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Proximate composition and antinutritional factors in rice bean (*Vigna umbellata*)

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Abstract. Thirteen promising strains of Rice bean (*Vigna umbellata*) were analysed for their proximate compositions and antinutritional factors. Protein content in these varieties ranged from 17.50 to 23.10 per cent, ash from 3.06 to 4.48 per cent, ether extract from 2.4 to 3.9 per cent and crude fibre from 1.70 to 4.25 per cent. Trypsin inhibitor activity ranged from 112.63 to 163.98 units/g and polyphenols ranged from 0.58 to 1.19 per cent. Phytohemagglutinating activity was present in all the strains, except one, RB-32. Oligosaccharides, viz., raffinose, stachyose and verbascose, ranged from 0.32 to 0.91, 0.95 to 1.98 and 1.40 to 2.58 per cent, respectively. Attempts have been made to compare the results with a standard variety each of cowpea (*Vigna unguiculata*), moong (*Vigna radiata*) and mash (*Vigna mungo*).

Introduction

Most of the protein requirements of the rural Indian population are met by legumes which constitute an important source of protein. In spite of the fact that traditional pulses with better yield are being grown continuously, there has been no spectacular increase in the yield during about the last 25 years. As a result, the demand for pulses has not yet been met. New pulses with high yield and better nutritional quality are being identified for introduction in different areas of the world. Rice bean (*Vigna umbellata*) has been identified, as a suitable addition to the presently existing pulses [1]. However, there is no systematic work done so far on the antinutritional factors and the toxic constituents commonly present in pulses. It is with this objective that thirteen promising strains of rice bean have been analysed for some antinutritional and toxic constituents.

Materials and methods

Thirteen promising strains of rice bean and one standard variety each of cowpea (FS-68), moong (K-851) and mash (T-9) were obtained from the Department of Plant Breeding, Haryana Agricultural University, Hisar, ground to pass a 100 mesh screen, and stored in air tight polythene containers. Proximate composition, viz., ash, protein, ether extract and crude fibre were determined by the methods described in AOAC [2]. NFE was calculated by difference. Trypsin inhibitor activity was estimated by the original method of Kunitz [3] as modified by Roy and Rao [4]. One unit of trypsin inhibitor activity was expressed as 1 μ mole of trypsin inhibited in 20 min of incubation at 37 °C. Oligosaccharides (viz., raffinose, stachyose and verbascose) were extracted by the method of Dubois [6] after separating them by descending paper chromatography using propanol:ethanol:water (70:10:20) as the solvent. The method described by Singh [7] was employed for extracting total polyphenols and their content was determined as tannic acid equivalents following the method of Swain and Hillis [8].

Phytohemagglutinating activity was determined semiquantitatively by the method described by Vasishta et al. [9]. One g of finely divided legume seed flour was shaken vigorously with 10 ml of 0.15 N NaCl solution for 2 h, on a wrist action mechanical shaker. The contents were centrifuged at 10,000 **g** for 20 min. Clear supernatant (0.4 ml) was poured in depression (pit) 1, on a microtitration plate. Normal saline solution (0.9% NaCl, 0.2 ml) was placed in each of the next 19 depressions. An aliquot (0.2 ml) of the contents was transferred from depression 1 to depression 2. Likewise, 0.2 ml of the contents were transferred from depression 2 to depression 3, after thoroughly mixing the contents in depression 2. This process of successive transfer was continued until the contents from depression 18 had been transferred to depression 19. An aliquot (0.2 ml) from depression 19 was finally discarded so that the contents in each of the 20 depressions measured 0.2 ml. Thus, starting from depression 1, the extract was diluted two fold in each successive depression. The extract in depression 19 was diluted 144-fold. The depression 20 which lacked the extract, served as a blank. Trypsinized rabbit erythrocyte suspension (0.2 ml) was added to each of the 20 depressions. The plates were incubated for 1 h at 37 °C, in a warm air oven. At the end of the incubation period, contents in each depression were observed by eye, for agglutination in the form of a gel-clot at the base of the depression, in comparison with the blank depression. The trypsinized erythrocytes suspension was prepared fresh every day from normal healthy rabbit blood, according to the method described by Sage and Green [10].

Results and discussion

Based on the colour of the seed coat, the strains could be grouped into two categories viz. yellowish green (RB_4, RB_{17}, RB_{26}, RB_{37}, RB_{45}, RB_{49}, RB_{53} and RB_{56}) and reddish brown (RB_{32}, RB_{39}, RB_{40}, RB_{44} and RB_{46}).

The proximate composition of rice bean along with that of cowpea, moong and mash is included in Table 1. Perusal of the data suggests that rice bean is as good a source of protein as cowpea, moong and mash. The protein content in rice bean genotypes varied from 17.50 to 23.10 per cent; ash content from 3.06 to 4.60 per cent; ether extract from 2.3 to 3.9 per cent and crude fibre from 1.70 to 4.25 per cent with average values of 20.19, 4.05, 2.86 and 2.98 per cent, respectively. When these results are compared with those of cowpea, moong and mash as well as with the data available on traditional pulses, it was observed that rice bean compared very well with other pulses

Table 1. Proximate composition of different rice bean varieties and a standard variety of cowpea, moong and mash*

Variety	Moisture (%)	Protein (%)	Ash (%)	Ether extract (%)	Crude fibre (%)	NFE (%)
RB- 4	7.60	18.55	4.16	2.8	2.05	64.84
RB-17	7.90	17.85	4.48	3.6	3.40	62.78
RB-26	7.74	18.45	4.16	3.9	4.05	61.70
RB-32	7.40	21.53	4.14	2.7	2.20	63.03
RB-37	8.10	22.05	4.26	2.5	3.30	59.79
RB-39	7.10	21.70	4.04	2.4	2.50	63.26
RB-40	8.10	23.10	3.06	3.0	2.75	59.99
RB-44	7.20	21.18	4.40	2.2	3.20	60.82
RB-45	8.88	21.00	3.80	2.3	2.35	61.67
RB-46	7.04	17.50	4.08	3.3	4.25	63.83
RB-49	7.16	18.73	3.84	3.4	3.60	63.27
RB-53	7.06	20.30	4.60	3.1	1.85	63.09
RB-56	7.52	20.48	3.80	3.5	1.70	63.00
Mean	7.60 ± 0.54**	20.19 ± 1.8	4.06 ± .39	2.98 ± .54	2.86 ± .83	62.39 ± 1.5
Cowpea FS-68	7.52	21.00	3.40	3.60	4.50	59.98
Moong K-851	7.60	22.85	3.58	3.7	2.80	64.47
Mash T-9	7.46	22.75	3.00	2.7	2.90	61.19

*Values are averages of two replicates.
**Means ± S.E.M.

Table 2. Trypsin inhibitor activity (TIA) and polyphenol content of rice bean varieties and a standard variety of cowpea, moong and mash

Variety	TIA (Units/g) Av* ± S.E.	Polyphenol (g/100 g) Av* ± S.E.
RB- 4	146.20 ± 5.123	0.99 ± 0.017
RB-17	128.69 ± 4.457	1.04 ± 0.029
RB-26	163.98 ± 5.135	1.00 ± 0.029
RB-32	116.21 ± 7.456	0.66 ± 0.024
RB-37	134.79 ± 6.342	1.06 ± 0.024
RB-39	128.02 ± 6.520	0.64 ± 0.014
RB-40	112.63 ± 4.138	0.62 ± 0.012
RB-44	132.37 ± 4.860	0.66 ± 0.014
RB-45	148.59 ± 2.901	0.99 ± 0.017
RB-46	114.23 ± 4.590	0.58 ± 0.008
RB-49	151.66 ± 5.135	1.12 ± 0.024
RB-53	118.87 ± 4.925	1.14 ± 0.033
RB-56	149.12 ± 5.795	1.19 ± 0.027
Mean	134.26 ± 6.36	0.90 ± 0.017
Cowpea FS-68	154.42 ± 3.780	1.13 ± 0.020
Moong K-851	149.65 ± 4.593	1.06 ± 0.010
Mash T-9	98.34 ± 3.250	1.07 ± 0.036

* The values are averages of three replicates.

as far as its protein, ash, ether extract and crude fibre contents are concerned. However, rice bean has not been well investigated for its chemical composition. Only a single report by Singh et al. [11] is available in the literature; the values obtained in the present investigation are in good agreement with the values obtained by these workers. Results clearly indicate that rice bean strains, no doubt, differed considerably in their protein content, but may be regarded as an appreciable source of protein similar to other popular pulses such as cowpea, moong and mash.

However, it is necessary to determine in this crop the antinutritional factors commonly present in pulses so as to reach some conclusion regarding its nutritional status among other popular pulses. Data on trypsin inhibitor activity and polyphenol content as tannic acid equivalents are included in Table 2. Trypsin inhibitor activity varied from 112.63 to 163.98 units/g with an average of 134.26 units/g. Investigation of one standard variety each of cowpea, moong and mash showed these inhibitor activities to be 154.42 units, 149.65 units and 98.34 units/g; respectively. Though the trypsin inhibitor activity has been studied in a number of pulses as well as in rice bean [12], the results obtained in the present investigation cannot be compared because the expression of trypsin inhibition activity, nature and concentration of the substrate etc. were different. However, based on the investiga-

Table 3. Flatus factors (raffinose, stachyose and verbascose) in rice bean varieties and a standard variety of cowpea, moong and mash

Variety	Raffinose (%) Av* ± S.E.	Stachyose (%) Av* ± S.E.	Verbascose (%) Av* ± S.E.
RB- 4	0.32 ± 0.024	1.09 ± 0.040	1.67 ± 0.055
RB-17	0.44 ± 0.020	1.11 ± 0.046	1.40 ± 0.086
RB-26	0.33 ± 0.040	1.35 ± 0.061	1.49 ± 0.052
RB-32	0.91 ± 0.050	1.66 ± 0.064	2.51 ± 0.115
RB-37	0.59 ± 0.043	0.95 ± 0.033	1.44 ± 0.076
RB-39	0.83 ± 0.037	1.98 ± 0.063	1.88 ± 0.121
RB-40	0.81 ± 0.037	1.96 ± 0.066	2.57 ± 0.101
RB-44	0.86 ± 0.059	1.76 ± 0.083	2.58 ± 0.102
RB-45	0.44 ± 0.040	1.07 ± 0.058	1.58 ± 0.052
RB-46	0.90 ± 0.080	1.88 ± 0.061	2.37 ± 0.095
RB-49	0.40 ± 0.016	1.09 ± 0.041	1.71 ± 0.058
RB-53	0.46 ± 0.047	1.16 ± 0.032	1.56 ± 0.056
RB-56	0.34 ± 0.041	1.35 ± 0.058	1.63 ± 0.071
Mean	0.58 ± 0.020	1.42 ± 0.017	1.87 ± 0.039
Cowpea FS-68	0.73 ± 0.053	1.69 ± 0.044	2.02 ± 0.073
Moong K-851	0.66 ± 0.060	1.58 ± 0.052	1.89 ± 0.075
Mash T-9	0.82 ± 0.040	1.63 ± 0.081	2.15 ± 0.101

* The values are averages of three replicates.

tions carried out under the same experimental conditions on pigeonpea, cowpea, moong and mash, it may be inferred that the antitryptic activity in rice bean is at par with the antitryptic activity in other pulses. In fact there are strains like RB_{40}, RB_{46} and RB_{32} with very low antitryptic activity as compared to cowpea and moong. Only RB_{26} had an antitryptic activity (163 units/g) which was even higher than that of cowpea – the crop which had the maximum antitryptic activity among the standard crops used.

The total polyphenol content in the rice bean genotypes ranged from 0.58 to 1.19 per cent. Average content of polyphenols in rice bean (0.90 per cent) was lower than that of the standard crops (cowpea, moong and mash; 1.13, 1.06 and 1.07 per cent polyphenols, respectively). Yellowish green varieties, in general, had higher contents of polyphenols than the reddish brown varieties.

Amounts of total soluble sugars and oligosaccharides (raffinose, stachyose and verbascose) in rice bean genotypes and in cowpea, moong and mash are listed in Table 3. Total soluble sugars varied from 3.32 to 7.57 per cent whereas the raffinose, stachyose and verbascose contents ranges from 0.32 to 0.91 per cent; 0.95 to 1.98 per cent and 1.40 to 2.58 per cent, respectively. The standard variety each of cowpea, moong and mash had total soluble sugars, raffinose, stachyose and verbascose contents of 7.48, 0.73, 1.69 and 2.02 per cent; 7.15, 0.66, 1.58 and 1.89 per cent and 6.83, 0.82, 1.63 and 2.15 per cent, respectively.

The food legumes are regarded as notorious inducers of flatulence when they are consumed in large quantities. It has been reported that two oligosaccharides, raffinose and stachyose are the causative factors for flatulence and uncomfortable feeling often experienced upon ingestion of soybean products [13]. From the results reported here, it appears that rice bean genotypes contain significant amounts of flatus-producing oligosaccharides as is the case with other popular pulses [14–16]. However, the average contents of raffinose (0.58 per ceet), stachyose (1.42 per cent) and verbascose (1.87 per cent) were lower than those of the standard cowpea, moong and mash tested.

Results from the semiquantitative test for phytohemagglutinating activity clearly showed that the original extracts from rice bean genotypes (see materials and methods) could agglutinate the rabbit erythrocytes only up to a dilution of 4 to 16 times. Genotype RB-32 was devoid of the phytohemagglutinating activity. However, original extracts from cowpea, moong and mash agglutinated the rabbit erythrocytes up to a dilution of 32 to 64 times. Studies carried out under the same exerimental conditions on lentil [9] also resulted in much higher phytohemagglutinating activity as their extracts could agglutinate the rabbit erythrocytes up to a dilution of 4096 times.

Based on the data presented here it is suggested that rice bean can be considered to be a potential source of good quality protein like other popular pulses commonly consumed by people in this country.

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