



AMYLASE ACTIVITY AND CARBOHYDRATE CONTENT OF THE DIFFERENT PLANT PARTS IN *CAJANUS CAJAN* (L.) DURING CROP GROWTH

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ABSTRACT

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 for the present study on amylase activity, starch content, soluble sugars and reducing sugars of the whole plant and its different parts of the vegetative, flowering and seed maturation phase. Amylase activity in the genotypes, the ST1 of long duration and the T21 of medium duration genotypes expressed greater and lower values respectively at all phases of crop growth. The starch content of the whole plant recorded the maximum values in the genotype PDM1, the total leaves were maximum in the Pusa agheti and stem and root was maximum in the PDM1. Of all the genotypes, the PDM1 of long duration, exhibited greater values of soluble sugar content in the whole plant. Among the different parts studied, stems showed more soluble sugar content followed by leaves and roots. Out of all the genotypes studied the PDM1 recorded the maximum values of reducing sugar content in the whole plant and its different parts on both the expressions. The non-reducing sugar content of the different parts studied was the stems showed greater values than the leaves and roots on per part basis in the genotype PDM1 expressed the maximum values than the medium and short duration genotypes.

INTRODUCTION

The potential yield of a crop is dependent on the genotype, the environment in which it grows and the management it receives. These factors are closely linked with different physiological processes and their interrelationships of a genotype (Evans, 1975). In grain crops, the

economically important part of the plant is the mature seed. Varietal differences in physiological and biochemical changes of developing pods and seeds have been described in a number of legumes such as pea (Flinn and Pate, 1968), soyabean (Egli, 1975; Wein and Ackah, 1978; Egli et

al., 1981,1984; Fraser *et al.*, 1982; Guldan and Brun, 1985; Swank *et al.*, 1987), french bean (Carr and Skene, 1961; Crookston *et al.*, 1974), field bean (Barratt, 1982), urd bean (Anita Saha, 1987), mungbean (Dhillon and Nainawatee, 1990), bambarra ground nut (Sreeramulu *et al.*,1992) and pigeonpea (Singh *et al.*, 1980; Balakrishnan *et al.*, 1984; Singh *et al.*, 1984). Carbohydrates are the main constituents of many legume seeds. During the maturation of seeds, these constituents are stored in the cells of the cotyledons (Black, 1983). Although legume seed proteins have been the subject of numerous studies in the past, only limited information is available on various legume starches (Leach *et al.*, 1959; Schoch and Maywald, 1968; Lai and Varriano-Marston, 1979; Yang *et al.*, 1980; Tjahjadi and Breene, 1984). Generally the whole seed in pigeonpea genotypes contains 48 to 59 per cent of starch (ICRISAT, 1976). Little information is available on the nutritional aspects of pigeonpea genotypes belonging to different maturity groups. Singh (1984) reported that protein and starch together constituted 75 per cent of total dhal (split seeds without seeds coat) weight. Only a small variation was observed between genotypes in starch and soluble sugar content of early, medium and late maturity groups. It was also observed that the starch content is less in high protein genotypes when compared to the others. On the other hand, soluble sugar content varied among pigeonpea genotypes (Singh *et al.*, 1990). Although genetic differences in the morphology and yield have been reported, the physiological basis for genotypic variation of crop growth and yield formation remains obscure in pigeonpea. Therefore, the present study is undertaken to fill this lacuna to a certain extent.

MATERIAL AND METHODS

Twelve genotypes of pigeonpea (*Cajanus cajan* (L.) Millspaugh) were selected for the investigation which was

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° 35' north and longitude 83° 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 10 X 10 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 2:

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.

Amylase: Amylase activity was estimated by the method of Filner and Varner (1967) as followed by Kapoor and Sachar (1979).

One g of fresh leaves were homogenised in 15 ml of 50 mM phosphate buffer pH 6.5. The homogenate was centrifuged at 10,000 x g for 20 min. The supernatant containing crude enzyme extract was collected and used for measuring the starch hydrolyzing activity. The change in absorbance of 0.01 at 610 nm/min/mg protein represented one enzyme unit. The protein content of the enzyme extract was determined by the method Lowry *et al.*, (1951).

Carbohydrate fractions

Starch and Total soluble sugars: The total soluble sugars and starch were estimated according to the method of Mc Cready *et al.* (1950) as modified by Clegg (1956). Soluble sugars were separated by alcohol extraction and the residue containing starch was brought into solution with perchloric acid.

Reducing sugars: Total reducing sugars were estimated according to the phenolsulphuric acid method of Dubois *et al.* (1956) as followed by Smyth and Dugger (1980). The absorbance of the characteristic yellow colour was measured at 490 nm using ECIL's Junior Spectrophotometer GSB 66B. Standard reference curve was prepared using Analar glucose.

Non-Reducing sugars: The reducing sugar content subtracted from the total soluble sugars was considered as non-reducing sugars.

RESULTS

Amylase: Genotypic variation of the amylase activity of the 10th leaf of the pigeonpea during its crop growth was shown in figures 1a, b. On per leaf as well on per unit fresh weight bases amylase activity tend to increase from the vegetative to flowering phase followed by a sharp decline at the seed maturation phase in all the genotypes. Among the different groups, the long duration genotypes exhibited greater values than the

medium and short duration genotypes in both the expressions. Among the genotypes the ST1 of long duration and the T21 of medium duration genotypes expressed greater and lower values respectively at all phases of crop growth.

Carbohydrate fractions

Starch

Changes in starch content of the whole plant and its different parts of the 12 pigeonpea genotypes during the crop growth were shown in figures 2a, b, 3a, b, 4a, b, 5a, b. A gradual decrease in the starch content of the whole plant and its different parts from the vegetative to seed maturation phase was evident in all the genotypes. However, on per organ basis, the short duration genotypes recorded lower values than medium and long duration genotypes (Figs. 2a, 3a, 4a, 5a). The starch content of the whole plant recorded the maximum values in the genotype PDM1 (Fig 2a). Of all the genotypes the starch content of total leaves was maximum in the Pusa agheti (Fig. 3a). Starch content of the stem and root was maximum in the PDM1 (Figs. 4a and 5a). On unit dry weight basis an equal and continuous decrease of starch content from the vegetative to the seed maturation phase was observed in the whole plant and other parts of the genotypes studied (Figs. 4.15b, 4.16b, 4.17b, 4.18b). Among the different parts, leaves recorded greater values of starch content followed by stem and root.

Total soluble sugars: Genotypic variation in the total soluble sugar content of the whole plant, leaves, stem and root of pigeonpea was shown in figures 6a, b; 7a, b; 8a, b and 9a, b. On per plant/part basis, an increase of total soluble sugars content from the vegetative to the flowering phase was observed. It was followed by a decrease at the seed maturation phase (Figs. 6a, 7a, 8a, 9a). Of all the genotypes, the PDM1 of long duration, exhibited greater values of soluble sugar content in the whole plant. Among the different parts studied, stems showed more soluble sugar content followed by leaves and roots.

Further, the medium and long duration genotypes recorded greater values than the short duration genotypes.

On unit dry weight basis also a similar trend was observed in the whole plant and its parts. In contrast, the total leaves (Fig. 7b) recorded higher soluble sugar content followed by the stems (Fig. 8b) and roots (Fig. 9b). The PDM1 of long duration genotypes recorded maximum values of soluble sugar content in the whole plant, total leaves, stem and root among all the genotypes studied.

Reducing sugars: The figures 10a, b; 11a, b; 12a, b and 13a, b represents the reducing sugar content of the whole plants, total leaves, stems and roots of different genotypes during the crop growth of pigeonpea. On per part basis as well as on unit dry weight basis, reducing sugars closely exhibited a trend similar to the total soluble sugars. On per part basis the stems showed higher values of reducing sugar content followed by the leaves and roots. On unit dry weight basis, the total leaves recorded more values than the stems and roots. Among the genotypes and on per part basis the medium and long duration genotypes expressed higher values than the short duration genotypes. Out of all the genotypes studied the PDM1 recorded the maximum values of reducing sugar content in the whole plant and its different parts on both the expressions.

Non-reducing sugars

The non-reducing sugar content of the whole plants of pigeonpea genotypes showed a gradual decrease from the vegetative phase to the seed maturation phase on both the expressions. On per plant basis, all the long duration genotypes and the PDM1 in particular exhibited greater values than the rest of the genotypes (Fig.14a). However, on per unit dry weight basis genotypic variation was less prominent. However, the PDM1 always recorded greater values at all phase of crop growth (Fig.14b).

The non-reducing sugar content per plant total leaves exhibited a gradual decrease

from the vegetative to the seed maturation phase in all the genotypes (Fig.15a). The genotype Pusa agheti recorded higher values of the non-reducing sugar content among the leaves of all the genotypes at all the growth phases of pigeonpea crop. On unit dry weight basis it was observed that there was a slight increase of non-reducing sugar content of leaves at the flowering phase followed by a slight decrease towards the seed maturation phase in all the genotypes studied (Fig.15b). The non-reducing sugar content of the stems showed a slow increase in all the genotypes except in the case of PDM1 and LRG30 of the long duration genotypes, which exhibited a slight decrease towards the harvest (Fig.16a). Among the genotypes studied, the stem of PDM1 recorded greater values of non-reducing sugar content. On unit dry weight basis, the non-reducing sugar content showed a gradual decrease in the stems at the seed maturation phase in all the genotypes selected for study (Fig.16b). At the vegetative phase the values among the genotypes exhibited a range from 24.00 to 29.41 mg/g dry wt. At this phase the PDM1 of long duration genotypes and the ICPL87 of short duration genotypes recorded the highest and the lowest values respectively. The non-reducing sugar content of roots on per part basis showed a slight decreasing trend from the vegetative to the seed maturation phase in all the genotypes studied (Fig.17a,b). The long duration genotypes always recorded greater values. The genotype PDM1 expressed the maximum values than the medium and short duration genotypes. On unit dry weight basis the non-reducing sugar content of the roots showed a decline until the seed maturation phase in all the genotypes. Among the different parts studied the stems showed greater values than the leaves and roots on per part basis and the leaves exhibited greater values than the stems and roots on per unit dry weight basis.

Table 2

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

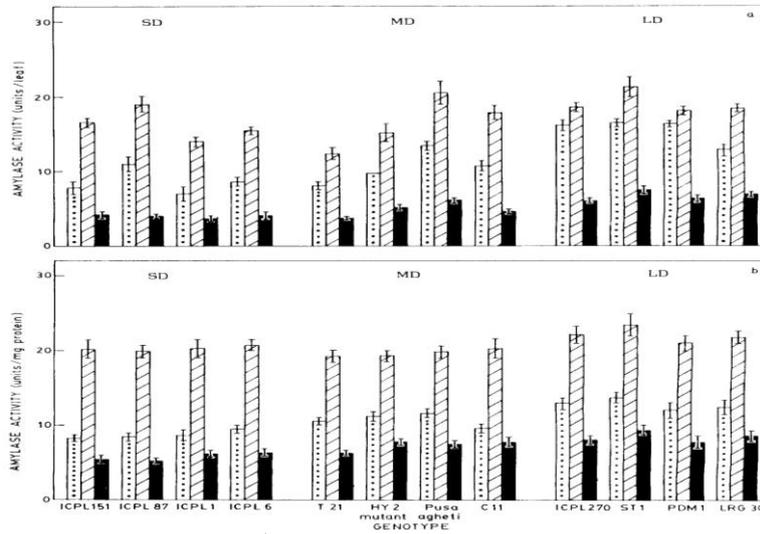


Fig.1: Amylase activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

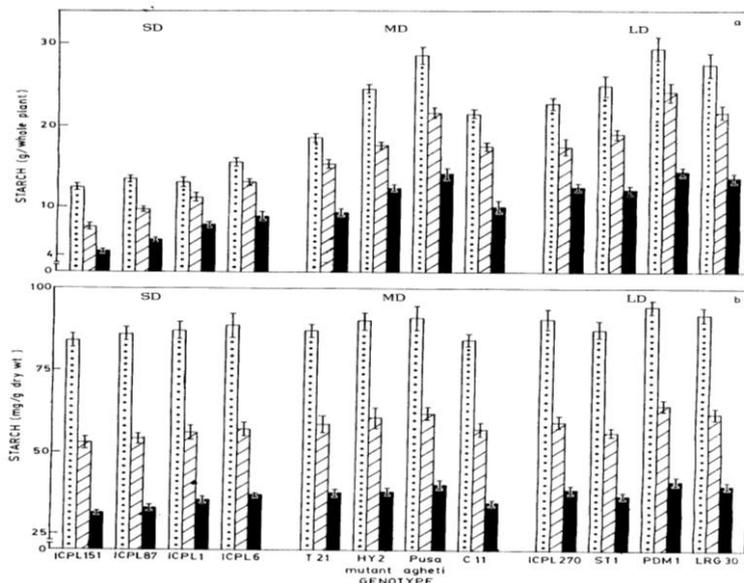


Fig.2: Starch content of the whole plants during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

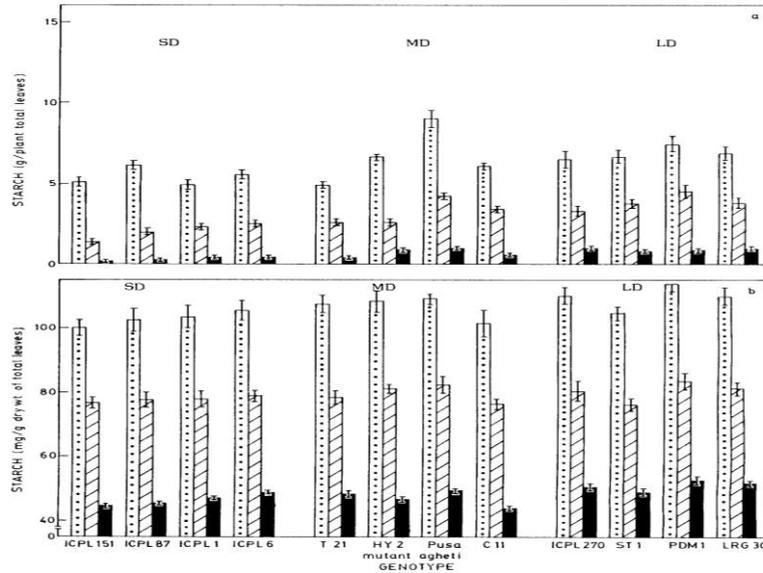


Fig.3: Starch content of the plant total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase, ▨ Flowering phase, ■ Seed maturation phase

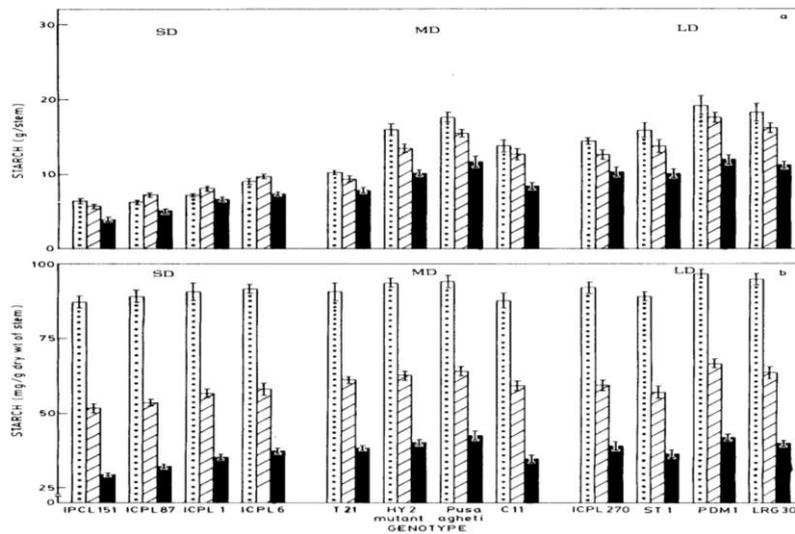


Fig. 4: Starch content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase, ▨ Flowering phase, ■ Seed maturation phase

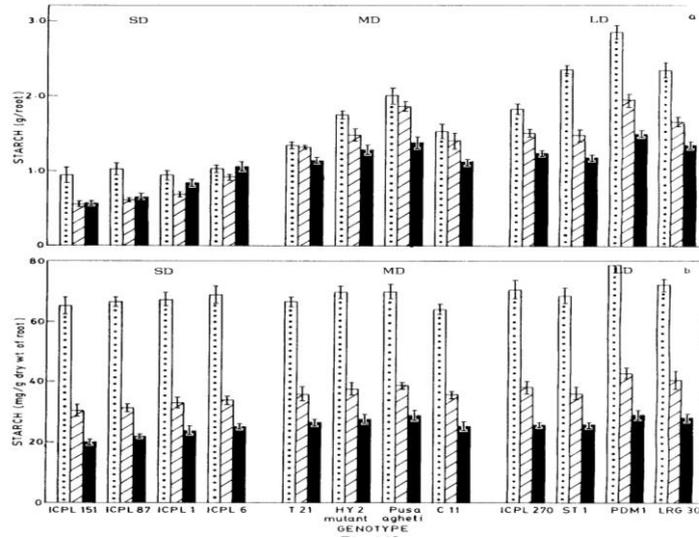


Fig. 5: Starch content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase,
 Flowering phase,
 Seed maturation phase

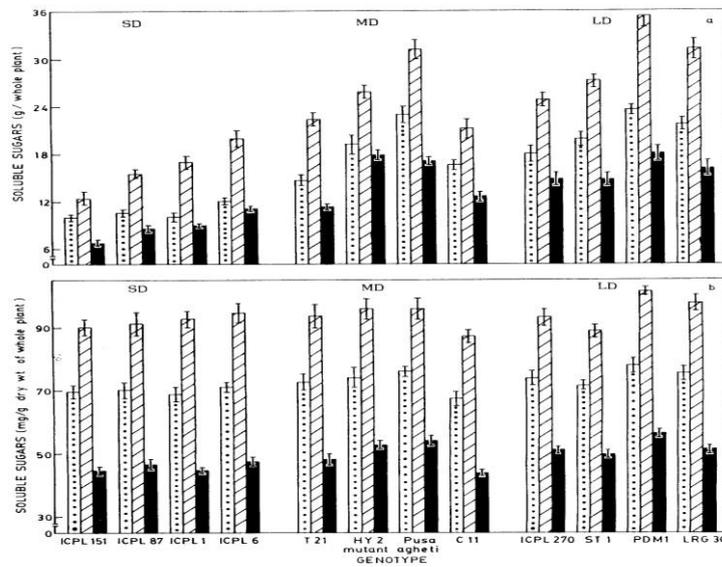


Fig.6: Soluble sugar content of the whole plants during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase,
 Flowering phase,
 Seed maturation phase

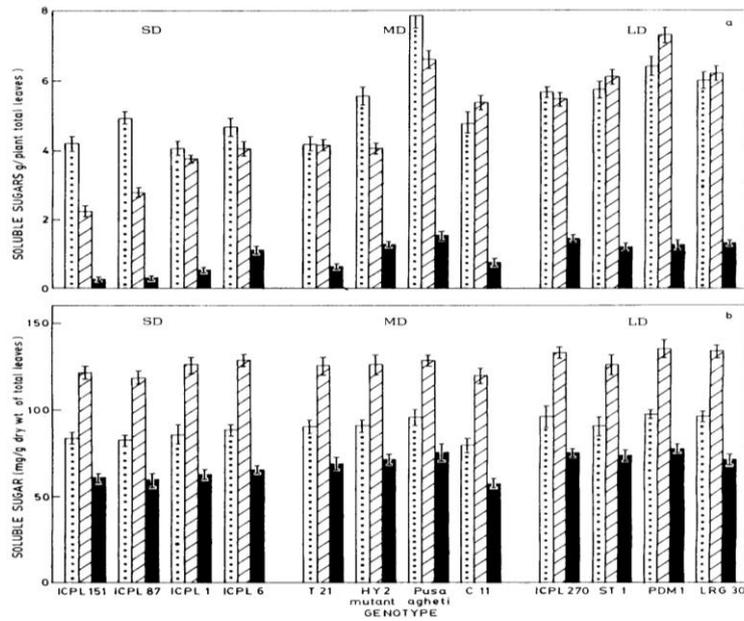


Fig.7: Soluble sugar content of the plant total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase ; ▨ Flowering phase ; ■ Seed maturation phase

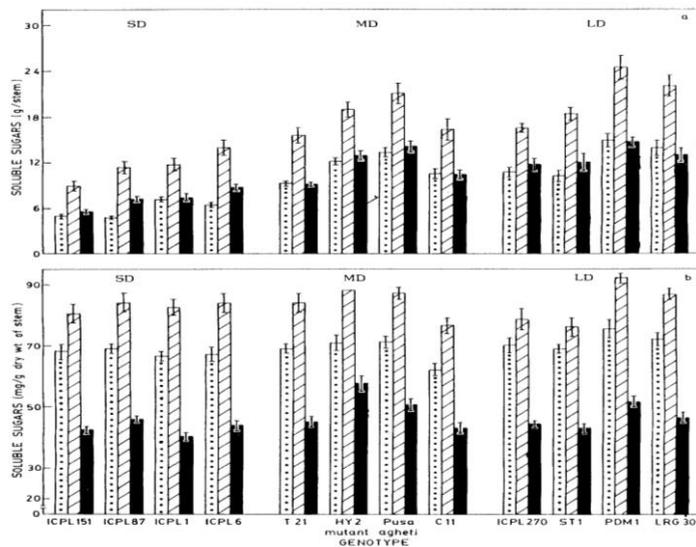


Fig.8: Soluble sugar content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase ; ▨ Flowering phase ; ■ Seed maturation phase

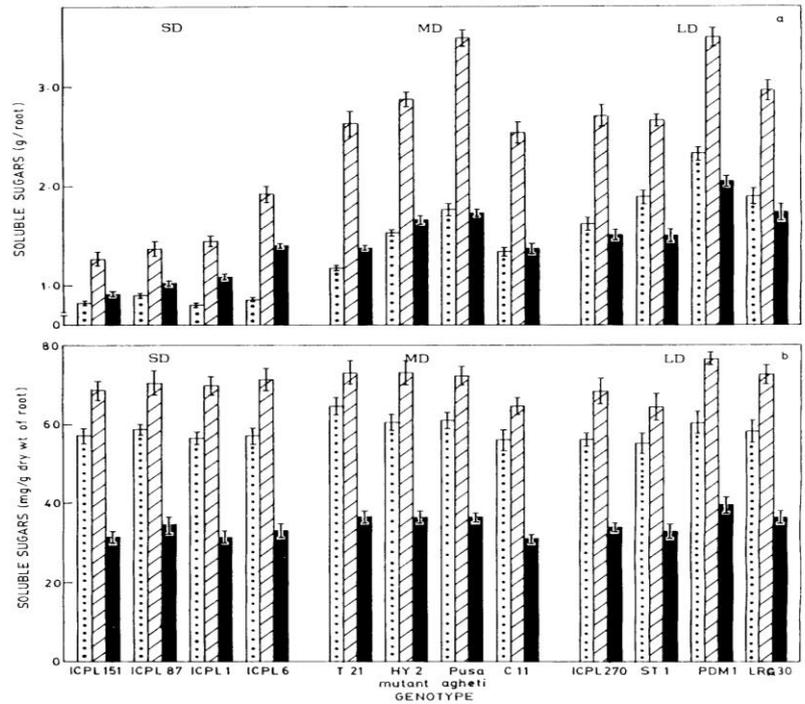


Fig.9: Soluble sugar content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase, ▨ Flowering phase, ■ Seed maturation phase

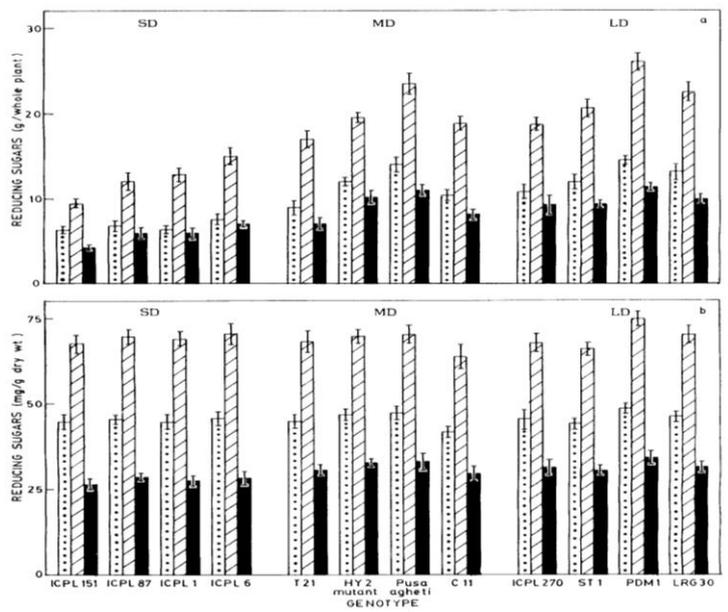


Fig.10: Reducing sugar content of the whole plants during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase, ▨ Flowering phase, ■ Seed maturation phase

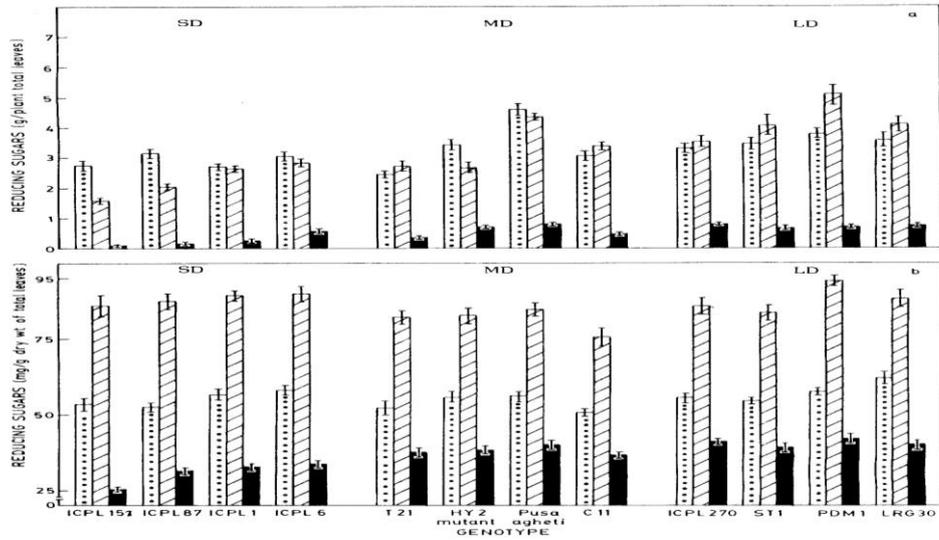


Fig.11: Reducing sugar content of the total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase, ▨ Flowering phase, ■ Seed maturation phase

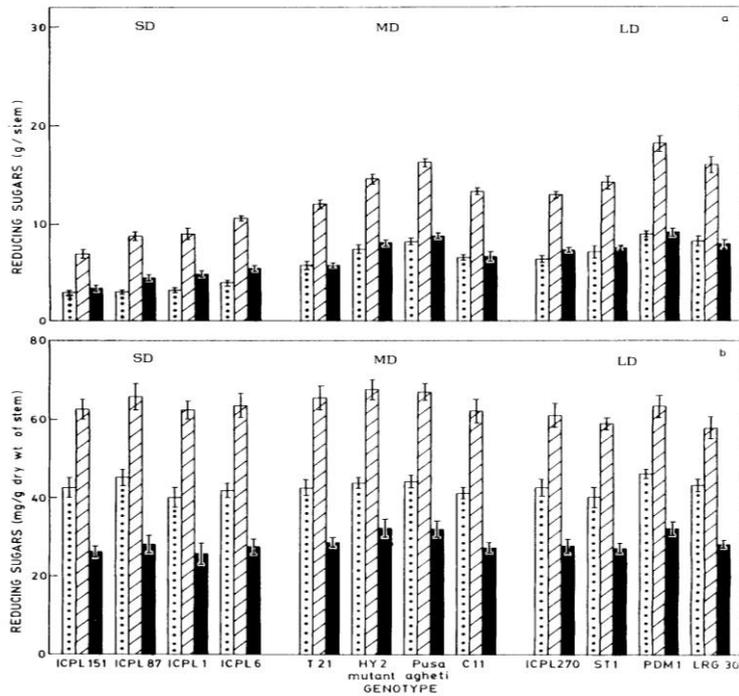


Fig.12: Reducing sugar content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase, ▨ Flowering phase, ■ Seed maturation phase

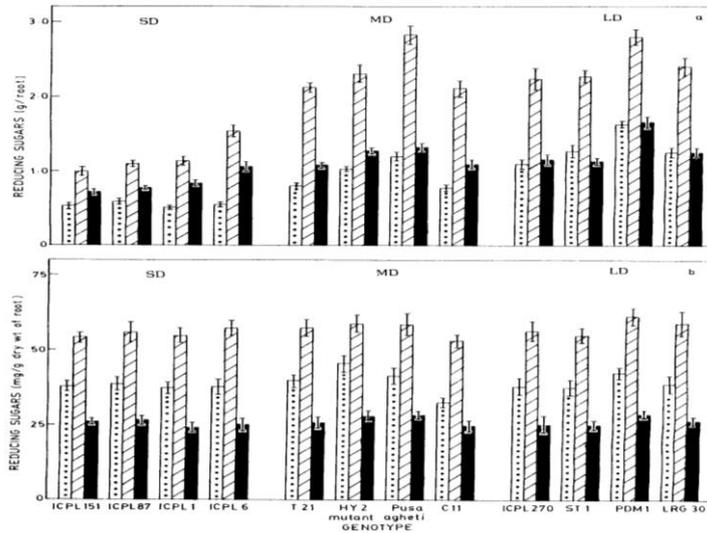


Fig.13: Reducing sugar content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase,
 Flowering phase,
 Seed maturation phase

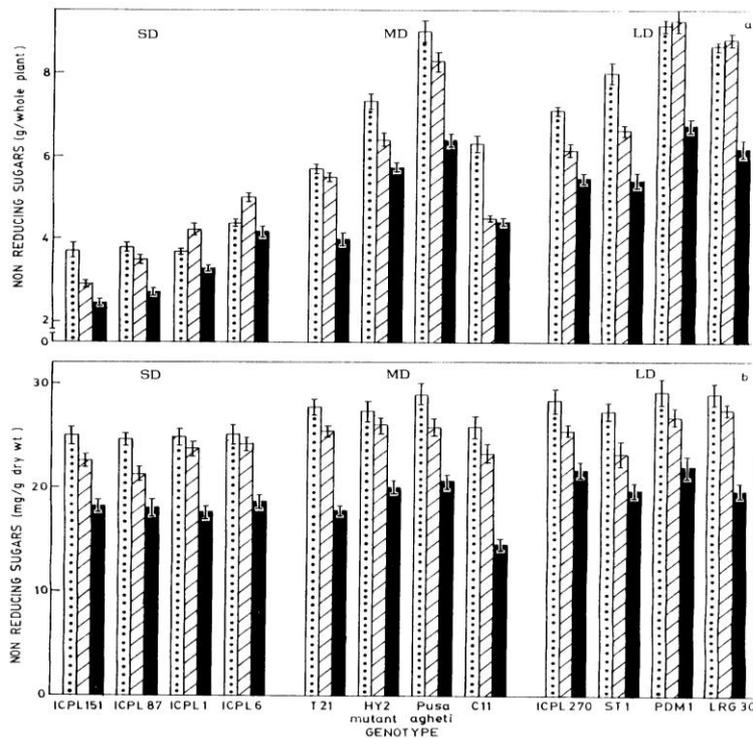


Fig.14: Non-Reducing sugar content of the whole plants during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase,
 Flowering phase,
 Seed maturation phase

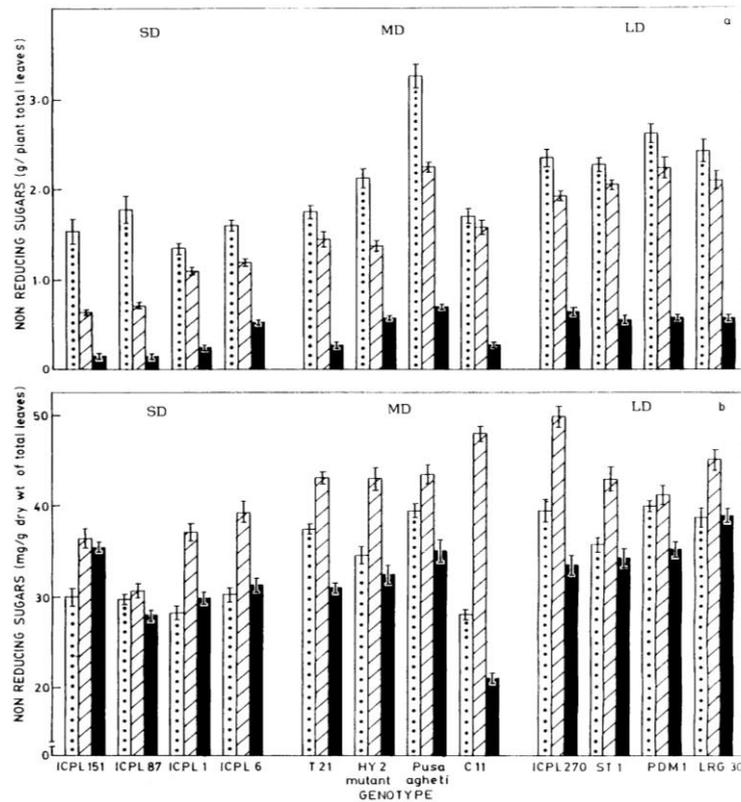


Fig.15:Non-Reducing sugar content of the total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

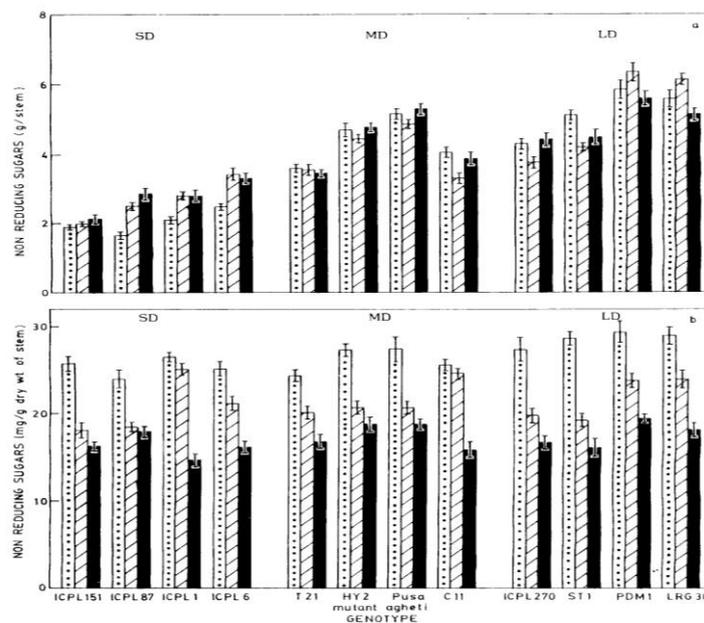


Fig.16:Non-Reducing sugar content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

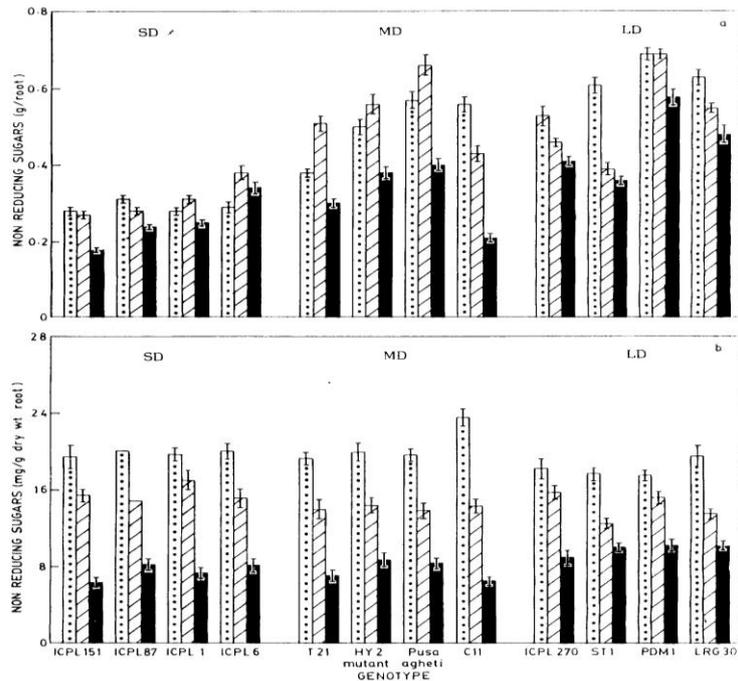


Fig.17:Non-Reducing sugar content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

☐ Vegetative phase ; ▨ Flowering phase ; ■ Seed maturation phase

DISCUSSION

Amylase activity of all the pigeonpea genotypes increased from the vegetative to the flowering phase followed by a rapid decrease at the seed maturation phase (Fig. 1a, b). The higher amylase activity during the vegetative and flowering phases results in increased soluble sugar and reducing sugar contents during those phases of crop growth (Figs. 6a, b to 13a, b). Crop yield may be limited by some physiological process such as photosynthesis and carbohydrate partitioning. Pigeonpea, when compared to other legumes possess massive stems and abundant foliage which constitute 80 per cent of plant dry weight at the time of harvest. Therefore, a substantial portion of carbohydrates and other substances could be retained in them. The amount of starch and non-reducing sugar content in whole plant and its different parts decreased with increasing crop age of pigeonpea. The short duration genotypes recorded lower values than the medium and long duration genotypes (Figs.2a, b; 3a, b; 4a, b; 5a, b;14a, b; 15a,

b; 16a, b; 17a, b). High starch content of stems and roots of the long duration genotypes throughout the flowering and seed maturation phases was associated with high dry matter accumulation but with low yields in these genotypes. This may be due to the inability of proper mobilization and distribution of starch and non-reducing sugars within the growing crop plant. In addition, low number of pods in these genotypes perhaps did not provide the required “sink” demand for storage of the photosynthates being produced (Adams *et al.*, 1978). The total soluble sugars and reducing sugars exhibited an increase from vegetative to flowering phase followed by a decrease at the seed maturation phase of the whole plant and its different parts of pigeonpea genotypes studies (Figs. 6a, b to 13a, b). The decrease in all the carbohydrate fractions after the flowering phase towards the seed maturation phase may be related to the cessation of dry matter accumulation after the flowering phase. The decrease in carbohydrate fractions with seed maturation was recorded in sorghum

(Sinha *et al.*, 1990) and in meadowfoam lines (Fiez *et al.*, 1991). The data on pigeonpea indicated that the growth of the long duration genotypes showed a positive relationship with photosynthetic rate, respiration and dry matter accumulation. Among the genotypes of the long duration, PDM1 exhibited higher photosynthetic rate, leaf area, biomass production, carbohydrate content and the consequent high yield. Among the medium duration genotypes, higher photosynthetic rates were associated with higher yields in T21. Although the Pusa agheti exhibited higher photosynthetic rates, it exhibited lower seed yields indicating the poor partitioning of photosynthates to sink region. Among the short duration genotypes greater photosynthetic rates were associated with higher yields and lower biomass production. The genotypes such as the ICPL87 possessing greater photosynthetic rates, low photorespiration and with better nonstructural carbohydrate partitioning produced higher yields in pigeonpea.

CONCLUSION

The amylase activity of the 10th leaf increased from the vegetative phase to the flowering phase followed by a decrease at the seed maturation phase. The long duration genotypes recorded greater amylase activity than the medium and short duration genotypes. Some of the studies were also carried out on the whole plants and their different parts of pigeonpea genotypes. A gradual decrease in starch and non-reducing sugar content of the whole plants and their different parts were observed in all the genotypes. The soluble sugar and reducing sugar contents showed an increase from the vegetative to the flowering phase followed by a decrease at the seed maturation phase. The short duration genotypes recorded lower values of carbohydrate fractions than the medium and long duration genotypes on per part basis. However, on unit dry weight basis the differences were less prominent.

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