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Free Amino Acid and Remobilized Nitrogen Composition in Leaves of *Cajanus cajan* (L.) at Three Different Phases of Crop Growth

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Abstract

Twelve genotypes of pigeonpea (*Cajanus cajan* (L.) Millspaugh) which were divided into three groups based on the duration for flower initiation i.e. Short duration (ICPL151, ICPL87, ICPL1, ICPL6), Medium duration (T21, HY2 mutant, Pusa agheti, C11) and Long duration (ICPL270, ST1, PDM1, LRG30) were selected and was raised at the Experimental Farm of the Department of Botany, Andhra University, Waltair, Visakhapatnam, A.P., India for the present study on Total free amino acids, Free amino acid composition, Remobilized nitrogen, Nitrogen remobilization efficiency and Total nitrogen harvest index of the pigeonpea genotypes at three different phases of crop growth i.e. vegetative, flowering and seed maturation phase. Total free amino acid content in the genotypes the ICPL151 of short duration, the Pusa agheti of medium duration and the ST1 of long duration recorded higher values at the seed maturation phase in their respective groups. Quantitative differences in the free amino acid composition of different genotypes at the flowering phase was recorded the absence of cysteine and valine in all the medium duration, long duration and ICPL6 of short duration genotypes. Lysine was present in greater quantities in the short duration genotypes than in the medium and long duration genotypes, whereas methionine was absent in all long duration genotypes. Glutamic acid and proline were present in greater quantities in the medium and long duration genotypes when compared to the short duration genotypes. Maximum and minimum values of remobilized nitrogen, nitrogen remobilization efficiency and total nitrogen harvest index were recorded in the ICPL87 of short duration and the ST1 of long duration genotypes respectively.

Keywords
Free amino acid composition, genotypes, Remobilized nitrogen,Total nitrogen harvest index, pigeonpea.

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INTRODUCTION

In grain crops, the economically important part of the plant is the mature seed. Varietal differences in physiological and biochemical changes of developing pods and seeds have been described in a number of legumes such as urd bean (Anita Saha, 1987), mungbean (Dhillon and Nainawatee, 1990), bambarra ground nut (Sreeramulu *et al.*, 1992) and pigeonpea (Singh *et al.,* 1980; Balakrishnan *et al.*, 1984; Singh *et al.,* 1984). Proteins are the main constituents of many legume seeds. During the maturation of seeds, these constituents are stored in the cells of the cotyledons (Bewley and Black, 1983). Pigeonpea (*Cajanus cajan* (L.) Millspaugh), the material selected for the present investigation is the second most important pulse crop in India. The seeds of pigeonpea are rich in protein content (18 to 24%) and constitute principal source of vegetable protein to the people of India.

Nitrogen containing compounds play an important role in plant growth and development (Beevers, 1976; Bray, 1983). In addition to proteins, the nitrogen content of seed comprises certain amount of free amino acids, amides, amines, nucleic acids, alkaloids and other related substances. Ontogenic and age-dependent changes in protein and free amino acids have been reported in leaves (Mckee, 1958; Weinberger, 1975; Vianna and Metavier, 1980). Protein and amino acid metabolism of leaves in relation to flowering has been investigated in photoperiod sensitive *Sinapsis* (Kinet, 1975), *Xanthium* (Sherwood *et al*., 1971), Spinach (Bault and Greppin, 1976) and soybean (Housley *et al*., 1979). Day-neutral plants, especially those with axillary flowers, have been generally neglected in studies of leaf metabolism in relation to the flowering process. Nabeesa and Neelakandan (1984, 1985, 1987) reported the metabolism of free amino acids and proteins in the leaves and flower buds of *Abelmoschus esculentus* at different stages of growth.

Pandey and Pant (1979) reported the distribution of non-protein N, protein N and different protein fractions in early, medium and late maturing pigeonpea genotypes and concluded that the levels of different nitrogenous constituents could not be used to characterise the various genotypes. The seed protein content differed significantly among the pigeonpea genotypes (Singh and Eggum, 1984; Jain et al., 1986). Nevertheless, the information available on the nitrogen remobilization of pigeonpea in relation to flowering and yield formation is scanty. Therefore, the present study was undertaken in order to investigate the free amino acid composition, remobilized nitrogen, Nitrogen remobilization efficiency and total nitrogen harvest index in relation to the genotypic variation 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 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 farmyard manure and fertilizers were supplied at the rates shown in the following table:

Manure/Fertilizer	Kgs/ha	No.of doses	Stages
Farmyard 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.

Free amino acid composition

Changes in free amino acid composition was studied using LKB Automatic amino acid analyzer. The analysis was made on the 10th leaf of each of the 12 genotypes at the flowering stage only.

Extraction of free amino acids

For amino acid analysis 1 gram of fresh leaves were homogenized in 80 % ethanol. The alcohol was evaporated in vacuo and the residue was dissolved in pH 2.2 citrate buffer and made up to a known volume.

Running of amino acid analyzer:

The amino acids were located and analysed on a cation exchange resin with buffer of carefully defined salt concentrations and pH as described in the handbook and applications for LKB Biochrom automatic amino acid analyzer which was used for this analysis. Sodium salts were used for buffer preparation. The eluate from the ion exchange column is passed through a Teflon coil placed in a boiling water bath. Before entering the coil, the column effluent is mixed with acetate buffer containing the reduced ninhydrin. This compound reacts with the amino acids forming a dye complex. The absorption determined in a flow photometer was registered on the chart of a recorder. The quantification and identification of the different amino acids were carried out using standard amino acid mixture consisting of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, ammonia and arginine. The LKB Standard concentration is 2.5 µM/ml except for cysteine which is present in 1.25 µM/ml.

Peak evaluation

The quantitative evaluation of the amino acids on a good chromatogram was performed by calculating the area under the peaks manually as described in the handbook and application for LKB Biochrom Automatic Amino acid analyzer.

Remobilized nitrogen (RN), nitrogen remobilization efficiency (NRE) and Total nitrogen harvest index (TNHI)

The Remobilized nitrogen (RN), Nitrogen remobilization efficiency (NRE) and Total nitrogen harvest index (TNHI) were calculated according to the Leffel *et al*., (1992) using the following formulae:

Remobilized nitrogen (RN) = Plant total nitrogen at vegetative phase- Plant total nitrogen at seed maturation phase

$$\textbf{Nitrogen remobilization efficiency (NRE)} = \frac{\textbf{Plant total nitrogen at vegetative phase - Plant total nitrogen at seed maturation phase}}{\textbf{Plant total nitrogen at vegetative phase}} \times 100$$

$$\textbf{Total nitrogen harvest index (TNHI)} = \frac{\textbf{Plant total pod nitrogen}}{\textbf{Plant total nitrogen}} \times 100$$

RESULTS

Total free amino acids

Figures 1 a and b represent the total free amino acid content of the 10th leaf of pigeonpea genotypes. The free amino acid content showed a gradual decrease from vegetative to flowering phase followed by a slight increase at seed maturation phase in all the genotypes studied (Fig. 1a). Among the genotypes the ICPL151 of short duration, the Pusa agheti of medium duration and the ST1 of long duration recorded higher values at the seed maturation phase in their respective groups. On unit fresh weight basis, there was a gradual increase in the free amino acid content up to the seed maturation phase (Fig. 1b). The total free amino acid content values among all the genotypes studied varied from 2.16 to 3.50 mg/g fresh wt. The ST1 of long duration genotypes and the HY2 mutant of medium duration genotypes expressed the highest and lowest values among the respective groups at the seed maturation phase.

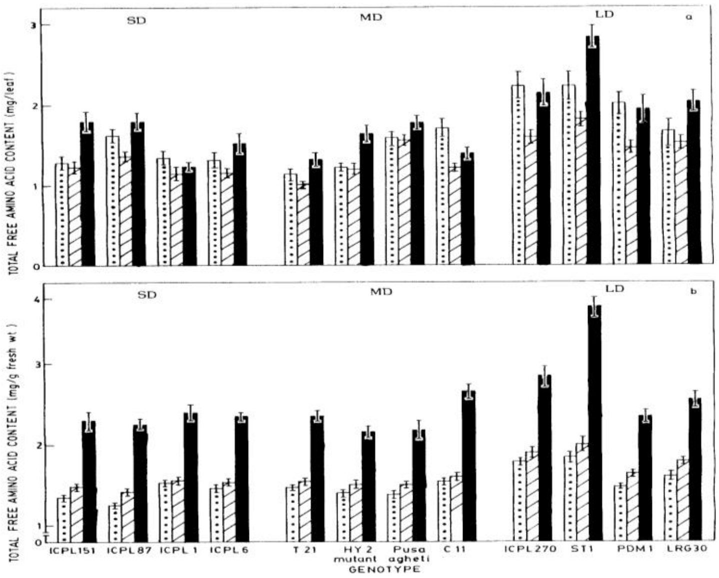

Fig. 1: Total free amino acid content of the 10th leaf during the crop growth of pigeonpea genotypes. (Vertical bars represent S.E.)

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

Free amino acid composition

The free amino acids were analysed using LKB Biochrom automatic amino acid analyser. The amino acids were identified, and the concentrations were determined as described in Handbook and Applications of LKB Biochrom Automatic Amino acid analyser. The concentrations of the different amino acids were expressed as µ moles per gram fresh wt of leaves at the flowering phase of crop growth. The free amino acid composition in the 10th leaf of all the 12 pigeonpea genotypes at the flowering phase of crop growth is presented in Tables-1a, b, c.

Table-1a: Amino acid composition of the 10th leaf of short duration genotypes of pigeonpea at the flowering phase (µ moles/g fresh wt)

Genotypes				
Amino acid	**ICPL 151**	**ICPL87**	**ICPL1**	**ICPL6**
Aspartic acid	0.913	0.896	0.733	1.467
Threonine	0.438	0.568	0.292	0.194
Serine	1.356	1.436	1.188	0.792
Glutamic acid	1.734	1.850	1.499	1.187
Proline	0.217	0.203	0.295	0.273
Glycine	0.958	0.846	0.842	1.034
Alanine	1.296	1.709	1.060	1.495
Cysteine	0.368	0.397	0.290	--
Valine	0.194	0.143	0.176	--
Methionine	0.081	0.305	0.062	0.118
Isoleucine	0.046	0.047	0.513	0.178
Leucine	0.340	0.464	0.337	0.032
Tyrosine	0.805	1.080	0.740	0.754
Phenylalanine	1.721	1.564	1.693	1.935
Histidine	1.920	1.886	1.750	1.920
Lysine	3.292	3.750	3.876	3.544
Ammonium sulphate	2.835	2.082	2.464	2.563
Arginine	0.528	0.527	0.406	0.884
Total	19.042	19.753	18.216	18.370

The results showed marked quantitative differences in the free amino acid composition of different genotypes at the flowering phase. The data showed the absence of cysteine and valine in all the medium duration, long duration and ICPL6 of short duration genotypes. Methionine was absent in all long duration genotypes. However, ICPL151, ICPL87 and ICPL1 of short duration genotypes exhibited traces of cysteine and valine in the 10th leaf at the flowering phase of the crop growth. Lysine was present in greater quantities, in the short duration genotypes than in the medium and long duration genotypes. Greater quantities of glutamic acid and proline were present in the medium and long duration genotypes when compared to the short duration genotypes. Ammonium sulphate was present in greater quantities in the long duration genotypes than in the short duration genotypes. The ICPL87 of short duration, the T21 medium duration and the PDM1 of long duration genotypes recorded greater quantities of free amino acids in their respective groups.

Table-1b: Amino acid composition of the 10th leaf of medium duration genotypes of pigeonpea at the flowering phase (µ moles/g fresh wt)

	Genoypes			
Amino acid	**T21**	**HY2 mutant**	**Pusaagheti**	**C11**
Aspartic acid	1.369	1.467	0.978	1.691
Threonine	0.365	0.443	0.753	0.466
Serine	1.530	0.982	0.643	0.924
Glutamic acid	1.953	2.095	2.148	2.100
Proline	1.521	1.878	1.782	1.930
Glycine	2.823	3.197	3.293	1.905
Alanine	0.766	0.592	0.624	0.649
Cysteine	--	--	--	--
Valine	--	--	--	--
Methionine	0.208	0.203	0.169	0.176
Isoleucine	0.172	0.124	0.155	0.157
Leucine	0.280	0.202	0.268	0.302
Tyrosine	1.059	1.347	1.157	1.185
Phenylalanine	1.067	1.045	1.128	0.972
Histidine	1.910	1.720	1.360	1.565
Lysine	0.415	0.840	0.786	0.634
Ammonium sulphate	2.733	2.705	2.670	3.001
Arginine	1.691	0.234	1.223	1.920
Total	19.862	19.074	19.137	19.577

Table-1c: Amino acid composition of the 10th leaf of long duration genotypes of pigeonpea at the flowering phase (µ moles/g fresh wt)

	Genotypes			
Amino acid	**ICPL270**	**ST1**	**PDM1**	**LRG30**
Aspartic acid	0.424	1.304	0.896	1.369
Threonine	2.803	3.246	2.623	2.556
Serine	0.435	0.554	0.365	0.396
Glutamic acid	3.613	3.218	3.031	3.798
Proline	1.613	1.542	1.728	1.561
Glycine	0.416	0.541	0.763	0.716
Alanine	0.656	0.706	0.644	0.523
Cysteine	--	--	--	--
Valine	--	--	--	--
Methionine	--	--	--	--
Isoleucine	0.052	0.034	0.280	0.019
Leucine	0.464	0.343	0.509	0.473
Tyrosine	0.964	0.821	0.877	1.173

Phenylalanine	1.377	1.214	1.552	1.359
Histidine	1.280	0.810	1.810	1.260
Lysine	0.332	0.221	0.373	0.284
Ammonium sulphate	2.811	2.737	2.797	2.633
Arginine	1.054	1.374	1.268	0.906
Total	18.297	18.665	19.516	19.026

Free amino acid composition

The free amino acids were analysed using LKB Biochrom automatic amino acid analyzer. The amino acids were identified, and the concentrations were determined as described in Handbook and Applications of LKB Biochrom Automatic Amino acid analyser. The concentrations of the different amino acids were expressed as µ moles per gram fresh wt of leaves at the flowering phase of crop growth. The free amino acid composition in the 10th leaf of all the 12 pigeonpea genotypes at the flowering phase of crop growth is presented in Tables-1a, b, c. The results showed marked quantitative differences in the free amino acid composition of different genotypes at the flowering phase. The data showed the absence of cysteine and valine in all the medium duration, long duration and ICPL6 of short duration genotypes. Methionine was absent in all long duration genotypes. However, ICPL151, ICPL87 and ICPL1 of short duration genotypes exhibited traces of cysteine and valine in the 10th leaf at the flowering phase of the crop growth. Lysine was present in greater quantities, in the short duration genotypes than in the medium and long duration genotypes. Greater quantities of glutamic acid and proline were present in the medium and long duration genotypes when compared to the short duration genotypes. Ammonium sulphate was present in greater quantities in the long duration genotypes than in the short duration genotypes. The ICPL87 of short duration, the T21 medium duration and the PDM1 of long duration genotypes recorded greater quantities of free amino acids in their respective groups.

Remobilized nitrogen (RN)

Genotypic variation in the remobilized nitrogen was given in the Table-2. The amount of remobilized nitrogen varied between 75.40 to 158.40 kgs/hectare among the pigeonpea genotypes studied. The ICPL87 of short duration and the ST1 of long duration genotypes recorded the maximum and minimum values respectively. The higher yielding genotypes remobilized more nitrogen than the lower yielding genotypes.

Table-2: Remobilized nitrogen, nitrogen remobilization efficiency and total nitrogen harvest index of pigeonpea genotypes

Genotype	Remobilized nitrogen kgs/hectare	Nitrogen Remobilization Efficiency (%)	Total Nitrogen Harvest index (%)
Short duration			
ICPL151	100.80	41.86	14.87
ICPL 87	158.40	52.38	21.00
ICPL1	76.20	32.19	15.01
ICPL6	88.80	33.63	15.01
Medium duration			
T21	135.80	44.67	13.15
HY2 mutant	120.80	32.14	10.38
Pusa agheti	100.40	26.60	8.67
C11	130.90	39.12	9.60
Long duration			
ICPL270	95.20	26.50	9.04
ST1	75.40	21.20	8.06
PDM1	130.50	32.82	8.69
LRG30	129.60	33.00	8.29

Nitrogen remobilization efficiency (NRE)

The nitrogen remobilization efficiency of all the pigeonpea genotypes were showed in Table-2. The ICPL87 of short duration, T21 of medium duration and LRG30 of long duration recorded higher values in their respective groups. The highest per cent NRE was registered in the ICPL87 of short duration and lowest per cent in the ST1 of long duration genotypes.

Total nitrogen harvest index (TNHI)

Total nitrogen harvest index varied greatly among the genotypes studied. They varied from 8.06 to 21 per cent among the genotypes studied (Table-2). The short duration genotypes recorded higher values than the medium and long duration genotypes. The maximum TNHI was noticed in the ICPL87 of short duration and the minimum in the ST1 of long duration genotypes.

DISCUSSION

Amino acid synthesis and utilization is associated with nitrogen nutrition of plants. Higher availability of nitrogen in the form of NO_3^- leads to higher synthesis of several amino acids such as aspartic acid, threonine, serine, glutamic acid, alanine and arginine (Atkins *et al*., 1979). Some interesting variation in amino acid composition among the genotypes of pigeonpea were observed. Cysteine and valine were absent in the medium and long duration genotypes. Methionine was also absent in all the long duration genotypes (Table-1 c). The methionine is a precursor for the formation of ethylene which promotes leaf fall. The absence of methionine in long duration genotypes can be attributed to the formation of ethylene and ultimately higher per cent leaf fall and also higher amounts of NH_3 in these genotypes. Proline was present in greater amounts in the medium and long duration genotypes and could be attributed to the lower leaf moisture content in these genotypes. Genotypic differences in the proline accumulation under lower leaf moisture contents were reported in chickpea (Jirali *et al*., 1990).

The remobilized nitrogen, nitrogen remobilization efficiency, total nitrogen harvest index were more in higher yielding than lower yielding pigeonpea genotypes (Table-2). Maximum values of remobilized nitrogen, nitrogen remobilization efficiency and total nitrogen harvest index in the ICPL87 were associated with its greater seed protein content and greater seed yield.

CONCLUSION

Highest and lowest values of free amino acid composition was expressed in ST1 of long duration genotypes and the HY2 mutant of medium duration genotypes at the seed maturation phase. The ICPL87 of short duration, the T21 medium duration and the PDM1 of long duration genotypes recorded greater quantities of free amino acids in their respective groups. The total nitrogen harvest index was greater in the short duration genotypes than the medium and long duration genotypes. The ICPL87 of short duration, the T21 of medium duration and the PDM1 of long duration genotypes exhibited greater values of the remobilized nitrogen and nitrogen remobilization efficiency in their respective groups.

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