Characterization of seed storage proteins in high protein genotypes of cowpea [*Vigna unguiculata* (L.) Walp.]

Prachi Gupta¹, Rohtas Singh², S. Malhotra¹, K.S. Boora³ and H.R. Singal¹

¹Department of Biochemistry, CCS Haryana Agricultural University, Hisar - 125 004, India

²Centre for Plant Biotechnology, CCS Haryana Agricultural University, New Campus, Hisar - 125 004, India

³Department of Biotechnology & Molecular Biology, CCS Haryana Agricultural University, Hisar - 125 004, India

ABSTRACT

Twenty one genotypes and two check varieties viz. CS-88 and V-240 of cowpea [*Vigna unguiculata* (L.) Walp.] were screened for total proteins. The total protein content ranged from 22.4 (HC-3) to 27.9 % (HC-98-64) in 21 genotypes whereas in check varieties it was 25.6 (V-240) and 26.0 % (CS-88). Seven genotypes viz. HC-6, HC-5, CP-21, LST-II-C-12, CP-16, COVU-702 and HC-98-64 having high protein content (26.7 to 27.9 %) were selected for further characterization of their seed storage proteins. Globulins were the major protein fraction ranging from 55.6 (LST-II-C-12) to 58.8 % (CP-16 and HC-6) of total protein. Glutelins was the second major fraction ranging from 14.4 to 15.6 % followed by albumins (8.2 to 11.9 %) and prolamins (2.3 to 5.0 %). Content of free amino acids also showed variations amongst genotypes with COVU-702 having maximum and LST-II-C-12 having minimum content. Essential amino acid analysis revealed that S-amino acids (cysteine and methionine) were the first limiting amino acids followed by tryptophan. From the results presented here it could be suggested that two genotypes viz. LST-II-C-12 and HC-5 be used in breeding programmes aimed at developing high protein moth bean varieties with good quality. [*Physiol. Mol. Biol. Plants* 2010; 16(1) : 53-58] E-mail : rohtas_bot@rediffmail.com

Keywords : Genotypes, cowpea, protein fractions, seeds, *Vigna unguiculata*

INTRODUCTION

Seeds predominantly, legumes and cereals, represent the primary source of proteins for animal feeding and human nutrition, because they provide reasonably sufficient quantity of proteins and other nutrients at a relatively much cheaper rate. (Gupta and Kapoor, 1979). On an average, legume seeds contain 20-27 per cent protein (Wang and Daun, 2004; Horax *et al.*, 2004) and occupy the second place after cereals as the source of calories and proteins in the human diet.

Among the legumes, cowpea (*Vigna unguiculata* (L.) Walp.) is a tropical legume of African origin and was introduced from Africa to Indian subcontinent approximately 2000-3500 years ago (Allen, 1983). It is

very palatable, highly nutritious and relatively free from metabolites or other toxins (Quass, 1995). It can be used at all stages of growth as a vegetable crop. Green cowpea seeds can be used as fresh vegetable, or may be canned or frozen. Its young leaves, pods and peas contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding (Nielsen *et al.*, 1997; Rachie, 1985). Despite the importance of cowpea as a food crop in tropical and subtropical regions, little work has been carried out on characterization of its major seed storage proteins in comparison to the other legume species such as *Glycine max* or *Phaseolus vulgaris*. This prompted us to analyze different genotypes along with two check varieties of cowpea for protein fractions and their essential amino acid content. The data so generated would help the plant breeders to select varieties for further breeding programs aimed at achieving cowpea genotypes of high protein content with better quality.

Correspondence and reprint requests : Rohtas Singh
Telephone: 00-91-1662-227088; Fax: 00-91-1662-271369

MATERIALS AND METHODS

Plant materials

The experimental material comprised of twenty one genotypes of cowpea [*Vigna unguiculata* (L.) Walp.] and two check varieties viz. CS-88 and V-240 from different agro-climatic zones of Haryana, Punjab, Delhi, Gujarat, Zodhpur, Coimbatour, Trombay and South Vailore. Brief description of the genotypes is given in Table 1. These were obtained from Forage Section of the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar-125004, India. The seeds were oven dried at 60 °C for 48 h, ground in Udy-cyclone mill using 80 mesh screen and used for further analysis. All estimations were made in duplicate in each

Table 1. Brief description of the genotypes used.

S.No.	Code	Decode	Location
1	HC-3	HC-3	Hisar
2	HC-98-64	HC-98-64	Hisar
3	HC-6	HC-6	Hisar
4	LST-II-C-12	LST-II-C-12	Gujarat
5	CP-16	DCP-15	Delhi
6	CP-21	GC-121	Gujarat
7	CP-26	GC-26	Gujarat
8	LST-II-C-2	HC-2-2	Haryana
9	COVU-702	COVU-702	Coimbatour
10	GC-119	GC-119	Gujarat
11	CP-11	PCP-9708	Punjab
12	CP-13	CPD-72	Delhi
13	CP-27	GC-27	Gujarat
14	CP-22	GC-115	Gujarat
15	CP-17	HC-98-45	Hisar
16	CP-12	CAZC-13	Zodhpur
17	HC-5	HC-5	Hisar
18	CP-18	HC-98-46	Hisar
19	CP-19	TC-501	Trombay
20	CPD-80	CPD-80	Delhi
21	CP-20	TC-502	Trombay
22	CS-88	CS-88	Hisar
23	V-240	V240	South Vailore

of the three extracts prepared separately from three different lots of every sample.

Total protein

Total protein (N x 6.25) in seed flour and in different fractions viz. albumins, globulins, prolamins and glutelins was estimated by determining the total nitrogen content by Micro-Kjeldhal method (AOAC, 1984).

Protein fractionation

Different fractions viz. albumins (water-soluble), globulins (salt-soluble), prolamins (alcohol-soluble) and glutelins (alkali-soluble) were prepared by following the classical method of Osborne (1924) using solubility criteria. The solvents used for extraction of these fractions were distilled water, 1 % NaCl, 60 % ethanol and 0.4 % NaOH solution, respectively.

Total free amino acids

The seed flour of each sample was treated with 60 percent ethanol, refluxed for 15 min on a water bath at 60 °C and centrifuged at 3000 rpm for 15 min. This step of refluxing and centrifugation was repeated three times with residue, supernatants pooled together and used for estimation of total free amino acid content by following the method of Yemm and Cocking (1955).

Essential amino acids

The amino acid composition was determined by the method given in Technical note 50 (Dionex Corporation) using the automatic amino acid analyzer (AAA-Direct), which separates amino acids on a high performance anion-exchange column and directly detects them by integrated amperometry.

Statistical analysis

Statistical analysis (standard error and correlation analysis) of the results was done using three replicates following the statistical software system (SPSS) of Nie *et al.* (1970), which stood for the Statistical Package for the Social Sciences.

RESULTS AND DISCUSSION

Protein content

Total crude protein content as presented in the Table 2 ranged from 22.4 (HC-3) to 27.9 % (HC-98-64) in 21 genotypes of cow pea, whereas in the check varieties V-240 and CS-88 the protein content was 25.6 and 26.0

Table 2. Protein content of cowpea (*Vigna unguiculata*) genotypes (g/100g flour). Mean $\pm$ SE, n = 3.

Genotype	Protein
HC-3	22.4 $\pm$ 0.13
HC-98-64	27.9 $\pm$ 0.05
HC-6	26.7 $\pm$ 0.15
LST-II-C-12	27.5 $\pm$ 0.05
CP-16	27.7 $\pm$ 0.22
CP-21	27.5 $\pm$ 0.18
CP-26	24.5 $\pm$ 0.43
LST-II C-2	26.0 $\pm$ 0.13
COVU-702	27.7 $\pm$ 0.22
GC-119	25.7 $\pm$ 0.11
CP-11	24.1 $\pm$ 0.04
CP-13	24.0 $\pm$ 0.12
CP-27	24.2 $\pm$ 0.07
CP-22	24.3 $\pm$ 0.04
CP-17	23.2 $\pm$ 0.17
CP-12	24.6 $\pm$ 0.22
HC-5	26.9 $\pm$ 0.30
CP-18	24.5 $\pm$ 0.74
CP-19	25.6 $\pm$ 0.09
CPD-80	25.4 $\pm$ 0.07
CP-20	23.9 $\pm$ 0.11
CS-88	26.0 $\pm$ 0.00
V-240	25.6 $\pm$ 0.05

%, respectively. The results are in close agreement with those reported by Wang and Daun (2004) where the protein content has been reported to vary from 20.2 to 26.7% in different legumes. However, Horax *et al.* (2004) reported that in cowpea, protein content varied from 20 to 25 %. These wide variations in protein content may be due to combination of genetic and environmental factors (Wang and Daun, 2004).

In the recent years, considerable interest has been shown in breeding high protein legumes with better nutritional quality in terms of essential amino acids. The amino acid make up largely depends upon the relative distribution of the various protein fractions like albumins, globulins, prolamins and glutelins. Therefore, among the mothbean genotypes investigated during present investigations, seven genotypes viz. HC-6, HC-5, CP-21, LST-II-C-12, CP-16, COVU-702 and HC-98-64 were identified as high protein genotypes (26.7 to 27.9 % protein) and used to prepare different proteins fractions.

Protein fractionation

Remarkable variations were noticed in the contents of different protein fractions of cow pea genotypes (Table 3). In all the seven genotypes, globulins constituted the bulk of mothbean seed proteins. The content varied from 55.6 % (LST-II-C-12) to 58.8 % (CP-16) of the total proteins. The values are slightly lower than those reported for cowpea by Rosario *et al.* (1981). They have observed the globulin content to range from 63 to 67 per cent. However the values obtained during present investigations are within the range of 50-85 % as has been reported for different legumes (Mahajan *et al.*, 1988; Dhawan *et al.*, 1991; Bala *et al.*, 1994; Mendoza

Table 3. Content of protein fractions in cowpea (*Vigna unguiculata*) genotypes (g/100g flour). Mean $\pm$ SE, n = 3. Values in parentheses indicate the % of total protein.

Genotype	Protein			
	Albumin	Globulin	Prolamin	Glutelin
HC-98-64	3.31 $\pm$ 0.009 (11.9)	16.3 $\pm$ 0.058 (58.4)	1.40 $\pm$ 0.052 (5.0)	4.03 $\pm$ 0.007 (14.4)
HC-6	3.01 $\pm$ 0.006 (11.3)	15.7 $\pm$ 0.067 (58.8)	0.67 $\pm$ 0.007 (2.5)	4.16 $\pm$ 0.009 (15.6)
LST-II-C-12	2.80 $\pm$ 0.003 (10.2)	15.3 $\pm$ 0.058 (55.6)	1.04 $\pm$ 0.006 (3.8)	4.21 $\pm$ 0.006 (15.3)
CP-16	3.30 $\pm$ 0.009 (11.9)	16.3 $\pm$ 0.033 (58.8)	0.64 $\pm$ 0.006 (2.3)	4.01 $\pm$ 0.006 (14.5)
CP-21	3.28 $\pm$ 0.009 (11.9)	15.7 $\pm$ 0.058 (57.0)	0.67 $\pm$ 0.003 (2.4)	4.27 $\pm$ 0.006 (15.5)
COVU-702	3.08 $\pm$ 0.006 (11.1)	16.2 $\pm$ 0.088 (58.5)	0.72 $\pm$ 0.003 (2.6)	4.03 $\pm$ 0.006 (14.5)
HC-5	2.20 $\pm$ 0.003 (8.2)	15.6 $\pm$ 0.088 (57.9)	0.69 $\pm$ 0.006 (2.6)	4.12 $\pm$ 0.003 (15.3)

et al., 2001). Glutelins was the next major fraction and found to be in the range of 14.4 to 15.6 % of the total proteins. The values observed in the present investigations are much higher than those reported for other legumes e.g. 8 % in soybean (Kapoor and Gupta, 1977) and 2 % in blackgram (Padhye and Salunkhe, 1979).

Next to globulins and glutelins, was the albumins fraction which varied between 8.2 to 11.9 % (Table 3). Rosario *et al.* (1981) have reported that cowpea seeds had 8-12 % albumins content. However, the general range of albumins in legume seeds is 10-15 % of the total proteins (Mahajan *et al.*, 1988; Dhawan *et al.*, 1991; Bala *et al.*, 1994). The prolamins fraction was the least concentrated protein in cow pea and ranged from 2.3 to 5.0 % (Table 3). Similarly, blackgram (Padhye and Salunkhe, 1979), soybean (Kapoor and Gupta, 1977) and ricebean (Singh *et al.*, 1985) have been reported to contain less than 3 % prolamin content.

Free amino acid content

The total free amino acid content ranged between 1.7 to 3.7 mg/100g flour (Table 4) with maximum in COVU-702 and minimum in LST-II-C-12. Similar observations with regard to free amino acid content have been recorded by Awolunate (1983) in mungbean, cowpea and soybean. Very low amount of free amino acids in mature cowpea seeds, as has been observed in the

Table 4. Content of total free amino acids of cowpea (*Vigna unguiculata*) genotypes (mg/100g flour). Mean $\pm$ SE, n = 3.

Genotype	Free amino acids
HC-98-64	2.9 $\pm$ 0.001
HC-6	2.0 $\pm$ 0.000
LST-II-C-12	1.7 $\pm$ 0.003
CP-16	3.5 $\pm$ 0.002
CP-21	3.2 $\pm$ 0.001
COVU-702	3.7 $\pm$ 0.000
HC-5	2.8 $\pm$ 0.002

present investigations, suggests there utilization for protein synthesis during seed development.

Essential amino acid content

Data in Table 5 reveal that histidine, cysteine, methionine, isoleucine, leucine, threonine, lysine and tryptophan varied from 1.85 to 2.47, 0.84 to 1.08, 1.28 to 2.06, 4.17 to 5.46, 6.45 to 8.50, 3.89 to 5.12, 7.30 to 8.74 and 1.00 to 1.33 g/100g protein, respectively. HC-6 had maximum content of total essential amino acids of 33.43 g/100g protein while the minimum was found to be 27.52 g/100g protein in HC-98-64 and 27.50

Table 5. Essential amino acid composition of different genotypes of cowpea (*Vigna unguiculata*) (g/100g protein). Mean $\pm$ SE, n = 3.

Amino Acid	Genotype						
	HC-98-64	HC-6	LST-II-C-12	CP-16	CP-21	COVU-702	HC-5
Histidine	1.92 $\pm$ 0.092	2.42 $\pm$ 0.173	2.47 $\pm$ 0.110	1.99 $\pm$ 0.185	2.05 $\pm$ 0.040	1.85 $\pm$ 0.133	2.23 $\pm$ 0.225
Cysteine	0.96 $\pm$ 0.318	1.08 $\pm$ 0.115	1.00 $\pm$ 0.566	0.84 $\pm$ 0.375	1.05 $\pm$ 0.087	1.00 $\pm$ 0.681	0.98 $\pm$ 0.670
Methionine	1.28 $\pm$ 0.046	1.75 $\pm$ 0.421	2.06 $\pm$ 0.733	1.67 $\pm$ 0.086	1.29 $\pm$ 0.060	1.40 $\pm$ 0.023	2.05 $\pm$ 0.283
Cys + Met	2.24 $\pm$ 0.225	2.83 $\pm$ 0.514	3.06 $\pm$ 0.058	2.51 $\pm$ 0.508	2.34 $\pm$ 0.156	2.40 $\pm$ 0.658	3.03 $\pm$ 0.439
Isoleucine	4.17 $\pm$ 0.358	5.46 $\pm$ 0.485	4.23 $\pm$ 0.427	4.37 $\pm$ 0.318	4.58 $\pm$ 0.069	4.05 $\pm$ 0.318	4.45 $\pm$ 0.312
Leucine	6.49 $\pm$ 0.046	7.87 $\pm$ 0.069	6.98 $\pm$ 0.127	8.21 $\pm$ 0.167	8.50 $\pm$ 0.028	6.45 $\pm$ 0.548	7.05 $\pm$ 0.381
Threonine	4.13 $\pm$ 0.358	5.12 $\pm$ 0.075	4.47 $\pm$ 0.318	3.94 $\pm$ 0.035	4.00 $\pm$ 0.196	3.89 $\pm$ 0.497	4.83 $\pm$ 0.179
Lysine	7.30 $\pm$ 0.087	8.40 $\pm$ 0.572	7.43 $\pm$ 0.548	8.74 $\pm$ 0.445	8.21 $\pm$ 0.439	7.83 $\pm$ 0.335	7.90 $\pm$ 0.237
Tryptophan	1.27 $\pm$ 0.098	1.33 $\pm$ 0.185	1.25 $\pm$ 0.092	1.18 $\pm$ 0.115	1.29 $\pm$ 0.000	1.00 $\pm$ 0.242	1.02 $\pm$ 0.156
Total essential Amino acid	27.52 $\pm$ 1.917	33.43 $\pm$ 0.976	29.90 $\pm$ 2.034	30.94 $\pm$ 2.309	30.97 $\pm$ 0.214	27.50 $\pm$ 1.804	30.51 $\pm$ 3.256

g/100g protein in COVU-702. Similarly, higher S-amino acids (cysteine and methionine) content was found in the genotype LST-II-C-12. Lysine content was found to be the highest in CP-16. The values reported here are in general agreements with those reported earlier in cowpea (Oluwatosin, 1997; Hussain and Basahy, 1998), mungbean (Gonzalez *et al.*, 1964), soybean (Leverton, 1967), and pea (Wang and Daun, 2004).

Correlation between protein fractions and essential amino acids

The correlation analysis (Table 6) revealed that S-amino acids (cysteine and methionine) were low in the varieties having high globulins whereas genotypes having high prolamins were low in lysine indicating a negative correlation between globulins and S-amino acids and between prolamins and lysine.

On the basis of results obtained in present investigation, it is suggested that the genotypes LST-II-C-12 and HC-5 having reasonably high content of protein and essential amino acids (particularly, cysteine and methionine) can be exploited in the breeding programme for the development of mothbean varieties with high protein content, S-amino acids (cysteine and methionine) and lysine contents.

ACKNOWLEDGMENTS

The authors are thankful to Dr. V.K. Choudhary, Professor and Head, Department of Biotechnology and

Table 6. Correlation between protein fractions (g/100g flour) and essential amino acids (g/100g protein). Mean $\pm$ SE, n = 3.

Genotype	Globulin	Prolamin	Cyst +Met	Lysine
HC-98-64	16.3 $\pm$ 0.058	1.40 $\pm$ 0.052	2.24	7.30
HC-6	15.7 $\pm$ 0.067	0.67 $\pm$ 0.007	2.83	8.40
LST-II-C-12	15.3 $\pm$ 0.058	1.04 $\pm$ 0.006	4.07	7.43
CP-16	16.3 $\pm$ 0.033	0.64 $\pm$ 0.006	2.51	8.74
CP-21	15.7 $\pm$ 0.058	0.67 $\pm$ 0.003	2.34	8.21
COVU-702	16.2 $\pm$ 0.088	0.72 $\pm$ 0.003	2.40	7.83
HC-5	15.6 $\pm$ 0.088	0.69 $\pm$ 0.006	4.03	7.90

$r = -0.786$ (significant at 5 % level) between globulins and cys+met; $r = -0.818$ (significant at 5 % level) between prolamins and lysine

Molecular Biology, for providing necessary facilities. The CCS, Haryana Agricultural University, Hisar-125004 (Haryana) India, is also acknowledged for financial assistance, given to senior author in the form of merit scholarship.

REFERENCES

- Allen DJ (1983). The Pathology of Tropical Food Legumes. John Wiley and Sons, Chichester.
- AOAC (1984). Official Methods of Analysis, 14th edn. Association of Official Analytical Chemists. Washington, DC.
- Awolumat EO (1983). Accumulation and quality of storage protein in developing cowpea, mungbean and soybean seeds. *J. Sci. Food Agric.* 34: 1351-1357.
- Bala A, Satija DR and Gupta VP (1994). Variation and association analysis among protein quality traits in chickpea. *Indian J. Genet. Pl. Breed.* 54: 105-109.
- Dhawan K, Malhotra S, Dahiya BS and Singh D (1991). Seed protein fractions and amino acid composition in gram (*Cicer arietinum*). *Plant Fd. Human Nutr.* 41: 225-232.
- Dionex Corporation. Determination of the amino acid content of peptides by AAA-Direct. Technical Note 50. Sunnyvale, CA.
- Gonzalez ON, Banzon EA, Liggayu RG and Quintio PH (1964). Isolation and chemical composition of mungbean (*Phaseolus aureus* Roxb.) protein. *Philip. J. Sci.* 93: 47-56.
- Gupta VP and Kapoor AC (1979). Chemical evaluation of protein quality of various grain legumes. *Indian J. agric. Sci.* 50: 393-398.
- Horax R, Hettiarachchy NS, Chen P and Jalaluddin M (2004). Preparation and characterization of protein isolate from cow pea (*Vigna unguiculata* (L.) walp). *J. Fd. Sci.* 69: 114-118.
- Hussain MA and Basahy AY (1998). Nutrient composition and amino acid pattern of cowpea (*Vigna unguiculata* (L.) Walp, Fabaceae) grown in the Gizan area of Saudi Arabia. *Int. J. Fd Sci. Nutr.* 49: 117-124.
- Kapoor AC and Gupta YP (1977). Chemical evaluation and electrophoretic pattern of soya protein. *J. Fd. Sci. Agric.* 42: 1558-1561.
- Leverton RM (1967). Soybean protein in mixed foods. In: *Proc. Int. Conf. Soybean protein foods*, U. S. Dept. Agric., pp. 75-80.
- Mahajan R, Malhotra SP and Singh R (1988). Characterization of seed storage proteins of urdbean (*Vigna mingo*). *Plant Fd. Human Nutr.* 38: 163-173.
- Mendoza EMT, Adachi M, Bernardo AEN and Utsumi S (2001). Mung bean (*Vigna radiate* (L.) Wilczek) globulins: purification and characterization. *J. Agric. Food Chem.* 49: 1152-1158.
- Nie NH, Hull CH and Bent DH (1970). *Statistical Package for the Social Sciences*. McGraw-Hill. Eds 1 & 2 1970, 1975. New York.

- Nielsen S, Ohler T, Mitchell C (1997). Cowpea leaves for human consumption: production, utilization, and nutrient composition. In: *Advances in cowpea research* (Ed. Singh, B.; Mohan Raj, D.; Dashiell K. and Jackai L.), Intr. Inst. Trop. Agric. (IITA) and Japan Intr. Res. Center Agric. Sci. (JIRCASS), Ibadan, Nigeria, pp 326-332.
- Oluwatosin OB (1997). Genetic and environmental variation for seed yield, lipid and amino acid composition in cowpea [*Vigna unguiculata* (L.) Walp]. *J. Sci. Fd Agric.* 74: 107-116.
- Osborne TB (1924). The vegetable proteins. Longmans, Green and Co, London.
- Padhye VW and Salunkhe DK (1979). Biochemical studies on black gram (*Phaseolus mungo* L.) seeds. Amino acid composition and subunit constitution of fractions of the proteins. *J. Fd. Sci.* 44: 606-610.
- Quass CF (1995). Guidelines for the Production of Cowpeas. National Department of Agriculture, Pretoria.
- Rachie KO (1985). Introduction. In: *Cowpea Researches, Production and Utilization* (Ed. Singh, S.R and Rachie, K.O.), Wiley, New York. pp. 108.
- Rosario RRD, Lozano Y and Neol MG (1981). The chemical and biochemical composition of legume seeds. 2. Cowpea. *Philippine-Agriculturist.* 64: 49-57.
- Singh SP, Misra BK, Sikka KC, Chandel KP and Pant KC (1985). Studies on some nutritional aspects of ricebean (*Vigna umbellata*). *J. Fd. Sci. Tech.* 22: 180-185.
- Turkova V and Klovova E (1985). Comparison of seed proteins in some representatives of the genus *Vigna*. *Biol. Plant.* 27: 70-73.
- Wang N and Daun JK (2004). Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (*Pisum sativum*). *J. Sci. Fd Agric.* 84: 1021-1029.
- Yemm EW and Cocking EC (1955). The determination of amino acids with ninhydrin. *Analyst.* 80: 209-213.