Biochemical evaluation of some promising varieties/genotypes of underutilized food legumes of Himachal Pradesh

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ABSTRACT

Studies on biochemical analysis of some important varieties/genotypes of underutilized food legumes of Himachal Pradesh were carried out to evaluate their nutritional significance. The dried mature seeds of promising ten adzuki bean, *Vigna angularis* (Willd) Ohwi and Ohasi and fourteen rice bean, *Vigna umbellata* (Thunb) Ohwi and Ohashi genotypes were analyzed for moisture, crude protein, crude fat, crude fibre, ash, carbohydrate, methionine, tryptophan, in vitro protein digestibility, tannins, cooking time, calcium, iron and zinc. On the basis of genotypic rating in respect of desirable nutritional quality attributes taken together EC-030256, EC-290652 and EC-340249 genotypes of adzuki bean and LRB-452, PRR-2 and LRB-448 genotypes of rice bean were found superior over others in that order.

Keywords: Adzuki bean; rice bean; genotypes; legumes; quality traits

INTRODUCTION

Underutilized crops at preliminary stages of domestication are those which are adapted to stressed environments, provide food and nutritional cover to about 10 per cent population inhabiting in remote, tribal and backward areas, possess enumerable unique traits of nutrition, impart resistance to biotic and abiotic stresses and thus offer a good scope for diversification of agriculture. Besides major pulses such as chickpea, pigeon pea, green gram, black gram, lentil and peas there are other several legumes which are cultivated because of their being intrinsic resilient and associated with important useful traits. The important ones include rice bean, faba bean, adzuki bean and winged bean. Many of them are nutritionally better as compared to major pulses.

Adzuki bean, *Vigna angularis* (Willd) Ohwi and Ohashi belongs to Leguminosae family and has a wide variety of uses. The dried seeds are used for human food either cooked whole or made into meal and used in soups, cakes or confectionary. Flour is used for making shampoos and face creams. Sprouted beans are used as vegetable. Its seeds have medicinal properties and are reported to be used in the treatment of kidney trouble, constipation, boils, abscesses, threatened miscarriage, difficult labour, retained placenta and non-secretion of milk. Its leaves are said to be helpful in lowering fever and sprouts are used to treat threatened abortion caused by injury.

Rice bean, *Vigna umbellata* (Thunb) Ohwi and Ohashi is the native of southeast Asia. In India the distribution of the crop is confined to northeastern hills, western and eastern Ghats and parts of Himachal Pradesh (Chandel et al 1978). It is one of the promising beans having high yield potential. The nutritional profile of rice bean is very high due to the high content of proteins and essential amino acids (tryptophan and methionine) as compared to the other traditional pulses (Sharma et al 2003, Awasthi et al 2011). The mature seeds are also rich in important minerals and water soluble vitamins like thiamin, riboflavin and niacin. The

vegetative parts of the crop serve as nutritive forage for animals. Thus the suitability of rice bean both for food and fodder makes it a dual purpose crop (Chandel et al 1988).

Since adzuki bean and rice bean appear to be new additions to the group of pulses the pertinent information on biochemical constituents of dietary significance of promising newly identified genotypes grown in Himachal Pradesh conditions is still scanty. Thus the present investigation was carried out to evaluate important nutritional quality attributes of these important underutilized food legumes of hills in order to identify nutritionally superior multipurpose genotypes for further crop improvement.

MATERIAL and METHODS

Seed samples of ten genotypes of adzuki bean and fourteen genotypes of rice bean procured from NBPGR Regional Station, Phagli, Shimla, HP were evaluated for various biochemical parameters by following standard procedures. The finely ground seed samples were stored in air tight containers for biochemical analysis.

Genotypes were analyzed in triplicate for moisture, crude protein, ash and crude fibre as per Anon (1990) and crude fat (ether extract) as per Anon (1975). Carbohydrate content was computed as a difference of 100 – moisture + crude protein + crude fat (ether extract) + ash + crude fibre as given by Gopalan et al (2004). The essential amino acids (methionine and tryptophan), in vitro protein digestibility and total tannins were estimated by the methods of Horn et al (1946), Mertz et al (1975), Akeson and Stahmann (1964) and Makkar et al (1993) respectively.

Cooking quality of rice bean was determined by recording the cooking time. Five gram of sample was put in a beaker with excess of deionized water (20 ml) and kept in boiling water. Seeds were tested for doneness by pressing them between layers of filter paper and cooking time was recorded. Minerals viz calcium, iron and zinc were digested using the di-acid wet digestion (HNO_3, $HClO_4$, 3:1) and estimated by using atomic absorption spectrophotometer (Model ContrAA 700) following the procedure of Lindsay and Norvell (1969). The data were analyzed statistically by using analysis of variance as given by Panse and Sukhatme (1984).

RESULTS and DISCUSSION

The data on variation in biochemical constituents of various genotypes of adzuki bean and rice bean are depicted in Tables 1 and 2. Among various adzuki bean genotypes the moisture content was found to vary significantly from 5.1 (EC-340246) to 11.2 (EC-340249) per cent. In rice bean genotypes values for moisture content varied significantly from 5.0 (LRB-448) to 11.8 (LRB-460) per cent. The range of variation in moisture content in the present study is in close proximity with earlier reports of Hira et al (1988) in rice bean genotypes.

Significant variation in crude protein content was observed from 20.5 (IC-341946) to 23.8 (Toturu Local) per cent in adzuki bean genotypes. In rice bean genotypes crude protein content varied from 17.5 (LRB-160) to 21.0 (LRB-467) per cent. The trend of variation is in agreement with the observations of Saikia et al (1999), Sadana et al (2006) and Myrna et al (1991). Narasinga Rao et al (1989) also reported that values for protein content in rice bean were comparable to some cultivated legume seeds like cowpea (24.1%), green gram (24.0%), Bengal gram (17.1%), lentil (25.1%), moth bean (23.6%) and peas (19.7%) but were lower than soy bean (43.2%).

Though present in small amounts in adzuki bean seeds crude fat (ether extract) content was observed to vary significantly from 0.29 to 0.52 per cent. The genotype IC-341946 contained more crude fat (ether extract) content followed by the genotypes IC-341948 and HPU-51. EC-290251exhibited the lowest crude fat (ether extract) content. In rice bean genotypes crude fat (ether extract) content varied from 0.31 (IC-141077) to 0.62 (PRR-2) per cent. The variability pattern for crude fat (ether extract) content is in consonance with the findings of Saikia et al (1999) and Sadana et al (2006) who have reported 0.46 to 2.2 per cent crude fat (ether extract) content in this crop.

Values in respect of ash and crude fibre content ranged significantly from 3.9 (IC-341946) to 5.5 (EC-030256) per cent and 4.4 (EC-340246) to 5.7 (EC-290652) per cent in adzuki bean respectively. The released varieties viz HPU-51 and Toturu Local contained 4.2 and 4.8 per cent ash content and 4.7 and

Table 1. Variation in biochemical parameters of adzuki bean genotypes

Parameter	EC-290251	EC-290652	EC-030256	EC-034625	EC-340246	EC-340249	IC-341946	IC-341948	HPU-51*	Toturu Local*	SEm±	$CD_{0.05}$
Moisture (%)	9.6	8.4	7.2	10.9	5.1	11.2	7.5	6.3	10.1	10.2	0.54	1.32
Crude protein (%)	21.4	21.6	23.6	22.3	22.8	23.2	20.5	21.8	23.6	23.8	2.85	8.40
Ether extract (%)	0.29	0.33	0.40	0.20	0.35	0.28	0.52	0.48	0.44	0.35	0.05	0.16
Ash (%)	5.2	4.4	5.5	4.9	5.1	5.3	3.9	4.6	4.2	4.8	0.33	0.98
Crude fibre (%)	4.7	5.7	4.9	4.5	4.4	5.4	5.3	5.1	4.7	5.6	0.32	0.93
Carbohydrates (%)	58.8	59.6	58.4	57.2	62.3	54.6	62.3	61.7	57.0	55.6	2.43	7.17
Methionine (g/100 g protein)	1.18	0.95	1.49	0.80	0.97	0.70	1.03	1.15	1.04	0.83	0.09	0.27
Tryptophan (g/100 g protein)	0.79	0.88	0.80	0.79	0.71	0.78	0.72	0.78	0.65	0.77	0.07	0.20
IVPD (%)	74.2	84.3	80.6	78.5	81.6	84.6	81.8	81.0	85.4	83.0	1.21	3.58
Calcium (mg/100 g)	315.3	336.5	329.3	321.8	322.0	338.9	320.3	335.2	301.8	300.8	1.32	3.89
Iron (mg/100 g)	7.8	8.1	8.5	7.8	8.2	8.5	9.5	7.8	7.2	6.5	0.49	1.43
Zinc (mg/100 g)	2.8	3.3	3.9	4.1	3.4	3.0	3.1	2.9	2.6	3.5	0.35	1.03
Total tannins (mg/100 g)	470	685	510	685	722	609	641	832	681	571	6.34	18.7
Cooking time (minutes)	44	45	46	41	42	43	40	39	45	46	3.16	9.31

*Released varieties, IVPD= In vitro protein digestibility

Table 2. Variation in biochemical parameters of rice bean genotypes

Parameter	PRR-1*	PRR-2*	BC-1	RL 3	LRB-160	LRB-10	LRB-311	LRB-448	LRB-452	LRB-460	LRB-467	IC-141077	IC-563980	VRB-3	SEm ±	$CD_{0.05}$
Moisture (%)	10.4	10.0	9.0	10.1	9.6	7.7	7.2	5.0	6.1	11.8	10.2	11.7	8.5	10.1	0.64	1.44
Crude protein (%)	18.8	17.7	19.7	18.4	17.5	19.6	17.7	19.7	18.2	20.1	21.0	19.6	18.8	19.3	0.86	2.48
Ether extract (%)	0.58	0.62	0.50	0.54	0.48	0.39	0.45	0.34	0.44	0.34	0.47	0.31	0.46	0.34	0.05	0.14
Ash (%)	4.1	4.2	4.3	3.8	3.9	4.0	5.0	5.2	5.8	4.7	4.8	4.3	5.6	4.9	0.43	1.23
Crude fibre (%)	5.5	4.8	5.9	5.9	5.7	6.3	6.5	5.1	6.2	5.2	4.8	5.7	4.6	6.6	0.45	1.29
Carbohydrates (%)	60.6	62.7	60.6	61.3	62.8	62.0	63.2	64.7	63.3	57.9	58.7	58.4	62.0	58.8	1.44	4.16
Methionine (g/100 g protein)	0.95	1.46	1.03	1.26	1.07	0.76	0.90	1.20	0.86	0.86	0.96	0.72	0.79	0.81	0.06	0.18
Tryptophan (g/100 g protein)	1.33	1.00	0.81	0.92	1.03	0.52	0.75	0.62	0.65	0.55	0.55	0.68	0.74	0.46	0.07	0.20
IVPD (%)	84.8	84.0	85.6	84.4	88.4	86.4	89.3	90.0	87.5	82.7	83.3	81.7	88.8	88.4	2.37	6.88
Calcium (mg/100 g)	318.3	383.0	333.5	353.3	364.0	311.3	315.8	302.8	354.8	302.0	345.0	307.0	325.8	340.0	5.69	16.5
Iron (mg/100 g)	4.0	4.6	7.9	5.7	5.4	5.8	6.5	6.3	9.1	5.3	4.6	6.2	6.5	4.2	0.39	1.12
Zinc (mg/100 g)	3.6	4.2	3.8	4.1	3.8	2.5	3.3	3.4	4.5	2.7	3.2	2.5	2.9	3.1	0.30	0.88
Total tannins (mg/100 g)	650	630	510	570	520	530	560	620	540	530	560	610	560	630	5.69	16.5
Cooking time (minutes)	56	56	53	47	48	42	53	49	53	54	53	46	48	45	3.09	8.94

*Released varieties, IVPD= In vitro protein digestibility

5.6 per cent crude fibre respectively. In rice bean genotypes ash and crude fibre content varied from 3.8 (RL-3) to 5.8 (LRB-452) per cent and 4.6 (IC-563980) to 6.6 (VRB-3) per cent respectively. Saikia et al (1999), Sadana et al (2006), Myrna et al (1991) and Kanwar et al (1997) revealed variation in crude fibre content from 4.8 to 6.0 per cent in Indian edible legumes viz Bengal gram, cowpea, green gram, moth bean and pea.

Variation in carbohydrate content of adzuki bean was from 54.6 to 62.3 per cent EC-340246 and IC-341946 showing the highest content. The released varieties HPU-51 and Toturu Local showed 57 and 55.6 per cent carbohydrate content. In case of rice bean carbohydrate content was from 57.9 (LRB-460) to 64.7 (LRB-448) per cent. Myrna et al (1991) reported variation in this parameter from 61.09 to 64.73 per cent in these crops.

Variation in methionine and tryptophan content in adzuki bean was observed from 0.70 (EC-340249) to 1.49 g/100 g protein (EC-030256) and 0.65 (HPU-51) to 0.88 g/100 g protein (EC-290652) respectively. The released varieties HPU-51 and Toturu Local exhibited 1.04 and 0.83 g methionine and 0.65 and 0.77 g tryptophan/100 g protein. In rice bean genotypes methionine and tryptophan content varied from 0.72 (IC-141077) to 1.46 g (PRR-2) and 0.46 (VRB-3) to 1.33 g/100 g protein (PRR-1) respectively. The results of the present study are corroborated with the findings of Mohan and Janardhanan (1994) and Bhagmal (1994) who reported variation from 1.58 to 2.88 g for methionine and 0.79 to 1.10 g/100 g protein for tryptophan content in rice bean.

Values for in vitro protein digestibility of dry mature seeds of adzuki bean ranged from 74.2 to 85.4 per cent. The values in HPU-51, EC-340249 and EC-290652 were found to be the highest over other genotypes. In rice bean values for in vitro protein digestibility ranged from 81.7 to 90 per cent. The values in LRB-448, LRB-311 and IC-563980 were found to be the highest over other genotypes. The released varieties viz PRR-1 and PRR-2 showed 84.8 and 84.0 per cent in vitro protein digestibility. Hira et al (1988) and Myrna et al (1991) observed the range of variation from 82 to 90.30 per cent for this parameter in rice bean genotypes. In adzuki bean calcium content ranged from 300.8 (Toturu Local) to 338.9 (EC-340249), iron from 6.5 (Toturu Local) to 9.5 (IC-341946) and zinc from 2.6 (HPU-51) to 4.1 (EC-034625) mg/100 g. In rice bean calcium content ranged from 302.0 (LRB-460) to 383.0 (PRR-2), iron from 4.0 (PRR-1) to 9.1 (LRB-452) and zinc from 2.5 (LRB-10, IC-141077) to 4.5 (LRB-452) mg/100 g. Awasthi et al (2011) earlier reported wide variation in calcium, iron and zinc content in rice bean genotypes to range from 265 to 385, 3.6 to 7.4 and 2.4 to 4.2 mg/100 g respectively.

Total tannins content varied from 470 (EC-290251) to 832 (341948) mg/100 g in whole seeds of adzuki bean. The released varieties viz HPU-51 and Toturu Local showed 651 and 571 mg/100 g total tannins. In rice bean total tannins content varied from 510 to 650 mg/100 g. The lowest value for tannins content was noticed in the genotype BC-1 whereas PRR-1 showed the highest value. Saikia et al (1999) reported 513 to 572 mg/100 g tannins in mature seeds of four cultivars of rice bean. Relatively higher levels of tannins in different rice bean genotypes observed in this study may be attributed to seed testa, colour, genotypic make up and variable agro-climatic conditions.

In adzuki bean cooking time varied from 39 (IC-341948) to 46 (EC-030256, Toturu Local) minutes. In rice bean it varied from 42 (LRB-10) to 56 (PRR-1 and PRR-2) minutes. Hira et al (1988) also reported average variation in cooking time from 52 to 55 minutes in rice bean genotypes.

To identify overall superior varieties/genotypes grading/ranking was done in respect of various nutritional quality attributes viz protein, fibre, carbohydrates, essential limiting amino acids - methionine and tryptophan, in vitro protein digestibility, cooking time, calcium, iron, zinc (in descending order) as well as anti-nutritional factor ie tannins content (in ascending order). On the basis of genotypic rating the genotypes EC-030256, EC-290652 and EC-340249 of adzuki bean and LRB-452, PRR-2 and LRB-448 of rice bean were found superior over others in that order (Tables 3 and 4).

The study indicated wide variation in individual biochemical parameters of nutritional significance which seems to be useful to the plant breeders and those engaged in adzuki bean and rice bean improvement. Moreover the multipurpose varieties/ genotypes identified in the present study based on genotypic rating might be used after conducting biological feeding experiments for further dietary intake purpose for optimum human nutrition.

Table 3. Varietal grading/ranking of adzuki bean genotypes in search of versatile nutritionally superior multipurpose genotypes

Parameter	EC-290251	EC-290652	EC-030256	EC-034625	EC-340246	EC-340249	IC-341946	IC-341948	HPU-51	Toturu Local
Crude protein (%)	8	7	2	5	4	3	9	6	2	1
Crude fibre (%)	7	1	6	8	9	3	4	5	7	2
Carbohydrates (%)	4	3	5	6	1	9	1	2	7	8
Methionine (g/100 g protein)	2	7	1	9	6	10	5	3	4	8
Tryptophan (g/100 g protein)	3	1	2	3	7	4	6	4	8	5
IVPD (%)	10	3	8	9	6	2	5	7	1	4
Total tannins (mg/100 g)	1	7	2	7	8	4	5	9	6	3
Cooking time (minutes)	6	7	8	3	4	5	2	1	7	8
Calcium (mg/100 g)	8	2	4	6	5	1	7	3	9	10
Iron (mg/100 g)	5	4	2	5	3	2	1	5	6	7
Zinc (mg/100 g)	9	5	2	1	4	7	6	8	10	3
Total*	63	47	42	62	57	50	51	53	67	59
Cumulative grading	9	2	1	8	6	3	4	5	10	7

*Genotypes graded in the descending order for nutritionally desirable characters and in the ascending order for nutritionally undesirable characters

Table 4. Varietal grading/ranking of rice bean genotypes in search of versatile nutritionally superior multipurpose genotypes

Parameter	PRR -1	PRR -2	BC -1	RL -3	LRB -160	LRB -10	LRB -311	LRB -448	LRB -452	LRB -460	LRB -467	IC -141077	IC -563980	VRB -3
Crude protein (%)	6	9	3	7	10	4	9	3	8	2	1	4	6	5
Crude fibre (%)	7	10	5	5	6	3	2	9	4	8	10	6	11	1
Carbohydrates (%)	8	5	8	7	4	6	3	1	2	12	10	11	6	9
Methionine (g/100 g protein)	7	1	5	2	4	12	8	3	9	9	6	13	11	10
Tryptophan (g/100 g protein)	1	3	5	4	2	12	6	10	9	11	11	8	7	13
IVPD (%)	8	10	7	9	4	6	2	1	5	12	11	13	3	4
Total tannins (mg/100 g)	1	2	10	5	9	8	6	3	7	8	6	4	6	2
Cooking time (minutes)	1	1	3	6	5	9	3	4	3	2	3	7	5	8
Calcium (mg/100 g)	9	1	7	4	2	11	10	13	3	14	5	12	8	6
Iron (mg/100 g)	12	10	2	7	8	6	3	4	1	9	10	5	3	11
Zinc (mg/100 g)	5	2	4	3	4	12	7	6	1	11	8	12	10	9
Total*	65	54	59	59	58	89	59	57	52	98	81	95	76	78
Cumulative grading	6	2	5	5	4	10	5	3	1	12	9	11	7	8

*Genotypes graded in the descending order for nutritionally desirable characters and in the ascending order for nutritionally undesirable characters

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REFERENCES

Akeson WR and Stahmann MA 1964. A pepsin pancreatin digest index of protein quality evaluation. Journal of Nutrition **83:** 257- 261.

Anonymous 1975. Official methods of analysis. Association of Official Analytical Chemists. 6th edn, Washington, DC.

Anonymous 1990. Official methods of analysis. Association of Official Analytical Chemists. 15th edn, Washington, DC.

Awasthi CP, Thakur M, Dua RP and Dhaliwal Y 2011. Biochemical evaluation of some promising varieties/ genotypes of rice bean, *Vigna umbellata* (Thunb) Ohwi and Ohashi. Indian Journal of Agricultural Biochemistry **24(1):** 39-42.

Bhagmal 1994. Underutilized grain legumes and pseudocereals: their potentials in Asia. Regional Office for Asia and the Pacific (RAPA), FAO, Bangkok, Thailand, 162p.

Chandel KPS, Arora RK and Pant KC 1988. Rice bean- a potential grain legume. NBPGR Scientific Monograph # 12, NBPGR, New Delhi, India.

Chandel KPS, Joshi BS, Arora RK and Pant KC 1978. Rice bean- a new pulse with higher potential. Indian Farming **28(9):** 19-22.

Gopalan C, Rama Sastri BV and Balsubramanian SC 2004. Nutritive value of Indian foods (NVIF). National Institute of Nutrition (ICMR), Hyderabad, Andhra Pradesh, India, pp 47-85.

Hira CK, Kanwar JK, Gupta N and Kochhar A 1988. Cooking quality and nutritional evaluation of the rice bean (*Vigna umbellata*). Journal of Food Science and Technology **25(3):** 133-136.

Horn MJ, Jone DD and Blum ANE 1946. Colorimetric determination of methionine in proteins and foods. Journal of Biological Chemistry **116(1):** 313-320.

Kanwar KC, Kanwar V and Shah S 1997. Friendly fibers. Science Reports **34:** 9-14.

Lindsay WL and Norvell WA 1969. Equilibrium relationships of Zn^{2+}, Fe^{3+}, Ca^{2+} and H^{+} with EDTA and DTPA in soils. Proceedings, Soil Science Society of America **33:** 62-68.

Makkar HPS, Blummel M, Borowy NK and Becker K 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitating methods. Journal of the Science of Food and Agriculture **61(2):** 161-165.

Mertz ET, Jambunathan R and Mishra, PS 1975. Protein quality. Agricultural Research Bulletin # 7, Pardue University, USA, pp 9.

Mohan VR and Janardhanan K 1994. Chemical composition and nutritional evaluation of raw seeds of six rice bean varieties. Journal of Indian Botanical Society **73:** 259-263.

Myrna S Rodriguez and Evelyn Mae T Mendoza 1991. Nutritional assessment of seed proteins in rice bean, *Vigna umbellata* (Thunb) Ohwi and Ohashi. Plant Foods for Human Nutrition **41(1):** 1-9.

Narasinga Rao BS, Deosthale YG and Pant KC 1989. Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research (ICMR), Hyderabad, Andhra Pradesh, India.

Panse VG and Sukhatme PV 1984. Statistical methods for agricultural workers. ICAR, New Delhi, India.

Sadana B, Hira CK, Singla N and Grewal H 2006. Nutritional evaluation of rice bean, *Vigna umbellata* strains. Journal of Food Science and Technology, Mysore **43(5):** 516-518.

Saikia P, Sarkar CR and Borua I 1999. Chemical composition, antinutritional factors and effect of cooking on nutritional quality of rice bean, *Vigna umbellata* (Thunb) Ohwi and Ohashi. Food Chemistry **67(4):** 347-352.

Sharma BK, Das A and Banjarbarnah KM 2003. Underutilized life support crop species: production and research in northeastern hill region of India. Programme-I, NATP Household Food and National Security.