



CHANGES IN THE ACTIVITIES OF ANTIOXIDANT ENZYMES AND HILL REACTION DURING SEEDLING DEVELOPMENT OF *CAJANUS CAJAN* (L.) GENOTYPES

B. Sujatha*, V. Durga Bhavani, B. Priyadarshini, M.V.V.P. Kumar and L.B. Divya Jyothi

Department of Botany, Andhra University, Visakhapatnam-530003, A.P., India.

*Corresponding Author Email: sujathaau@yahoo.co.in

ABSTRACT

Pigeonpea (Cajanus cajan (L.) Millspaugh), the material selected for the present investigation is the second most important pulse crop in India. Twelve genotypes of pigeonpea 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 current study on apparent photosynthesis, hill reaction activity, photophosphorylation, organic acids, ascorbic acid content and catalase activity in the 10th leaf. The apparent photosynthesis was observed greater in the ICPL87 of short duration, the T21 of medium duration and the PDM1 of long duration genotypes. The hill reaction activity of the 10th leaf of pigeonpea genotypes ICPL87, Pusa agheti and PDM1 of short, medium and long duration genotypes exhibited greater values in their respective groups. Variation in cyclic and non-cyclic photophosphorylation in ICPL87 and ST1 recorded the greatest and lowest values among all the genotypes studied. Among the genotypes, ICPL6 of short duration, the Pusa agheti of medium duration and the ICPL270 of long duration genotypes exhibited maximum values of free organic acid content in their respective groups. Among all the genotypes studied the PDM1 and T21 showed the maximum and the minimum values of ascorbic acid content and ICPL1 and ICPL6 of short duration genotypes showed a decrease in catalase activity respectively.

KEY WORDS

Apparent photosynthesis, ascorbic acid, catalase, hill reaction, photophosphorylation, pigeonpea genotypes.

INTRODUCTION

Photosynthesis of leaves is the major determinant of biomass production in higher plants, although higher rates of photosynthesis were not always correlated with higher yields, since other factors such as allocations of assimilates and respiratory losses may also be important factors in biomass accumulation (Rawson *et al.*, 1983). They also found that apparent photosynthesis tends 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.*

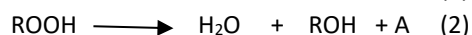
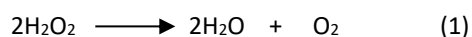
(1982) and found high and significant correlation between canopy apparent photosynthesis and seed yield in soybean.

Dark respiration is a key physiological process of plant in growth and maintenance. It may be considered that respiration is a good indicator of physiological activity. Nevertheless, the links between respiration and growth are not well characterised (Gerik and Eastin, 1985). Photorespiratory loss of carbon is of common occurrence particularly in C₃ plants which reduces net photoassimilation rate considerably (Forrester *et al.*, 1966). Photorespiration increases at high light

intensities, high temperature, high O₂ and low CO₂ concentrations (Downton and Tregunna, 1968; Hew *et al.*, 1969; Jolliffe and Tregunna, 1973) and therefore, the conditions available in the tropics encourage high rates of photorespiration.

Ascorbic acid plays an important role in plant metabolism. Ascorbic acid is involved in plant growth

differentiation and development. It is a constituent of oxidation-reduction system and thus plays an active part in important plant processes (Chinoy, 1962, 1967, 1968, 1969a and b; Chinoy *et al.*, 1971). Catalase is an enzyme presents in nearly all plant cells which catalase the following reactions (Luck, 1963).



It is not clear whether the role of catalase in the organism is to decompose hydrogen peroxide (equation 1) or to catalase a peroxidation reaction (equation 2). Yokayama (1956) reported that catalase activity is linked with mitochondrial respiration. However, a close relationship between glycolate oxidase and catalase activity was also noticed. Several workers have reported the involvement of these two enzymes in photorespiratory activity and also their presence together in the microbodies i.e., peroxysomes (Tolbert *et al.*, 1968, 1969; Fair *et al.*, 1973a and b). Genotypic differences of catalase activity in the leaves of sunflower genotypes showed that efficient species has low catalase activity (Sairam and Srivastava, 1984). Basing

on the earlier reports much work remains to be investigated on hill reaction activity, photophosphorylation and some antioxidative enzymes of different pigeonpea genotypes. 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 investigations 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° 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:

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.

Apparent photosynthesis

Apparent photosynthesis was measured by dry weight increase method. Twenty-five leaf discs were taken from one side of the lateral leaflets of the 10th leaves of different genotypes at each phase of study at 6.00 a.m. Immediately the fresh weights of the leaf discs were recorded and were kept in hot air oven until constant dry weight were obtained. Again, in the evening at 6.00 p.m. twenty-five leaf discs from the opposite side of the leaflets from which the leaf discs were collected in the morning. As described earlier their fresh and dry weights were also determined. The difference in the dry weights of the evening and morning leaf discs were considered as apparent photosynthesis and was expressed on unit leaf area.

Hill reaction activity

Hill reaction activity of the isolated chloroplasts was determined according to the method of Trebst (1972). Five grams of washed and surface moisture blotted out leaves (10th leaf) of different pigeonpea genotypes were cut separately into several small pieces. The pieces were ground in a chilled mortar with about 60 ml of chilled 0.4 M sucrose, 0.05 M KH₂PO₄-Na₂HPO₄ buffer (pH 7.2) and 0.01 M KCl. The green juice was filtered through eight layered cheese cloth, and centrifuged at about 1000 x g for 2 min. The supernatant was discarded and the pellet (chloroplast) was resuspended in about 10 ml of the sucrose phosphate buffer solution. All the preparations were carried out at 0 °C.

The reduction of 2,6-DCIP was measured by adding 5 ml of the chloroplast suspension and 0.5 ml of 5x10⁻⁴ M DCIP (0.145 mg DCIP/ml water) and enough of the sucrose phosphate buffer solution was used to bring the final volume to 10 ml. The test tubes containing the above reaction mixture were exposed to 1000-watt bulb at a distance of about 15 cm. To serve as the dark control, one tube was covered with aluminum foil to avoid light. All the tubes were put in a large beaker of water to maintain the desired temperature (usually about 20 °C). The disappearance of the blue colour is measured as a change in absorbance at 600 nm as a consequence of the reduction of DCIP by the electrons

that are obtained from the oxidation of water by the operation of photosystem II of photosynthesis was determined using 150-20 UV-VIS spectrophotometer (Hitachi, Japan). The concentration of chlorophyll was determined by the method of Harborne (1973). The Hill reaction activity of chloroplasts was expressed as micromoles of DCIP reduced per milligram chlorophyll per hour.

Photophosphorylation

Rates of ATP formation by isolated chloroplasts were determined by following the incorporation of ³²PO₄ into ATP. One gram of the leaf material (10th leaf) was used for chloroplast isolation. The chloroplast isolation medium consists of 50 mM Tris HCl buffer pH 8.0; 0.33 M Sorbitol; 5.0 mM DTT; 1.0 mM EDTA; 1.0 mM MgCl₂; 1.0 mM MnCl₂ and 0.5% bovine serum albumin (Anderson *et al.*, 1971). After centrifugation the chloroplast pellet was suspended in a mixture of 20 mM Tris-HCl Buffer, pH 8.0, 0.33 M sorbitol, 1.0 mM MgCl₂ and 0.5% bovine serum albumin. The assay medium for photophosphorylation (3 ml) contained 30 mM Tris-HCl buffer, pH 8.0; 5 mM MgCl₂; 10 mM NaCl; 0.5% bovine serum albumin; 1 mM ADP; 2 mM phosphate (containing ³²P with 10⁵-10⁶ cpm); 0.05 M pheazine methosulphate (for cyclic) or 5 mM ferricyanide (non-cyclic) and chloroplasts equivalent to 10 µg chl/ml. The illumination was 500 µE m⁻²sec⁻¹ for 5 minutes at 25 °C in air after which the light was switched off with simultaneous addition of 0.3 ml 20% (w/v) trichloroacetic acid (TCA). After centrifugation at 1200 x g for 15 min the supernatant was assayed for its radioactivity (Losada and Arnon, 1964). One ml of supernatant was mixed with 1 ml of magnesia mixture. A drop of 0.2% (w/v) phenolphthalein was added to check the alkalinity of the solution. Since the precipitation of inorganic phosphate ideally required alkaline medium. The mixture was allowed to stand for one hour at room temperature. The precipitate containing unesterified radioactive phosphate was removed by filtration with a whatmann no.1 filter paper under suction. The precipitate on the filter paper was washed twice with 1:10 magnesia mixture. An aliquot of filtrate (containing radioactive ATP) was taken on a planchet, dried under infrared lamp and examined for the amount of radioactivity in gas flow proportional counting system. The ATP formed was calculated by comparing the radioactivity in the filtrate with the total radioactivity present in the reaction mixture.

Organic acids

Total free organic acids of the 10th leaf of all the 12 genotypes were determined according to the method of Ting and Dugger (1968). Early in the day, 1 g leaf material was taken, chopped into fine slices and boiled for 30 min with glass distilled water free of carbon dioxide. The homogenate was centrifuged at 5000 xg for 15 min and the supernatant was made up to a known volume. Ten ml of aliquots of the supernatant were titrated against 0.01 N NaOH using phenolphthalein as indicator and the results were expressed as milliequivalents of acid per 100 g fresh weight as well as per part.

Ascorbic acid

Ascorbic acid content was estimated according to the method of Roe (1964). One gram of fresh leaves was macerated thoroughly and rapidly with 10 ml of 5% metaphosphoric acid using mortar and pestle. The maceration was completed within 1 or 2 minutes and the homogenate was made up to 20 ml with 5 % metaphosphoric acid. It was filtered through filter paper. Duplicate samples of 10 ml aliquots were titrated with DCIP reagent until a pink end point. Which persists for 15 seconds was obtained. The quantity of ascorbic acid was calculated using the following formula:

$$L \times S \times D/A \times 1/W = \text{mg AA per 1 g material}$$

Where

L = ml of DCIP reagent used in the titration.

S = mg of AA reacting with 1 ml of DCIP reagent

D = Volume of the extract in ml

A = The aliquot titrated in ml

W = Weight of the sample in grams

Catalase (E.C.1.11.1.6)

Catalase activity was estimated by the permanganate method of Povolotskaya and Sedenka (1956) as followed by Gopalachari (1963) with slight modification. Five hundred mg of fresh leaves (10th leaf) were weighed and was ground in a precooled glass mortar with few ml of cold phosphate buffer pH (7.0). The extract was filtered through glass wool and made up to 25 ml with the same buffer solution. Two ml of the enzyme extract was taken in a conical flask and 1 ml of 0.045 M

hydrogen peroxide was added. Exactly after 5 min, one ml of 12% H₂SO₄ was added to stop the enzyme activity and titrated with 0.05 N potassium permanganate. The end point was denoted by the pink colour of the solution which lasts for 30 sec. A blank was run simultaneously as above substituting 2 ml of the enzyme extract with 2 ml phosphate buffer. The difference in the titre values gave the activity of catalase and was expressed as mg H₂O₂ destroyed per minute per g plant tissue by using the following equation:

$$\text{mg of H}_2\text{O}_2 \text{ destroyed in 5 minutes by 1 g plant tissue} = 25/2 \times 0.85 \times V/M$$

where

V = difference between blank and sample titre values

W = fresh weight in grams

25 = total volume of extract taken in ml

2 = extract taken in ml

The factor 0.85 represents 1 ml of 0.05 N KMnO₄ equal to 0.85 mg of H₂O₂

RESULTS

Apparent Photosynthesis

Figure 1 represents the genotypic variation in the apparent photosynthesis of the 10th leaf of pigeonpea. Greatest apparent photosynthesis was observed at the vegetative phase of crop growth followed by a decline up to the seed maturation phase. Among the genotypes,

the ICPL87 of short duration, the T21 of medium duration and the PDM1 of long duration genotypes showed greater values throughout the growth period in their respective groups. The rate of apparent photosynthesis varied greatly and conspicuously with the phase of crop growth rather than genotypic variation.

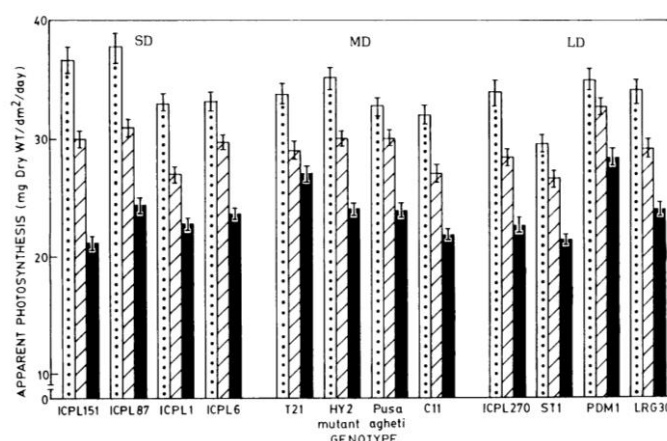


Fig 1: Apparent photosynthesis of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

Hill reaction activity

The changes in the Hill reaction activity of the 10th leaf of pigeonpea genotypes were observed in figure 2a, b. On per part as well as on unit fresh weight basis the ICPL87, Pusa agheti and PDM1 of short, medium and long duration genotypes exhibited greater values in their respective groups. Among all the genotypes studied the ICPL87 and ST1 recorded the greatest and the lowest values of Hill reaction activity respectively throughout the crop growth of pigeonpea.

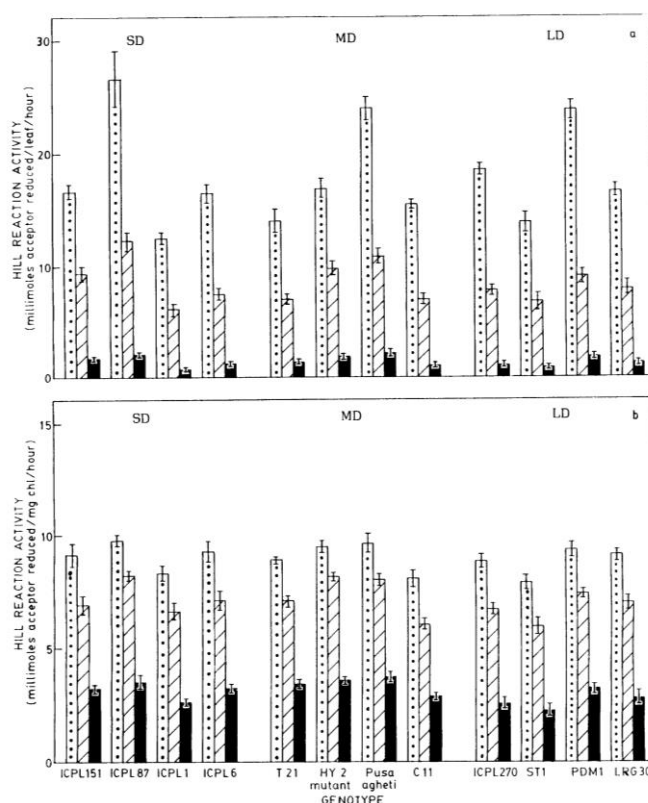


Fig 2: Hill reaction activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

Photophosphorylation

The photophosphorylation activity of the 10th leaf exhibited considerable variation during the crop growth of different genotypes of pigeonpea. Rates of ATP formation by isolated chloroplasts were determined by following the incorporation of ³²PO₄ into ATP. Figures 3a, b represents the cyclic photophosphorylation activity, in which the values showed a decrease with crop growth phase. The greater values were recorded at vegetative phase and lower values were recorded at the

seed maturation phase. On per leaf basis the values ranged from 6.15 x 10³ to 9.80 x 10³ cpm/leaf/h. The ICPL87 and ST1 recorded the greatest and lowest values at the vegetative phase among all the genotypes studied. However, on per mg chlorophyll basis the values ranged from 3.40 to 3.91 x 10³ cpm/h. The HY2 mutant of the medium duration and the ICPL151 of the short duration genotypes recorded lowest values in their respective groups at the vegetative phase.

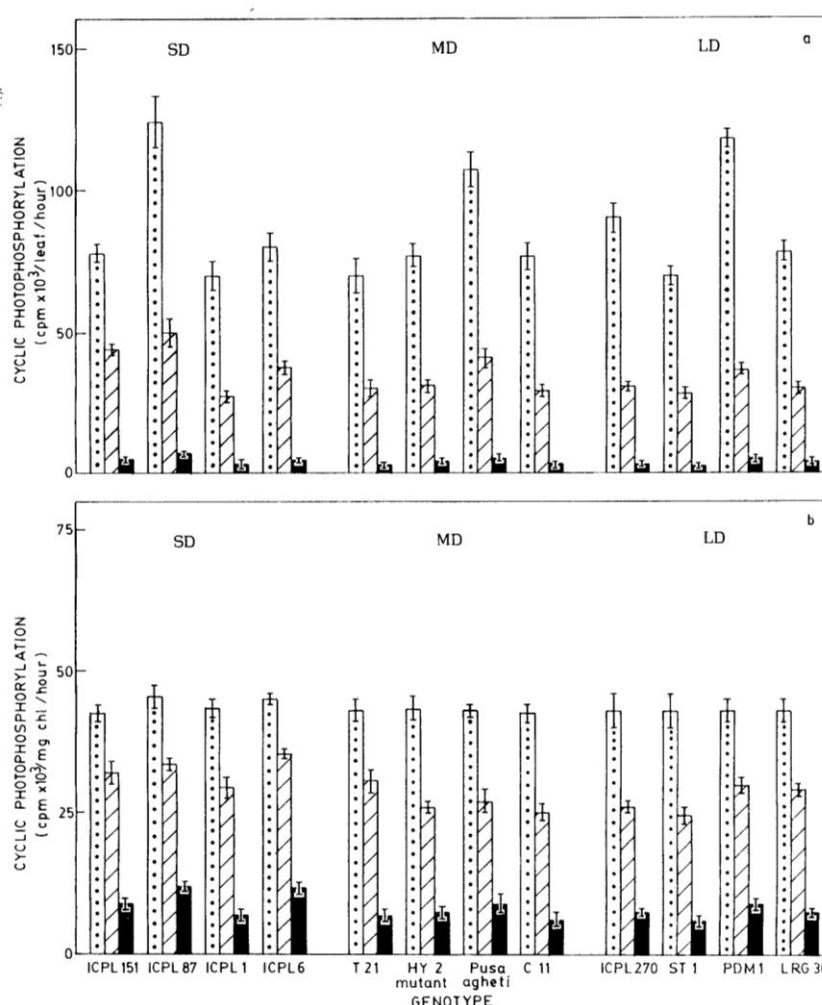


Fig 3: Cyclic photophosphorylation of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase ;
  Flowering phase ;
  Seed maturation phase

Figures 4a, b represents the genotypic variation in non-cyclic photophosphorylation activity of the 10th leaf of pigeonpea genotypes. The non-cyclic photophosphorylation activity also showed a trend similar to that observed for cyclic photophosphorylation. The non-cyclic photophosphorylation activity recorded greater values than cyclic photophosphorylation.

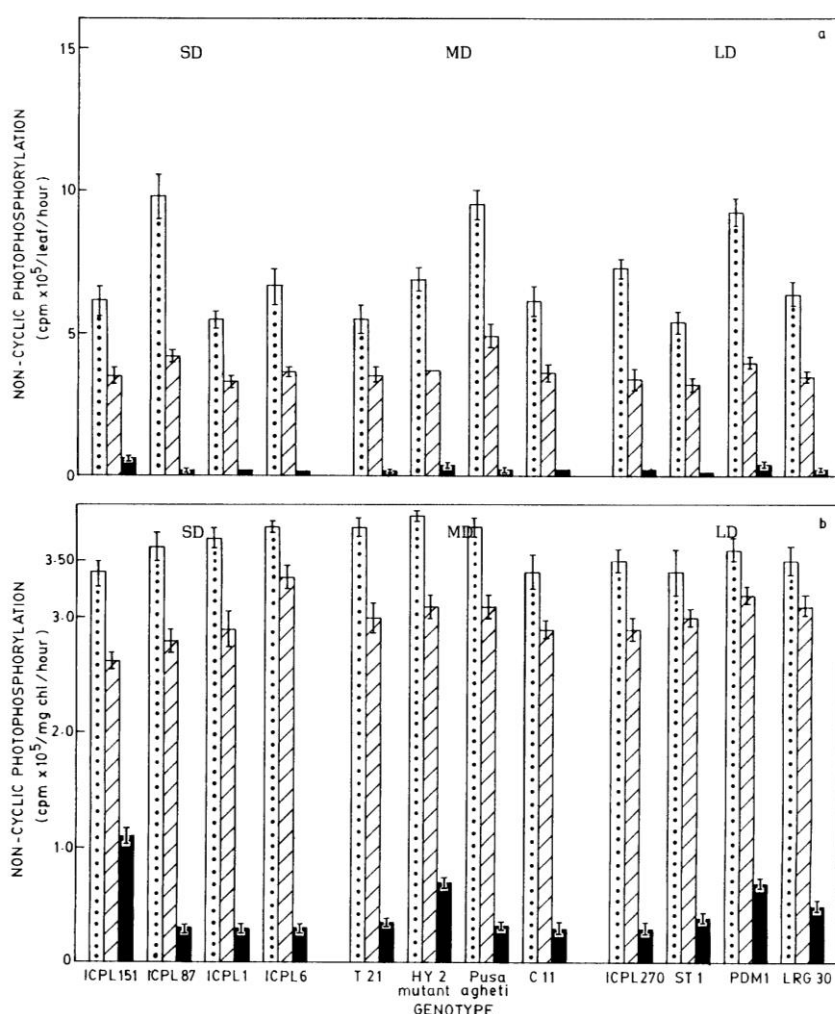


Fig 4: Non-cyclic photophosphorylation of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

Organic acids

The genotypic variation in the free organic acids content of the 10th leaf of all the genotypes were shown in figures 5 a, b. On per leaf basis, free organic acid content showed a decrease from the vegetative phase to the flowering phase followed by a slight increase towards the end of the seed maturation phase in all the short and medium duration genotypes except for the C11. The long duration genotypes showed a gradual decrease in the organic acid content with increasing crop age except for the LRG30 which showed an increasing trend from the vegetative phase to the seed maturation phase. Among the total genotypes, long duration genotypes exhibited higher values than the medium and short

duration genotypes (Fig. 5a). The PDM1 of long duration and the T21 medium duration genotypes exhibited the maximum and minimum organic acid content at the vegetative phase respectively. However, at the seed maturation phase the genotypic variation of the free organic acid content was not so conspicuous. On fresh weight basis, an increase in the organic acid content was observed in the 10th leaf of all the genotypes (Fig. 5b). At the seed maturation phase the ICPL6 (5.01 meq/g fresh wt) of short duration, the Pusa agheti (5.23 meq/g fresh wt) of medium duration and the ICPL270 (5.35 meq/g fresh wt) of long duration genotypes exhibited maximum values of free organic acid content in their respective groups.

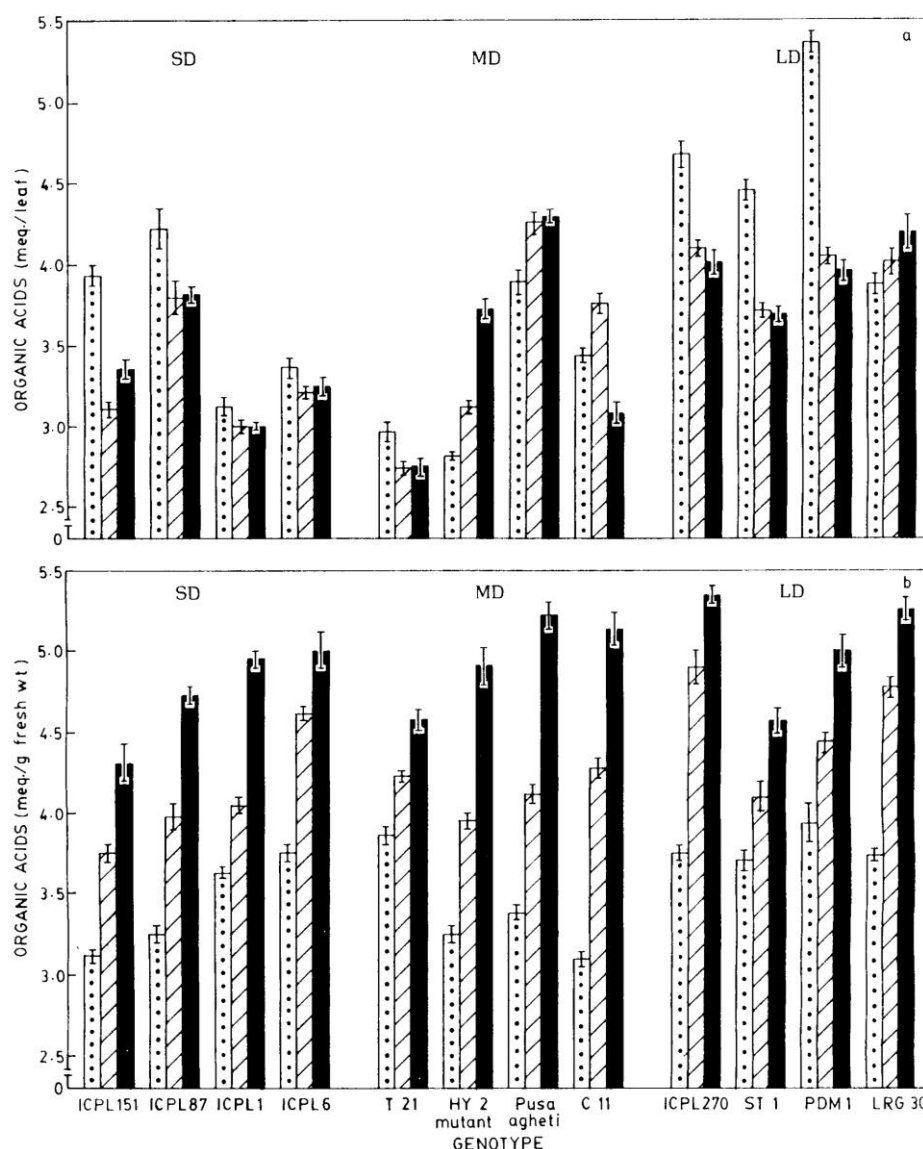


Fig 5: Organic acid content of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.).

 Vegetative phase ;
  Flowering phase ;
  Seed maturation phase

Ascorbic acid

Figures 6a, b represents the genotypic variation of the ascorbic acid content of the 10th leaf of pigeonpea. A gradual rise in the ascorbic acid content from the vegetative to the seed maturation phase was observed in all the genotypes. At the seed maturation phase the ascorbic acid content ranged from 1.86 to 3.2 mg/leaf. Among all the genotypes studied the PDM1 and T21 showed the maximum and the minimum values of

ascorbic acid content respectively (Fig. 6a). On fresh weight basis also changes in ascorbic acid content exhibited a trend similar to that observed for per leaf basis (Fig 6b). However, the maximum quantity of ascorbic acid with a value of 3.96 mg/g fresh wt and the minimum with a value of 2.75 mg/g fresh wt were observed at the seed maturation phase of the C11 of medium duration and the ICPL6 of short duration groups respectively.

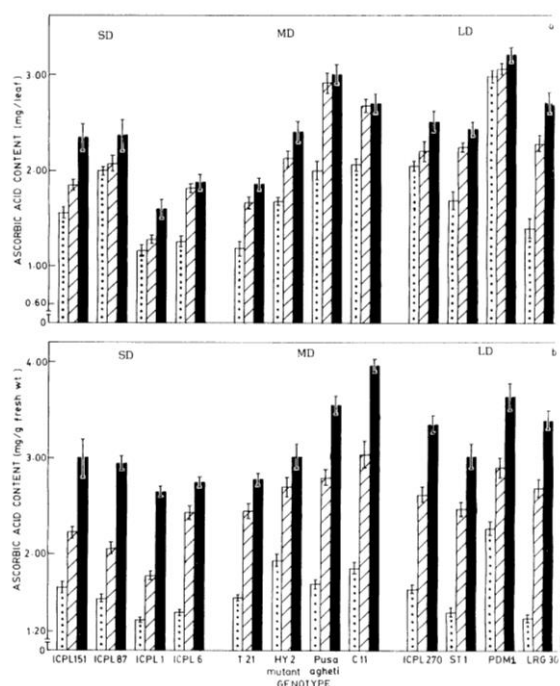


Fig 6: Ascorbic acid content of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

Catalase

Changes in the catalase activity of the 10th leaf of the 12 pigeonpea genotypes during the crop growth was presented in figures 7a, b. The ICPL1 and ICPL6 of short duration genotypes showed a decrease in catalase

activity from the vegetative phase to the seed maturation phase (Fig. 7a). On unit fresh weight basis, the ST1 and the ICPL87 recorded the greater and lower activities respectively (Fig. 7b).

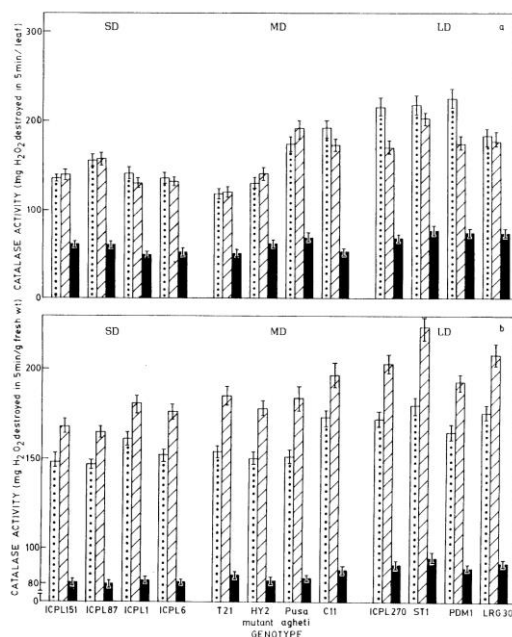


Fig 7: Catalase activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

 Vegetative phase,
  Flowering phase,
  Seed maturation phase

DISCUSSION

Photosynthetic activity of the leaves is the most important physiological character which decides the production potential of any crop plant (Arjunan *et al.*, 1990). Research programmes were aimed at obtaining higher photosynthetic efficiency by selecting genotypes with higher photosynthetic rates per unit leaf area and with most photosynthetically effective canopy. The apparent photosynthesis was measured in the 10th leaf from the shoot tip. The apparent photosynthesis exhibited a gradual decrease with crop age in all the pigeonpea genotypes. Greater values were noticed at the vegetative phase followed by a decrease towards the seed maturation phase (Fig. 1a, b). The rate of apparent photosynthesis varied greatly with crop age rather than with genotype (Rawson and Constable, 1981). Apparent photosynthesis in soybean genotypes also positively correlated with leaf area, specific leaf weight and chlorophyll content (Kokubun and Wantabe, 1983). Although, the ICPL6 and the Pusa agheti of short and medium duration genotypes showed high leaf area they did not exhibit higher apparent photosynthesis when compared to the ICPL87 and T21 of the short and medium duration genotypes. This may be due to their efficient plant architecture to perceive light (Rawson and Constable, 1981).

The Hill reaction activity decreased with increasing crop age in all the pigeonpea genotypes. Among the genotypes, the ICPL87 short duration recorded higher values followed by the Pusa agheti and the PDM1 of medium and long duration genotypes in their respective groups (Fig. 2a, b). The photophosphorylation rates of the 10th leaf of all the genotypes decreased with increasing crop age. The uptake of ³²PO₄ values in photosystem I recorded the highest values in the ICPL87 and the lowest values in the ST1 of all the genotypes studied. The activity of photosystem II also showed a trend similar to that observed for photosystem I. However, the incorporation of ³²PO₄ into ATP in photosystem II recorded greater values than photosystem I. Increased activity of hill reaction, photosystem I and II resulted in higher photosynthetic rates leading to higher yields in pigeonpea genotypes. Photorespiration in leaves of terrestrial C₃ plants leads to considerable loss of carbon (Ludwig and Calvin, 1971). Glycolate oxidase plays an important role in photorespiration and the measurement of its activity may provide information on the photorespiratory

activity or its rate (Zelitch, 1959, 1966; Fair and Cresswell, 1973a, b; 1974 a, b) (Figs. 3a, b; 4a, b). An increase in the organic acid content was observed in the 10th leaf of all the genotypes. 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 (Fig. 5a, b), which could further be utilized in the production of amino acids (Sinha, 1965; Splittstoesser, 1966).

Ascorbic acid is universally present in plants and is actively involved in the plant growth, differentiation and development. It is a constituent of oxidation-reduction system and plays an active part in important plant processes (Chinoy *et al.*, 1971). The content of ascorbic acid increased in all the pigeonpea genotypes from the vegetative to the seed maturation phase. Comparatively, the short duration genotypes exhibited lower ascorbic acid content than the medium and long duration genotypes (Fig. 6a, b). This may be due to their lower respiratory rates, low biomass production and higher yields of these genotypes.

The catalase activity registered higher values in long duration pigeonpea genotypes than medium and short duration genotypes. The maximum catalase activity of the 10th leaf was recorded at the flowering phase in all the pigeonpea genotypes (Fig. 7a, b). The high catalase 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 catalase activity, which in turn utilized the photosynthates to increase yield. High catalase activity in the low yielding genotypes were reported for sunflower (Sai Ram and Srivastava, 1984) and rice (Chakraborti and Saha, 1983).

CONCLUSION

The apparent photosynthesis, hill reaction activity, photophosphorylation and organic acids 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 ascorbic acid content of the 10th leaf of all the genotypes increased from the vegetative phase to the seed maturation phase. Comparatively the short duration genotypes exhibited lower ascorbic acid content than the medium and long duration genotypes. The catalase

activity 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 catalase activity than the medium and short duration genotypes.

REFERENCES

- Anderson, J.M., N.K. Boardman and D. Spencer., 1971. Photophosphorylation by intact bundle sheath chloroplasts from maize. *Biochem. Biophys. Acta.*, 245: 255-258.
- Arjunan, A., N. Natarajaratnam, M. Nagarajan, R. Sadasivam and K. Balakrishnan., 1990. Photosynthesis and productivity in Rice Cultivars. *Photosynthetica*, 24(2): 273-275.
- Chakrabarti, S. and Saha, S., 1983. Photorespiration in high and low yielding cultivars of rice. *Plant Physiol. Biochem.*, 10(1): 81-88.
- Chinoy, J.J., 1962. Formation and utilization of ascorbic acid in the shoot apex of wheat as factors of growth and development. *Ind. J. Plant Physiol.*, 5: 172-201.
- Chinoy, J.J., 1967. Role of ascorbic acid in crop production. *Poona Agric. Coll. Mag.*, 57: 1-6.
- Chinoy, J.J., 1968. Physiological and physiogenetical studies in relation to crop production in India. *Vidya.*, 11: 138-173.
- Chinoy, J.J., 1969a. Role of free radical of ascorbic acid and charge transfer in revival of plants after undergoing water stress. *Natl. Inst. Sci. India.*, May 6-7, Abst. Pp. 34-35.
- Chinoy, J.J., 1969b. A new concept of flowering on the basis occurring in the shoot apex and the leaf of wheat. *Ind. J. Plant Physiol.*, 12: 67-80.
- Chinoy, J.J., Y.D. Singh and K. Gurumurthi., 1971. Some aspects of physiological role of ascorbic acid in plants. *Ind. Agric.*, 15: 33-48.
- Downton, W.J.S. and E.B. Tregunna., 1968. Carbon dioxide compensation – its relation to photosynthetic carboxylation reactions, systematics of the Gramineae and leaf anatomy. *Can. J. Bot.*, 46: 207-215.
- Fair, P., J. Tew and C.F. Cresswell., 1973a. Enzyme activities associated with carbon dioxide exchange in illuminated leaves of *Horedum vulgare* L. I. Effect of light period, leaf age and position on carbon dioxide compensation point. *Ann. Bot.*, 37: 831-844.
- Fair, P., J. Tew and C.F. Cresswell., 1973b. Enzyme activities associated with carbon dioxide exchange in illuminated leaves of *Horedum vulgare* L. II. Effect of external concentration of carbon dioxide and oxygen. *Ann. Bot.*, 37: 1035-1039.
- Fair, P., J. Tew and C.F. Cresswell., 1974 a. Enzyme activities associated with carbon dioxide exchange in illuminated leaves of *Horedum vulgare* L. III. Effect of concentration and form of nitrogen supply on carbon dioxide compensation points. *Ann. Bot.*, 38: 39-43.
- Fair, P., J. Tew and C.F. Cresswell., 1974b. Enzyme activities associated with carbon dioxide exchange in illuminated leaves of *Horedum vulgare* L. IV. Effect of light intensity on the carbon dioxide compensation point. *Ann. Bot.*, 38: 45-52.
- Forrester, M.L., G. Krotkov and C.D. Nelson., 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. *Soybean. Plant Physiol.*, 41: 422-427.
- Gerik, T.J. and J.D. Eastin., 1985. Temperature effects on dark respiration among diverse *Sorghum* genotypes. *Crop Sci.*, 25: 957-961.
- Gopalachari, N. C., 1963. Changes in the activities of certain oxidizing enzymes during germination and seedling development of *Phaseolus mungo* and *Sorghum vulgare*. *Ind. J. Exp. Biol.*, 1: 98-100.
- Harborne, J.B., 1973. Nitrogen compounds. In: *Phytochemical Methods*-Chapman and Hall Ltd., London, pp. 204-208.
- Hew, C.S., G. Krotkov and D.T. Canvin., 1969. Effects of temperature on photosynthesis and CO₂ evolution in light and darkness by green leaves. *Plant Physiol.*, 44: 671.
- Jolliffe, P.A. and E.B. Tregunna., 1973. Environmental regulation of the oxygen effect on apparent photosynthesis in wheat. *Can. J. Bot.*, 51: 841.
- Kokubun, M. and K. Watanabe., 1983. Analysis of the yield – determining process of field-grown soybeans in relation to canopy structure. VII. Effects of source and sink manipulation during reproductive growth on yield and yield components. *Japanese Journal of Crop Sci.*, 52: 215-219.
- Losada, M. and D.I. Arnon., 1964. Enzymes systems in photosynthesis. In: K. Poach, B. D. Saniwal and M.V. Tracey (eds.). *Modern Methods of plant Analysis*, Vol.7, Springer-Verlag, Berlin. PP. 168-615.
- Luck, H., 1963. Catalase. In: *Methods of Enzymatic Analysis*. Ed. H.U. Bergmeyer, Verlag Chemie, GMBH, pp.895-897. *Academic Press, New York*.
- Ludwig, L.J. and D.T. Canvin., 1971. An open gas-exchange system for the simultaneous measurement of CO₂ and ¹⁴CO₂ fluxes from leaves. *Can. J. Bot.*, 49: 1299-1313.
- Povolotskaya, K.L. and D. M. Sedenka., 1956. A method for collective determination of ascorbic, polyphenol and peroxidase activities. *Biokhimiya (Leningr)*, 21: 133-136.

- Rawson, H.M. and G.A. Constable., 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.
- Rawson, H.M., Hindmarsh J.N., Fischer R.A. and Stockman Y.M., 1983. Changes in leaf photosynthesis with plant ontogeny and relationship with yield per ear in wheat cultivars and 120 progenies. *Aust. J. Plant Physiol.*, 10: 504-514.
- Roe, J. H., 1964. Chemical determination of ascorbic, dehydroascorbic and diketogluconic acids. *Methods of Biochemical Analysis I.* (Ed. D. Glick). Interscience Publishers, New York., PP. 115-139.
- Sairam, R.K. and G.C. Srivastava., 1984. Physiological studies on seed setting in sunflower II. Photorespiration. *Indian J. Plt. Physiol.*, XVII. No. 3: 276-280.
- Sinha, S.K. 1965. Carbon dioxide fixation by the germinating sunflower cotyledons. *Indian J.Plant Physiol.*, 8:111-117.
- Splittstoesser, W.E. 1966. Dark CO₂ fixation and its role in the growth of plant tissue. *Plant Physiol.*, 41:755-759.
- Ting, I.P. and W.M. Dugger., 1968. Non-autotrophic carbon dioxide metabolism in Cacti. *Bot. Gaz.*, 129: 9-15.
- Tolbert, N.E., A. Olser, R.K. Yamazaki, R.H. Hageman and T. Kasaki., 1969. A survey of plants for leaf peroxysomes. *Plant Physiol.*, 44: 135-147.
- Tolbert, N.E., A. Olser, T. Kasaki, R.H. Hageman and R.K. Yamazaki., 1968. Peroxysomes from spinach leaves containing enzymes related to glycolate metabolism. *J. Biol. Chem.*, 243: 5179-5184.
- Trebst, A., 1972. Measurement of Hill reaction and photoreduction. *Methods Enzymol.*, 24: 146-165.
- Wells, R., L.L. Schulze, D.A. Ashley, H. R. Boerma and R.H. Brown., 1982. Cultivar differences in canopy apparent photosynthesis and their relationship to seed yield in soybeans. *Crop Sci.*, 22: 886-890.
- Yokayama, K., 1956. The relation of catalase activity to mitochondrial respiration. *Proc. Plant Physiol. Meetings Respiratory metabolism.* P. XXVI.
- Zelitch, I., 1959. The relationship of glycolic acid to respiration and photosynthesis in tobacco leaves. *J. Biol. Chem.*, 234: 3077-3081.
- Zelitch, I., 1966. Increased rates of net photosynthetic CO₂ uptake caused by the inhibition of glycolate oxidase. *Plant Physiol.*, 41: 1623-1631.

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***Corresponding Author:**

B. Sujatha*

Email: sujathaau@yahoo.co.in