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A COMPARATIVE STUDY ON THE CHEMICAL COMPOSITION OF WILD AND CULTIVATED GERMPLASM OF *PHASEOLUS LUNATUS* L.

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ABSTRACT : Wild Lima beans (*Phaseolus lunatus* L.) were compared with cultivated seed sample (LBS.1) in proximate composition, seed protein fractions, amino acid profiles of total seed proteins, mineral composition, *in vitro* protein digestibility (IVPD) and certain anti-nutritional factors. The wild beans contained more protein (22.84% vs. 20.05%), ash (3.42 vs. 2.91%), crude fiber (5.48% vs. 4.54%), crude lipid (2.63% vs. 2.55%), less carbohydrates (NFE) (65.63% vs. 69.95%) and the energy level of the seed (1576.60 vs. 1599.14 $kJ100g^{-1}$ DM) than the cultivated seeds. Globulins formed the major bulk of seed proteins in both the seed samples. The essential amino acid profile of total seed proteins compared favorably with FAO/WHO (1991) requirement pattern, except that there were deficiencies of sulphur containing amino acids in the varieties. Wild seeds were found to be a rich source of potassium, calcium, magnesium and phosphorus. The wild seed samples presented a less free phenolics (0.66 %), tannins (0.90%), higher content of L-DOPA (0.91 %), and less trypsin inhibitor activity (17.80 TIU mg^{-1} protein) than the cultivated seeds. Lower levels of phytohaemagglutinating activity for human erythrocytes of "O" blood group than for "A" and "B" blood groups were found. From the chemical point of view, domestication seems to be positive; however, the better protein nutritive quality of the wild beans should be further confirmed by biological assays.

Key words: Phaseolus lunatus, Proximate and Mineral Composition, Protein fractions and anti-nutritional factors

INTRODUCTION

Plants are the most predominant harvesters of solar energy and they constitute primary resources of carbohydrates, vitamins, proteins, essential fatty acids and utilizable energy for human food production. Most of the world's population depends on approximately twenty different food crops, which are generally divided into cereals, vegetables (including legumes), fruits and nuts. In the context of human PEM, the most important groups are cereal grains and food legumes, including oil-seed legumes. Legumes are cheaper than animal products like meat, fish and eggs; therefore they are consumed worldwide as major sources of protein and especially in developing or poor countries where consumption of animal protein may be limited because of economics, social, cultural or religious factors (Oboh, 2006). There is a fierce competition between human and livestock for the same food or land on which the food crop is grown. The high cost of producing and maintaining livestock which are the primary source for balanced diets in the developing world constitutes a major problem. Protein-energy malnutrition is among the most serious problems which tropical developing countries are facing today. This can be attributed mainly to the ever-increasing population as well as to the enhanced dependence on a cereal-based diet, scarcity of fertile land, and degradation of natural resources (Deshpande 1992; Steiner 1996; FAO 2000). It has been estimated that 800 million malnourished people exist in some of the least developed countries (Myers 2002). Apart from this, high prices of available staple foods and policy constraints on food imports are also contributing factors that have been worsening the food situation in the developing countries (Weaver 1994).

Even though several common proteinaceous edible legumes (soybean, cowpea, and others) are available on the market, in most instances, production rate compared with consumption (as food and feed) has remained unmet, and an ever-increasing demand has been witnessed (Ali and Kumar 2000). Also, switching by most of the world's population to a protein-rich vegetarian-based diet from animal-based protein has created unwarranted scarcity to plant resources. In this regard, legumes have been highlighted as an effective substitute to animal protein as well as being cost effective (Famurewa and Raji 2005). Therefore, in the present study, an attempt has been made to a comparative study on the chemical composition of wild and cultivated Lima beans (*Phaseolus lunatus* L.) with a view to assessing their nutritional quality.

MATERIALS AND METHODS

Preparation of seed flour

The wild lima bean (*Phaseolus lunatus* L.) seed samples employed in the present study were collected from Nadugani of Nilgiri Biosphere Reserve (NBR) Western Ghats, Tamil Nadu, India. The cultivated lima bean var. LBS.1 was procured from Tamilnadu Agricultural University Coimbatore India.

Proximate composition

Seed moisture content, on a percent basis, was determined by drying 50 transversely cut seeds in an oven at 80°C for 24h. The air-dried samples were powdered separately in a Wiley mill (Scientific Equipment, Delhi, India) to 60-mesh size and stored in screw capped bottles at room temperature for further analysis. Nitrogen content was estimated by the micro-Kjeldahl method (Humphries, 1956) and the crude protein content was calculated (N x 6.25). Crude lipid content was determined using Soxhlet apparatus (AOAC, 2005). Ash was determined by heating 2g of the dried sample in a silica dish at 600°C for 6h (AOAC, 2005). Total dietary fibre (TDF) was estimated by the non-enzymatic-gravimetric method proposed by Li and Cardozo (1994). To determine the TDF, duplicate 500mg ground samples were taken in separate 250ml beakers. To each beaker 25ml water was added and gently stirred until samples were thoroughly wetted, (i.e. no clumps present); the beakers were then covered with Al foil and allowed to stand 90min without stirring in an incubator maintained at 37°C; after that, 100ml 95% ethanol were added to each beaker and allowed to stand for 1hr at room temperature (25±2°C). The residue was collected under vacuum in a pre-weighed crucible containing filter aid. The residue was washed successively with 20ml of 78% ethanol, 10ml of 95% ethanol and 10ml acetone. The crucible containing the residue was dried >2 h at 105°C and then cooled > 2h in a desiccator and weighed. One crucible containing residue was used for ash determination at 525°C for 5h. The ash-containing crucible was cooled for > 2h in a desiccator and weighed. The residue from the remaining duplicate crucible was used for crude protein determination by the micro-Kjeldahl method as already mentioned. The TDF was calculated as follows.

$$TDF\% = 100 \times \frac{Wr - [(P+A)/100]\,Wr}{Ws}$$

Where *Wr* is the mg residue, *P* is the % protein in the residue; *A* is the % ash in the residue, and *Ws* is the mg sample.

The nitrogen free extract (NFE) was obtained by difference (Muller and Tobin, 1980). The energy value of the seed (kJ) was estimated by multiplying the percentages of crude protein, crude lipid and NFE by the factors 16.7, 37.7 and 16.7, respectively (Siddhuraju *et al.*, 1996).

Amino acid analysis

The total seed protein was extracted by a modified method by Basha *et al.,* (1976). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). A protein sample of 30mg was hydrolysed by 6N HCL (5ml) in an evacuated sealed tube, which was kept in an air oven maintained at 110°C for 24 hours. The sealed tube was broken and the acid removed completely by repeated flash evaporation after the addition of deionized H2O. Dilution was effected by means of citrate buffer pH 2.2 to such an extent that the solution contained 0.5mg protein ml-1. The solution was passed through a millipore filter (0.45µM) and derivatized with O-phthaldialdehyde by using an automated pre-column (OPA). Amino acids were analyzed by a reverse – phase HPLC (Method L 7400, HITACHI, Japan) fitted with a denali C: 18 5 micron column (4.6X 150mm). The flow rate was 1ml min^{-1} with fluorescence detector. The cystine content of protein sample was obtained separately by the Liddell and Saville (1959) method. For the determination of tryptophan content of proteins, aliquots containing known amounts of proteins were dispersed into glass ampoules together with 1 ml 5M NaOH. The ampoules were flame sealed and incubated at 110°C for 18 hours. The tryptophan content of the alkaline hydrolysates were determined calorimetrically using the method by Spies and Chambers (1949) as modified by Rao *et al.* (1974). The contents of the different amino acids were expressed as g100g-1 proteins and were compared with FAO/WHO (1991) reference pattern. The essential amino acid score is calculated as follows:

$$\text{Essential amino acid score} = \frac{\text{Grams of essential amino acid in 100g of the test protein}}{\text{grams of essential amino acid in 100g of FAO / WHO (1991) reference pattern.}} \times 100$$

Analysis of minerals

Five hundred milligrams of the ground legume seed was digested with a mixture of 10ml concentrated nitric acid, 4ml of 60% perchloric acid and 1ml concentrated sulphuric acid. After cooling, the digest was diluted with 50ml of deionised H_2O, filtered through Whatman No. 42 filter paper and filtrates were made up to 100ml with deionised H2O in a glass volumetric flask. All minerals, except phosphorus, were analyzed from a triple acid-digested sample by atomic absorption spectrophotometry, ECIL (Electronic Corporation of India Ltd., India) (Issac and Johnson, 1975). The phosphorus content in the triple acid digested extract was determined calorimetrically (Dickman and Bray, 1940).

Determination of *in vitro* protein digestibility (IVPD)

The determination of *in vitro* protein digestibility was determined using the multi-enzyme technique (Hsu *et al.*, 1977). The enzymes used for IVPD were purchased from Sigma Chemical Co., St. Louis, MO, USA. Calculated amounts of the control (casein) and sample were weighed out, hydrated in 10ml of distilled water and refrigerated at 5°C for 1hr. The samples containing protein and enzymes were all adjusted to pH 8.0 at 37°C. The IVPD was determined by the sequential digestion of the samples with a multi-enzyme mixture [trypsin (porcine pancreatic trypsin–type IX with 14190 BAEE unites per mg protein), α-chymotrypsin (bovine pancreatic chymotrypsin–type II, 60 units per mg powder) and peptidase (porcine intestinal peptidase - grade III, 40 units per g powder)] at 37°C followed by protease (type IV from *Streptomyces griseus*) at 55°C. The pH drop of the samples from pH 8.0 was recorded after 20min of incubation. The IVPD was calculated according to the regression equation $Y = 234.84 – 22.56\ X$, where Y is the % digestibility and X the pH drop.

Analysis of anti-nutritional compounds

The anti-nutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971) and L-DOPA (3, 4-dihydroxyphenylalanine) (Brain, 1976) were quantified. Trypsin inhibitor activity was determined by the enzyme assay Kakade *et al.* (1974) by using benzoil-DL-arginin-*p*-nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Quantitative determination of phytoheamagglutinating (Lectin) activity

Lectin activity was determined by the method of Almedia *et al.* (1991). One gram of air-dried seed flour was stirred with 10ml of 0.15N sodium chloride solution for 2hr and the pH 4.0 was adjusted. The contents were centrifuged at 10,000 x g for 20min. and the supernatants were collected separately. Protein content was estimated after Lowry *et al.* (1951) method.

Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3min at low speed (3,000 g for 10 min at room temperature). Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24drops of phosphate – buffered saline. Human blood (blood groups A, B and O) was procured from the blood bank of Micro Clinical Laboratory, Coimbatore.

The determination of lectin was done by the method of Tan *et al.* (1983). Clear supernatant (50µl) was poured into the depression (pit) on a microtitration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25µl) were added to each of the twenty depressions. The plates were incubated for 3hours at room temperature. After the incubation period, the titer values were recorded. One Haemagglutinating unit is defined as the least amount of heamagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. The phytoheamagglutinating activity was expressed as heamagglutinating units (HU)/mg protein.

Statistical analysis

Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used for analysis [MSTAT – 'C' software (version 1.4.1 Michigan State University, MI, USA)] of any significant difference in chemical compositions among the fifteen wild / under-exploited legumes. Significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

The results of proximate analysis are shown in Table 1. The wild lima bean seeds registered higher crude protein than the cultivated seeds. In most of the food crops, genetic variability for protein content is always considered as an important factor in improvement of protein quality by selection and breeding (Singh and Eggum, 1984). Therefore this observation could be used for breeding programmes when a higher protein concentration is often emphasized. Crude fat content of wild seed sample is found to be high compared to cultivated seeds of the present study which is agreement with an earlier investigation in the same species (Vadivel and Janardhanan, 1999).

The relatively higher ash content of the wild seeds (3.48%) generally indicates higher concentration of minerals than that of cultivated samples. However, its ash content is found to be low compared to earlier reports in the same species (Rajaram and Janardhanan, 1993). High energy content of wild seed sample could be attributed to the presence of high levels of both crude protein and crude lipid. In general, wild seed sample registered higher crude protein, crude lipid, crude fibre, ash and energy levels than cultivated Lima bean. This is in consonance with earlier reports for lima bean (De la Vega and Sotelo, 1986) and pigeon pea (Singh and Eggum, 1984).

Table: 1. Proximate composition of wild and cultivated seeds of *Phaseolus lunatus*[1, 2] (g 100 g^{-1})

Components	Wild germplasm	Cultivated germplasm
Moisture	11.78 ± 0.05[a]	12.07 ± 0.04[b]
Crude protein (Kjeldahl N x 6.25)	22.84 ± 0.07[c]	20.05 ± 0.06[a]
Crude lipid	2.63 ± 0.09[b]	2.55 ± 0.06[a]
Crude fibre	5.48 ± 0.07[c]	4.54 ± 0.04[a]
Ash	3.42 ± 0.03[b]	2.91 ± 0.03[a]
Nitrogen Free Extractives (NFE)	65.63	69.95
Calorific value (kJ 100g-1DM)	1576.6	1599.14

1 - Values are the means of triplicate determinations and ± - Standard error
2 - Mean values in the same row sharing different superscript are significantly different (P < 0.05)

Like other grain legumes, the globulin protein fraction constituted 54.63 and 55.16% of the total seed proteins of wild and cultivated lima beans respectively (Table 2). The amino acid profiles of the purified seed proteins and the essential amino acid scores are presented in Table 3. The amount of threonine, leucine, cystine and methionine seem to be deficient whereas valine, isoleucine, tyrosine, phenylalanine, lysine and histidine contents appear to be present in more than adequate levels compared to FAO / WHO (1991) requirement pattern. The deficient threonine content range of *Phaseolus lunatus* is found to be higher than that of *Cicer areitinum* (Singh *et al.,* 1988), *Vigna mungo* (Gupta and Wagle, 1978) and *V. radiata* (Ignacimuthu and Babu, 1987).

Table 2. Total protein and different protein fractions of wild and cultivated seeds of *Phaseolus lunatus* [1, 2] (g 100 g^{-1})

Protein fraction	Wild germplasm		Cultivated germplasm	
	seed flour	seed protein	seed flour	seed protein
Total protein	17.48 ± 0.02[d]	100	14.33 ± 0.04[a]	100
Albumins	4.99 ± 0.02[c]	28.55	4.14 ± 0.02[b]	28.87
Globulins	9.55 ± 0.08[c]	54.63	7.91 ± 0.03[a]	55.16
Prolamins	0.85 ± 0.04[a]	4.86	0.95 ± 0.05[a]	6.63
Glutelins	2.09 ± 0.02[b]	11.96	1.34 ± 0.02[b]	9.34

1 - Values are the means of triplicate determinations and ± - Standard error
2 - Mean values in the same row sharing different superscript are significantly different (P < 0.05)

Table 3. Amino acid profiles of the wild and cultivated seeds of *Phaseolus lunatus*

Amino acid	Wild germplasm		Cultivated germplasm		FAO/WHO (1991) requirement pattern
	g 100 g^{-1} protein	EAA Score	g 100 g^{-1} protein	EAA Score	
Glutamic acid	13.9		13.4		
Aspartic acid	9.3		9		
Serine	4		4.2		
Threonine	3.2	94.12	3.1	91.18	3.4
Proline	2.4		2.5		
Alanine	4		4		
Glycine	3.6		3.7		
Valine	5.4	154.29	5.8	165.71	3.5
Cystine	Trace	28	Trace	36	2.5
Methionine	0.7		0.9		
Isoleucine	8.7	310.71	8.4	300	2.8
Leucine	5.1	77.27	5.6	84.85	6.6
Tyrosine	1.6	107.94	1.8	120.63	6.3
Phenylalanine	5.2		5.3		
Lysine	7.4	127.59	7.5	129.31	5.8
Histidine	2.6	136.84	2.2	115.79	1.9
Tryptophan	N.D		N.D		1.1
Argnine	6.4		6.6		

N. D - Not Deducted

Table 4. Mineral composition of wild and cultivated seeds of *Phaseolus lunatus*[1, 2](mg 100 g^{-1})

Components	Wild germplasm	Cultivated germplasm
Sodium	37.84 ± 0.06^{d}	30.84 ± 0.07^{a}
Potassium	1892.05 ± 0.64^{d}	1698.68 ± 0.52^{a}
Calcium	352.56 ± 0.71^{a}	720.88 ± 0.26^{d}
Magnesium	225.64 ± 0.33^{d}	155.74 ± 0.52^{b}
Phosphorus	247.91 ± 0.51^{c}	177.81 ± 0.37^{b}
Iron	2.09 ± 0.03^{b}	2.25 ± 0.05^{c}
Copper	1.58 ± 0.03^{b}	1.12 ± 0.07^{a}
Zinc	0.54 ± 0.06^{b}	0.19 ± 0.04^{a}
Manganese	4.75 ± 0.04^{bc}	5.16 ± 0.05^{c}

1 - Values are the means of triplicate determinations and ± - Standard error

2 - Mean values in the same row sharing different superscript are significantly different ($P < 0.05$)

Table 5. Data of invitro protein digestibility and anti-nutritional factors of wild and cultivated seeds of *Phaseolus lunatus*[1, 2](g 100 g^{-1})

Particulars	Wild germplasm	Cultivated germplasm
IVPD	69.47 ± 0.05[c]	68.39 ± 0.06[b]
Total free phenolics	0.66 ± 0.05[b]	0.78 ± 0.04[b]
Tannins	0.90 ± 0.04[b]	1.02 ± 0.02[b]
L-DOPA	0.91 ± 0.03[ab]	0.80 ± 0.05[a]
Tripsin inhibitor activity[3]	17.80 ± 0.16[a]	20.30 ± 0.05[b]

Phytohaemagglutinating activity [4, 5]

Name of the protein fraction	Erythrocytes from human blood group	Haemagglutinating activity (HU/mg protein)	
Albumins	A	6	4
Albumins	B	9	7
Albumins	O	3	0
Globulins	A	52	50
Globulins	B	5	3
Globulins	O	0	4

1 - Values are the means of triplicate determinations and ± - Standard error
2 - Mean values in the same row sharing different superscript are significantly different ($P < 0.05$)
3 - Tripsin inhibitor unit (TIU mg^{-1} protein)
4 - Values of two different experiments
5 - Haemagglutinating units (HU/mg protein)

The mineral profiles of both the lima bean seed samples are shown (Table 4). The mineral composition reveals that the wild seeds appeared to be a rich source of potassium, phosphorus, calcium sodium, magnesium and iron compared to the same species (Vijayakumari et *al*., 1993). The IVPD of wild seed sample of lima bean seem to be higher than that of cultivated species (Table 5).

Anti-nutrients are those which interfere with the assimilation of nutrients contained in foods. Food legumes are important sources of dietary proteins in developing countries, but their acceptability and utilization have been limited due to the presence of relatively high concentrations of anti-nutritional compounds (Nwoacki, 1980). The data on anti-nutritional compounds are given in Table 5. The total free phenolics, trypsin inhibitor activity and tannin contents of wild seed samples seems to be lower than that of cultivated seed sample. The contents of L-DOPA in wild seed sample appeared higher than cultivated seed sample of the present study and some earlier report in *P. chilensis* (Rajaram and Janardhanan, 1991). It has been demonstrated that in *Mucuna pruriens*, the level of L-DOPA and trypsin inhibitor activity is significantly eliminated by dry-heat treatment (Siddhuraju *et al.,* 1996) and cooking and autoclaving (Vijayakumari *et al.*, 1996). Phytohaemagglutinating activity of both wild and cultivated seed samples of lima bean registers higher activity with respect to, “A” blood group of human erythrocytes. All the accessions had low levels of phytohaemagglutinating activity with respect to erythrocytes of, “O” blood group. This is in good agreement with earlier reports in the other *Mucuna* species (Vijayakumari *et al.*, 1996).

To sum up the wild seeds exhibited higher food components and mineral composition. However, dry-heat and autoclaving are known to be inactivate completely but the trypsin inhibitors and phytohaemagglutins. Based on aforesaid facts, the wild lima bean may be used in the breeding programmes.

Conclusion

In conclusion, through hybridization and selection, the genes responsible for the desirable nutritional quality observed in wild seed sample might be incorporated into other genotypes which possess the desirable agronomic traits. Eventually, a superior cultivar for human use can be developed.

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