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Nutritional Quality Evaluation of Newly Developed High-Protein Genotypes of Pigeonpea (*Cajanus cajan*)*

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ABSTRACT

Two high-protein genotypes of pigeonpea (Cajanus cajan *L*), *HPL 8 and HPL 40, were analysed for their nutritional quality characteristics, and the results were compared with those of normal-protein genotypes (C 11 and ICPL 211). The protein content of the high-protein genotypes was higher on average by nearly 20 % but their starch content, the principal constituent of the seed, was lower by about 8 %. The higher fraction (about 7 %) of globulin, the major storage protein, was associated with a lower glutelin fraction in the high-protein genotypes. The amino acid composition (g per 100 g protein) of the high-protein genotypes was comparable with those of the normal-protein genotypes. However, the sulphur-containing amino acids methionine and cystine were noticeably higher (about 25 %) in high-protein genotypes when results were expressed in g per 100 g sample. No large differences in true protein digestibility, biological value and net protein utilisation were observed between HP and NP genotypes. True protein digestibility was significantly increased by cooking in both whole-seed and dhal samples. The values for utilisable protein were considerably higher in high-protein genotypes, suggesting their superiority from the nutritional point of view.*

Key words: Pigeonpea, high-protein genotypes, chemical composition, protease inhibitors, biological evaluation.

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INTRODUCTION

Pigeonpea (*Cajanus cajan* L), also called red gram, has occupied an important place in human nutrition as a rich source of protein in the diet of consumers in India. India accounts for about 85% of the world's supply of pigeonpea. Other countries where pigeonpea is an important legume are Kenya, Malawi, Uganda, Thailand, Indonesia and the Philippines. In India, pigeonpea is mostly consumed in the form of dhal (decorticated split cotyledons) after dehusking and cooking in water to a desirable softness, whereas in some African countries whole seeds of pigeonpea are consumed after boiling. Green pods of pigeonpea are also harvested and the developing green seeds are used as a vegetable in India and some African, Latin American and south-east Asian countries.

Attention has been paid worldwide to improving the nutritional quality of grain legumes (Bressani 1973; Bliss and Hall 1977; Eggum and Beames 1983; Salunke *et al* 1986). Some information on the nutritional aspects of pigeonpea is available, and efforts have been made to identify factors affecting its nutritional quality (Singh *et al* 1981; Singh and Eggum 1984). The protein content of commonly grown pigeonpea cultivars ranged between 17·9 and 24·3 g per 100 g for whole grain samples, and between 21·1 and 28·1 g per 100 g for dhal (Singh and Eggum 1984). At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), attempts have been made to improve the protein quality of pigeonpea, and some high-protein genotypes of pigeonpea have been developed by using wild species as a high-protein source (Reddy *et al* 1979; Saxena *et al* 1987). This paper reports on various aspects of the nutritional quality of high-protein (HP) genotypes in comparison with two normal-protein (NP) genotypes.

EXPERIMENTAL

Seed material

The experimental seed material for the present study consisted of two high-protein (HP) genotypes (HPL 8 and HPL 40) and two normal-protein (NP) genotypes (C11 and ICPL 211). C 11 is a released commercial variety. These genotypes were grown at ICRISAT Center, Patancheru, India, during the rainy season of 1986.

Decortication and cooking

Whole-seed samples were decorticated to prepare dhal (decorticated dry split cotyledons) by using a Praire Regional Laboratory (PRL) mill. About 1 kg each of whole seed and dhal samples were cooked for 15 min at 1·05 kg cm^{-2} pressure in a pressure cooker. After cooking, the whole content, including the broth, was dried in the oven at 50°C. Raw and cooked samples were ground in a Udy cyclone mill to pass through a 0·4-mm screen.

Chemical analysis

Nitrogen content in pigeonpea samples was determined using a Technicon auto analyser (Singh and Jambunathan 1981), and nitrogen values were converted into

protein by multiplying by a factor of 6·25. For amino acid analysis and protein fractionation, finely ground samples were defatted in a Soxhlet apparatus using *n*-hexane. Previously published methods were used for the determination of ash, fat and crude fibre (AOAC 1975) and soluble sugars and starch (Singh and Jambunathan 1980). Moisture content was determined by drying the samples overnight in an oven at 110°C. All these constituents were analysed in duplicate and average values are reported. All results were expressed on moisture-free basis.

Seed protein fractionation

Seed proteins were fractionated into albumin, globulin, glutelin and prolamin by successive extractions with different solvents as described earlier (Singh *et al* 1981). Defatted flour samples were successively extracted with 0·5 M sodium chloride solution in 0·01 M phosphate buffer (pH 7·0), 0·1 M sodium hydroxide and 70% ethanol to separate total protein into albumin and globulin, glutelin and prolamin fractions, respectively.

Amino acid analysis

Defatted samples (50 mg) were refluxed in 50 ml of 6 M HCl for 24 h. After refluxing, acid was removed in a rotary flash evaporator. The residue was washed with water to remove HCl and taken in a known volume of citrate buffer (pH 2·2). An aliquot of each sample was used for analysis in a Beckman 119-CL amino acid analyser. As a result of refluxing in 6 M HCl, tryptophan was destroyed and hence not determined.

Trypsin and chymotrypsin inhibitors

The trypsin inhibitor activity (TIA) was assayed according to Kakade *et al* (1969). Trypsin inhibitor was extracted by shaking 200 mg of defatted material with 10 ml of 0·1 M phosphate buffer (pH 7·6) at room temperature for 1 h. Extracts were assayed for TIA. Chymotrypsin inhibitor activity (CIA) was assayed according to Kakade *et al* (1970). Chymotrypsin inhibitor was extracted as described above, except that 0·1 M borate buffer (pH 7·6) was used.

Biological evaluation of protein quality

True protein digestibility (TD), biological value (BV), net protein utilisation (NPU) and utilisable protein (UP) were determined by conducting rat feeding experiments using metabolic cages obtained from Lab Products Inc, New Jersey, USA. Groups of five Wistar male rats, weighing about 70 g, were used in these experiments. Each rat was daily fed 10 g diet (dry weight basis) containing 150 mg nitrogen. At the end of the 5-day feeding period, unconsumed diet weight was recorded and total nitrogen intake was calculated. The remaining procedures were followed and calculation of TD, BV, NPU and UP values was made according to Eggum (1973).

Statistical analysis

For all chemical analysis, excepting amino acids, two replicates were used for the determination of each constituent. For biological evaluation, five replicates of randomly chosen rats were used to determine biological value, protein digestibility and net protein utilisation as discussed above. Standard error was determined by

one-way analysis of variance (Snedecor and Cochran 1967) and statistical tests are based on this.

RESULTS AND DISCUSSION

Chemical composition

The protein content of dhal of the HP genotypes (HPL 8 and HPL 40) is significantly higher (20%) than that of the NP genotypes (ICPL 11 and ICPL 211) as shown in Table 1. The present study shows that the genotypic differences are quite large, although the possibility of small environmental effects on the protein content of these genotypes could not be ruled out. The protein content of some HP genotypes of pigeonpea, including HPL 40, has been reported to vary from 27·0 to 29·8 g per 100 g (Saxena *et al* 1987). As expected the starch content of HP genotypes was lower than that of the others and a similar trend was observed for fat content. On the other hand, soluble sugars, ash and crude fibre showed variable results among these genotypes (Table 1).

From the consumer's point of view, small-seeded pigeonpeas are not preferred. One-hundred-seed mass of HP genotype HPL 8 was comparable with those of the NP genotypes (Table 1). However, 100-seed mass of HPL 40 was slightly lower, and this might have been due to environmental effects. The 100-seed mass of this genotype has been reported to be similar to those of the other genotypes of pigeonpea evaluated under identical conditions (Saxena *et al* 1987). Also, values for the seed coat percentage of HP genotypes did not differ significantly, suggesting that these genotypes might be acceptable for dehulling in terms of dhal yield.

Protein fractionation and amino acids

Considerable differences were observed in the concentrations of the major protein fractions of these genotypes, globulin and glutelin (Table 2). The globulin fraction was noticeably higher in HP genotypes than in NP genotypes, and the reverse was true for the glutelin fraction. The storage proteins, globulins, constitute the major proportion of the legume seed proteins. Since these proteins are deficient in sulphur-

TABLE 1
Chemical composition of dhal of high and normal protein genotypes[a]

Genotype	*100-seed mass (g)*	*Protein*	*Starch*	*Soluble sugars*	*Fat*	*Ash*	*Crude fibre*
		(g per 100 g)					
HPL 8	10·7	28·7	54·3	4·3	2·6	4·9	1·4
HPL 40	9·3	31·1	55·6	5·1	2·5	5·1	1·1
C 11	11·0	24·8	58·7	4·8	2·9	4·9	1·2
ICPL 211	12·7	23·1	59·3	4·2	3·1	5·0	1·4
SE	±0·34	±0·09	±0·30	±0·06	±0·02	±0·03	±0·03

[a] Averages of two determinations and expressed on dry weight basis.

TABLE 2
Protein fractions of dhal sample of high and normal protein genotypes[a]

Genotype	*Protein (g per 100 g)*	*Albumin*	*Globulin*	*Glutelin*	*Prolamin*	*Total*
		(g per 100 g protein)				
HPL 8	28·7	9·1	63·5	20·2	2·9	95·7
HPL 40	31·1	8·0	66·2	19·7	3·2	97·1
C 11	24·8	7·7	60·5	23·3	3·6	95·1
ICPL 211	23·1	8·6	60·3	22·8	2·1	94·5
SE	±0·09	±0·34	±1·08	±0·75	±0·06	—

[a] Averages of two determinations and expressed on dry weight basis.

TABLE 3
Amino acid composition (g per 100 g protein) of high and normal protein genotypes

	HPL 8	*HPL 40*	*C 11*	*ICPL 211*	*SE*
Lysine	5·5	5·8	5·8	6·0	±0·07
Histidine	3·2	3·2	3·2	3·3	±0·03
Arginine	5·7	6·3	5·8	5·6	±0·02
Aspartic acid	8·7	8·7	8·7	8·9	±0·14
Threonine	2·0	2·9	3·0	3·0	±0·11
Serine	4·1	4·0	4·1	4·3	±0·07
Glutamic acid	20·5	20·0	21·2	21·3	±0·21
Proline	3·7	4·1	4·4	4·8	±0·12
Glycine	3·4	3·2	3·4	3·3	±0·05
Alanine	3·6	3·7	3·9	4·0	±0·03
Cystine	0·8	0·8	0·7	0·7	±0·01
Valine	3·6	3·7	3·9	4·1	±0·08
Methionine	1·0	1·0	1·1	1·1	±0·02
Isoleucine	3·4	3·2	3·5	3·6	±0·03
Leucine	6·4	6·4	6·7	7·0	±0·08
Tyrosine	2·6	2·5	2·7	2·7	±0·03
Phenylalanine	8·3	7·9	8·1	8·7	±0·09
Protein (g per 100 g)[a]	29·9	32·5	25·7	24·2	±0·09

[a] Analysis of defatted dhal samples (N × 6·25, dry weight basis).

containing amino acids, their limitations in the nutrition of humans and other monogastric animals are well known (Millerd 1975). The higher levels of sulphur-containing amino acids in the glutelin than in the globulin fraction of pigeonpea have led to the suggestion that cultivars with a higher ratio of glutelin to globulin should be identified to improve their seed protein quality (Singh and Jambunathan 1982). These small relative changes in the protein fractions of these genotypes did not, however, result in changes in the limiting essential amino acids, methionine and cystine (Table 3). The levels of various essential and non-essential amino acids of these genotypes did not show large differences (Table 3). Although tryptophan is an essential amino acid of pigeonpea, and nutritionally important, this amino acid was

not determined in the present study as it was destroyed during refluxing in 6 M HCl. Like other plant proteins, amino acid composition serves as a first approximation of the protein quality of pigeonpea proteins (Singh and Eggum 1984). No marked differences were observed in sulphur-containing amino acids of the HP and NP genotypes.

Trypsin and chymotrypsin inhibitors

In common with other grain legumes, pigeonpea seeds contain considerable amounts of protease inhibitors (Singh 1988). Trypsin and chymotrypsin inhibitors of raw and cooked samples of the HP and NP genotypes are shown in Table 4. Trypsin inhibitor activity (TIA) did not reveal marked differences in the HP and NP genotypes, although differences among the genotypes were significant ($P < 0.01$). TIA was remarkably reduced as a result of cooking in all the genotypes. Chymotrypsin inhibitor activity (CIA) was slightly higher in the raw sample of HP genotypes than in that of NP genotypes. However, CIA was not detected in cooked samples, indicating that CIA was completely destroyed in the heat treatment. But this did not happen in the case of TIA.

Biological evaluation of protein quality

Protein digestibility is of increasing interest in grain legumes in general, and pigeonpea in particular (Singh and Eggum 1984). Rat feeding trials were conducted using these genotypes, and the present authors also examined the effect of cooking on protein digestibility of raw and cooked whole-seed and dhal samples of these genotypes. The results of these experiments are summarised in Tables 5 and 6. True protein digestibility (TD) increased significantly ($P < 0.01$) with cooking and the effect was more pronounced in whole seed than in dhal samples (Tables 5 and 6). Interestingly, the biological value (BV) of the cooked samples decreased in both whole seed and dhal, whereas net protein utilisation (NPU) of the cooked samples

TABLE 4

Trypsin inhibitor activity (TIA) and chymotrypsin inhibitor activity (CIA) of raw and cooked dhal samples of high and normal genotypes

Genotype	*TIA*				*CIA*		
	Raw		*Cooked*		*Raw*		*Cooked*
	a	*b*	*a*	*b*	*a*	*b*	
HPL 8	7·2	25·1	0·4	1·5	3·5	12·2	ND
HPL 40	5·4	17·4	0·7	2·3	3·8	12·4	ND
C 11	4·8	19·4	0·4	1·7	2·2	8·9	ND
ICPL 211	6·9	24·8	0·3	1·3	2·4	10·4	ND
SE	±0·34	±0·75	±0·08	±0·18	±0·06	±0·26	—

[a] Enzyme units inhibited per mg meal.
[b] Enzyme units inhibited per mg protein.
ND = Not detected.

TABLE 5

Biological evaluation of raw and cooked whole seed samples of high and normal protein genotypes[a]

Genotype	Raw					Cooked				
	Protein[b]	TD	BV	NPU	UP	Protein[b]	TD	BV	NPU	UP
	(g per 100 g)									
HPL 8	25·6	58·5	68·7	40·2	10·3	24·4	79·4	68·5	54·4	13·3
HPL 40	27·3	58·0	70·5	40·9	11·2	27·6	75·8	66·4	50·3	13·9
C 11	21·9	59·5	64·3	38·3	8·4	22·2	75·6	62·5	47·3	10·5
ICPL 211	21·0	60·6	64·0	38·8	8·1	20·9	74·9	64·5	48·3	10·1
SE	±0·48	±1·08	±1·13	±0·64	±0·23	±0·32	±1·35	±1·07	±1·01	±0·31

[a] TD = True protein digestibility, BV = biological value, NPU = net protein utilisation (TD × BV/100), UP = utilisable protein (protein × NPU/100).
[b] Protein = N × 6·25 (dry weight basis).

TABLE 6

Biological evaluation of cooked and raw sample of dhal of high and normal protein genotypes[a]

Genotype	Raw					Cooked				
	Protein[b]	TD	BV	NPU	UP	Protein[b]	TD	BV	NPU	UP
	(g per 100 g)									
HPL 8	28·7	71·5	75·8	54·2	15·6	27·6	83·7	67·0	56·1	15·5
HPL 40	31·1	69·8	73·6	51·4	16·0	30·8	82·9	65·3	54·1	16·7
C 11	24·8	72·3	73·6	53·2	13·2	23·9	84·3	66·7	56·2	13·5
ICPL 211	23·1	70·8	76·4	54·1	12·5	22·8	85·7	62·9	53·9	12·3
SE	±0·28	±0·98	±1·14	±1·23	±0·34	±0·26	±2·14	±1·68	±1·06	±0·25

[a] TD = True protein digestibility, BV = biological value, NPU = net protein utilisation (TD × BV/100), UP = utilisable protein (protein × NPU/100).
[b] Protein = N × 6·25 (dry weight basis).

increased; this may have been due to an increase in the protein digestibility. A decrease in BV of cooked samples of both whole seed and dhal might be attributable to heat treatment, which causes considerable nutritional damage to methionine, the most important amino acid in grain legumes (Shemer and Perkins 1975).

A comparison of the TD of raw samples of whole-seed and dhal samples of these genotypes indicated large differences. The average TD was nearly 60% for whole seed (Table 5), whereas it increased to over 70% in dhal samples (Table 6). The reduced TD of whole seed may be due to higher polyphenol and fibre contents as the majority of these compounds are concentrated in the seed coat. Polyphenols decrease protein digestibility in animals including humans, probably by making protein partially unavailable or by inhibiting digestive enzymes and increasing faecal nitrogen (Singh 1984; Bressani *et al* 1988). However, these polyphenols may not have a great nutritional implication as they are removed by the processing of pigeonpea (Rao and Doesthale 1982; Singh 1988).

Although TD, BV and NPU values have shown some differences among these genotypes, no noticeable differences in these protein quality attributes were observed among the HP and NP genotypes. More importantly, the values for utilisable protein (UP) were considerably higher in the HP genotypes than in the NP genotypes. Higher UP values for the HP genotypes are attributed to their higher protein content. This indicated that the HP genotypes are nutritionally better than the NP genotypes as the former contain more utilisable protein.

CONCLUSIONS

These results show that the levels of various nutritional attributes of the HP and NP genotypes are quite comparable, and that it is possible to improve pigeonpea protein content and its quality by breeding. Further, the HP genotypes may be preferred from the nutritional point of view over the NP genotypes as, *per se*, they would provide more utilisable protein and sulphur-containing amino acids. To enhance the nutritive value, utilisation and productivity of the crop, the development of high-protein cultivars with desirable agronomic traits should be emphasised in breeding programmes.

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