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Research Article

NITROGEN METABOLISM AND NITRATE REDUCTASE ACTIVITY IN DIFFERENT PARTS AT THREE PHASES OF CAJANUS CAJAN (L.) GENOTYPES

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ABSTRACT

In the present study 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, on the total nitrogen, soluble nitrogen, insoluble nitrogen, total protein and nitrate reductase activity in the whole plants, leaves, stems and roots of the pigeonpea genotypes at three different phases of crop growth i.e. vegetative, flowering and seed maturation phase. The total nitrogen, insoluble nitrogen and protein content of the stems and roots showed an increasing trend from the vegetative to the seed maturation phase in all the pigeonpea genotypes. Among the different parts studied the leaf recorded higher values of total nitrogen, insoluble nitrogen and protein content than the stems and roots. The soluble nitrogen content of the whole plant, stems and roots exhibited an increase from the vegetative to the flowering phase and declined at the seed maturation phase and the leaf exhibited a decreasing trend towards the seed maturation phase. Maximum value of total nitrogen exhibited in ICPL87 at the vegetative phase and maximum amount of insoluble nitrogen exhibited in PDM1 at the flowering phase in crop growth. At the vegetative phase ICPL87 of short duration genotypes exhibited higher quantity of protein content in the whole plant and total leaves. A continuous decrease of nitrate reductase activity was observed in all the genotypes studied, except in the ICPL87 of short duration group recorded highest nitrate reductase activity.

INTRODUCTION

The importance of nitrogen metabolism in growth, dry matter accumulation and yield of legume crop plants has been well documented (Herridge and Pate, 1977; Pate and Herridge, 1978; Rao *et al*., 1984; Cure *et al*., 1985; Salado Navarro *et al*., 1985; Sinclair, 1986; Leffel *et al*., 1992). Grain legumes accumulate large quantity of nitrogen in seeds. It has been suggested that leaf nitrogen is remobilised for seed development and thus causes a decline in photosynthesis and enhances leaf senescence (Sinclair and Dewitt, 1975, 1976). Analysis of source and sink relationship in mungbean revealed that sink itself was instrumental in hastening the decrease in photosynthesis and leaf senescence by affecting directly mobilisation and utilization of leaf nitrogen (Rao and Ghildiyal, 1985). Nitrogen therefore, seems to have an important role in source-sink relationship of grain legumes. The shoot nitrogen content closely related to the shoot biomass accumulation. However, the rate of nitrogen accumulation declines during pod or seed growth of pigeonpeas (Sheldrake and Narayanan, 1979; Dalal, 1980; Rao *et al*., 1984; Devries, 1986). Khanna-Chopra and Sinha (1980) also reported the relationship between nitrogen accumulation and dry matter accumulation during growth and development of field grown pigeonpea. Nandawal *et al*. (1992) reported that the partitioning of nitrogen was dependent on the growth habit, nitrogen fixation rate and differences in leaf water potential of pigeonpea genotypes. The experimental data of Lawn and Troedson (1990) indicated that 28 to 56 per cent of the nitrogen accumulated by pigeonpea shoots is recovered in the seeds. The recovery of nitrogen is less in long duration crops, presumably because of the relatively smaller harvest index in such crops and the relatively greater amount of nitrogen immobilised in stems, roots and fallen leaves, in pigeonpea (Kumar Rao and Dart, 1987). Remobilization of the nitrogen from the leaves can account for a major proportion of the nitrogen required for seed protein (Sheldrake and Narayanan, 1979; Kumar Rao and Dart, 1987).

The utilization of the soil nitrogen varies with the stage of crop growth (Russel, 1973). There are evidences in favour and against the suggestion that nitrogen uptake ceases subsequent to the onset of reproduction. Nitrate is the major form of

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nitrogen available to the plants and the enzyme nitrate reductase catalyses the reduction of nitrate leading to the availability of ammonical form of nitrogen for incorporation into amino acids and proteins (Evans and Nason, 1953; Beevers *et al*., 1965).

The process of nitrate reduction is believed to be the rate limiting step in protein synthesis as shown by Beevers and Hageman (1969) and Klepper *et al*., (1971). Dykstra (1974) however has suggested that nitrate reductase activity should not be considered as an index of nitrogen assimilation though it may be a useful index for growth potential when nitrate is not a limiting factor for enzyme induction. A close relationship between nitrate reductase activity and dry matter accumulation in grasses and cereals was shown by Good man *et al*., (1974). Since nitrate reductase is a substrate inducible enzyme (Beevers *et al*., 1965), its level is regulated, within limits by the levels of nitrate in the plant tissue (Croy and Hageman, 1970). This was substantiated by the positive relation between nitrate content and nitrate reductase activity in leaf tissue of wheat (Elrich and Hageman, 1973) and soybean (Liu and Hadley, 1971). Grain protein has also shown a positive correlation with leaf nitrate reductase activity in corn leaves (Deckard *et al*., 1973) and wheat leaves (Elrich and Hageman, 1973).

Genotypic variations in nitrate reductase activity has been reported for several crops viz., wheat (Rao *et al*., 1977; Nair and Abrol, 1982), sunflower (Deshmukh and Srivastava,1983), sugarcane (Solomon *et al*., 1987 and Kaur *et al*., 1992) finger-millet (Nataraju *et al*., 1990) and other higher plants (Naik *et al*., 1982). Genotypic differences, in their ability to accumulate the reduced nitrogen during growth has been attributed to differences in nitrate reductase activity (Elrich and Hageman, 1973; Rao *et al*., 1977; and Naik *et al*., 1982). Ved Prakash (1981) examined the relationship between nitrate reductase activity and protein content of grains of high and low protein lines of wheat. He found that the pattern of nitrate reductase activity observed at different growth stages was mostly similar in all the cultivars but the degree of activity was strikingly different. Enzyme activity was noticed to be high in high protein and low in low protein lines. Dechard *et al*., (1973) noted in corn the highest correlation between nitrate reductase activity of the leaves and grain yield as well as protein content during the ear initiation and its developmental stages. Nitrate reductase activity in relation to yield in sunflower was studied by Deshmukh and Srivastava (1983). They noticed that nitrate reductase activity at pre-flowering stage has significant positive correlation with seed yield under normal conditions. Sai Ram and Singh (1989) found that nitrogen conversion efficiency (NCE) had high positive correlation with nitrate reductase activity in barley and wheat genotypes. However, the information regarding nitrate reductase activity among genotypes in pigeonpea was almost meagre when compared to other crop plants. An understanding of the nitrogen compounds and their utilization in different genotypes are necessary to develop selection criteria for use in the breeding for higher yields of protein content. In the present work it is intended to find out the nitrogen metabolism, protein content and nitrate reductase activity on different genotypes of pigeonpea.

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° 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.

Total Nitrogen

Total nitrogen was determined according to the method of Markham (1942). One gram of dried and powdered material was taken in a 25 ml micro-kjeldhal flask taking care not to all the material to stick to the sides of the flask. One gram of catalyst (a mixture of 1 g copper sulphate, 9 g potassium sulphate and 1 g selenium dioxide) was added for aiding digestion. Three ml of nitrogen-free analar sulphuric acid and 1 ml of hydrogen peroxide were also added to the sample and it was digested on a hot plate until a clear colourless solution was obtained. The volume of the solution was made up to 25 ml in a volumetric flask after digestion. Blank with reagents alone was also carried out simultaneously. Five ml of the aliquot of the digest was transferred to the distillation unit and 10 ml of 40% sodium hydroxide was added. This solution was distilled water for 20 min in the microkhjeldhal distillation apparatus. The ammonia liberated was absorbed into 2 ml of boric acid indicator mixture kept below in a conical flask. The completion of the distillation was recognized by the change in pH of the indicator in the receiver. The indicator solution was pink in the beginning and turned green at the end of complete distillation. The solution containing the indicator was titrated against N/100 HCl until pink colour appears. The amount of nitrogen present in the sample was calculated thus:

1ml of N/100 HCl = 0.14 mg of nitrogen.

Preparation of Boric Acid Indicator Mixture

The boric acid indicator mixture was prepared by mixing 10 g of boric acid 200 ml of absolute alcohol and 20 ml of indicator solution (indicator solution was prepared by mixing 0.033 g of bromocresol green and 0.666 g of methyl red in 100 ml of absolute alcohol) in a litre flask and the final volume was made to 1 litre with distilled water. The pH of the solution was then adjusted to 5.0 to 5.1.

Soluble Nitrogen

Soluble nitrogen was recorded as the difference between the total nitrogen and the TCA insoluble nitrogen.

Insoluble Nitrogen and Total Protein

The plant material was macerated with 15 ml of 10% trichloroacetic acid (TCA) at 4° C and centrifuged at 2000 xg for 30 minutes. The precipitate was washed with 5 ml of 10% TCA was added and centrifuged. To the precipitate 5 ml of 10% TCA was added and it was then incubated for 30 minutes at 80° C to remove the nucleic acids and centrifuged. The nitrogen content of the precipitate obtained was determined as described for total nitrogen. Total protein was estimated as 6.25 times the insoluble nitrogen (Steward, 1960).

Nitrate Reductase Activity (E.C.1.6.6.1)

Nitrate reductase activity of the 10th leaf of all the 12 genotypes were estimated according to the method of Jaworski (1971) as modified by Dykstra (1974). Five hundred mg of fresh leaves were cut into the fine pieces and placed in a 5 ml of incubation medium consisting of 0.1 M K_2HPO_4, 0.2 M KNO_3, 0.5% W/V PVP (polyvinyl pyrrolidone) soluble and 5% isopropanol having pH of 7.5. The material along with incubation medium was kept in dark for 2 h at room temperature. Then the reaction was stopped by adding 1 ml of 0.02% (w/v) N,1-napthylethylenediamine-2HCl and 1 ml of 1.0% (w/v) sulphanilic acid in 1.5 N HCl. After 20 min the absorbance of the solution was read at 540 nm on ECIL'S Junior spectrophotometer GS 866C. The nitrate reductase activity was expressed as µ moles of nitrite formed per g tissue per hour. The standard curve was prepared by using analar $NaNO_2$.

RESULTS

Total Nitrogen

The total nitrogen content of the whole plants, leaves, stems and roots of the pigeonpea genotypes studied were presented in figures 1a, b; 2a, b; 3a, b and 4a, b. Total nitrogen content of the whole plants showed a gradual decrease from the vegetative phase to the seed maturation phase. The nitrogen content of the plant attained a maximum value at the vegetative phase in all the genotypes. The total nitrogen content varied from 3.16 to 6.21 g/plant among all the genotypes; in which Pusa agheti recorded the maximum value at the vegetative phase of crop growth (Fig.1a). On unit dry weight basis the total nitrogen content at the vegetative phase varied from 24.42 to 19.75 mg/g dry wt among the genotypes. The genotype ICPL87 showed maximum values at the vegetative phase.

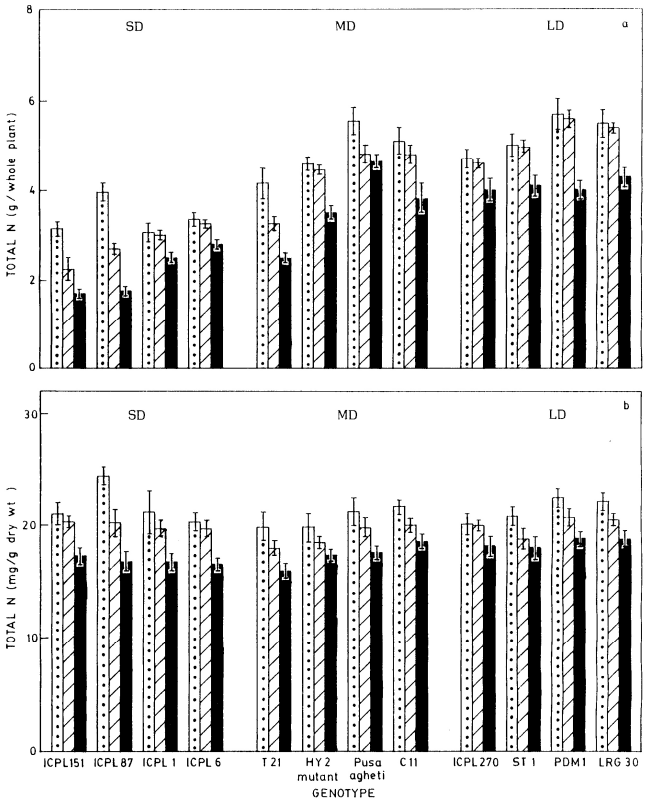

Fig 1 Total nitrogen content of the whole plant during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

The total nitrogen content of total leaves also exhibited a trend similar to that of the whole plant total nitrogen content. Among all the genotypes, the Pusa agheti and ICPL87 exhibited highest values on per part basis and per unit dry weight basis respectively at the vegetative phase of crop growth (Fig. 2a, b).

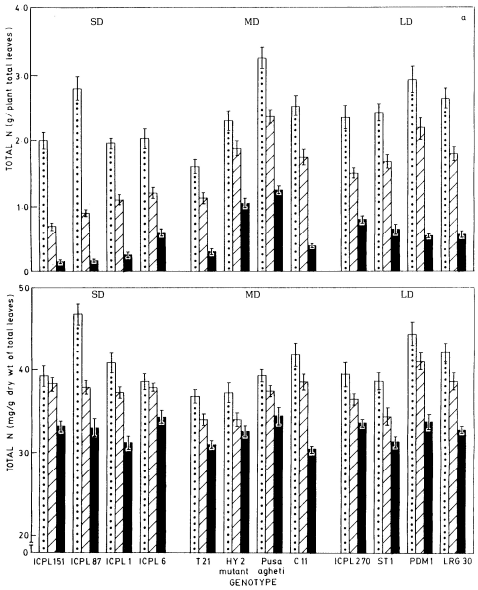

Fig 2 Total nitrogen content of the plant total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

The total nitrogen content of the stems and roots showed a gradual increase on per part basis up to the seed maturation phase (Figs. 3a and 4a). On the other hand, on unit dry weight basis it showed a gradual decrease from the vegetative phase to the seed maturation phase (Figs. 3b and 4b). On per part basis total nitrogen content of stems varied from 1.61 to 3.06 g at the seed maturation phase. Interestingly the stems of the medium and long duration genotypes exhibited greater values than the short duration genotypes (Fig. 3a). The total nitrogen content of stems of the PDM1 of the long duration and the ICPL151 of the short duration genotypes showed the maximum and minimum values respectively at the seed maturation phase. On per unit dry weight basis the total nitrogen content of the stems showed a range of values from 14.20 to 11.50 mg/g dry wt. The stems of ICPL87 of the short duration genotypes exhibited highest values of total nitrogen content (Fig. 3b).

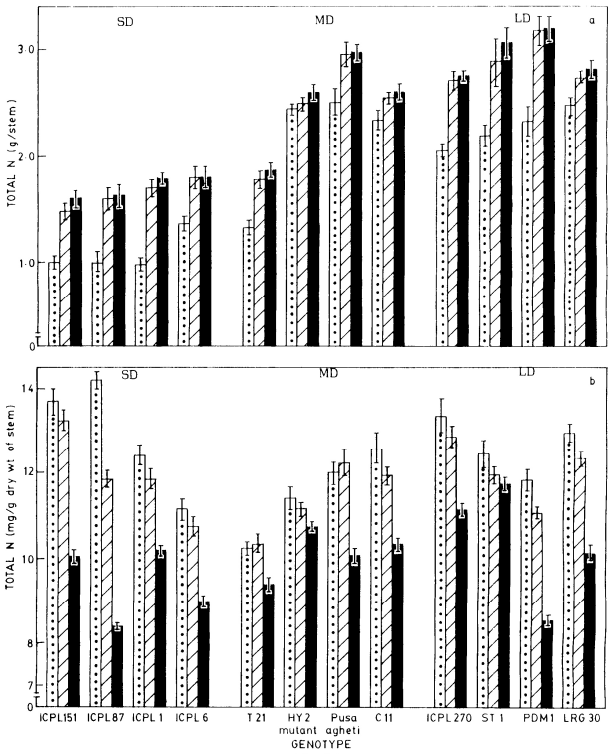

Fig 3 Total nitrogen content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

The total nitrogen content of the roots varied from 0.24 to 0.47 g reaching the maximum values on per part basis at the seed maturation phase. Among the genotypes studied the PDM1 of long duration group exhibited greatest value. Again, medium and long duration genotypes showed greater values than short duration genotypes (Fig. 4a). On per unit dry weight basis maximum values of total nitrogen were recorded at the vegetative phase in which the ICPL87 exhibited maximum value of 12.25 mg/g dry wt (Fig. 4b). Among the different parts studied the leaves showed greater values of total nitrogen content.

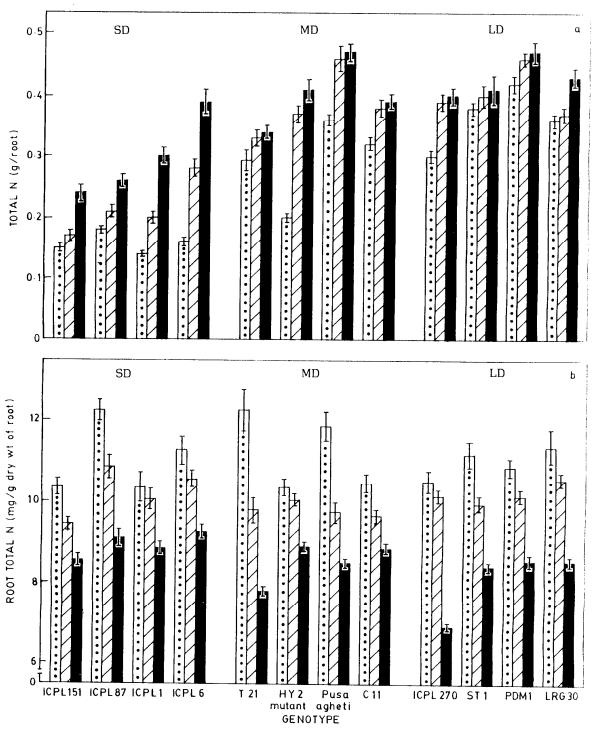

Fig 4 Total nitrogen content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

Soluble Nitrogen

Among the genotypes studied, the whole plant soluble nitrogen content showed a gradual increase from the vegetative to flowering phase followed by a decline at the seed maturation phase. The long duration genotypes exhibited higher values than the medium and short duration genotypes (Fig. 5a). The soluble nitrogen content on unit dry weight basis also exhibited a trend similar to that observed for per whole plant basis in all the genotypes studied (Fig.5b).

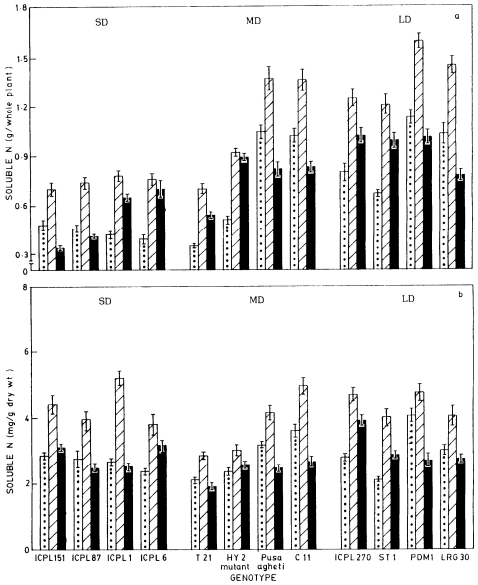

Fig 5 Soluble nitrogen content of the whole plant during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

The genotypic variation, of the soluble nitrogen content of the plant total leaves during the crop growth of pigeonpea was shown in the figure 6a,b. The soluble nitrogen content of the total leaves of a given genotype remained more or less constant till the flowering phase followed by a sharp decrease at the seed maturation phase. However, in the ICPL1, ICPL6 of short duration, the HY2 mutant of medium duration and the ICPL270 and ST1 of long duration genotypes showed some slight increase up to the flowering phase followed by a decrease at the seed maturation phase (Fig.6a). Among all the genotypes studied the long duration genotypes recorded greater values. Again among the long duration genotypes the PDM1 recorded the greatest amount at the vegetative and flowering phases of crop growth. On unit dry weight basis, there was a continuous increase of soluble nitrogen content of plant total leaves up to the flowering phase followed by a decrease at the seed maturation phase in all the genotypes. Among the genotypes studied the C11 of medium duration genotype exhibited the maximum value of 8.27 mg/g dry wt at the flowering phase of the crop growth (Fig. 6b).

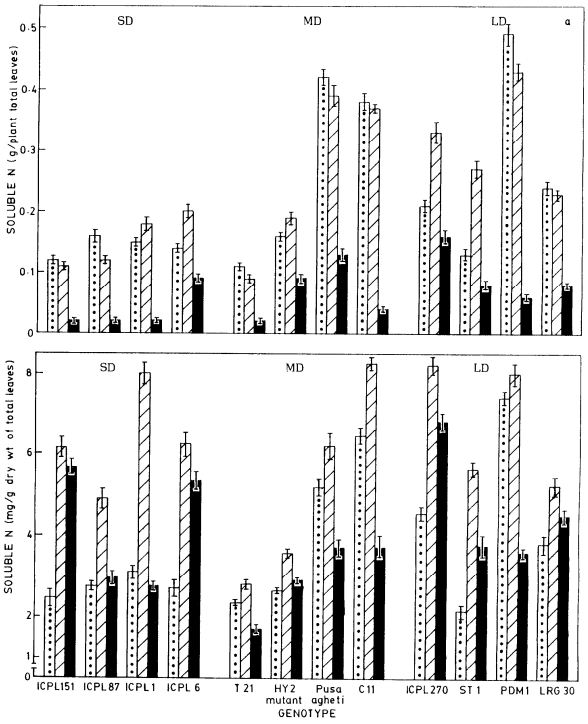

Fig 6 Soluble nitrogen content of the plant total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

A gradual increase of stem soluble nitrogen content from vegetative phase to the flowering phase followed by a decrease at the seed maturation phase was observed in all the genotypes on both the types of expressions i.e., on per stem and on per unit dry weight basis. Among the genotypes studied, the medium and long duration types showed higher values than the short duration genotypes (Fig. 7a). Among all the genotypes studied the PDM1 (1.06 g/stem) exhibited the maximum amount at the flowering phase of crop growth of pigeonpea. On unit dry weight basis, the ICPL87 (4.20 mg/g dry wt) of short duration genotypes exhibited the highest amount of soluble nitrogen content when compared to all other genotypes at the vegetative phase of crop growth of pigeonpea (Fig.7b).

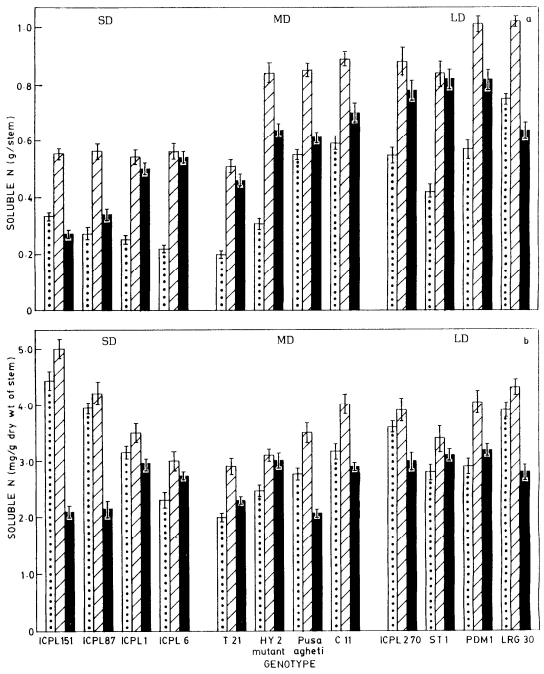

Fig 7 Soluble nitrogen content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

Soluble nitrogen content of the roots, on both the expressions followed a trend similar to that of the stems in all the genotypes studied. On per part as well as on unit dry weight bases the ST1 of the long duration genotypes recorded greatest values at the flowering phase among all the genotypes studied (Fig. 8a, b). Among the different parts studied the total leaves exhibited higher soluble nitrogen content of the plant followed by stems and roots.

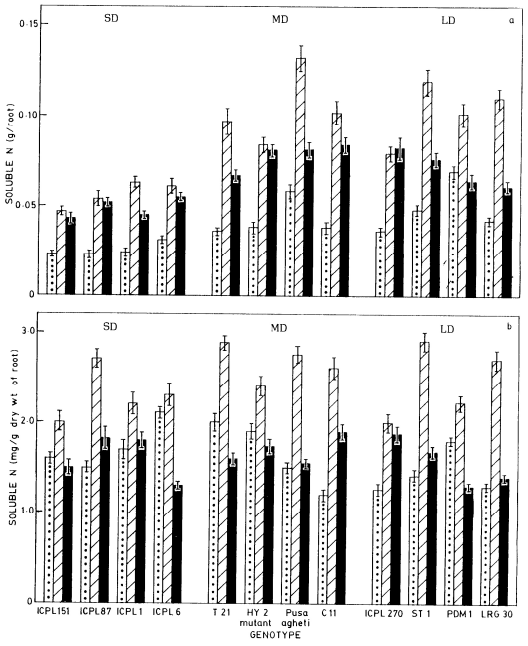

Fig 8 Soluble nitrogen content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

In soluble Nitrogen

Changes in the whole plant insoluble nitrogen content during the crop growth of all the 12 genotypes showed a gradual decrease from the vegetative to the seed maturation phase on both the expressions (Fig. 9a, b). However, on whole plant basis the medium and long duration genotypes exhibited higher values than the short duration genotypes. On unit dry weight basis, the ICPL87 of short duration genotypes exhibited the maximum value of 21.7 mg/g dry wt at the vegetative phase over the rest of the genotypes.

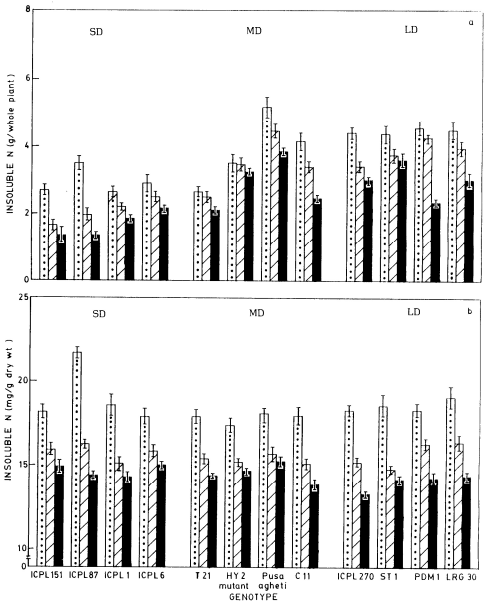

Fig 9 Insoluble nitrogen content of the whole plant during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

The insoluble nitrogen content of the total leaves also exhibited a gradual decrease on per part and on per unit dry weight bases in all the genotypes studied (Fig.10a, b).

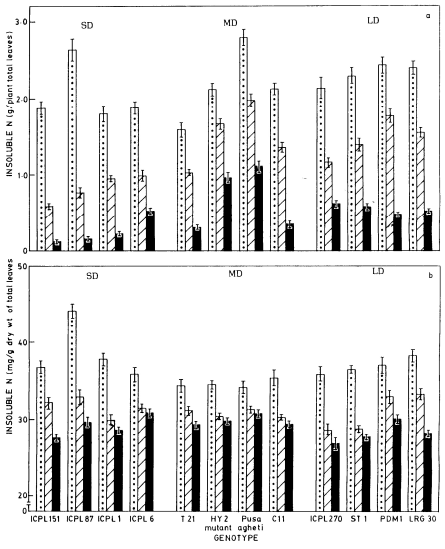

Fig 10 Insoluble nitrogen content of the plant total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

Among the genotypes studied, the ICPL87 recorded the highest amount of insoluble nitrogen content on both the expressions. The insoluble nitrogen content on per stem and root showed a gradual increase upto the seed maturation phase in all the genotypes studied (Fig.11a and 12a). The medium and long duration genotypes recorded greater values than the short duration genotypes. On the other hand, on per unit dry weight basis both the stems and roots showed a gradual decrease of insoluble nitrogen content from the vegetative phase to the seed maturation phase (Fig.11b and 12b). Out of all the genotypes studied, the PDM1 of long duration and ICPL87 of short duration recorded greater values on per part and on per part unit dry weight bases respectively both in the stems and roots. Among the different parts studied, the leaves recorded greater values than the stem and root.

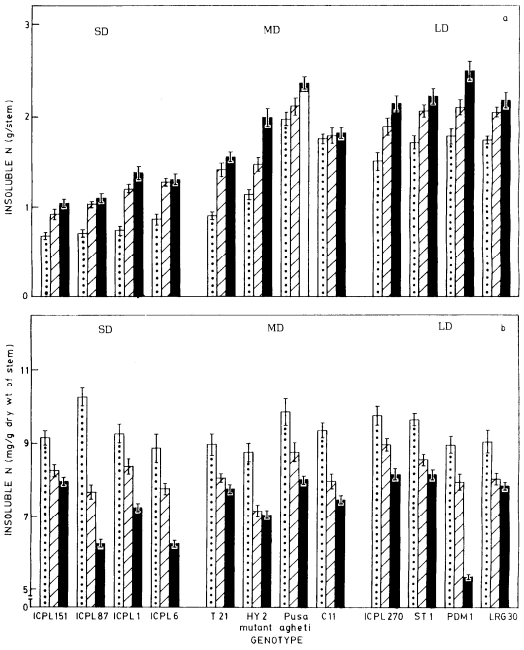

Fig 11 Insoluble nitrogen content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

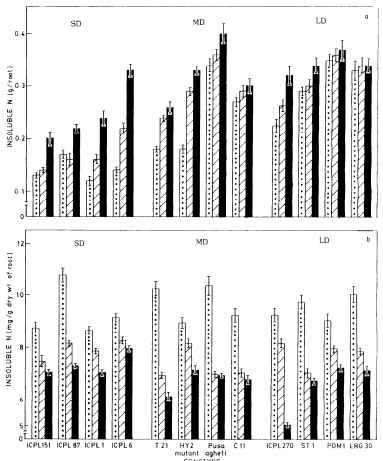

Fig 12 Insoluble nitrogen content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

Total Protein

A decline in the protein content of the whole plant and its total leaves was observed with increasing crop age in all the pigeonpea genotypes studied (Fig.13a, b and 14a, b). On per plant basis, both the whole plant and total leaves of medium

and long duration genotypes recorded greater values than short duration genotypes. The protein content of the whole plant of the ICPL87 (21.97 g/plant) of short duration, the Pusa agheti (32.37 g/plant) of medium duration and the PDM1 (28.54 g/plant) of long duration genotypes expressed greater values in their respective groups at the vegetative phase (Fig.13a). The protein content of the leaves of ICPL87 (16.48 g/plant total leaves), Pusa agheti (17.53 g/plant total leaves) and PDM1 (15.28 g/plant total leaves) of short, medium and long duration genotypes respectively recorded greater values at vegetative phase in their respective groups (Fig. 14a). On the other hand, on per unit dry weight basis the ICPL87 of short duration genotypes exhibited higher quantity of protein content in the whole plant and plant total leaves at the vegetative phase of crop growth (Fig. 13b and14b).

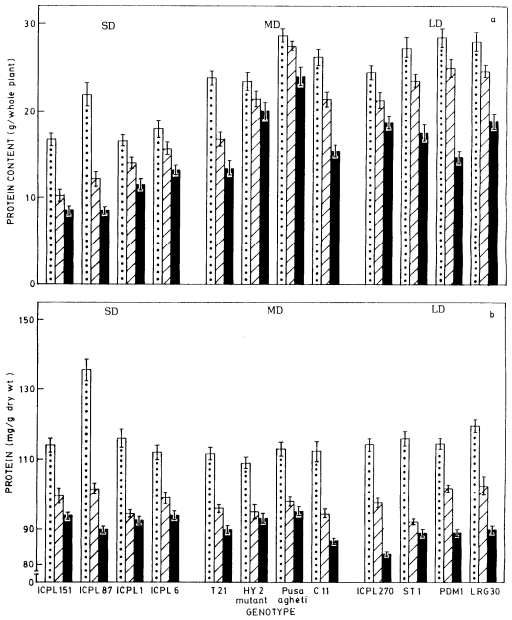

Fig 13 Protein content of the whole plants during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase, Flowering phase, Seed maturation phase

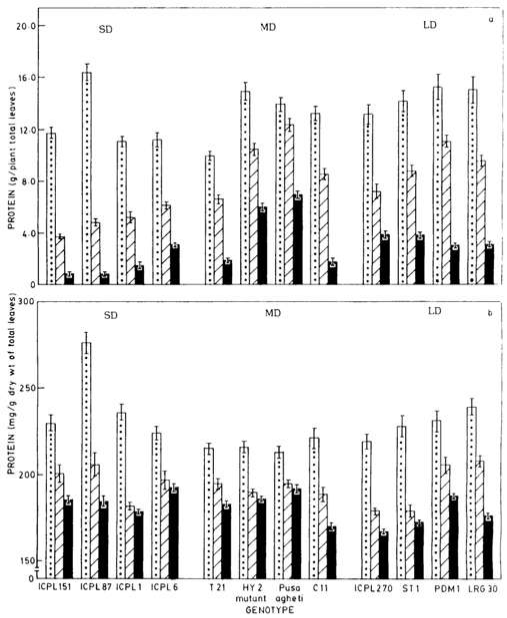

Fig 14 Protein content of the plant total leaves during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase, Flowering phase, Seed maturation phase

Protein content of the stem and root expressed on per part basis followed a slight increasing trend until the seed maturation phase in all the genotypes studied (Fig.15a and 16a). The long duration genotypes exhibited higher values of total protein content than the short duration genotypes in both the stems and roots. Among the total genotypes the PDM1 of long duration and the Pusa agheti of medium duration exhibited higher values at the seed maturation phase. The protein content on unit dry weight basis showed a gradual decrease from the vegetative to the seed maturation phase in all the 12 genotypes studied. The ICPL87 expressed the higher values of protein content in both the stems and roots of all the genotypes studied (Fig.15b and 16b).

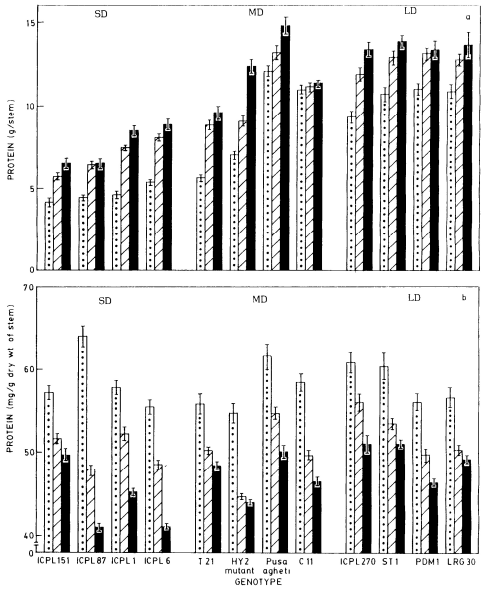

Fig 15 Protein content of the stems during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase, Flowering phase, Seed maturation phase

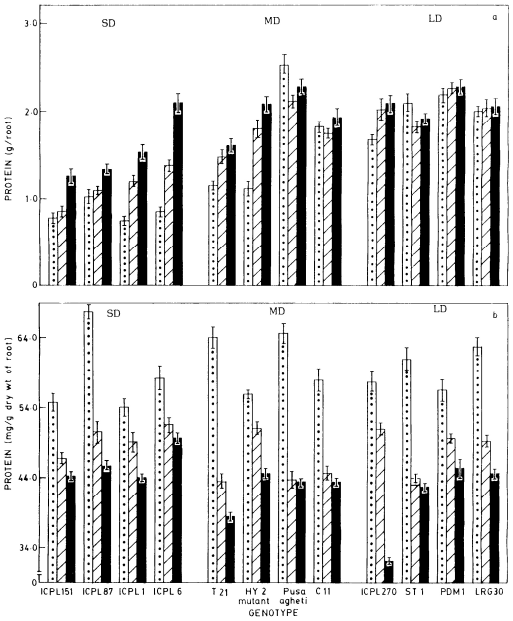

Fig 16 Protein content of the roots during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase, Flowering phase, Seed maturation phase

Nitrate Reductase Activity

Genotypic variation in relation to the nitrate reductase activity of the 10th leaf of pigeonpea was presented in Fig. 17a, b. On per part basis as well as on per unit fresh weight basis a continuous decrease of nitrate reductase activity was observed in all the genotypes studied. Among the genotypes studied, the ICPL87 (296.70 n moles/leaf and 259 n moles/g/fresh wt) recorded greater values throughout the crop growth of pigeonpea in both the expressions.

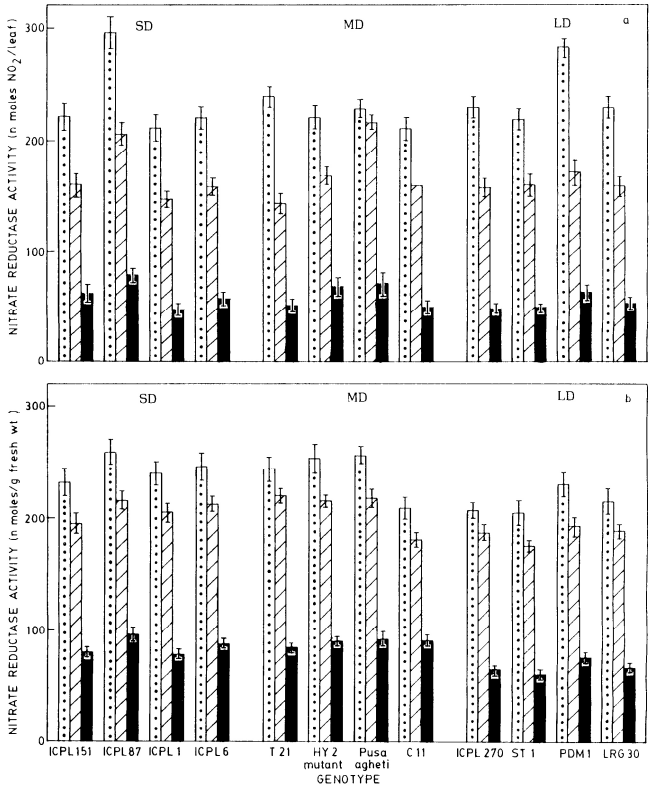

Fig 17 Nitrate reductase activity of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

Vegetative phase ; Flowering phase ; Seed maturation phase

Correlation Coefficients between leaf Total Nitrogen, leaf Protein, Nitrate Reductase Activity and Seed yield of Pigeonpea Genotypes

The leaf total nitrogen and leaf protein content showed a significant positive correlation with seed yield at vegetative phase and a negative correlation at later phases of crop growth in all the pigeonpea genotypes. The nitrate reductase activity showed a significant positive correlation at all phases of crop growth in the short duration pigeonpea genotypes. However, the medium and long duration genotypes exhibited a negative association of nitrate reductase activity with seed yield at the seed maturation phase of crop growth (Table-1).

Table 1 Correlation coefficients between leaf protein, total nitrogen and nitrate reductase activity and seed yield of pigeonpea genotypes

Yield	Vegetative phase	Flowering phase	Seed maturation phase
Short duration			
Leaf total nitrogen	0.998**	-0.234	-0.304
Leaf protein content	0.998**	-0.083	-0.307
NR activity	0.996**	0.998**	0.891**
Medium duration			
Leaf total nitrogen	0.506	-0.334	-0.973**
Leaf protein content	0.622*	-0.998**	-0.964**
NR activity	0.598	0.953**	0.933**
Long duration			
Leaf total nitrogen	0.793**	0.861**	-0.886**
Leaf protein content	0.938**	0.868**	-0.766**
NR activity	0.804**	0.773**	-0.907**

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

DISCUSSION

Nitrogen is an essential element contributing to the important biochemical constituents that are involved in the crop growth and yield formation. The pigeonpea genotypes studied exhibited a gradual decrease in the total nitrogen, insoluble nitrogen and protein content in the whole plant and total leaves throughout the crop growth (Figs. 1a, b; 2a, b; 9a, b; 10a, b; 13a, b; 14a, b). In our previous studies the decline in photosynthetic rate from vegetative to maturation phase may presumably related to the mobilization of nitrogen out of the senescencing leaves. Interestingly the leaf total nitrogen and protein contents showed linear relationship with photosynthetic rate at all phases of crop growth. The declines in leaf nitrogen content associated with photosynthetic rates were observed in soybean (Sinclair and Dewit, 1975; Boon-Long *et al*., 1983; Koch and Schrader, 1984; Salado-Navarro *et al*., 1985 and Leffal *et al*., 1992) and in mungbean (Rao and Ghildiyal, 1985).

The total nitrogen, insoluble nitrogen and protein content of the stems and roots showed an increasing trend from the vegetative to the seed maturation phase when expressed on per part basis in all the pigeonpea genotypes. An opposite decreasing trend was observed on unit dry weight basis in these parts (Figs. 3a, b; 4a, b; 11a, b; 12a, b; 15a, b; 16a, b). The long duration genotypes exhibited higher values of nitrogen content in the whole plants, stems and roots than the short duration genotypes. The recovery of nitrogen content in the seeds is less in long duration genotypes, presumably because of the relatively higher biomass accumulation and smaller harvest index. Thus, greater amount of nitrogen was immobilized and retained in the stems, roots and fallen leaves of the pigeonpea (Kumar Rao and Dart, 1987). We can conclude that partitioning of nitrogen towards seeds in the short duration genotypes was higher than the long duration pigeonpea genotypes. Higher dry matter accumulation leading to lower partitioning of nitrogen to grains and ultimately lower yields was reported in wheat genotypes (Nair and Abrol, 1979).

Among the different parts studied the leaf recorded higher values of total nitrogen, insoluble nitrogen and protein content than the stems and roots. The protein content of the per plant total leaves of the ICPL87 of the short duration genotypes recorded highest value commensurating with its high seed

protein content. Thus, remobilization of nitrogen from leaves can account for a major proportion of the nitrogen required for seed protein. Further, less vegetative competition in determinate (ICPL87) than indeterminate genotypes during reproductive development could result in greater quantities of available photosynthate for nodule maintenance and higher N fixation (Egli and Legette, 1973). Further, the earlier demand of sink strength in these short duration genotypes exhibited earlier leaf nitrogen depletion at the vegetative phase; faster nitrogen partition and dry matter allocation into seeds. High seed nitrogen demand in high seed- protein lines was reported in soybean (Leffel *et al*., 1992). Further, the ICPL87 of short duration pigeonpea genotypes also recorded higher values of leaf nitrogen and leaf protein content throughout the crop growth period. This ability to sustain higher leaf nitrogen and protein content until the late phases of reproduction might have contributed to higher seed yield in this genotype.

The soluble nitrogen content of the whole plant, stems and roots exhibited an increase from the vegetative to the flowering phase and declined at the seed maturation phase (Figs. 5a, b; 7a, b; 8a, b). The soluble nitrogen content of the leaf exhibited a decreasing trend towards the seed maturation phase on per plant total leaves basis and an opposite effect was seen when expressed on per unit dry weight basis (Fig. 6a, b). Leaf senescence prior to and during the flowering phase may be related to the nitrogen demand for reproductive growth. Further, the increase in soluble nitrogen content can be attributed to the increase in free amino acids derived from the breakdown of proteins at the flowering phase presumably for translocation to the developing seeds. In addition to the reduction of nitrate to ammonia, other factors markedly influence the amount of grain protein and the translocation of reduced nitrogen from the vegetative parts to the developing seed. Therefore, it becomes all the more important that a genotype must be able to make good use of the period preceding the onset of the process of senescence in terms of translocation and reduction of nitrate.

Genotypic variation in nitrate reductase activity under a given set of environmental conditions was noted in several crops (Rao *et al*., 1977; Naik *et al*., 1982; Nair and Abrol, 1982; Singh and Mahendra Singh, 1985; Soloman *et al*., 1987; Nataraju *et al*., 1990; Kaur *et al*., 1992). The nitrate reductase activity exhibited considerable variation among the pigeonpea genotypes both during early and later stages of crop growth. The maximum nitrate reductase activity of the 10^{th} leaf of pigeonpea genotypes were noticed at the vegetative phase followed by a decrease with advancing crop age (Fig.17a, b). The higher nitrate reductase activity at the vegetative phase may be attributed to the internal signals within the plants for making more of nitrate available for reduction. The nitrate reductase activity also declined rapidly during the pod filling stage indicating that the capacity of the plants to use nitrate diminishes at this phase of development in soybean (Harper, 1974). Of all the genotypes studied, the ICPL87 of short duration group recorded highest nitrate reductase activity throughout the crop growth period commensurating with the higher seed protein content and higher grain yields. This may be attributed to the capacity of the plant to take up and utilize the soil nitrate in an efficient manner. The nitrate reductase activity showed a significant positive correlation with the seed yield at all the stages of crop growth in the short duration pigeonpea genotypes. In the medium and long duration genotypes the nitrate reductase activity exhibited positive correlation at the vegetative phase only (Table-1). Further, the nitrate reductase activity showed a linear relationship with the leaf total nitrogen content and leaf protein content at the vegetative phase of crop growth. Therefore, it was noticed that nitrate reductase activity during early reproductive growth of the crop is important and bears a positive correlation with seed yield. However, this does not exclude the significance of nitrate reductase activity at the later part of crop growth of pigeonpea. The positive correlation of nitrate reductase activity at preflowering stage with seed yield was reported in wheat (Elrich and Hageman, 1973) in corn (Dechard *et al*., 1973) in grasses and cereals (Goodman *et al*., 1974) and in sunflower genotypes (Deshmukh and Srivastava, 1983). The nitrate reductase activity also showed a linear relationship with seed protein content in pigeonpea genotypes. The high nitrate reductase activity associated with the high grain protein lines was reported in wheat (Croy and Hageman, 1970; Nair and Abrol, 1982).

CONCLUSION

A gradual decrease in the total nitrogen, insoluble nitrogen and protein content of the whole plants and total leaves of all the pigeonpea genotypes were observed with advancing crop age. The total nitrogen, insoluble nitrogen and protein contents of the stems and roots showed an increase from the vegetative phase to the maturation phase when expressed on per part basis in all the pigeonpea genotypes. The soluble nitrogen of the whole plant stems and roots exhibited an increase from the vegetative to the flowering phase followed by a decline at the seed maturation phase in all the genotypes. The leaves of all the genotypes recorded higher values of soluble nitrogen content followed by the stems and roots. The nitrate reductase activity decreased from the vegetative phase with advancing crop age in all the pigeonpea genotypes. Throughout the crop growth period, the ICPL87 of short duration genotypes recorded higher values of nitrate reductase activity.

References

Beevers, L., LC. Schrader, D. Flesher and RH. Hageman, 1965. The role of light in the induction of nitrate reductase in radish cotyledons and maize seedlings. *P1. Physiol.,* 40: 691-698.

Beevers, L and RH. Hageman., 1969. Nitrate reduction in higher plants. *Ann. Rev. P1. Physiol.,* 20: 495-522.

Boon-Long, P., DB. Egli and JE. Leggett, 1983. Leaf N and photosynthesis during reproductive growth in soybeans. *Crop Sci.,* 23: 617-620.

Croy, LI. and RH. Hageman, 1970. Relationship of NR activity to grain protein in wheat. *Crop. Sci.,* 10: 280-285.

Cure, JD., CD. Raper, RP. Patterson and WP. Robarge, 1985. Dinitrogen fixation in soybean in response to leaf water stress and seed growth rate. *Crop Sci.,* 25: 52-58.

Dalal, RC., 1980. Nutrient element concentration and dry matter production by field grown Pigeonpea (*Cajanus cajan (L.) Millsp*.). *Tropical Agriculture (Trinidad),* 57: 107-112.

Dechard, EL., RJ. Lambert and RH. Hageman, 1973. Nitrate reductase activity in corn leaves as related to yield of grain and grain protein. *Crop Sci.,* 13: 343-346.

Deckard, EL., RJ. Lambert and RH. Hageman, 1973. Nitrate reductase activity in corn leaves as related to yield of grain and grain protein. *Crop Sci.,* 13: 343-346.

Deshmukh, PS and GC. Srivastava, 1983. Nitrate reductase activity in relation to yield in sunflower. *Indian J. Plant Physiol.,* Vol. XXVI, No. 2. Pp. 143-147.

Devries, JD., 1986. Water relations and nitrogen fixation of three grain legumes during water stress. M.Sc. Thesis, University of Florida, U.S.A. 107 pp.

Dykstra, GF., 1974. Nitrate reductase activity and protein concentration of two Populus clones. *Plant Physiol.,* 53: 632-634.

Egli, DB and JE. Legette, 1973. Dry matter accumulation patterns in determinate and indeterminate soybeans. *Crop Sci.,* 13: 220-222.

Eilrich, GL and RH. Hageman, 1973. Nitrate reductase activity and its relationship to accumulation of vegetative and grain nitrogen in wheat. *Crop Sci.,* 13: 59-65.

Evans, HJ and A. Nason, 1953. Pyridine nucleotide nitrate reductase from extracts of higher plants. *P1. Physiol.,* 28: 233-254.

Goodman, PJ., M. Fothergill and DM. Haugues, 1974. Variation in nitrate reductase and nitrite reductase in some grasses and cereals. *Ann. Bot.,* 38: 31-37.

Harper, JE., 1974. Soil and symbiotic nitrogen requirements for optimum soybean production. *Crop. Sci.,* 14: 255-260.

Herridge, DF and JS Pate., 1977. Utilization of net photosynthate for nitrogen fixation and protein production in an annual legume. *Ibid. 60:* 759-764.

Jaworski, EG., 1971. Nitrate reductase assay in intact plant tissue. *Biochem. Biophys. Res. Commun.,* 43: 1274-1279.

Kaur, S., RS. Kanwar and O. Singh, 1992. Seasonal variation in nitrate reductase activity and tillering in sugarcane genotypes. *Indian J. Plant Physiol.* XXXV(2): 162-166.

Khanna-Chopra, R and SK. Sinha, 1980. Allantoin content and Nitrogen Accumulation in Relation to Dry Matter Accumulation and Yield in *Cajanus cajan* cv. Prabhat. In: Proceedings of the International Workshop on pigeonpeas, Vol. 2, 15-19 December, Patancheru, Hyderabad, India, ICRISAT, 397-401.

Klepper, L., D. Flesher and RH. Hageman, 1971. Generation of reduced nicotinamide adenine dinucleotide for nitrate reductase in green leaves. *P1. Physiol.,* 48: 580-590.

Kumar Rao, JVDK and Dart, PJ., 1987. Nodulation, nitrogen fixation and nitrogen uptake in Pigeonpea (*Cajanus cajan (L.)* Millsp.) of different maturity groups. *Plant and Soil,* 99: 255-256.

Koch, KE and LE. Schrader, 1984. ^{14}C-Photosynthate partitioning and translocation in soybeans during reproductive development. *Plant Physiol.,* 75: 1040-1043.

Leffel, RC., PB. Cregan, AP. Bolgiano and DJ. Thibeau, 1992. Nitrogen metabolism of normal and high-seed protein soybean. *Crop. Sci.,* 32: 747-750.

Lawn, RJ and RJ. Troedson, 1990. Pigeonpea: Physiology of yield formation. In: The Pigeonpea, eds., Nene, Y.L., Hall S.D., Sheila V.K. and C.A.B. International, Wallingford, Oxon OX10 8D, UK and ICRISAT, Patancheru, A. P.

Liu, MC and HH. Hadley, 1971. Relationships of nitrate reductase activity to protein content in related nodulating and non-nodulating soybeans. *Crop. Sci.,* 11: 467-471.

Markham, R., 1942. A steam distillation apparatus suitable for micro-kjeldhal analysis. *Biochem. J.,* 36: 790-791.

Nair, TVR and YP. Abrol, 1979. Physiological studies on the leaf blades of wheat (*Triticum aestivum* L.) in relation to nitrate assimilation and grain protein accumulation. *J. Agric. Sci.,* (Camb): 93: 473-484.

Nair, TVR and YP. Abrol, 1982. Nitrate reductase activity in the flag leaf blade and its relationship to protein content and grain yield in wheat. *Indian J. Plant Physiol.,* 25: 111-112.

Naik, MS., YP. Abrol, TVR. Nair and CS. Rama Rao, 1982. Nitrate assimilation, its regulation and relationship to reduced nitrogen in higher plants. *Phytochemistry,* 21(3): 495-504.

Nandawal, AS., S. Bharti, I. Singh and BS. Kundu, 1992. Nitrogen partitioning and its efficiency in pigeonpea under drought and during recovery. *Indian J. Plant Physiol.,* XXXV (4): 364-370.

Nataraju, SP., M. Udaykumar, BR. Gurumurthy and K. Umesha, 1990. Genotypic variations in Nitrate reductase activity, Nitrate flux and reducing sugars in Finger millet (*Eleucine coracana Gaertn*). *Indian J. Plant Physiol.,* XXXIII (2): 145-149.

Pate, JDS and DF. Herridge, 1978. Partitioning and utilization of net photosynthates in an annual legume. *Ibid.* 29: 401-412.

Rao, KP., DW. Rains, CO. Qualset and RC. Huffafer, 1977. Nitrogen nutrition and grain protein in two spring genotypes differing in nitrate reductase activity. *Crop. Sci.,* 17: 283-286.

Rao, AS., YP. Luthra, IS. Sheoran and R. Singh, 1984. Partitioning of carbon and nitrogen during growth and development of Pigeonpea (*Cajanus cajan L.*). *J. Exp. Bot.,* 35(155): 774-784.

Rao, TR and MC. Ghildiyal, 1985. Analysis of photosynthetic source and sink relationship in mung bean [*Vigna radiata L.* (Wilczek)]. *Indian J. Plant Physiol.,* Vol. XXVIII No. 2 p. 135-144.

Russel, EW., 1973. The food of plants. In: Soil, conditions and plant growth. The Book Society and Longman, pp. 23-30.

Sai Ram, RK and SS. Sing, 1989. Screening of barley and wheat genotypes efficient in nitrogen uptake and conversion. *Indian J. Plant Physiol.,* XXXII(3): 244-247.

Salado-Navarro, LR., K. Hinson and TR. Sinclair, 1985. Nitrogen partitioning and dry matter allocation in soybean with different seed protein concentration. *Crop. Sci.,* 22: 451-455.

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

Sinclair, TR and CT. Dewitt, 1975. Photosynthate and nitrogen requirements for seed production by various crops. Science, 189: 565-567.

Sinclair, TR. and CT. Dewitt, 1976. Analysis of the carbon and nitrogen limitations to soybean yields. *Agron. J.,* 68: 319-324.

Singh VP and Mahendra Singh., 1985. Nitrate reductase activity and its relationship with Grain protein and grain

yield of wheat. *Indian J. Plant Physiol.,* Vol. XXVIII, No.3, pp.235-245.

Sinclair, TR., 1986. Water and nitrogen limitations in soybean grain production I. Model development. *Field Crop Res.,* 15: 125-141.

Solomon, S., K. Singh and S. Bhatnagar, 1987. Distribution of nitrate reductasev activity during tillering in sugarcane. *Indian J. Sugarcane Techn.,* 4: 132-136.

Steward, FC., 1960. In Plant Physiology. A Treatise, Vol. IVA Metabolism Organic nutrition and Nitrogen metabolism Academic Press, New York.

Ved Prakash, 1981. Study of Nitrate reductase activity in different protein lines of wheat during vegetative and grain development period. *Indian J. Plant* Physiol., Vol. XXIV pp. 283-290.

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