Studies on *Vigna umbellata* Land Races from NE India for Nutritional Quality and Characterization through Seed Protein Profiling

Gautam K Handique, Kigwe Seyie[1] and AK Handique*
Department of Biotechnology, Guwahati University, Guwahati-781014, Assam, India
[1]*Department of Botany, Nalbari College, Nalbari-781335, Assam, India*

Thirty land races of the seed legume *Vigna umbellata*, collected from the hilly state of Nagaland, North-East India were screened for nutritive value. Crude protein content varied from 14.66% to 26.88%. Total carbohydrate varied from 44.3% to 57.6%; ash content from 3.6% to 6.32%. Lipid content varied from 1.0% to 2.04% and crude fibre varied from 3.8% to 4.4%. Calorific values were in the range of 271.04 kcal/100 gm to 316.04 kcal/100 gm. SDS-PAGE analysis of seed protein revealed a total of 35 protein bands. Dendrogram revealed the presence of four major clusters. Diversity was evident from the fact that while RB-27, 28, 29 and 30 had 21 protein bands each, RB-11 had only 6 protein bands. Our study indicates that rice bean is a promising pulse crop for future because of its wide adaptability and high nutritive values.

Key Words: Rice bean, *Vigna umbellata*, Nutritive value, SDS-PAGE, Seed protein

Introduction

Search for new pulse crops is gaining momentum and getting priority because conventional pulses are stagnating in productivity and not in a position to meet the ever growing demand. Land races represent the natural gene pool for a crop species from which superior or desired cultivars are sorted out for use in genetic improvement programs. In India, the hilly states of North-East, particularly Nagaland, is the home for many little known, underutilized but promising seed legumes, the foremost among which is *Vigna umbellata*, referred to as rice bean with large number of land races which are yet to be assessed properly. In Nagaland the villagers have been cultivating rice bean for centuries as single crop or as mixed crop with maize. Most villages are isolated and land locked which help in perpetuation of a large number of land races over centuries. Grain legumes or pulses are the primary sources of plant protein for millions of people in tropical and sub-tropical countries and in countries like India and pulses are integral part of diet for rich poor alike. In view of the growing concern for nutritional security study of non-conventional grain legumes deserve priority since it is a foregone conclusion that animal protein can not cater to the needs of growing population in underdeveloped and developing countries. In a country like India which is the biggest producer as well as biggest importer of pulses, production of pulses has been stagnating for the last half a century (Gadgil *et al.*, 1999). On the other hand since population is steadily increasing, per capita grain availability has been dropping alarmingly. Considering a recommended requirement of 40 gm pulses per day/person, India's projected requirement for 2010 is 22.61 m tonnes and for 2020 the requirement is 26.76 m tonnes. But even in the initial years of 21st century India's pulses production have been about 13 m tonnes or below except in 2003-04 when it was 15.23 m tonnes. Two main reasons are cited for the stagnation of pulses production. Most pulse crops are recalcitrant and have limited genetic variability which make genetic improvement difficult. Secondly, pulse crops require specific agro-climate and soil type for which further expansion of pulse growing area is not possible (Asthana, 2000).

In this context *Vigna umbellata* (Thunb) Ohwi and Ohashi assumes great importance for its nutritional quality, high grain yield and multipurpose utility as feed, fodder, cover crop and green manure. Moreover, unlike most conventional pulses it can be grown under wide range of agro-climatic condition and soil type (Chandel, 1980). The present study was undertaken to collect and evaluate the land races of Nagaland with respect to major nutritive values and their characterization through seed protein profiling in an effort to short list the promising land races for future and further works.

Materials and Methods

Seeds of different land races of rice bean were collected through visit to various villages of the hills of Nagaland of North-East India. The altitudes of collection areas were in the ranges of 300 msl to 1700 msl. Under field condition of Nagaland rice bean mature and become ready for harvest in the month of November. The land races were

* *Corresponding Author*

primarily based on seed colour and morphology. Collections were made from farmers field as well household granaries and catalogued as RB–1, RB–2 etc. A total of 30 land races were collected and grown in the experimental garden of the School of Agricultural Sciences and Rural Development, Medziphema (altitude 305 msl), where climate is sub- tropical, mostly humid with moderate temperature and medium to high rainfall. The land races were grown in randomized blocks with three replications. All the land races were climbers and bamboo poles were erected to give mechanical support. The mature seeds were harvested in the month of November.

Chemical analysis for nutritional components was done on dry weight basis. For this the sample was finely grounded and dried in an oven at 60°C till constant weight was recorded. Crude protein was estimated using microkjeldahl method. Total carbohydrate of the grains was determined using the method described by Clegg (1956). Lipid content was estimated by extracting the sample with petroleum ether in a Soxhlet Apparatus for 8 hours. Subsequently the solvent was evaporated away to get the lipid as residue. Crude fibre of the grains was determined as per the method of Sadasivam and Manickam (1996). For ash content the sample was ashed in a muffle furnace at 600°C for four hours and the difference in weight was recorded from which ash content was calculated. Three replications were made for each sample. Calorific values were computed as per the formula given by Sherman (1952).

Seed protein profile for the cultivars were analysed by SDS-PAGE technique as outlined by Laemmli (1970). The seeds were washed with distilled water and then blotted dry. 200 mg of seed sample were grounded in a pre-chilled morter with ice cold 0.5 M Tris buffer, pH 6.5. The extract was centrifuged in a refrigerated centrifuge at 8000 rpm for 10 minutes at 4°C. Sample weight to extract volume was adjusted to 1:5 ratio and the seed protein was resolved in 15% polyacrylamide gel. From the protein profile similarity indices were generated using Nei and Li co-efficient (Nei and Li, 1979). The similarity matrix was used to generate the dendrogram by UPGMA using NTSYS pc.v.2.02j.

Results and Discussion

Considerable variability were observed with respect to protein content, which was evident from the fact that protein content varied from 14.66% in RB-14 to 26.88% in RB-16 (Table 1). Frequency distribution analysis showed that 7 land races had 17% protein; 5 land races had 21% while 4 land races had 18% protein (Fig. 1). Thus the variation among the land races has been found to be highly significant. Among the major nutritional components, total carbohydrate constituted the major fraction and like protein, carbohydrate content also exhibited considerable variation. This is evident from the fact that it varied from 44.3% in RB-16 to 57.6% in RB-17. However, 23 land races out of 30 in the present study had 50% or more total carbohydrate. Among the major nutritional components of the grain, lipid occurred in the lowest quantity. Unlike crude protein and carbohydrate, lipid content did not exhibit appreciable variation. The range of variation was from 1.0% in RB-7, 13, 18 and 30 to 2.04 in RB-5 with the exception of RB-16 with 0.8%. Total minerals in the form of ash content exhibited appreciable variation. RB-12 with 3.6% recorded lowest while RB-16 with 6.32% recorded highest ash content. Among all the nutritional components, crude fibre exhibited least variation as evident from the range of variation which was 3.8% to 4.4%. Total calorific value exhibited significant variation and varied in the range of 271.04 kcal/100 gm in RB-14 to 316.04 kcal/100 gm in RB-29.

Seed protein profile exhibited remarkable variability. Total 35 protein bands were detected ranging in size from 109.0 to 13.5 Kd. Proteins with size range 109.0 to 59.5 Kd were considered as High Molecular Weight (HMW) proteins; those in the size range of 50.4 to 23.0 Kd were considered as Medium Molecular Weight (MMW) proteins and the remaining as Low Molecular Weight (LMW) proteins. Highest number of protein bands were observed for RB-27, 28, 29 and 30; each with 21 bands. Second highest number were recorded for RB-18, 22 and 23; each with 19 protein bands. Lowest number of protein bands were observed in RB-11 with 6 bands which include no low molecular weight protein. Proteins with medium molecular weight were comparatively more abundant. The protein with size 29 Kd exhibited highest frequency, being present in 25 land races. Proteins with size 33.6 and 26.2 Kd exhibited second highest frequency, being present in 20 land races. Lowest frequency was observed for the protein with 50.4 Kd which was detected in only 3 land races. Among the HMW group, the protein with size 91.8 Kd was the most frequent as it was present in 20 land races, followed by the protein 83.0 Kd which was present in 17 land races.

On the other hand, the protein with highest molecular weight of 109.0 Kd was found in only 4 land races.

Table 1. Chemical composition of the grains of rice bean with respect to major chemical constituents. The values are in terms of gm/ 100 gm dry weight

Cultivars	Crude Protein	SD ±	Total Carbohydrate	SD ±	Lipid	SD ±	Ash	SD ±	Crude fibre	SD ±	Calorific Value
RB-1	21.69	0.743	48.00	1.212	1.33	0.030	5.46	0.039	4.04	0.091	290.73
RB-2	20.22	0.590	47.46	1.103	1.73	0.027	4.02	0.045	3.90	0.100	286.05
RB-3	17.02	0.094	54.86	0.928	1.50	0.000	3.90	0.036	4.10	0.075	300.78
RB-4	17.17	0.392	50.03	1.049	1.70	0.062	4.00	0.0	4.33	0.065	283.98
RB-5	19.43	0.478	48.83	0.987	2.04	0.057	5.11	0.040	4.38	0.120	291.28
RB-6	17.11	0.346	54.00	1.154	1.04	0.038	4.03	0.0	4.05	0.083	293.44
RB-7	16.41	0.297	57.20	1.016	1.00	0.033	3.85	0.050	4.28	0.0	303.44
RB-8	15.57	0.438	57.60	0.930	1.60	0.025	3.76	0.047	4.30	0.100	307.08
RB-9	18.07	0.363	55.06	1.396	1.31	0.018	3.90	0.040	4.00	0.062	303.98
RB-10	17.17	0.124	53.66	1.262	1.42	0.021	3.90	0.036	4.13	0.071	295.68
RB-11	21.33	0.453	53.04	0.887	1.14	0.0	5.20	0.057	3.90	0.083	307.22
RB-12	17.86	0.392	53.25	1.016	1.60	0.064	3.60	0.0	4.06	0.111	298.64
RB-13	19.85	0.407	53.87	1.622	1.02	0.053	4.04	0.0	4.22	0.080	303.60
RB-14	14.66	0.085	50.40	1.098	1.24	0.018	3.88	0.039	4.31	0.076	271.04
RB-15	16.74	0.127	56.41	1.452	1.66	0.042	4.26	0.047	4.14	0.0	306.96
RB-16	26.88	0.381	44.30	1.085	0.80	0.0	6.33	0.053	3.81	0.068	291.92
RB-17	14.73	0.356	57.60	0.864	1.66	0.045	4.00	0.0	4.42	0.070	303.72
RB-18	19.64	0.524	57.00	1.272	1.03	0.042	4.41	0.054	4.31	0.096	315.56
RB-19	18.98	0.247	52.63	1.201	1.54	0.025	3.86	0.046	4.27	0.102	299.82
RB-20	18.56	0.103	56.28	1.621	1.36	0.027	4.39	0.0	3.93	0.069	310.74
RB-21	20.23	0.624	46.80	0.990	1.30	0.023	4.93	0.040	4.00	0.110	279.82
RB-22	23.74	0.235	48.22	0.959	1.43	0.040	5.60	0.043	4.03	0.091	300.71
RB-23	21.06	0.367	46.81	1.047	1.63	0.025	5.40	0.066	4.11	0.080	285.84
RB-24	23.58	0.478	50.60	0.827	1.58	0.035	3.81	0.042	4.00	0.078	310.22
RB-25	22.30	0.416	53.26	1.048	1.33	0.020	4.04	0.060	3.84	0.097	313.70
RB-26	17.01	0.609	54.80	1.567	1.40	0.0	4.10	0.0	4.04	0.073	299.84
RB-27	21.77	0.556	52.80	0.927	1.77	0.047	5.12	0.063	3.92	0.062	313.58
RB-28	21.70	0.117	45.02	0.990	2.02	0.0	5.30	0.050	4.21	0.0	284.80
RB-29	18.56	0.103	56.41	1.101	1.81	0.023	4.13	0.0	4.03	0.116	316.04
RB-30	17.32	0.107	57.88	0.991	1.00	0.0	4.30	0.044	3.81	0.060	309.48
CD 5%	1.19		1.94		0.25		1.20		1.12		2.048
CD 1%	1.46		2.59		0.34		1.60		1.49		2.759

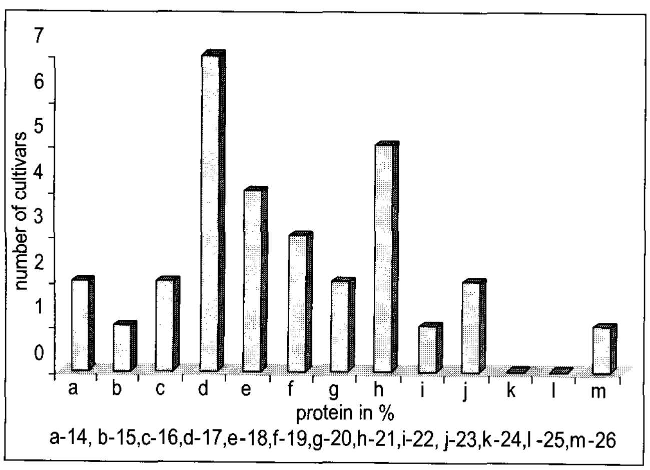

Fig. 1: Frequency distribution for protein content among the 30 cultivars of rice bean

Proteins with low molecular weight were less abundant in distribution. The most prominent among LMW proteins were the ones with molecular weight 22.4, 18.5 and 14.3 Kd; each of which were present in 11 land races. A protein band of 55.0 Kd was found in RB-28 which was not detected in others. Likewise another protein band of 15.5 Kd was found to be unique for RB-2.

Grain legumes are considered as a major source of plant protein and hence nutritional quality of a pulse crop is mainly judged on the basis of its protein content. In India the most commonly used pulses which are part of every day diet for majority population are lentil, mung bean, black gram, pigeon pea and chick pea and their average protein content are – 25.1, 24.5, 24.0% (Gopalan *et al.*, 1989); 22.9 and 22% (Chaturvedi and Ali, 2002),

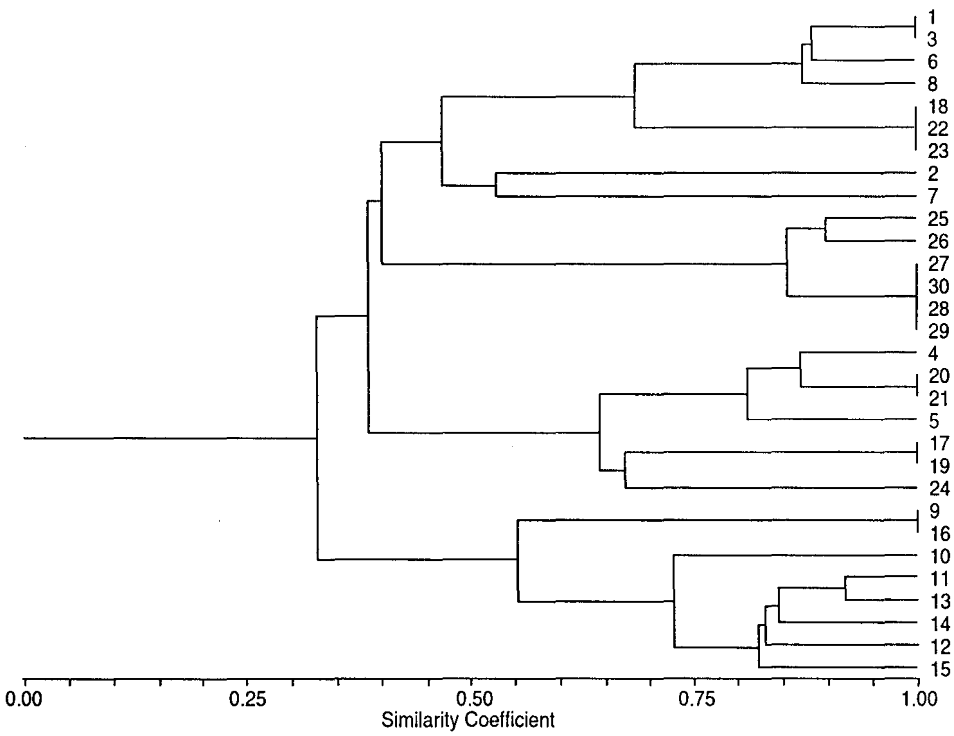

Fig. 2: Dendrogram of the thirty rice bean cultivars based on seed protein profile

respectively. The present study showed that while some land races were inferior, some were at par and at least one was superior to the conventional pulses in matter of protein content. Like protein wide range of variation was observed for total carbohydrate also which varied from 44% to 57%. In major pulses, carbohydrate content varies as – lentil 57.6%, black gram 46.3%, green gram 51.7% and chick pea 54.6% (Belitz and Grosch, 1999). In the present study, 5 land races had 57%; another 5 land races had 53% while only one land race had 44% carbohydrate. Therefore, most land races of rice bean are at par with the major pulses in matter of carbohydrate content. For major pulses total mineral in the form of ash content varies from 3.0% in Chick pea to 3.8% in Green gram (Belitz and Grosch, 1999). As against this ash content in the present study varied from 3.6% to 5.6% and as many as 8 land races were found to have over 5% ash content. It is therefore, clear that rice bean land races are superior to most conventional pulses with regard to ash content. Crude fibre for major pulses exhibit wide variation form 0.7% in Lentil to 4.1% in green gram and 5.3% in chick pea whereas the same varied from 3.8% to 4.4% among the rice bean land races. Lipid content among the land races were comparable to that of the major pulses and constituted the smallest and hence less significant nutritional component.

Seed protein profiling by SDS-PAGE has emerged as a reliable and powerful technique for resolving taxanomical and evolutionary problems; species and cultivar identification that are of great importance in many breeding studies (Naik and Kole, 2002). It has been demonstrated that composition of seed protein is highly constant and not influenced by environmental conditions, seasonal fluctuation and age of mature seeds (Ladizinsky and Hymowitz, 1979). In the present study, the land race RB-11 was characterized by only 6 protein bands while RB-27, 28, 29 and 30 were characterized by 21 protein bands, which reflect the degree of diversity among the land races studied. Dendrogram analysis revealed the presence of 4 major clusters and showed that while some land races were closely related, some others were distantly related which indicated the diversity prevalent (Fig. 2). On the other hand some land races viz. RB-27, 28, 29 and 30 were found to be same at molecular level in terms of seed protein profile although they appear different at morphological level. Seed protein profile has been successfully used by a number of workers for diverse purposes like characterization of cultivars, elucidating phylogenetic relationship and to develop molecular marker for specific trait and for identification of hybrid. Gomathinayagam and Ramaswamy (1994) used seed protein profile to find out interspecific and intraspecific relationship in cow pea and its related species. Chandran *et al.* (2002) worked out the intraspecific relationship among 70 cultivars of ground nut by the same process. Panigrahi (2001) identified species specific seed protein for pigeon pea and its related species *Cajanus cajanifolius.* Chand and Kole (2002) identified a seed protein as molecular marker for genotype resistant to Cercospora leaf spot in Mungbean while Pattnaik and Kole (2002) identified similar molecular marker for identifying mungbean susceptible to Mungbean Yellow Mosaic Virus. Seed proteins are known to be controlled by multi gene family (De Lumen, 1990) and their Mendelian monogenic inheritance has been established (Panella *et al.*, 1993; Naik and Kole, 2002). The polymorphism in seed protein profile observed in the present study therefore indicate that high degree of genetic diversity exists among the land races of rice bean. The floral morphology of rice bean is akin to most other *Vigna* species where self pollination is the rule. In India's North Eastern state of Nagaland, rice bean has been cultivated by villagers in isolated villages in hilly terrain for centuries as part of subsistence agriculture. Under such condition selection pressure is less which may be a reason for wide genetic diversity. Because of its ability to grow in diverse geographical and climatic condition, the genetic diversity as well as diversity in nutritional parameters can be exploited to breed superior cultivars to make it a promising pulse crop for future.

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