electrophoresis. II. Method of application to human serum proteins. In: Gel Electrophoresis. *Ann. N.Y. Acad. Sci*., 121: (A to Z): 404-427.

Dawson R and McIntosh, 1973. Varietal and environmental differences in the proteins of the ground nut (*Arachis hypogea*). *J. Sci. Food Agric*., 24:597-609.

De Jabrun PLM, Byth DE and Wallis ES., 1981. Imbibiton by and effects of temperature on germination of mature seeds of pigeonpea. In: procedings of the international workshop on pigeonpea. Vol.2: 15-19, Dec. 1980, ICRISAT, Hyderabad, India. Pp. 181-187.

Deshpande R and Nimbalkar JD., 1982. Effect of salt-stress on translocation of photosynthates in pigeonpea. *Plant Soil*, 65: 129-132.

Fiske CH and Subba Row Y., 1925. The colorimetric determination of phosphorus. *J. Biol. Chem*., 66:375-400.

Gopalan C, Rama Sastri BV and Balasubramanian SC., 1978. Nutritive value of Indian foods. National Institute Of Nutrition, Indian Council Of Medical Research, Hyderabad, India.

Goyal KC., 1992. Identification of some cotton cultivars using PAGE. *Indian J. Plant Physiol*., XXXV(4): 405-407.

Harrington JF., 1973. Seed storage and longivity in seed Biology (Ed.) I.T. Kozlowski 3: pp. 145-245. Academic press, New York and London.

Hynes MJ., 1968. Genetically controlled electrophoretic variants of a storage protein in *Pisum sativum*. *Aust. J. Biol. Sci*., 21:827-829.

Jackson ML., 1973. Soil chemical Analysis. Prentice Hall of India private Limited, New Delhi, pp.331-336.

Kadwe RS, Thakare KK and Radhe NN., 1974. A note on the protein content and mineral composition of twenty five varieties of pulses. *Indian J. Nutr. Diet*., 11:83.

Kalpana R., 1992. Physiological and biochemical changes during accelerated ageing of seeds of pigeonpea. Ph. D. thesis submitted to Andhra University, Waltair.

Kaplan SL and Koller H.R., 1974.Variation among soybean cultivars in seed growth rate during the linear phase of seed growth. Crop. Sci., 14: 613-614.

Karivaratharaju TV, Ramakrishnan V, Vadivelu KK and Ramaswamy KR., 1982. Effect of seed size and seed coat colour on seed quality and productivity of red gram (*Cajanus cajan* (L.). *Madras Agric. J*., 69(7): 421-430.

Khatra SS, Kumar V and Sidhu PS., 1986. Patterns of dry matter accumulation in developing fruit parts of early and late-maturing pigeonpeas (*Cajanus cajan* (L.) Millsp.). *Biologia Plantarum*, 28(4): 297-302.

Kumari P and Ram C., 1987. Effect of stages of maturity on seed quality of pigeonpea. *Seeds and Farms*, 13(8): 31-33.

Larsen AC., 1967. Electrophoretic differences in seed proteins among varieties of soyabean (*Glycine max* (L.) Merill). *Crop. Sci*., 7:311-313.

Littleton EJ, Dennett MD, Monteith JL and Elston J., 1979b. The growth and development of cowpeas (*Vigna unguiculata*) under tropical field conditions. 2. Accumulation and portions of dry weight. *J. Agric. Sci*., (Camb), 93: 321-327.

Lowry KL, Carton JE and Foard DE., 1974. Electrophoretic methods for detecting differences in seed proteins of soybean varieties and induced mutants. *J. Agric. Fd. Chem*., 22:1043-1045.

Manohar MS and Sachan SCP., 1974. Pod development and germination studies on pea (*Pisum sativum* L.). *Veg. Sci*., 1: 22-30.

Mayer AM and Poljakoff Mayber A, 1975. The germination of seeds. 2nd Ed. Pergamon press, oxford.

Mayer AM., 1973. The control of the initial stages of germination. Some biochemical investigations. *Seed Sci. and Technol*., 1:51-72.

Mehrota ON, HK Saxena and M Moosa, 1987. Physiological analysis of varietal differences in seed yield of Indian mustard (*Brassica juncea* L. Czern and Coss). *Indian J. Plant Physiol*., 19: 139-146.

Meiners CR, Derise NL, Lau HC, Crews MG, Ritchey SJ and Murphy EW., 1976. The content of nine mineral elements in raw and cooked mature dry legumes. *J. Agric. Food Chem*., 24: 1126-1130.

Narayanan A, Saxena NP and Sheldrake AR., 1981b. Varietal differences in seed size and seedling growth of pigeonpea and chickpea. *Indian J. Agric. Sci*., 51: 389-393.

Oliveira M, De A, Mathews S and Powell AA., 1984. The role of split seed coats in determining seed vigour in commercial seed lots of soyabean, as measured by the electrical conductivity test. *Seed Science and Technology*, 12: 659-668.

Powell AA and Matthews S., 1979. The influence of testa condition on the imbibition and vigour of pea seeds. *Journal of Experimental Botany*, 30:193-197.

Rawson HM and Constable GA., 1981. Gas exchange of pigeonpea: a comparison with other crops and a model of carbon production and its distribution within the plant. In: Proceedings of the International Work shop on pigeonpea, Volume 1 by ICRISAT. 15-19, December 1980. Pp. 175-189.

Savithri KS, Ganapathy PS and Sinha SK., 1978. Fruit and seed development in mung beans (*Phaseolus aureus* Roxb.). *J. Agri. Sci*., 90:551-556.

Setter TL, Mc David and Lcpez FB., 1984. Photosynthate partitioning in pigeonpea in response to defoliation and shading. *Crop Science*, 24: 221-224.

Sharma YK, Tiwari AS, Rao KC and Mishra A., 1977. Studies on chemical constituents and their influence on cook ability in pigeonpea. J. Food Sci. Technol., 14:38-40.

Sheldrake AR and Narayanan A., 1979.Comparisions of earlier- and later- formed pods of pigeonpea (*Cajanus cajan* (L.)Millsp.). *Ann. Bot*., 43: 459-466.

Sherrard JH, Lambert RJ, Messmer MJ, Below FE and Hageman RH., 1984: Search for useful physiological and biochemical traits in maize. In: crop breeding, a contemporary basis. Pergamon press, Oxford, pp.81-66.

Singh SP, Govil JN, Ram H., 1981. Effect on maturity time on seed germination and pod development in extra-early pigeonpea. *Pulse Crops Newsletter*, 1(2): 18-19.

Singh U, Jambunathan R and Narayanan A., 1980. Biochemical changes in developing seeds of pigeonpea (*Cajanus cajan*). *Phytochemistry*, 19(7): 1291-1295.

Thirathon A, Byth DE, Fischer KS and Whiteman PC., 1987a. Effect on sink development of changes in assimilate supply during different growth stages of short-season pigeonpea. In: Wallis, E. S. and Byth, D. E. (Eds), Food Legume Improvement for Asian Farming Systems: Proceedings of an International Workshop held in Khon Kean, Thailand, 1-5 Sept., 1986: ACIAR Proceedings No. 18 ACIAR Canberra, p.252.

Thomson JA and Schroeder HE., 1978. Cotyledonary storage proteins in *Pisum sativum* II. Hereditary variation in components of the legumin and vicilin fractions. *Aust. J. Plant Physiol*., 5: 281-294.

Thomson JA Schroeder HE and Dudman WF., 1978. Cotyledonary storage proteins in *Pisum sativum* I. Molecular heterogeneity. *Aust. J. Plant Physiol*., 5: 263-279.

Vanangamudi K, Vanangamudi M, Balakrishnan K, Palaniswamy V and Karivaratharaju TV., 1988. Physical, Physiological and biochemical evalution of graded seeds of pigeonpea cultivars. *Madras Agricultural Journal*, 75 (5-6): 228-230.

Yu MA and Bliss FA., 1978. Seed proteins of common bean. *Crop Sci*., 18:431-437.

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