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RESEARCH ARTICLE

PHOTOSYNTHETIC ENZYME ACTIVITIES IN LEAVES OF *CAJANUS CAJAN* (L.) AT THREE DIFFERENT PHASES OF CROP GROWTH

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ABSTRACT

Pigeonpea (Cajanus cajan (L.) Millspaugh) genotypes (twelve), of 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 investigation on different enzymes like Malate dehydrogenase, Glycolate oxidase, Total chlorophyll content, Photosynthetic rate, Ribulose bisphosphate carboxylase activity and Phosphenolpyruvate carboxylase of the 10th leaf at three selected phases of crop growth i.e. vegetative, flowering and seed maturation phase. The malate dehydrogenase activity recorded an increase from the vegetative to flowering phase followed by a decrease at the seed maturation phase. In all the genotypes glycolate oxidase activity of the 10th leaf exhibited an increase at the flowering phase followed by a decrease at the seed maturation phase. Total chlorophyll content was gradually decreased with age in all the genotypes. The photosynthetic rate was decreased from vegetative to seed maturation phase in all the genotypes. The greatest fixation rate was observed in the ICPL87 of short duration genotypes and the lowest rate was observed in the ST1 of long duration genotypes. The ribulose bisphosphate carboxylase activity was decreased from vegetative phase to the seed maturation phase. Among the genotypes the ICPL87 of short duration and the ST1 of long duration genotypes recorded the maximum and minimum values respectively at the vegetative phase of crop growth. Phosphoenolpyruvate carboxylase activity of the genotypes increased from vegetative to flowering phase followed by a decline on the seed maturation phase. The enzyme activity recorded greater values on reaching the flowering phase.

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INTRODUCTION

The dehydrogenation of malate is well-known as one of the energy yielding steps in krebs cycle and therefore considered important in studies on respiration (Chapman and Graham, 1974; Crookston *et al*., 1974). Genotypic differences in malate dehydrogenase activity is positively correlated with harvest index in dry beans (Peet *et al*., 1977). There is considerable evidence that glycolate oxidase (GAO) is the key enzyme in photorespiration (Jackson and Volk, 1970; Zelitch, 1973; Crookston *et al*., 1974). In addition to varietal differences, glycolate oxidase activity differed significantly with the stage of crop growth. It was reported that glycolate oxidase activity was highest at first flowering and lowest at early pod setting stage in the dry bean varieties (Peet *et al*., 1977). Sairam and Srivastava (1984) suggested that low yielding genotypes had high photorespiratory activity when compared to high yielding genotypes of sunflower.

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Chakrabarti and Saha (1983) also reported that high yielding genotypes photorespired less than low yielding genotypes of rice. The physiological basis for yield differences between low and high yielding soyabean genotypes in relation to leaf chlorophyll and other characters was studied by Singh *et al*. (1985). They suggested that specific leaf weight is most promising characteristic feature in soybeans for selection in improving grain yield. Significant genotypic differences in apparent photosynthesis were observed at vegetative, pod forming and seed development stages of early and late maturing genotypes of soybeans (Kokubun and Watanable, 1983). They also found that apparent photosynthesis tend to be correlated positively with leaf area, specific leaf weight and chlorophyll content both at vegetative and pod forming stages. Genotypic differences in canopy apparent photosynthesis was studied by Wells *et al*. (1982) and found high and significant correlation between canopy apparent photosynthesis and seed yield in soyabean. Murata (1961) and Stoy (1965) have suggested that leaf photosynthetic rate can be very important in determining growth rates. Crisswell and Shibles (1971) found that net photosynthesis in oat leaves was related to

specific leaf weight. Association between photosynthetic rate and leaf thickness was reported for sugarcane (Irvine, 1967, 1975). Delaney and Dobrenz (1974) obtained similar results with alfalfa. However, in cotton, leaf thickness was negatively correlated with net photosynthetic rate (EI-Sharkawy and Hesketh, 1965). Murthy and Singh (1979) studied genetic variations in relation to photosynthetic rate, chlorophyll content and ribulose bisphosphate carboxylase activity in wheat varieties. Chlorophyll and ribulose bisphosphate carboxylase activity in leaves of different wheat genotypes increased with advancing age while apparent photosynthesis decreased. They have also demonstrated that photosynthetic rates were associated with specific leaf characters. Heichel (1971) found that photosynthetic rates and stomatal frequencies were inversely related in two maize genotypes. It was also found in maize that the photosynthetic rates were lower in ageing leaves and in leaves situated nearer to the roots than in the leaves which were away from them. Ribulose bisphosphate carboxylase is a key enzyme in carbon fixation and perhaps, the most important biochemical factor controlling CO_2 uptake (Wareing, 1968; Crookston *et al*., 1974; Peet *et al*., 1977; Devlin and Witham, 1986). It has been suggested that differences in photosynthesis can be accounted for by differences in ribulose bisphosphate carboxylase activity of leaves (Bjorkman, 1968; Wareing *et al*., 1968; Bowes *et al*., 1972). A linear relationship was observed between net photosynthesis and ribulose bisphosphate carboxylase activity in wheat genotypes (Massacci *et al*., 1986).

A comparison between hexaploid and decaploid tall fescue indicated that both assimilate rates and the specific activity of ribulose bisphosphate carboxylase increased with ploidy. It has also been concluded that differences in ribulose bisphosphate carboxylase is associated with ploidy in wheat genotypes (Randall *et al*., 1977; Evan and Seeman, 1984). Photosynthetic rates and ribulose bisphosphate carboxylase activities were highest at early pod set stage, which was the only developmental stage where they are significantly correlated with biological yields in dry bean genotypes (Peet *et al*., 1977). It was also reported that high yielding genotypes has considerable amount of ribulose bisphosphate carboxylase activities and higher rates of CO_2 fixation at seed filling stage when compared to low yielding genotypes of sunflower (Srivastava and Sairam, 1983). Keeping in this view twelve genotypes of pigeonpea were taken to analyze the photosynyhetic activity of the 10^{th} leaf at three different phases of crop growth.

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° 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.

Malate Dehydrogenase (EC. 1.1.1.37)

The enzyme extract for the study of malate dehydrogenase activity was prepared according to the method followed by Crookston *et al*., (1974) and its assay was carried out by the method of Heddley and Stoddart (1971). Ten leaf discs (1 cm diameter) from the respective genotypes were homogenized in 5 ml of cold extraction mixture (0.04 M tris, PH 7.8; 0.01 M $MgCl_2$, 0.25 mM EDTA, 5.0 mM glutathione). The homogenate was centrifuged at 20,000 x g for 10 minutes and the resulting supernatant was used as enzyme extract. The assay mixture consisted of 1 ml of 200 µ moles of oxaloacetate, 1 ml of 0.75 µ moles of NADH and 0.9 ml of 0.1 M phosphate buffer pH 7.5. To this mixture 0.1 ml enzyme extract was added and the change in absorbance was followed for a period of 3 minutes at 340 nm. The readings were taken on schimadzu (UV-240) Spectrophotometer.

Glycolate oxidase (E.C.1.1.3.1)

The enzyme extract of glycolate oxidase was prepared according to the method followed by Crookston *et al*., (1974) and the assay was carried out by the method of Heddley and Stoddart (1971). The assay mixture consisted of 2.5 ml of 0.1 M KH_2PO_4 buffer (pH 7.4), 0.3 ml of 0.05 M phenylhydrazine HCl and 0.05 ml of 0.1 M glycolate. To this, 0.5 ml of enzyme extract was added and the production of phenylhydrazone was measured at 324 nm on Milton Roy Spectronic 1201UV-spectrophotometer.

Total chlorophyll content

Chlorophyll content was determined by the method of Harborne (1973). Two hundred milligrams of fresh leaf (10^{th} leaf) material of all the 12 genotypes were ground separately in a mortar using 80% acetone in the presence of a small quantity of acid washed sand and a pinch of calcium carbonate. The completely homogenized material was centrifuged and the supernatant was diluted suitably to a

known volume with 80% acetone without exposing to light. The absorbance of the solution was read at two wavelengths 645 nm and 663 nm using 150-20 UV-VIS-Spectrophotometer (Hitachi, Japan). The amount of total chlorophyll content was calculated as mg of chlorophyll content per gram of leaf tissue according to the following formula:

$$\frac{(20.2 \times A_{645} + 8.02 \times A_{663})}{1000 \times W} \times V$$

Where A represents the absorbance of the chlorophyll extract at the specific indicated wavelength; V, the final volume of the 80% acetone chlorophyll extract and W the fresh weight in grams of the tissue.

Photosynthetic rate

Photosynthetic rates of the 10th leaf of different pigeonpea genotypes were determined by feeding leaf discs with $NaH^{14}CO_3$. The $^{14}CO_2$ fixation rate was determined by the method of Jones and Osmond (1973) as modified by Rao and Ghildiyal (1985). Four leaf discs (1 cm diameter) were placed in petridishes (5.0 cm diameter) having 5.7 ml water. Feeding was initiated by adding 0.3 ml of aqueous medium containing 5.0 µci $NaH^{14}CO_3$ (1.0 mci/m mole) to each petri dish. Feeding period maintained was 30 minutes kept in sunlight. After 30 minutes, 6 ml of 4 N HCl was added to stop the reaction. Insoluble material was hydrolysed in 2 N HCl at 80 ^oC for 2 hours. Final volume was adjusted to 25 ml. One ml of this solution was placed in scintillation vial dried at 65 ^oC and counted for ^{14}C activity in a liquid scintillation counter (ECIL LSS 34). The counts obtained represent total ^{14}C incorporation into soluble and insoluble fraction and provides a measure of the rate of photosynthesis.

Ribulose bisphosphate carboxylase activity (EC 4.1.1.39) and Phosphenolpyruvate carboxylase (EC 4.1.1.31)

The one ml of assay mixture of Ribulose bisphosphate carboxylase consists of 150 mM Tricine (pH 8.6), 50 mM $MgCl_2$, 40 mM 2-mercaptoethanol, 6.4 mM ribulose bisphosphate (sigma), 10 mM $NaH\ ^{14}CO_3$ (5µci/m mole). After a period of temperature equilibrium for 10 minutes the reaction was initiated by the addition of 0.25 ml activated enzyme extract to 1.0 ml of assay mixture and stopped it after 2 minutes at 30 ^oC with the addition of 0.5 ml of 0.6 N HCl saturated with 2,4 dinitrophenylhydrazine. The aliquots were placed in scintillation vials and after processing with the radioactivity was determined by automatic liquid scintillation system (ECIL LSS 34). The values were expressed in cpm/mg chl and cpm/leaf.

Assay of Phosphenolpyruvate carboxylase

One ml of assay mixture of Phosphenolpyruvate carboxylase contained 150 mM Tricine (pH 8.6), 50 mM $MgCl_2$, 40 mM 2-mercaptoethanol, 5 mM phosphoenolpyruvate (sigma), 5 mM sodium glutamate and 10 mM $NaH^{14}CO_3$. The reaction was initiated by adding 0.25 ml of activated enzyme extract to 1.0 ml of assay mixture. The reaction was stopped by the addition of 0.5 ml of 6 N HCl saturated with 2,4 dinitrophenylhydrazine. The aliquots were placed in scintillation vials and after proper processing the radioactivity was determined by automatic liquid scintillation system (ECIL LSS 34). The values were expressed in cpm/mg/chl. and cpm/leaf.

RESULTS

Malate dehydrogenase activity: Malate dehydrogenase is an important respiratory enzyme. The activity of the malate dehydrogenase of the 10th leaf of different pigeonpea genotypes was presented in figure 1. The activity of the enzyme in the leaf varied in relation to the crop growth. In all the genotypes, the malate dehydrogenase activity recorded an increase from the vegetative to flowering phase followed by a decrease at the seed maturation phase. The activity was greatest during flowering phase, which showed a range of variation from 401 to 480 µ moles X $10^{-2}/dm^2/h$ in the genotypes studied. The ST1 recorded the maximum and the ICPL87 the minimum malate dehydrogenase activities when compared to the rest of the genotypes at all the three stages of the growth. Comparatively long duration genotypes exhibited greater values than medium and short duration genotypes.

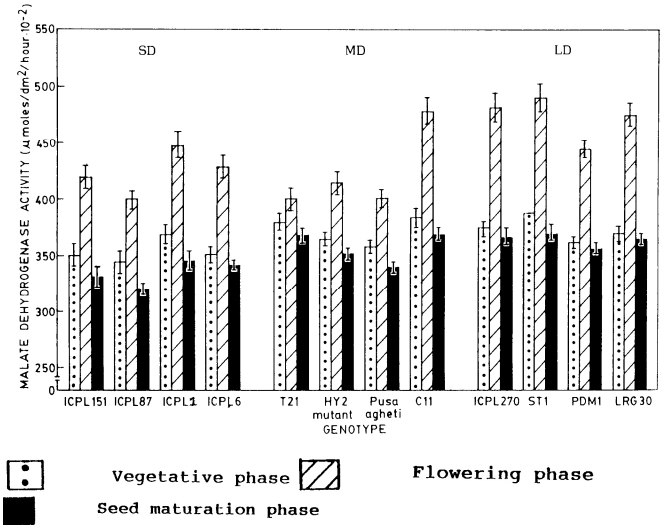

Fig. 1. Malate dehydrogenase activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Glycolate oxidase activity: The activity of the photorespiratory enzyme, glycolate oxidase of the 10th leaf exhibited an increase at the flowering phase followed by a decrease at the seed maturation phase in all the genotypes studied (Fig-2). At all the phases of crop growth the maximum activity of the enzyme was exhibited by the ST1 of long duration genotypes and the minimum value were exhibited by the ICPL87 of short duration genotypes. Comparatively the long duration genotypes exhibited higher values than the medium and short duration genotypes.

Total chlorophyll content: Changes in the total chlorophyll content of the 10th leaf of all the 12 genotypes during crop growth was presented in figures 3a, b. There was a gradual decrease in total chlorophyll content with age in all the genotypes. On per part as well as on unit fresh weight basis the ICPL87 of short duration recorded the greatest and the ST1 of long duration the lowest quantities of chlorophyll content throughout the crop growth period.

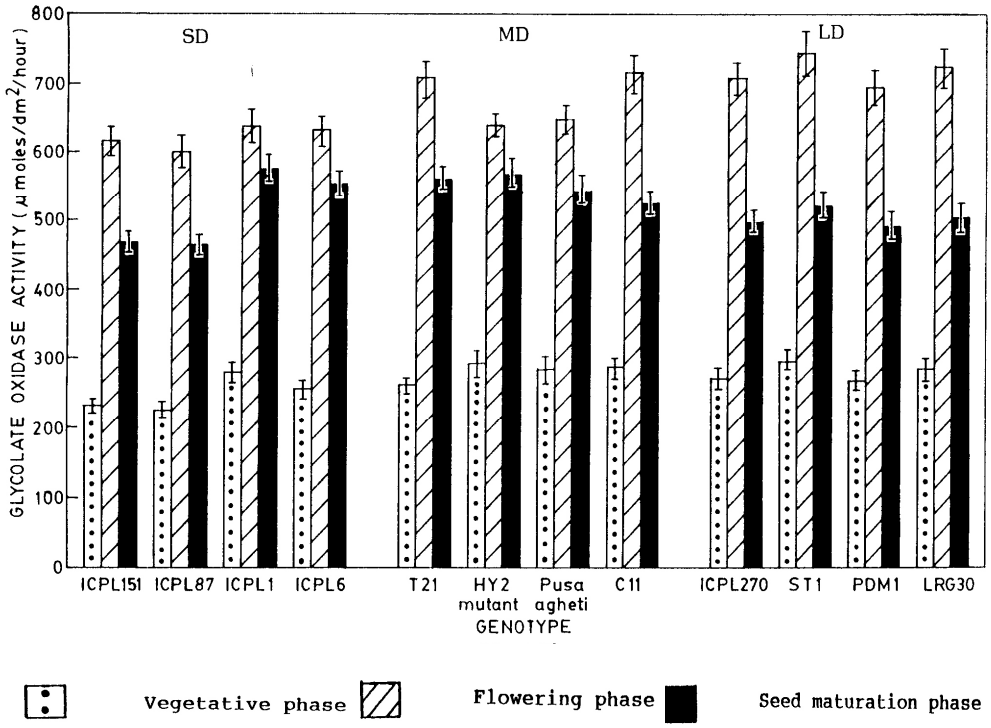

Fig. 2. Glycolate oxidase activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

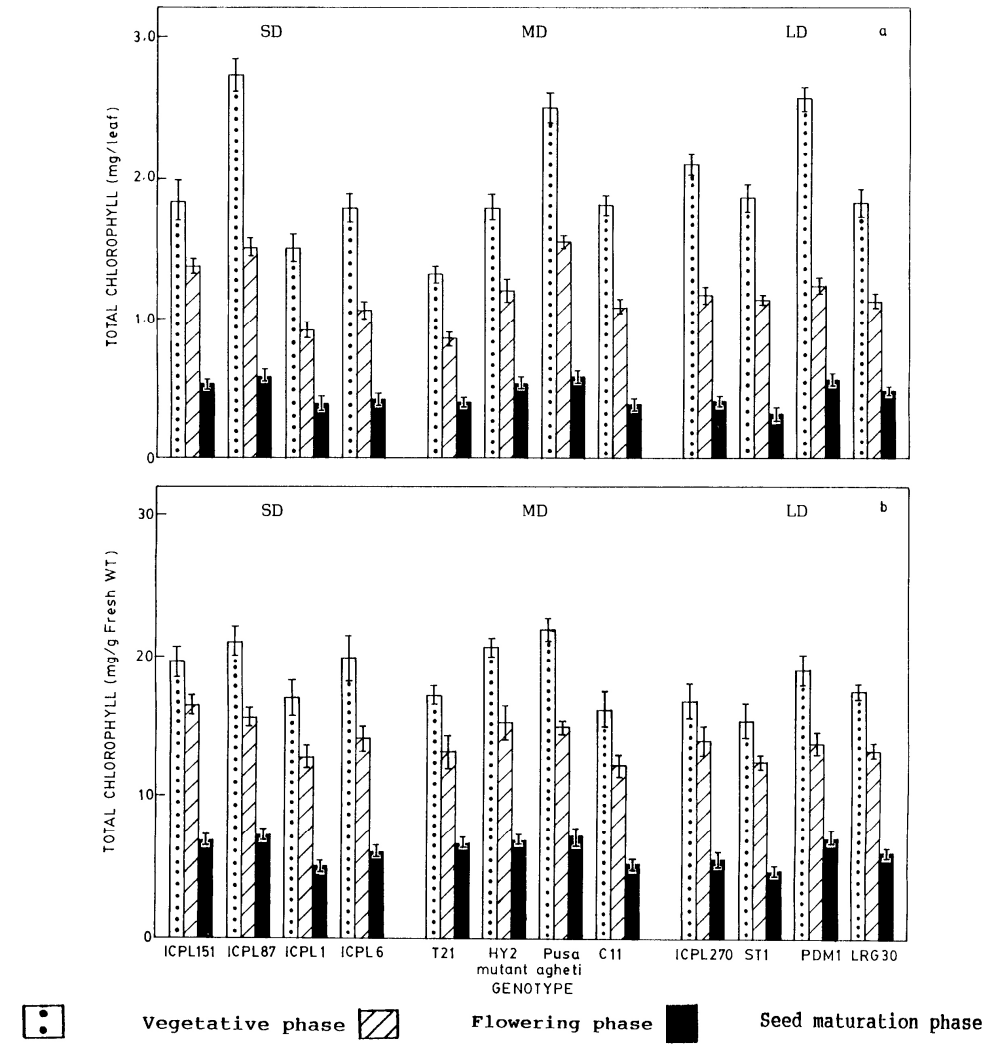

Fig. 3. Total chlorophyll content of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

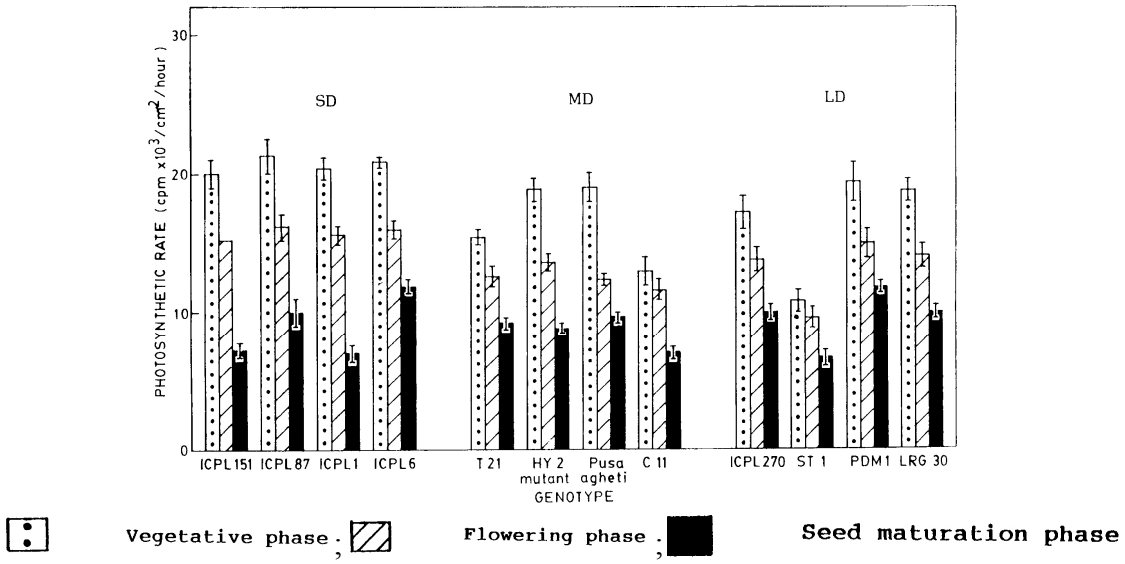

Fig. 4. Photosynthetic rate ($^{14}CO_2$ fixation rate) of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

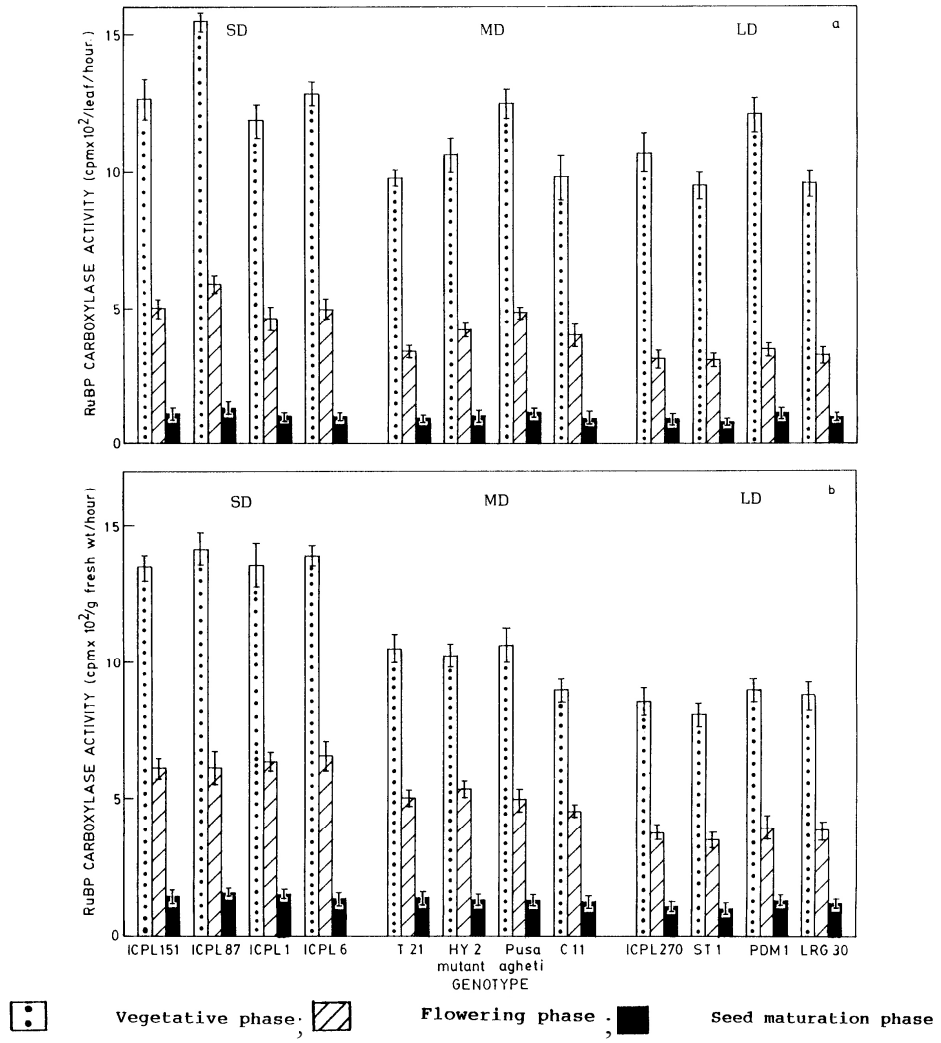

Fig. 5. Ribulose bisphosphate carboxylase (Rubisco) activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Photosynthetic rate: The photosynthetic rate as measured through the fixation of radioactive $^{14}CO_2$ by the 10th leaf of all the 12 genotypes was shown in Figure 4. The photosynthetic rate was decreased from vegetative to seed maturation phase in all the genotypes.

Among all the genotypes studied, the greatest fixation rate of 21.43 x 10^3 cpm/cm^2/h was observed in the ICPL87 of short duration genotypes and the lowest rate of 10.43 x 10^3 cpm/cm^2/h was observed in the ST1 of long duration genotypes.

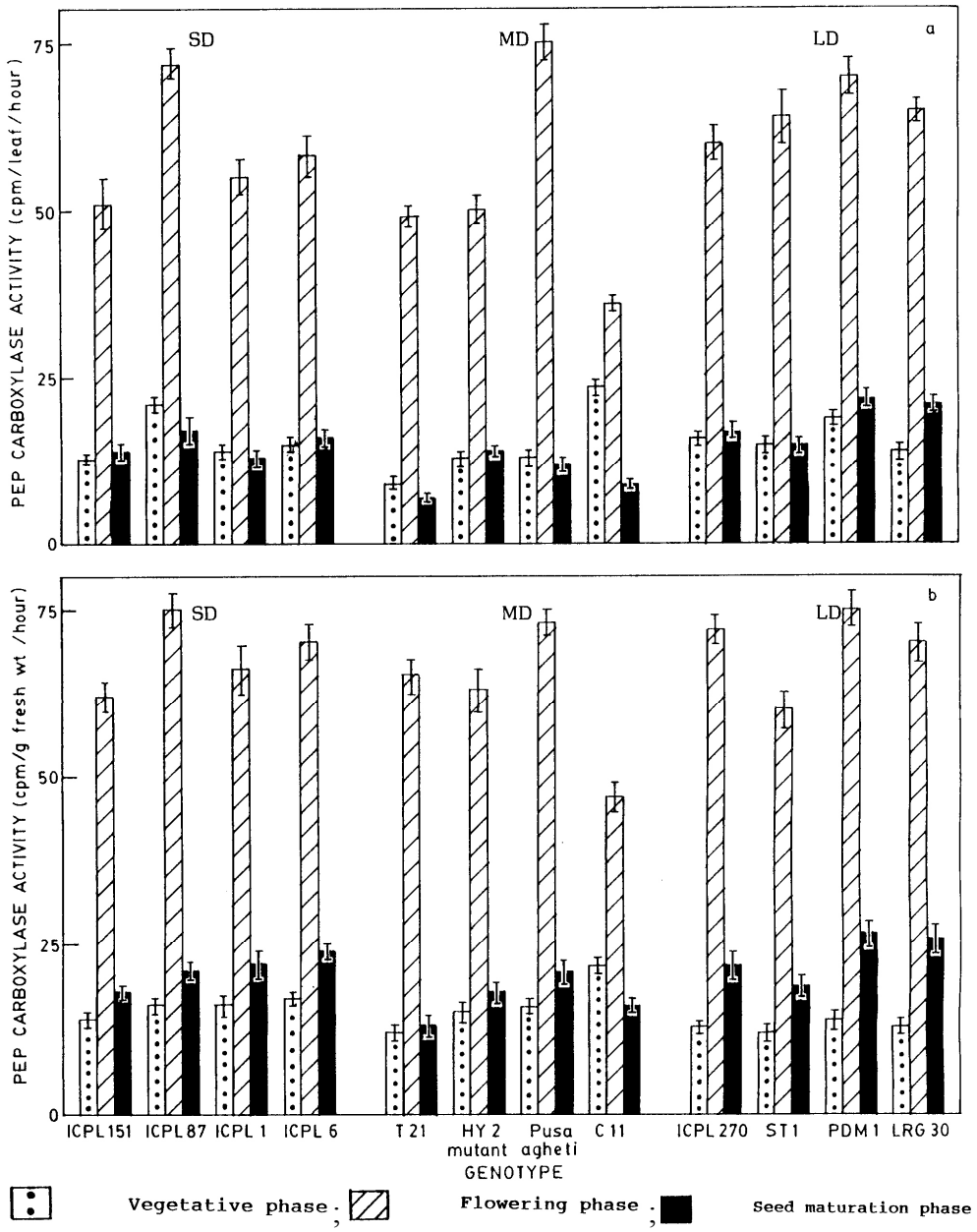

Fig. 6. Phosphoenol pyruvate (PEP) carboxylase activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

The greater rates of fixation were recorded in vegetative phase of the crop growth. Interestingly, short duration genotypes exhibited higher photosynthetic rates during vegetative and flowering phases than medium and long duration genotypes.

Ribulose bisphosphate carboxylase activity: The ribulose bisphosphate carboxylase activity on per leaf basis as well as on per unit fresh weight basis, recorded a decrease from vegetative phase to the seed maturation phase (Fig-5). Among the genotypes studied the ICPL87 of short duration and the ST1 of long duration genotypes recorded the maximum and minimum values respectively at the vegetative phase of crop growth. The short duration genotypes always exhibited greater values than medium and long duration genotypes.

Phosphoenolpyruvate carboxylase activity: Figure 6 represents the phosphoenolpyruvate carboxylase activity of the 10th leaf of pigeonpea genotypes. On per leaf as well as on unit fresh weight bases the enzyme activity of the genotypes increased from vegetative to flowering phase followed by a decline on the seed maturation phase.

The enzyme activity recorded greater values on reaching the flowering phase. The activity showed a range of variation from 41 to 86 cpm/leaf/min on organ basis and 47 to 83 cpm/g fresh wt/min on unit weight basis. Among the genotypes studied the maximum and minimum valueswere exhibited by the Pusa agheti and the C11 respectively. Interestingly both the genotypes belong to the medium duration type. Furthermore, phosphoenolpyruvate carboxylase activity was always recorded lower than ribulose biphosphate carboxylase in all the genotypes studied.

Correlation coefficients between some enzyme activities and seed yield: The correlation coefficients between some important enzyme activities and seed yield at three phonological phases of crop growth of short, medium and long duration pigeonpea genotypes were presented in table-1. In the short and long duration genotypes, the malate dehydrogenase and glycolate oxidase activities showed a negative association with seed yield at all three phases of crop growth.

Table 1. Correlation coefficients between some enzyme activities and seed yield of pigeonpea genotypes

	Phase		
Yield	Vegetative	Flowering	Seed maturation
Short duration			
Malate dehydrogenase	-0.657*	-0.083**	-0.862**
Glycolate oxidase	-0.665*	-0.885**	-0.606*
Total chlorophyll content	-0.983**	0.740**	0.704**
Photosynthetic rate	0.092	-0.336	-0.041
RuBp Carboxylase	0.992**	0.962**	0.825**
PEP Carboxylase	0.979**	0.957**	0.810**
Medium duration			
Malate dehydrogenase	0.875**	0.549	0.948
Glycolate oxidase	0.799**	-0.887**	0.830**
Total chlorophyll content	-0.913**	-0.973**	-0.827**
Photosynthetic rate	-0.775**	-0.261	-0.363
RuBp Carboxylase	0.963**	-0.965**	-0.782**
PEP Carboxylase	-0.269	-0.762**	-0.995**
Long duration			
Malate dehydrogenase	-0.917**	-0.899**	-0.889**
Glycolate oxidase	-0.551	-0.635*	-0.667*
Total chlorophyll content	0.588	0.553	0.982**
Photosynthetic rate	0.823**	0.798**	0.917**
RuBp Carboxylase	0.288**	0.811**	0.896**
PEP Carboxylase	0.498	0.803**	0.988**

** Significant at 1% level; * Significant at 5% level.

However, in the medium duration genotypes seed yield showed a positive association with malate dehydrogenase and glycolate oxidase activities at all stages of crop growth. All the other characters such as total chlorophyll content, photosynthetic rate, ribulose bisphosphate carboxylase (RUBP carboxylase) activity and phosphoenolpyruvate carboxylase (PEP carboxylase) activity showed a negative correlation with seed yield at all phases of crop growth in medium duration genotypes (Table-1). The short and long duration genotypes showed a significant positive correlation of total chlorophyll content, photosynthetic rate and Rubp carboxylase and PEP carboxylase activities with seed yield in most of the growth phases of pigeonpea crop.

DISCUSSION

Malate dehydrogenase plays an important role in the energy-yielding reaction of krebs cycle. Keeping this in view, the enzymatic activity of malate dehydrogenase was used to represent the respiratory activity. In all the pigeonpea genotypes the malate dehydrogenase activity in the 10^{th} leaf, increased from the vegetative to flowering phase followed by a decrease at the seed maturation phase. Among the genotypes, the enzyme activity recorded higher values in the long duration genotypes than the short and medium duration genotypes (Fig-1a, b). The maximum enzyme activity was recorded at the flowering phase in all the genotypes. This may be due to the increased metabolic activity during the transition to flowering and flower formation. The high malate dehydrogenase activity was also associated with high growth activity. High growth potential is known to be positively related to the high respiratory activity in barley (Mc Daniel, 1969) and wheat (Ching and Kronstan, 1972). Although high malate dehydrogenase activity associated with high biomass accumulation in long duration pigeonpea genotypes, the seed yields were low because of low efficiency in photosynthate partitioning to the seeds. The glycolate oxidase activity of the 10^{th} leaf of pigeonpea genotypes showed an increase from the vegetative to the flowering phase followed by a decline towards the seed maturation phase.

The long duration genotypes of the pigeonpea exhibited higher values than the medium and short duration genotypes. The short duration genotypes registered lowest values of glycolate oxidase activity among all the genotypes studied (Fig-2). Higher glycolate oxidase activity of the flowering phase was also observed in *Phaseolus vulgaris* (Fraser and Bidwell, 1974) and dry bean varieties (Peet *et al*., 1977). The high glycolate oxidase activity in the long duration pigeonpea genotypes resulted in low seed yield. This may be due to the enhanced photorespiratory activity during the critical period (flowering phase) of crop growth in long duration genotypes. The high yielding short duration genotypes had low glycolate oxidase activity, which in turn utilized the photosynthates to increase yield. High glycolate oxidase activity in the low yielding genotypes were reported for sunflower (Sai Ram and Srivastava, 1984) and rice (Chakraborti and Saha, 1983).

The intensity of chlorophyll concentration is considered to be an index of the degree of maturity of plant green tissue. The chlorophyll content was found to have positive relationship with the net photosynthetic rate and hence is reasonable to attribute that it plays a major role in controlling grain yield (Liu, 1980). The differences in the total chlorophyll content among the genotypes in relation to differences in the photosynthesis of barley genotypes were advocated by McCashin and Canvin (1979). The total chlorophyll content may be a better indicator than leaf area for the photosynthetic potential (Sestak, 1966; Patterson *et al*., 1977). The chlorophyll content of the 10^{th} leaf was higher at the vegetative phase than at the flowering phase and it further decreased at the seed maturation phase in all the pigeonpea genotypes. Among the genotypes, the ICPL87 of short duration, the T21 and the Pusa agheti of medium duration and the PDM1 of long duration type exhibited greater values of total chlorophyll content in their respective groups at all the phases of crop growth (Fig-3a, b). The accumulation of higher amounts of chlorophyll may have a relation with photosynthetic rates and consequent higher yields. The Pusa agheti genotype registered higher chlorophyll content and high photosynthetic rate, that produced high dry matter accumulation rather than specific seed yield.

The total chlorophyll content was found to be positively correlated with net photosynthetic rate leading to increased yields in wheat (Murthy and Singh, 1979), in rice (Padmaja Rao *et al*., 1986), in chick pea (Dhawan and Singh, 1983) and in mungbean genotypes (Rao and Ghildiyal, 1985). The photosynthetic rate was measured in the form of ^{14} Carbon dioxide fixation of the 10th leaf of all the pigeonpea genotypes (Fig-4). The decrease in photosynthetic rates with advancing crop age was observed in soybean genotypes (Jeffers and Shibles, 1969). The photosynthetic rates were higher at the vegetative phase than at the flowering phase in all the pigeonpea genotypes. The decrease in photosynthetic rate in relation to total chlorophyll content and photophosphorylation activities with advancing crop age suggests that all these traits are interdependent in this crop. Thus, the high photosynthetic rates at the vegetative phase of pigeonpea leads to the active growth during that period. Genotypic variation in the photosynthetic rates stemmed up from the variations of genetic potentials. Differences in mesophyll resistance may be the key factor for genotypic variation (Paz and Pallas, 1986). The genotypic variation in photosynthetic rates leading to variations in productivity were also noticed in rice (Takeda, 1961; Arjunan *et al*., 1990). Interestingly, the greater values of $^{14}CO_2$ fixation rates were observed in the short duration than the medium and long duration genotypes during the vegetative and flowering phases of pigeonpea crop growth. The greater variation in the photosynthetic rates among the genotypes were observed during the flowering phase. This might be due to the differential sink demands among the pigeonpea genotypes. The decline in photosynthetic rate after flowering could possibly be due to the mobilization of leaf nitrogen to the developing seeds as shown in soybean (Sinclair and De wit, 1975; Boon-Long *et al*., 1983; Koch and Schrader, 1984); and in mungbean (Rao and Ghildyal, 1985). Further, the high yielding genotypes of pigeonpea possess high photosynthetic $^{14}CO_2$ fixation rates than their low yielding counter parts. This linear relationship between photosynthetic rate and seed yield was observed particularly in the short duration pigeonpea genotypes. In contrast, photosynthetic rates showed linear relationship with the dry matter accumulation in the medium and long duration genotypes exhibiting low efficiency in photosynthate partitioning in the direction of seed filling. The higher photosynthetic rates were not always correlated with higher seed yield and higher biomass accumulation in pigeonpea genotypes due to their lower efficiency in photosynthate partitioning and respiratory losses (Rawson *et al*., 1983).

Pigeonpea genotypes exhibited significant variation in ribulose bisphosphate carboxylase activity at different growth stages. The ribulose bisphosphate carboxylase activity decreased in the 10th leaf of all the pigeonpea genotypes with advancing crop age. The short duration genotypes exhibited higher values of enzyme activity than the medium and long duration genotypes at the vegetative and flowering phases of crop growth (Fig-5 a, b). A linear relationship between photosynthetic rate and ribulose bisphosphate carboxylase activity was observed in all the genotypes of pigeonpea. A similar linear relationship between photosynthetic rate and ribulose bisphosphate carboxylase activity was noticed wheat genotypes (Sirohi and Ghildyal, 1975; Massacci *et al*., 1986). Further, the higher yielding genotypes ICPL87, T21 and PDM1 of the short, medium and long duration groups, exhibited higher enzyme activity even at seed filling stage when compared to their low yielding counter parts. High ribulose bisphosphate carboxylase activity at seed setting stage in high yielding genotypes was also noticed in sunflower (Srivastava and Sai Ram, 1983) and in Chickpea (Dhawan and Singh, 1983). The phosphoenolpyruvate carboxylase activity of the 10th leaf of all the pigeonpea genotypes showed an increase from the vegetative phase to the flowering phase followed by a decline at the seed maturation phase (Fig-6a, b). High activity of phosphoenolpyruvate carboxylase reduces the carbon loss by assimilating CO_2 released during the dark respiration or photorespiration (Hedley *et al*., 1975; Willner and Johnston, 1976; Basra and Malik, 1985; Nayyar *et al*., 1990). Phosphoenolpyruvate carboxylase activity registered higher values at the flowering phase. This has a closer relation with photorespiratory activity at the flowering phase. Further, it was noted that phosphoenolpyruvate carboxylase activity might be more of non-photosynthetic nature and was involved in the synthesis of free organic acids, which could further be utilized in the production of amino acids (Sinha, 1965; Splittstoesser, 1966).

Conclusion

The 10th leaf of pigeonpea genotypes was selected as a representative sample for certain studies at three selected phases of crop growth. The activity of the malate dehydrogenase increased from vegetative to flowering phase followed by a decrease at the seed maturation phase. The long duration genotypes recorded higher values than medium and short duration genotypes. The glycolate oxidase and catalase activities of the 10th leaf increased from the vegetative to the flowering phase followed by a decline at the seed maturation phase. The long duration genotypes recorded higher values of glycolate oxidase and catalase activities than the medium and short duration genotypes. The total chlorophyll content was also higher at the vegetative phase than the flowering and the seed maturation phases of crop growth. Among the genotypes, ICPL 87 of the short duration, T21 and Pusa agheti of the medium duration and PDM1 of the long duration genotypes exhibited higher values of total chlorophyll content in their respective groups at all the phases of crop growth. The chlorophyll content showed a positive correlation with photosynthetic rates and seed yield. The pusa agheti, even though registered higher chlorophyll content and higher photosynthetic rates, it exhibited greater dry matter accumulation rather than specific seed yield in the medium duration group. The photosynthetic rate as $^{14}CO_2$ uptake and the Rubisco activity, in the 10th leaf exhibited higher values at the vegetative phase of crop growth followed by a decrease towards the seed maturation phase in all the pigeonpea genotypes. The phosphoenolpyruvate carboxylase activity of the 10th leaf of all the pigeonpea genotypes registered greater values at the flowering phase. Among the total genotypes studied, the short duration genotypes showed greater values than the medium and long duration genotypes.

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