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INDUCED VARIATION IN PROTEIN QUANTITY AND QUALITY IN THE WILD AND CULTIVATED URD AND MUNG BEANS

S. IGNACIMUTHU* AND C. R. BABU

Department of Botany, University of Delhi
Delhi 110007

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ABSTRACT

Seeds of *Vigna sublobata* (wild), *V. radiata* cv. PS 16 and *V. mungo* cv. T 9 (both cultivars) were used for the induction of mutations with EMS and gamma rays. Four-dose treatments of each of these mutagens (EMS: 0.1, 0.2, 0.3 and 0.4%; gamma rays: 10, 20, 30, and 40 kR; EMS + gamma rays: 0.1% + 10 kR, 0.2% + 20 kR, 0.3% + 30 kR, and 0.4% + 40 kR) were employed. The M_2 and M_3 seeds were analysed for protein content and some selected mutant type M_3 seeds were analysed for amino acid content. The results suggest that the range of induced variation in the whole seed protein is narrow within the species, but within each mutant line, the range is very broad. Some of these high protein mutants have also high levels of lysine and limiting S-amino acids coupled with higher yield. The whole seed protein content showed genotype × environmental interactions both in the control and mutated genotypes. As far as protein and amino acids are concerned, the wild relative is as good as the cultivars.

Key words: Protein content, protein quality, *Vigna sublobata, V. radiata, V. mungo*.

Food legumes, with their protein-rich seeds, are a chief source of dietary amino acids for man and farm animals [1]. These crops form a major source of protein supplement in the rural diet in the Third World countries [2]. Consequently, the nutritional quality of food legumes has received the attention of agricultural scientists and biologists. The high lysine mutants of maize [3] and sorghum [4] have enhanced the nutritional quality of these cereals. Induced variations for protein quantity and quality have been utilized in the development of high-protein lines with higher levels of limiting amino acids in barley [5] and rice [6].

Vigna radiata (L.) Wilczek (mung bean) and *V. mungo* (L.) Hepper (urd bean) are the two major Asian pulses, while *V. sublobata* (Roxb.) Babu & Sharma is the putative progenitor of both these species. Investigations on mutagenesis for protein quantity and quality of the wild relatives are few, if any. The present paper reports the effect of induced mutagenesis in the variation patterns of protein quantity and quality in the cultivated urd and mung beans and their wild relative.

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*Present address: Department of Botany, St. Joseph's College (autonomous), Tiruchirapalli 620002.

MATERIALS AND METHODS

Seeds of *Vigna radiata* cv. PS 16, *V. mungo* cv. T 9 (obtained from Pulse Research Laboratory, IARI, New Delhi) and *V. sublobata* (harvested from the garden-grown progenies of natural population collected from Palney Hills of Western Ghats, Tamil Nadu) were used for mutation induction with ethylmethane sulphonate (EMS) and gamma rays, used in isolation and combination in the following doses: 0.1, 0.2, 0.3 and 0.4% EMS; 10, 20, 30, and 40 kR gamma rays; and 0.1% + 10 kR, 0.2% + 20 kR, 0.3% + 30 kR and 0.4% + 40 kR EMS and gamma rays, respectively. Seeds for EMS treatment were soaked in the aqueous (distilled water) solution of appropriate concentrations for 10 h, after 6 h of presoaking in distilled water. The treated seeds were thoroughly washed in running water for 12 h and the excess moisture was blotted off. The moisture content of the seed, before mutagen treatment, was adjusted to 10.5–11.0% by differential drying in an air oven [7]. ^{60}Co was used as the source of gamma rays for seed irradiation at IARI, New Delhi, at the dose rate of 2500 rads min^{-1}. For the combined treatment, seeds were first irradiated with gamma rays and then treated with EMS. A control was also maintained. Seeds from each M_1 plant were harvested separately and sown in M_2 as single plant progenies. Seeds protein was estimated by the Microkjeldahl method [8] in 20 phenotypes selected at random from M_2 and M_3 seeds, collected from M_1 and M_2 plants, respectively. Three-level analysis of variance was used to determine the induced variation in protein content. The components of variance, heritability

Table 1. Range, mean and standard deviation and coefficient of variation for whole seed protein content in M_2 and M_3 seeds of *V. sublobata* in different treatments

Treatment	M_2 seeds			M_3 seeds		
	range	mean ± SD	CV	range	mean ± SD	CV
Control	17.3–22.9	20.9±1.3	6.5	15.4–25.2	19.7±2.3	11.8
EMS:						
0.1%	18.2–23.0	20.3±1.2	6.0	14.9–24.8	19.5±2.6	13.7
0.2%	13.2–22.2	19.1±1.8	9.7	14.7–25.3	18.6±2.5	13.7
0.3%	17.3–23.6	19.8±1.8	9.3	14.7–24.4	20.2±2.5	12.3
0.4%	18.7–23.5	20.7±1.2	5.8	15.6–25.5	20.5±2.8	13.9
Gamma rays:						
10 kR	12.7–20.5	18.0±2.0	11.4	15.0–23.3	20.4±2.5	12.1
20 kK	13.1–25.1	19.5±2.4	12.7	15.0–24.9	20.3±2.6	12.8
30 kR	18.4–25.5	21.2±1.7	8.0	17.0–25.7	21.7±2.1	10.0
40 kR	17.1–25.5	20.6±2.0	9.7	16.9–25.1	21.7±2.5	11.6
EMS + gamma rays:						
0.1% + 10 kR	17.3–24.4	20.9±1.9	9.2	14.3–24.8	18.3±2.7	14.9
0.2% + 20 kR	16.8–25.5	20.4±2.3	11.2	13.9–22.6	17.7±2.2	12.4
0.3% + 30 kR	17.3–25.8	20.9±2.0	9.7	14.5–22.6	17.9±2.0	11.3
0.4% + 40 kR	16.2–22.1	18.6±1.5	8.4	14.1–23.1	17.5±2.1	12.1

(h^2) and genetic advance (GA) were also determined [9]. Some mutant M_3 seeds selected from M_2 generation (high protein, low protein and high yield) from all the three species were analysed for protein quality at the Technicon Sequential Multisample Automatic Amino Acid Analyser. The r values were calculated for some character associations.

Table 2. Range, mean and standard deviation and coefficient of variation for whole seed protein content in M_2 and M_3 seeds of *V. radiata* in different treatments

Treatment	M_2 seeds			M_3 seeds		
	range	mean ± SD	CV	range	mean ± SD	CV
Control	16.2–23.6	20.7±1.7	8.5	15.9–25.7	20.4±3.1	15.4
EMS:						
0.1%	19.5–22.9	21.2±0.9	4.6	15.6–27.9	22.5±3.3	14.6
0.2%	15.4–21.9	19.5±1.8	8.1	16.8–23.1	19.9±1.9	9.6
0.3%	17.7–25.5	20.6±1.8	9.1	15.6–21.6	18.8±1.7	9.0
0.4%	15.1–23.9	18.8±2.2	11.6	15.9–24.6	19.9±2.1	10.7
Gamma rays:						
10 kR	19.1–25.2	21.1±1.4	6.8	18.3–23.7	20.7±1.3	6.4
20 kR	18.6–24.9	21.4±1.9	7.3	15.8–24.6	20.6±2.3	11.4
30 kR	19.7–28.5	22.2±1.9	8.7	15.2–23.7	20.8±2.2	10.7
40 kR	19.3–25.0	21.6±1.6	7.4	15.4–29.5	20.9±3.6	17.2
EMS + gamma rays:						
0.1% + 10 kR	18.5–25.2	21.2±1.6	7.6	15.0–24.8	19.2±2.4	12.5
0.2% + 20 kR	16.9–23.5	20.8±1.3	6.5	16.3–23.9	21.6±2.1	9.6
0.3% + 30 kR	18.0–22.9	20.9±1.4	6.8	18.0–24.9	21.2±1.9	9.1
0.4% + 40 kR	18.3–24.7	20.9±1.4	6.9	16.1–22.4	19.8±1.7	8.7

RESULTS

The differences in the mean whole-seed protein content among the three species were marginal at all treatments both in M_2 and M_3 seeds (Tables 1, 2, 3). In the M_2 seeds of the wild relative, the mean protein content was lower than the controls, whereas the protein level of M_2 seeds of the cultivars increased marginally over the controls. The mean protein content of M_3 seeds of all the three species was higher than in M_2 seeds of some treatments. The F values for between species, between treatments, and species × treatments interaction were statistically significant ($P<0.001$) both in M_2 and M_3 seeds (Table 4). The environmental component was several times higher than the genetic component; h^2 was less than 25% and GA less than 1 in both M_2 and M_3 seeds (Table 5).

Table 6 gives the levels of essential and sulphur containing amino acids (S-amino acids) in the mutated and control plants of all three species. In *V. sublobata* mutant High Protein 2, the level of all amino acids (except S-amino acids) was higher than control. In mutant High Protein 1 of this species, cysteine content (1.7% of protein)

Table 3. Range, mean and standard deviation and coefficient of variation for whole seed protein content in M_2 and M_3 seeds of *V. mungo* in different treatments

Mutagen	M_2 seeds			M_3 seeds		
	range	mean ± SD	CV	range	mean ± SD	CV
Control	17.1–21.9	20.0±1.0	5.0	15.6–25.9	21.9±2.5	11.5
EMS:						
0.1%	18.8–22.1	20.8±0.8	4.2	18.3–25.1	21.2±1.9	9.4
0.2%	19.8–22.9	21.3±0.9	4.3	15.4–24.4	19.6±2.1	10.9
0.3%	18.3–21.9	20.0±1.0	5.0	17.1–23.5	20.5±1.7	8.5
0.4%	17.5–21.5	19.8±0.8	4.3	15.9–24.2	20.5±1.8	9.1
Gamma rays:						
10 kR	16.0–20.5	18.3±1.3	7.3	18.3–23.5	21.0±1.4	6.7
20 kR	19.3–22.4	20.8±0.9	3.8	15.1–28.3	20.3±3.2	15.9
30 kR	16.3–21.9	19.8±1.5	7.6	15.6–28.9	21.8±3.4	15.7
40 kR	18.2–20.8	19.3±0.7	3.9	15.9–22.8	20.5±1.7	8.5
EMS + gamma rays:						
0.1% + 10 kR	16.6–23.0	20.2±1.4	7.3	17.4–23.9	21.0±1.7	8.1
0.2% + 20 kR	16.8–22.7	19.8±1.2	6.2	16.9–26.3	21.1±2.1	9.9
0.3% + 30 kR	18.5–23.5	20.3±1.2	6.0	14.5–21.5	17.3±2.1	12.1
0.4% + 40 kR	16.8–21.9	19.4±1.0	5.5	14.1–23.3	17.9±2.5	14.3

was highest among all the variants of the three species; the methionine (1.2%) and lysine (7.6%) levels were also relatively higher as compared to mutant High Protein 2, but the level of other essential amino acids generally decreased. In mutants Low Protein 1 and 2, the leucine levels were high (7.5% and 7.5%, respectively). Arginine level of mutant High Yield 1 was the highest (9.4%) among all three species. In *V. radiata*, the histidine and lysine levels were highest in mutants Low Protein 1 and 2. Mutant High Protein 1 of *V. mungo* had highest lysine (9.9% of protein) and methionine (2.1%) content among all the mutants of the three species. Mutant High Yield 1 of this species showed the highest leucine content (8.6%) among all the mutants and controls analysed.

Table 4. Three-level analysis of variance of whole seed protein content in M_2 and M_3 seeds

Source of variation	M_2 seeds				M_3 seeds			
	d.f.	SS	MS	F	d.f	SS	MS	F
Between blocks	1	0.0	0.0	0.0	1	7.3	7.3	1.3
Between species	2	110.6	55.3	23.6*	2	138.1	69.0	12.8*
Block × species	2	18.4	9.2	3.9	2	1.8	0.9	0.1
Between treatments	12	216.0	18.0	7.7*	12	600.7	50.0	9.2*
Block × treatment	12	83.1	6.9	2.9	12	135.9	11.2	2.1
Species × treatment	24	353.3	14.7	6.3*	24	571.3	23.8	4.4*
Block × species × treatment	24	88.7	3.7	1.5	24	254.3	10.6	1.9
Error	702	1639.1	2.3		702	3785.4	5.3	
Total	779				779			

*Significant at $P < 0.001$.

Table 5. Components of variance, heritability (h^2), and genetic advance (GA) of whole seed protein content in M_2 and M_3 seeds (calculated from three-level analysis of variance)

Seeds	Vp	Vg	Ve	h^2	GA
M_2	2.49	0.16	2.33	6.43	0.13
M_3	5.46	0.17	5.39	3.14	0.03

Vp, Vg, Ve –Phenotypic, genotypic and environmental components of variance, respectively.

Table 7 gives the level of nonessential amino acids in the mutated and control genotypes of the three species. Mutant High Protein 1 of *V. sublobata* had highest glycine (7.7%) and alanine (6.0%) content; and mutant High Protein 2 had highest glutamine content (19.6%). In *V. radiata* and *V. mungo*, the levels of these amino acids did not differ markedly.

Table 6. Essential and S-amino acid content of the whole-seed protein in the control and protein mutants of the three *Vigna* species

Species, mutant	Protein %	Pods per plant	Amino acid content, % of protein									
			Arg	His	Isl	Leu	Lys	Thr	Val	Phe	Met	Cys
V. sublobata:												
Control	20.7	23	7.2	2.9	4.6	6.9	6.7	3.5	5.8	6.2	1.1	0.3
High Protein 1	21.3	22	7.1	3.9	3.8	6.0	7.6	3.5	6.1	5.6	1.2	1.7
High Protein 2	21.9	23	7.3	4.3	4.9	7.0	7.5	3.9	4.2	6.5	0.9	0.1
Low Protein 1	14.7	25	7.2	4.1	5.0	7.5	8.9	3.1	5.4	6.6	1.0	1.1
Low Protein 2	12.5	27	6.8	3.8	4.7	7.5	6.4	4.4	4.2	6.0	0.8	0.2
High Yield 1	22.2	33	9.4	3.9	5.1	6.9	8.9	4.0	4.4	5.8	1.3	0.2
High Yield 2	20.5	36	7.8	3.1	4.6	7.1	7.3	3.7	5.1	5.8	1.0	0.1
V. radiata:												
Control	21.3	25	6.8	3.2	5.1	7.5	6.8	3.4	5.7	6.3	1.3	0.4
High Protein 1	25.1	24	7.2	3.0	4.2	7.8	7.8	3.8	4.9	6.8	1.1	0.4
High Protein 2	23.8	28	8.6	4.7	4.6	6.8	8.4	3.3	5.1	5.7	1.3	0.3
Low Protein 1	18.9	27	5.2	5.3	4.4	7.2	8.4	3.8	4.4	6.5	1.4	0.3
Low Protein 2	18.3	26	7.1	5.4	4.4	7.2	8.7	3.5	5.6	6.6	1.3	0.1
High Yield 1	18.4	38	6.7	2.9	5.0	7.6	6.0	3.4	5.8	6.2	1.7	0.8
High Yield 2	16.4	39	7.0	3.7	4.9	7.6	7.2	3.9	5.0	6.6	1.1	0.2
V. mungo:												
Control	24.4	24	9.3	3.6	4.8	7.5	7.1	3.4	4.7	5.9	1.1	0.4
High Protein 1	25.9	22	7.2	4.9	4.8	7.3	9.9	3.6	4.6	6.8	2.1	0.4
High Protein 2	26.8	21	6.4	3.0	5.0	6.4	7.0	4.4	5.1	5.7	2.0	0.8
Low Protein 1	18.1	23	6.3	2.9	4.9	7.8	5.8	3.9	6.2	6.1	1.5	0.7
Low Protein 2	17.3	26	6.7	4.9	4.6	8.0	7.8	4.3	5.0	6.4	1.4	0.3
High Yield 1	23.5	29	6.1	3.5	4.8	8.6	6.5	3.8	5.2	6.4	1.4	0.4
High Yield 2	23.9	30	6.9	3.5	5.2	7.9	6.1	3.6	4.7	7.6	1.3	1.2

The relationship (r values) between protein and lysine content was positive and significant for the control but nonsignificant for mutants. Protein content showed positive relationship with methionine in the mutants but negative in the control. Lysine was negatively associated with methionine in the control but positively in the mutants (Table 8).

DISCUSSION

The protein content of the wild relative (*V. sublobata)* was comparable to that of cultivars. The broad range and high coefficient of variation for protein content in M_3 seeds of all three species (Tables 1, 2, 3) indicates induction of substantial variation for seed protein content, as was also reported from earlier studies [10, 11], although the mean protein content over treatments did not differ markedly. The increase in mean protein content in some treatments and decrease in others suggest that mutants with positive and negative effects were induced in different proportions with various treatments.

Table 7. Nonessential amino acid levels of whole seed protein in the control and mutated genotypes of three *Vigna* species

Species mutant	Protein, %	Pods per plant	Amino acid content, % of protein							Actual recovery (total amino acids)
			Arg	Ser	Glu	Pro	Gly	Ala	Tyr	
V. sublobata:										
Control	20.7	23	13.4	4.7	17.2	4.4	5.3	5.1	3.8	109.8
High Protein 1	21.3	22	10.3	4.1	16.2	5.5	7.7	6.0	2.9	90.9
High Protein 2	21.9	23	10.3	4.9	19.6	5.7	4.5	3.5	3.8	92.9
Low Protein 1	14.7	25	10.6	4.6	16.3	5.4	4.2	4.1	3.8	103.8
Low Protein 2	12.5	27	13.3	4.9	18.6	5.2	4.7	4.4	2.9	89.3
High Yield 1	22.2	33	11.8	4.8	16.1	4.6	3.9	3.9	3.0	105.0
High Yield 2	20.5	36	13.9	4.9	17.3	4.6	5.0	4.3	3.2	97.7
V. radiata:										
Control	21.3	25	13.5	4.4	18.8	3.4	4.4	4.4	3.0	97.6
High Protein 1	25.1	24	11.9	4.9	17.6	6.4	4.1	4.0	3.3	94.7
High Protein 2	23.8	28	11.7	4.4	17.2	5.6	4.1	4.2	2.8	90.4
Low Protein 1	18.9	27	12.7	4.9	18.1	5.2	3.8	4.2	2.9	112.8
Low Protein 2	18.3	26	11.4	4.9	16.0	5.1	3.8	4.7	2.8	109.9
High Yield 1	18.4	38	13.1	4.7	17.2	6.1	4.0	4.3	3.4	112.7
High Yield 2	16.4	39	12.5	4.5	17.6	5.7	3.5	4.1	3.4	113.6
V. mungo:										
Control	24.4	24	14.0	4.7	16.5	4.0	4.1	4.2	3.1	104.0
High Protein 1	25.9	22	12.8	5.2	14.6	3.9	3.6	3.8	3.1	98.6
High Protein 2	26.8	21	15.5	4.9	17.7	3.4	3.4	3.9	2.9	87.7
Low Protein 1	18.1	23	13.7	5.0	18.0	3.7	4.7	4.2	3.3	107.0
Low Protein 2	17.3	26	11.6	5.3	15.2	4.8	3.8	4.7	4.0	101.7
High Yield 1	23.5	29	12.1	4.6	17.9	4.6	4.5	5.0	3.2	107.6
High Yield 2	23.9	30	11.7	4.6	14.9	6.7	3.8	5.5	3.4	98.4

Protein content is known to be influenced by various endogenous and exogenous factors such as stem height, leaf area, time of maturation, seed size, seed number, temperature, water stress and nitrogen feeding levels [12]. The statistically significant F values between species indicate large variation in their protein level. Blixt [13] reported increase in protein content in induced mutants of legumes than in spontaneous mutants, cultivars, land races, cross derivatives, and wild relatives.

The nonmutagenized seed material of cultivars had higher essential amino acid content than the wild relative, but the level of nonessential amino acids was higher in the wild relative than in the cultivars. Similar observations have also been reported in *Cajanus* and its wild relative [14]. The amino acid levels in the wild relative are higher than the recommended FAO standards. The lysine, methionine and cysteine levels of some of the mutants of *V. sublobata* are much higher than those reported for the mutants of *V. mungo* and *Cicer arietinum* [15], *Cajanus* and *Atylosia* [14], strains of soybean [16], and *Phaseolus vulgaris, Vigna radiata* and *V. unguiculata* [17]. These isolated mutants can be directly evolved into new varieties or can be used in cross-breeding for nutritional upgrading of the existing cultivars. These mutant lines are being maintained for use in breeding programmes.

Table 8. Correlation coefficients (r) between whole seed protein, lysine, methionine and cysteine content in the controls and mutants of the three *Vigna* species

Character pair	Controls	Mutants
Protein v. lysine	0.91*	0.19
Protein v. methionine	−0.28	0.52
Methionine v. cysteine	0.58	0.14
Lysine v. methionine	−0.15	0.16

*Significant at $P < 0.05$.

The induced variability patterns for both essential and nonessential amino acid levels of all the mutants indicates the possibility that there is an interconversion of amino acids, as demonstrated in wheat [8]. The induced mutants showed positive correlation between lysine and protein content; however, negative correlations between these traits have been reported in *Cajanus* and *Atylosia* [14]. In *Pisum,* neither positive nor negative correlation was found [19]. There was no relationship between cysteine and methionine content in the mutants, although these characters are reported to be positively correlated in the cultivars of dry beans [20].

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