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Nutritional potential of *Vigna minima* (Roxb.) Ohwi and Ohashi:

I. Seed protein content and amino acid composition

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Abstract. The seed protein content and amino acid composition of 14 natural populations and their three-generation progenies (grown in different locations) belonging to *Vigna minima* (Roxb.) Ohwi & Ohashi and of *V. umbellata cv* IC 1568 have been investigated. The populations of *V. minima* were sampled from different ecozones of Western Ghats of Kerala and Tamil Nadu (India). The range of variation in protein levels is narrow, but the protein content of the coastal population is higher than the rice bean suggesting its breeding potential for high protein and salt tolerant lines of rice bean. Although the seed protein content shows genotype × environment interaction, there is a substantial genetic variability among the populations. The tenuous relationship between protein content and yield conponents suggest the presence of correlation breakers which can be utilized in breeding programmes of rice bean. There is a broad genetic base in the levels of essential amino acids, and the range of variation observed is higher than that recorded for different species of *Vigna* and *Phaseolus*. The wild relative is nutritionally as good as or superior to the cultigen.

Introduction

The legumes form the primary source of dietary amino acids for non-ruminate animals. Grain legumes provide one-fifth of all the plant proteins consumed by man on a global basis; in the tropics, where roots, tubers and starchy vegetables are the primary source of dietary calories, grain legumes contribute as much as two-fifths or more of protein in the diet [22].

In bridging the protein-calorie gap, one of the best approaches is the nutritional upgrading of food legumes by breeding [14, 26]. Until recently, little attention was paid to breeding for improvement in proteins. Studies in cereals and legumes showed substantial variability in seed protein content and relative amounts of amino acids [1, 3, 6, 7, 15, 16, 23, 24, 25, 28, 29, 33]. The scarce available data on the genetic variability in protein quality and quantity among germplasm collections, indeed do emphasize the need for extensive screening of wild relatives of crop plants for the discovery of novel variants with superior nutritional qualities and their

Table 1. Ecological aspects of the natural populations of *V. minima*

Ecozone	Region	Locality	Altitude (m)	Rainfall (mm)	Population code No.	General features of the locality
Western	Silent Valley	Silent Valley	990	4,543	WSS	60 km north of the nearest town Mannarkat. Forest floor close to the river front; wet and open
		Cholakad	795	2,800	WSC	16 km south of Silent valley. Exposed hill top; shady and wet.
		Dhoni	355	3,104	WPD	25 km south of Cholakad. Middle zone of the hills close to waterfall; wet and open
	Palghat	Congad	230	1,572	WPC	19 km north-west of Dhoni. Exposed hill top; dry and open
		Nelliampathy	517	3,901	WPN	47 km south-west of Congad. Foot hills; near dam site; wet and open
	Trichur	Wadakanchery	70	2,695	WTW	55 km north-west of Nelliampathy. Bottom lands close to cultivated paddy fields; wet and open.
		Nattika	0–2	2,821	WTN	32 km south-west of Wadakanchery. Close to sea; dry and open
		Kuthiran	210	3,003	WTK	30 km east of Nattika. Exposed slopes of hillocks; dry and open
		Sholayar	481	3,120	WTS	34 km south of Kuthiran, Foot hills; near water falls; wet and open

Eastern	Parambikolam	Parambikolam	1240	2,669	EPP	30 km north-east of Sholayar. Exposed hill tops; dry and open
		Rockpoint	1100	2,754	EPR	6 km north of Parambikolam. Slopes of hills close to roadside; dry and open
		Tunakadavu	667	2,950	EPT	8 km north-east of rockpoint. Bottom land, near dam site; marshy and open.
	Top slip	Charianchola	1150	3,532	ETC	7 km north of Tunakadavu. Hill tops; transition zone between semievergreen forests and grasslands; wet and open.
		Sethumadai	250	440	ETS	8 km east of Charianchola; bottom land; near cultivated lands; dry and open

subsequent utilization in the nutritional upgrading of cultigens by breeding.

Rice bean [*Vigna umbellata* (Thunb.) Ohwi & Ohashi] is extensively used in several parts of India as food and fodder [19]. The present paper reports the evaluation of populations of *Vigna minima* — the wild progenitor of rice bean — for seed protein content and amino acid composition, from the viewpoint of utilizing the observed variability in the nutritional upgrading of rice bean by breeding.

Materials and methods

Fourteen natural populations of *Vigna minima* (Roxb.) Ohwi & Ohashi inhabiting different ecozones of Western Ghats of Kerala and Tamil Nadu (India) were sampled (Table 1). The first generation progenies of these were grown in the experimental gardens of the University of Delhi, Delhi (DP_1), and the second (KP_2) and third generation (KP_3) progenies were raised in the experimental plots located at Kandassankadavu, Trichur region of Kerala State (India). Seeds harvested from natural populations and the three generation progenies were used for the estimation of seed protein content. Amino acid composition of whole seeds was determined for KP_2 progenies only. Seeds of *V. umbellata* (Thunb) Ohwi & Ohashi *cv* IC 1568 (Source: National Bureau of Plant Genetic Resources, Pusa Complex, New Delhi 110012, India) harvested from the plants grown in Kerala were similarly processed for comparison.

Seed protein content

For each population/progeny, air-dried, mature seeds were collected at random from the seed lot of each of the 5 phenotypes, again, chosen at random. Nitrogen content of the seed was determined by micro-Kjeldahl method [5, 13]. Protein per cent was calculated by multiplying the nitrogen value with 6.25.

Two-way analysis of variance was carried out [34] to study the location effect on protein levels; the different components of variance were estimated [8, 17, 18] and heritability coefficient (h^2) and genetic advance (G.A.) were calculated.

Pearson's product-moment correlation coefficient was used [34] to study the relationships among yield components (number of seeds per pod, seed weight, and seed size) and levels of protein (Figure 2).

Amino acid composition of seed proteins

Acid hydrolysis of ground (60 mesh size) and defatted seed material was performed in evacuated, sealed test tubes according to the procedure of Moore and Stein [27] with minor modifications (Chatterjee and Abrol, unpublished). the hydrolysate was flash evaporated at 45 °C under reduced

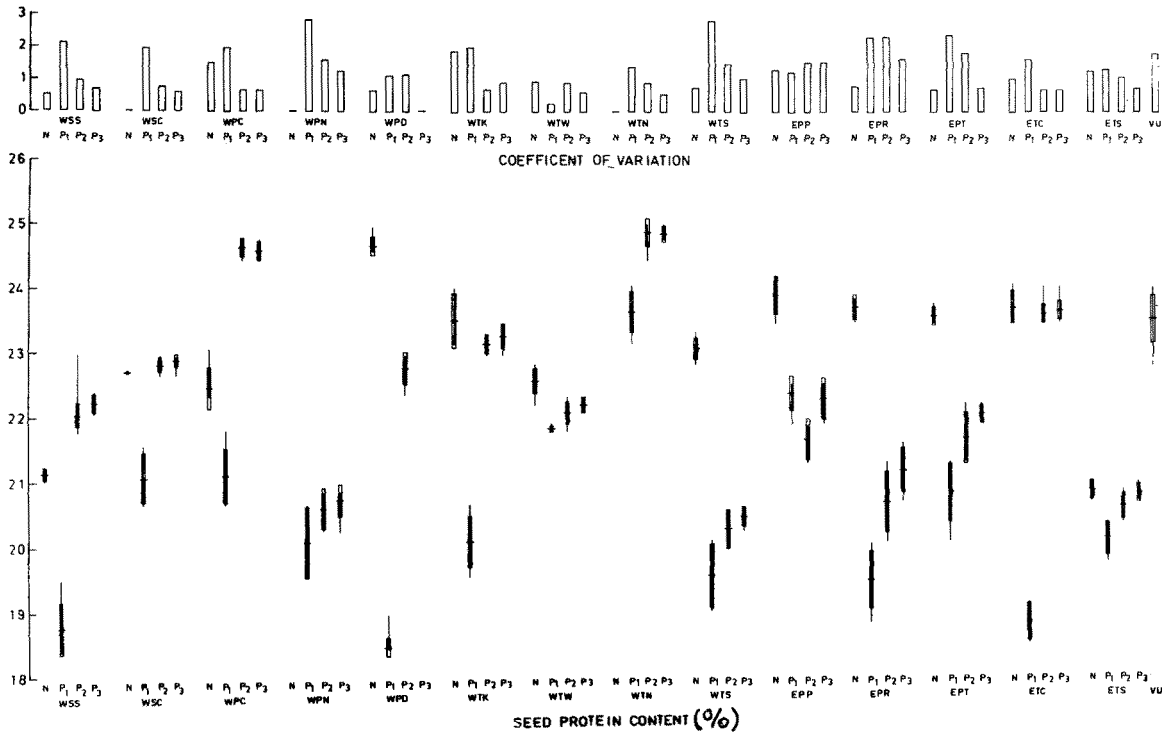

Figure 1. Graphic representation of variation in whole-seed protein (PNU) as evident by range, mean and its standard deviation, and coefficient of variation.

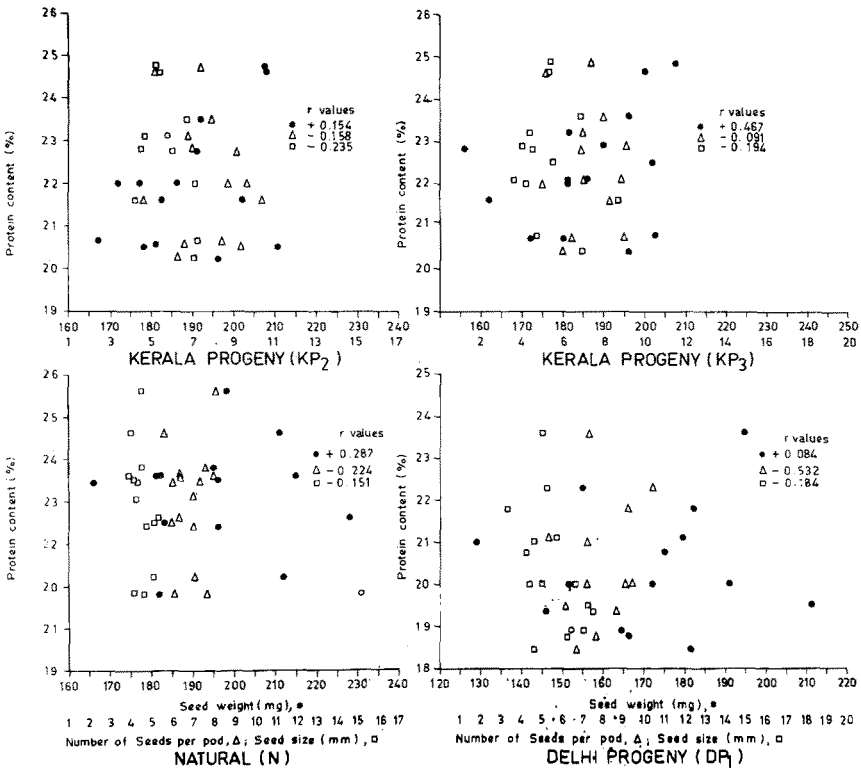

Figure 2. Scatter diagrams showing relationships among whole-seed protein, seed weight (●), number of seeds per pod (△), seed size (□) for natural populations and their progenies.

pressure and the residue finally taken up in sodium citrate buffer (0.1 M, pH 2.0) and made to a known volume. An aliquot from this was used to determine the amino acid composition using a Technicon Sequential Multisample (TSM) Amino Acid Autoanalyser. Quantification of the amino acids was done using norleucine as an internal standard. The standard deviations for the different amino acids were: Lysine 0.07, Histidine 0.04, Arginine 0.04, Aspartic acid 0.11, Threonine 0.08, Serine 0.07, Glutamic acid 0.52, Proline 0.20, Glycine 0.07, Alanine 0.06, Cystine 0.02, Valine 0.06, Methionine 0.02, Isoleucine 0.09, Leucine 0.10, Tyrosine 0.09, and Phenylalanine 0.16.

Results and discussion

The range of variation in seed protein content for the natural populations and their three-generation progenies grown at different locations was 18.5 to 25.7% suggesting a narrow genetic base (Figure 1). Similar observation

Table 2. Components of variance, heritability coefficient and genetic advance for seed protein content of natural populations of *V. minima* and their three — generation progenies grown in two different locations

Source	V_p	V_g	V_e	h^2	Genetic advance GA
Natural	2.03	1.95	0.08	96.06	1.98
DP_1	2.28	2.12	0.16	92.98	1.92
KP_2	2.25	2.17	0.08	96.44	1.99
DP_3	1.96	1.90	0.06	96.94	1.99

was made in other grain legumes [2, 4, 5, 9, 22, 30]. The population WTN and its progenies contained higher seed protein content (25%) than the cultigen. It was also tolerant to salinity because it inhabited the coastal tracts. Consequently, WTN could be utilized not only for breeding high protein lines of rice bean but also for developing salt tolerant cultivars.

The high genetic variance component, coupled with more than 95% h^2 and 1.97 G.A. (Table 2) strongly suggest that the variation in protein levels among populations was of genetic origin. However, the highly significant 'F' values (at $P < 0.01$) for locations and for population × location interaction (Table 3) demonstrate that seed protein content shows genotype × environment interactions. Similar observations were made in grain legumes [2, 4, 5, 20, 21, 35].

The fact that the third generation progenies of populations showed higher protein levels with a low coefficient of variation than the second generation progenies (Figure 1) grown under identical conditions, implied that selection played a significant role in increasing the protein levels. This was also evident from the high h^2 and G.A. values (Table 2). The fact that the seed protein profiles were population specific suggested that there was substantial variability in seed protein content.

Although the top-slip region recorded a high coefficient of variation, the western ecozone was the richest source of gene diversity. Consequent-

Table 3. Location effects on the whole-seed protein levels among the progenies grown in Delhi (DP_1) and Kerala (KP_2)

Source of variation	dF	MS	VC	VC (%)	'F'
Between populations	13	14.68	0.77	20.53	2.10^{ns}
Between locations	1	111.35	1.49	39.73	15.93**
Population × location	13	6.99	1.37	36.54	58.25***
Error	112	0.12	0.12	3.20	

ns = nonsignificant at $P > 0.05$
** = significant at $P < 0.01$
*** = highly significant at $P < 0.001$

Table 4. Amino acid composition (g/100 g protein) of the whole-seed protein in *V. umbellata* and different populations of *V. minima*, and the correlation coefficient (r) between protein and amino acid contents in *V. minima*.

Population	Protein (%)	Essential amino acids									General amino acids							
		Ile	Leu	Lys	Thi	Val	Phe	Tyr	Met	Cys	Arg	His	Asp	Ser	Glu	Pro	Gly	Ala
WSS	22.0	4.6	7.3	6.1	4.1	4.7	6.2	2.4	1.3	1.2	5.8	1.6	13.5	6.1	17.0	4.4	4.8	7.3
WSC	22.8	4.5	7.1	5.6	4.1	4.6	6.4	3.4	1.5	1.0	5.3	2.8	10.0	5.9	17.4	4.5	4.7	7.1
WPC	24.6	5.0	7.5	7.7	5.4	4.8	5.2	3.2	1.1	1.1	5.4	3.1	15.0	9.4	18.2	5.4	4.9	6.7
WPN	20.6	4.6	7.9	4.3	4.0	4.4	4.6	4.6	1.1	0.6	5.2	1.9	10.0	6.0	17.4	3.4	4.5	6.4
WPD	22.8	4.7	7.5	4.8	4.3	4.7	5.8	3.4	1.1	1.2	6.0	3.1	13.8	7.8	17.5	6.1	4.7	6.8
WTK	23.1	4.9	7.7	5.7	4.5	4.8	6.0	4.5	1.1	1.8	5.6	3.9	14.6	5.0	11.4	3.1	4.9	6.5
WTW	22.1	4.7	7.2	6.8	4.7	4.7	5.0	2.8	1.6	0.9	6.5	3.2	15.6	6.8	19.0	5.6	5.0	6.9
WTN	24.8	5.0	8.0	7.6	4.5	6.8	5.0	3.1	1.5	0.7	5.2	2.8	15.3	5.8	19.4	5.7	4.9	7.0
WTS	20.2	4.6	7.4	6.4	4.0	4.8	4.5	3.2	1.1	0.9	5.7	2.9	14.3	6.1	20.9	1.5	4.4	7.5
EPP	21.6	3.2	6.3	7.0	4.5	5.3	7.2	3.3	1.4	1.0	5.5	2.3	11.5	5.3	15.2	6.4	4.0	6.7
EPR	20.6	5.0	7.1	8.7	5.2	5.9	5.6	3.6	1.3	0.9	5.1	2.9	13.4	5.8	17.5	5.4	5.3	8.3
EPT	21.6	2.4	8.6	8.4	3.3	4.9	5.6	2.7	1.3	1.0	5.9	3.5	14.0	7.6	18.5	4.7	5.1	7.4
ETC	23.6	4.5	7.2	5.0	4.2	4.6	4.8	2.8	1.1	1.0	5.5	2.4	13.6	6.2	17.8	5.1	4.4	7.6
ETS	20.5	4.9	8.0	6.0	4.7	9.7	5.2	3.4	1.3	1.2	6.2	t	11.5	6.7	19.6	6.7	5.1	7.6
VU		4.6	7.8	7.7	4.1	4.4	5.5	3.2	0.9	1.0	6.3	3.3	13.5	5.6	17.0	3.6	3.8	6.6
r values		0.217	0.119	0.028	0.246	0.187	0.057	0.184	0.062	0.170	0.207	0.226	0.443	0.283	0.184	0.256	0.024	0.362

ly, the genetic diversity observed in gene pools of *V. minima* could be utilized in the selection schemes of plant breeding for generating transgressive variation.

The relationship of protein content with yield components was tenuous (Figure 2). This was contrary to published reports on several grain legumes, where stray negative relationship between per cent protein and yield components were observed [10, 12, 18, 21, 32, 35]. However, Blixt [5] reported correlation breakers in different lines of *Pisum*. In this context the gene pools of *V. minima* were unique since they contained several correlation breakers.

Amino acid profiles seemed to be population specific (Table 4). In most of the poulations of *V. minima* the levels of essential amino acids were markedly higher than the FAO/WHO pattern [11], except for sulphur amino acids. The levels of essential amino acids in some of the populations (EPR, EPP and WTK) were much higher than those of the cultigen. Consequently, the wild relative was nutritionally as good or superior to the cultigen. The range of variation in most of the essential amino acids was broad (Table 4) suggesting the existence of substantial genetic variability which could be utilized for the improvement of cultigen. Lysine content was much higher than the levels reported in different species of *Vigna*, *Phaseolus*, *Cajanus* and *Atylosia* [3, 30, 31]. Similarly, the methionine and cystine levels in the populations were substantially greater than those recorded in other wild reltives and their cultigens of *Vigna* and *Phaseolus* [3, 30].

The protein content did not show statistically, significant relationship ($P \geqslant 0.05$) with different amino acids in *V. minima*, and all the 'r' values were tenuous (Table 4).

In general, the range of variation in the levels of all essential amino acids observed among populations of *V. minima* was much greater than the total range of variation recorded in different species of *Vigna* and *Phaseolus*. These observations emphasize the need to evaluate the wild relatives for protein quality at the population level for the purpose of utilizing gene pools of wild relatives for the improvement of legumes in general.

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