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Variability for protein content and its association with yield and yield components in mungbean (*Vigna radiata L.*)

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ABSTRACT

Mungbean or green gram has high nutritive value with easily digestible protein, high amount of dietary fibres, antioxidant substances, vitamins and minerals. However, in a study conducted it was observed that main nutritive constituent of mungbean seeds, protein varied significantly among diverse germplasm including land races, released varieties, exotic and wild accessions. Wide range of variation was also observed for all the agronomic traits studied. Relative high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was observed for most important seed yield and important yield components viz, number of pod clusters per plant, number of pods per plant and 100-seed weight. Of the three different methods employed for rapid and accurate estimation of seed protein, Kjeldahl's method represented maximum protein content in all genotypes compare to remaining two methods Lowry and Bradford. The significant negative correlation of protein content with days to flowering seed size and seed yield per plant indicated that high yielding and higher seed sized genotypes had less protein as compared to smaller seeds while early maturing genotypes had higher protein.

1. Introduction

Grain legumes are an important part of human diet in almost every parts of the world. Among these, Mungbean (*Vigna radiata* L.) is one of the most commonly consumed food legumes in India. Besides, being a rich and less expensive source of protein, mungbean contains a large amount of carbohydrates, dietary fibres, essentials minerals, vitamins and folates[1]. Mungbeans nutrition includes a very impressive amount of a plant protein, with about 20–24 percent of their chemical structure being amino acids (protein). Mungbean is also rich in other essential amino acids, including leucine, isoleucine and valine, which can be combined with other plant sources (like whole grains or some vegetables) to make a "complete protein." Mungbean nutrition contains a range of phytonutrients that are considered anti-microbial and anti-inflammatory, and promote a healthy balance of bacteria within the digestive tract. The folate (vitamin B9) is an important vitamin for DNA synthesis, cell and tissue growth, hormonal balance, cognitive function, and even reproduction. Mungbean also provide about 36 percent of daily magnesium needs for the average adult man. Many adults are actually deficient in magnesium. The data released by the National Institute of Nutrition (NIN), Hyderabad[2] in 2017 suggests that the foods we eat today are less nutritious than what we used to consume just three decades ago. NIN has released such data after a gap of 28 years. NIN researchers have measured the values of 151 nutrients in 528 food items including pulses collected from markets across six geographical regions. All the food items and nutrients listed in the 2017 report showed a decline in quantity and quality as compared to the same food items measured in 1989. The analysis shows an alarming trend. There is a perceptible decrease in nutrition levels in all types of food and report says that those food items are 'Healthy no more'. The pulses are being depleted of their key nutrient protein, which plays an important role in building, repairing and maintaining tissues. Protein has reduced by 10.4 per cent in masoor (whole brown lentil) and 6.12 per cent in green gram, moong (whole green gram). Another study published in 2004 in the Journal of the American College of Nutrition[3], researchers with the University of Texas at Austin, analysed food composition data for 43 crops grown between 1950 and 1999. The six nutrients protein, calcium, iron, phosphorus, riboflavin and ascorbic acid-showed a significant decline in almost all the crops including pulses. Two reasons for declining food nutrition have been identified by Scientists across the world. One, intensive agricultural practices have stripped the soil of micronutrients. The second reason pertains to the impact of climate change on nutritional status of food crops.

Earlier studies[4] showed that seed size and protein content in pulses is significantly negatively correlated with each other. Here taking decision is very critical, whether we need attractive commercially viable large seeded pulses with less quality traits or short duration medium to small seeded varieties with high protein and micronutrients content. The present investigation is therefore, aims to ascertain the range of protein content in diverse munbean germplasm and the relationship between most important economic traits and protein content.

2. Experimental

Thirty mungbean genotypes including collections of wild and exotic accessions, landraces and released varieties originated from different geographical regions were chosen for present investigation. Sufficient variability in this selected panel of genotypes was selected with the consideration that this variability also may reflect in the biochemical composition of the seeds such as protein and amino acids. The thirty test lines were raised in

augmented block design with two lines of 4 meter rows for each genotype and after every five genotypes two checks Samrat and Virat were alternately sown during kharif 2012-13 and summer 2013-14. The observations on agronomic traits were recorded and fresh seeds were used to estimate the protein content. The seed protein contents in replicated samples of 30 genotypes were estimated by three different methods: Kjeldahl's method[5] based on total nitrogen content where as Lowry's[6] and Bradford's[7] methods based on soluble protein fractions.

Table 1: Analysis of variance for agronomic traits and protein content in mungbean

Source of variance	df	1	2	3	5	6	7	8	9	10	11	12
Replication	2	1.89**	76.1**	192.25**	1.0	0.35	0.12	4.95	1.72	0.12	0.3	0.65
Treatment	63	21.6**	101.5 **	161.3**	3.6**	0.64**	0.62**	2.25**	15.9**	0.60**	6.6**	4.50**
Error	126	2.00	5.1	28.6	0.9	0.30	0.24	1.3	7.9	0.06	1.1	0.86**

** Significant at 1% probability, * Significant at 5% probability; 1.Days to 50% flowering; 2. Days to maturity;3. Plant height (cm); 4. No. of clusters per plant;5. No of pods per cluster; 6. Pod length (cm); 7. No. of seeds per pod; 8. No of pods per plant;9. 100 seed weight (g);10. Protein content (mg g-1); 11. Seed yield per plant

Table 2: Genetic parameters of variations for seed yield and its components and protein content in mungbean

Traits	**Parameters**				
	Range			**Coefficient of variation (%)**	
	Minimum	**Maximum**	**Mean (X)**	**Genotypic**	**Phenotypic**
Days to 50% flowering	25.55	42.60	36.70	8.20	9.40
Days to maturity	65.52	85.00	75.60	7.98	8.54
Plant height (cm)	41.00	77.85	45.45	14.52	19.16
No. of clusters per plant	3.00	9.2	5.60	18.05	25.32
No of pods per cluster	2.2	5.2	3.10	11.25	22.00
Pod length (cm)	6.6	8.64	7.45	7.12	8.68
No. of seeds per pod	9.12	15.60	12.20	8.05	11.08
No of pods per plant	11.4	32.25	17.25	11.85	21.23
100 seed weight (g)	3.2	5.65	3.80	11.52	13.51
Protein content (mg g^{-1})	19.8	26.80	24.6	6.02	7.45
Seed yield per plant	3.75	10.45	7.52	30.35	24.55

Table 3: Percent protein determination by three different spectrophotometric methods in mungbean seeds

	Kjeldahl	**Lowry**	**Bradford**
V sylvestris IC 2770221	31.2±1.12	29.68±1.53	27.96±1.08
MGG 330	29.8±1.65	27.62±1.02	25.8±0.87
V. umbellata IC 251445	27.5±1.08	25.9±1.92	24.2±0.71
TARM 18	28.2±1.65	26.5±0.92	23.7±1.02
V. trilobata I C 349701	26.9±0.88	25.2±1.08	23.8±0.72
Sona mung (landrace)	28.1±1.10	25.7±0.55	24.2±1.65
Pratiksha (Nepal landrace)	24.7±2.04	22.3±1.14	21.8±1.02
EC 398889	25.5±1.08	23.6±1.10	22.3±0.88
IPM 2-3	23.7±2.04	23.1±1.02	21.4±0.55
IPM 205-7	23.6±1.65	21.6±0.87	21±1.14
IPM 2-14	25±1.12	24.7±1.02	22.4±1.15
IPM 409-4	23.8±1.65	21.5±1.14	20.40.54
SML 668	21.8±2.04	20.5±0.88	19.6±0.55
Samrat	23.8±1.10	22±0.87	22.1±1.92
GM 5	22.4±1.14	21±0.55	19.8±1.12
Pant Mung 5	22.8±1.53	20.8±1.02	19.2±0.54
MEHA	21.8±1.92	20±1.02	18.5±1.08
WGG 37	24.1±0.88	23.5±0.92	22.1±1.10
TARM 1	21.8±1.71	20.8±1.08	19.2±1.10
VBG 04-003	20.4±1.12	21.4±0.72	18.8±1.02
Saptari	22.1±2.04	19.2±0.55	17.5±0.87
HUM 16	22.2±1.10	21.3±1.12	20.2±0.92
KM 2241	22.1±1.14	21.1±0.70	18.9±1.14
K 851	20.8±1.92	18.2±0.88	18.5±1.15
DGGV 2	20.4±1.65	18.4±1.02	17.5±0.55
Pusa Vishal	21±0.88	21.1±0.92	18.8±1.92
CO 5	21.5±1.10	19.6±0.92	18.5±1.65
PUSA 9531	21.2±1.71	20±1.14	18.5±1.12
ML 818	20.2±1.14	19.8±0.92	19.1±0.72
MH 421	21.4±1.10	19.2±1.02	18.5±0.88

Table 4: Simple correlation of agronomic traits with protein content in mungbean

		2	3	4	5	6	7	8	9	10
1	Days to fl.intiation	0.78**	0.48**	-0.41*	0.4*	0.19	0.31	-0.21	-0.37*	-0.35*
2	Maturity duration	-	0.68**	-0.42**	0.21	0.37*	0.37*	-0.24	-0.21	-0.30
3	Plant height		-	-0.025	0.51**	0.45**	0.11	0.21	-0.34*	0.082
4	No. of clusters per plant			-	-0.03	0.42**	-0.66**	0.76**	-0.06	0.75**
5	No, of pods/plant				-	0.55**	0.45**	0.42**	0.072	0.4*
6	No of seeds/pod					-	-0.37*	0.46**	-0.17	0.41*
7	100 seed wt (g)						-	-0.12	-0.31*	-0.09
8	Seed yield/plant (g)							-	-0.09	-0.91**
9	Protein content (%)								-	0.29
10	Protein yield/pl. (g)									-
Abbreviations: fl. Flower; pl. plant										
*and ** denotes significant at P 0.05 (>0.325) and P 0.01 (>0.418) level respectively										

Kjeldahl method: Nitrogen content was determined by using Kel Plus distillation apparatus (modified Kjeldahl apparatus) after digestion of the samples. Accurately 1 g of sample was weighed and put in a digestion flask. 10 g potassium sulphate, 0.7 g mercuric oxide and 20 mL sulphuric acid were added. The flask was heated gently at an inclined angle until frothing subsides and then boiled until the solution clears. Boiling went on for an additional half hour. On cooling, about 90 mL. distilled water was added, re cooling was done; 25 mL sulphide solution was added and mixed. Small pieces of boiling chip added to prevent bumping and 80 mL of sodium hydroxide solution while tilting the flask so that two layers were formed. The condenser unit was connected rapidly, heated, and collected distilled ammonia in 50 mL boric acid/ indicator solution and 50 mL of distillate was collected. On completion of distillation, the receiver was removed and titrated against standard acid solution.

3. Calculation

Nitrogen content of sample (%) = mL acid X Normality of standard acid wt of sample (gm)×0.014 X 100

Crude protein content (%) = Nitrogen content X 6.25

Lowry's method: The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin Ciocaltea phosphomolybdic phosphotungstic acid to hetero-polymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. Powdered mungbean seeds (100 mg) for each genotype were kept overnight in 25 mL of 0.1 N NaOH to extract total proteins. A clear supernatant after centrifugation at 10,000 rpm for 20 min was used as a source for the estimation of total proteins.

Bradford method: The methods described by Bradford[7] uses a different concept-the protein's capacity to bind to a dye, quantitatively. The method is based on the ability of proteins to bind Coomassie brilliant blue dyes. The blue anion portion of the dye will be bound to arginyl and lysyl residues of the protein. The absorbance of the solution was measured at 595 nm. The results obtained were plotted into the regression equation of the BSA G250 standard to obtain the soluble protein content of samples.

4. Statistical analysis

To determine significant differences among the genotypes the one-way analysis of variance was done. The data were subjected to analysis of variance for each year and combined over both years and tested for significance. Statistical analysis was performed using SAS[8] version 9.1. All values were expressed as mean ± SEm. One-way analysis of variance (ANOVA) at $p<0.05$ was used for the analytical variation. Least significant difference (LSD) test as multiple comparison methods was used to determine differences between means of the sample with a level of significance of 0.05. Analysis of variance was used to determine the variation between the samples. The critical difference (CD) at 5% where the F-ratio was significant was calculated by the CPCS computer program I. Data were expressed as mean ± standard deviation (SD) for triplicates. Protein analysis between control and test was subjected to statistical analysis by t-test at 5 % level of significance. Correlations between yield traits and protein content were identified using Spearman's correlation (SPSS 17.0).

5. Results and Discussion

All the traits such as days to flower, maturity, primary branch, clusters, pod length, plant height, 100 seed weight, and seed yield were significantly different among the genotypes tested (Table 1). This clearly indicated the presence of considerable variability among the thirty genotypes of mungbean used in the present study for the important traits and provided an opportunity for further analysis and estimation of parameters of variability. Most importantly the variation for percent protein was also significant (<0.01) among the selected genotypes (Table 1). The range of variation for each trait is given in Table 2. Relative high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was observed for seed yield per plant g PCV (30.35) and GCV (24.55) followed by number of clusters per plant, plant height, number of pods plant and 100 seed weight. Interestingly sufficient variation as indicated by coefficient of variation studies was also reflected for protein content PCV (7.45) and GCV (6.02) in the population.

The protein is the major quality trait, which increases the seed quality. The crude protein content ranged approximately from 20.2 to 29.8% in most of the released varieties. Where as, the wild accessions and a landrace (Sona mung) recorded higher protein content ranging between 28.2 to 31.2%. Majority of the genotypes demonstrated the same kind of variation in soluble fraction of protein but soluble fraction of protein was less than crude protein (Table 3). The perusal of data revealed that soluble protein content in mungbean genotypes ranged from 18.2% to 29.68 % with an overall mean of 24.2%. The data revealed the maximum soluble protein content in wild species such as *V. sylvestris*, *V. umbellata*, *V. trilobata* followed by few released varieties such as MGG 330 and a landrace Sona mung; while the minimum value was obtained in ML 818 (19.6%).The variations in protein content in diverse sources of mungbean as mentioned here could be mainly attributed to genetic makeup of cultivars, their origin, method of analysis, cultural practices adopted to raise the crop along with some environmental factors which lead to differential synthesis of the protein. In accordance with the results, a large genetic variability in quantitative and qualitative traits has been reported in mungbean[9]. Payasi[10] in 2015 has also reported the variability in

quality traits. Low PCV and GCV in case of protein indicated that the trait is relatively conservative and less influenced by environment and although variation is significant among the population tested but genetic improvement in protein is difficult.

The comparative estimation using Kjeldahl and two other widely used spectrophotometric methods; the Lowry and Bradford method were carried out in order to find the most appropriate assay for total protein determination in a large range of mungbean genotypes. Irrespective of the different methods used, this trend remained same for all methodologies used for protein determination. The Kjeldahl method showed more accurate protein values and the smallest variation of protein than both the Lowry and Bradford method. Both Lowry and Bradford method showed almost similar sensitivity. The results showed that all the assays studied can be used for protein analysis, but Kjeldahl method provided more reliable protein values than Lowry and Bradford method due to its high accuracy, precision and good reproducibility. Kjeldahl method is still preferred since the spectrophotometric methods can only perform the determination of soluble protein in a sample and the quantification of insoluble protein is still being determined by Kjeldahl analysis. The major proportion of protein estimated was shared by soluble protein fraction as exhibited by most of the genotypes (Table 3). The mean crude protein content was about 26.2 while mean soluble protein content was 24.2, only with difference of 2%. Therefore, mungbean as a whole constitute mostly soluble and digestible protein.

The correlation matrix (Table 4) revealed that protein content was significantly and negatively correlated with most of the traits studied except pod length. Protein percent has significant negative correlation with days to flower initiation suggesting delayed flowering type genotype had less protein content per plant or late flowering mungbean contained less protein in their seeds, days taken for flower initiation is positively associated with pod length. Therefore, genetic enhancement for protein yield, efforts should be made to breed early flowering mungbean with, profuse pods, longer pods and more seeds in pods during selection. The Identification of component traits showing significant association with protein content is a prerequisite for indirect selection of genotypes for improving seed protein. The seed size was negatively correlated with protein content suggesting that genetic improvement for protein content needs seed size to be smaller. Similar relationship between seed size and protein content was reported in chickpea[11]. The study revealed that small seeded chickpea varieties have more protein content and much tastier as compared to large seeded chickpea, which are starchy and less protein. This finding also supported the earlier report by Malhotra and Singh[12]. Further exploration of germplasm including wild vigna relative landraes is required in order to identify the superior germplasm with high protein content for its utilization in breeding programm to breed high yielding and high protein maungbean varieties.

References

1. A. E. Mubarak, *Food Chem*., **2005**, 89, 489-495
2. R. Longvah, K. Ananthan, Bhaskarachary and K. Venkaiah, Indian Food Composition Tables published by National Innstitute of Nutritin, Hyderabad, 2017.
3. R. D. Davis, D. Melvin and D. Riordan, *Journal of the American College of Nutrition*, **2004**, 23, 669–682
4. K. H. Brown and N. W. Solomons, *Infect. Dis. Clin. N. Am.*, **1991**, 5, 297–317.
5. J. Kjeldahl, *Anal. Chem*., **1983**, 22, 366–382
6. O. H. Lowry, N. J. Rosebrough, *Journal of Biological Chemistry*, **1951**, 193 (1), 265-275.
7. M. M. A. Bradford, *Analytical Biochemistry*, **1976**, 72, 248-254.
8. SAS users Guide: statistics version (5th ed.). Cary, NC: SAS Institute Inc., **1985**.
9. M. B. Pandiyan, Subhalakshmi and S. Jebaraj, *Int. J. Plant Sci*., **2006**, 1, 72-75.
10. D. K. Payasi, *International Journal of Plant Breding and GeneticsI*, **2015**, 9,177-188.
11. A. Sagar, B. Mondol, S. K. Chaturvedi and P. S. Basu, Seed size and CO_2 enrichment influence seed protein content in chickpea (Cicer arietinum L.). Abstract. National Symposium on Pulses for Nutritional Security and Agricultural Sustainability at ICAR_IIPR, Kanpur. December, **2017**, 2-4, 2017
12. V. V. Malhotra and K. B. Singh, *Egypt J. Genet. Cytol*., **1976**, 5, 70:73