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Original scientific article

PROTEIN QUALITY PARAMETERS AND STORAGE PROTEIN PROFILING OF MUNGBEAN INTERSPECIFIC LINES (*Vigna radiata* L. Wilczek)

V. JAIN and S. SHARMA*

Department of Biochemistry, Punjab Agricultural University, Ludhiana, 141004, India

Jain V. and S. Sharma (2021*). Protein quality parameters and storage protein profiling of Mungbean interspecific lines (Vigna radiata L. wilczek)*. - Genetika, Vol 53, No.3, 1341-1356.

Present investigation reports the protein quality parameters in mungbean x ricebean (MR) and mungbean x urdbean (MU) interspecific lines along with mungbean checks. Interspecific lines contained 138.4-230.3 mg/g total soluble proteins, 0.84-2.63 mg/g free amino acids, 3.21-7.10 mg/g methionine, 0.08-0.15 mg/g cysteine and 0.67-1.32 mg/g tryptophan. Protein fractionation studies revealed 32.6-48.3 (albumins), 153.3-218.8 (globulins), 2.81-8.30 (prolamins) and 22.22-66.23 mg/g seed (glutelins). Among MU lines, mean albumin content was lower and globulins, prolamins and glutelins were significantly higher than that of mungbean checks. Globulins showed a positive correlation with total soluble proteins and tryptophan while albumins were negatively correlated to prolamins and positively correlated to methionine. High positive correlation was recorded between prolamins and glutelins, as well as prolamins and cysteine at 1% level of significance. Both prolamins and glutelins exhibited a negative correlation with methionine at $P<0.05$. Electrophoretic analysis of total proteins revealed presence of 6-11 bands in molecular weight ranging from 13-162 kDa. Cluster analysis of the resolved gel revealed 9 clusters at 95% homology with SML 2011, the most distant interspecific line among all genotypes. Overall, SML 1827 and SML 2033 were rich in total soluble proteins, free amino acids, methionine, tryptophan, albumins and globulins. The results suggest that electrophoretic profiles of mungbean interspecific lines provide valuable information with potential of being used in mungbean genetic improvement.

Keywords: Mungbean; Interspecific lines; Proteins; Electrophoresis; Cluster analysis.

Corresponding author: S. Sharma, Department of Biochemistry, Punjab Agricultural University, Ludhiana, 141004, India, E-mail: suchetasharma_pau@pau.edu, +91-9888412565

INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek), is among the pulse crops cultivated and consumed in South-East Asia, Australia, South America, and African countries. It is grown during *Kharif* and spring/summer season in North India and during *Rabi* or autumn/winter season in South India. Summer mungbean can be cultivated after harvesting of linseed, potato, pea, mustard or gram and is known to expedite the sustainable yield of rice-wheat cropping system of the Indo-Gangetic belt of northern India. The immature green pods of mungbean are used as a vegetable in rural areas while the whole or split seeds are used in the preparation of dal. It is easy to cook, free from flatulence and helps to overcome protein malnutrition. Its seeds are also used after sprouting to prepare salads or roasted to be eaten as snacks.

Mungbean is known as "green pearl" owing to its high nutrient content and better protein quality than other legumes such as pea, pigeon-pea, chickpea and black gram (ANISHA and PREMA, 2008). Mungbean proteins contain good proportions of essential amino acids isoleucine, leucine, valine and aromatic amino acids, but deficiency of sulfur amino acids, threonine, lysine and tryptophan, limits protein quality (SITAL *et al.*, 2011). The major mungbean seed storage proteins comprise of albumins, globulins and prolamins that account for approximately 85% of total proteins. About 60-70% of total seed proteins are comprised of salt-soluble globulins which are further classified into three main types, viz. legumin like (11-12S) vicilin like (8S) and basic type (7S). Vicilin fraction containing a low amount of sulfur-containing amino acids predominates over legumin fraction and both fractions constitute mungbean globulins in the ratio of 2:1 (YI-SHEN *et al.*, 2018). Mungbean storage proteins are known to exhibit antioxidative potential and antiproliferative effects, angiotensin-converting enzyme inhibition, antibacterial and antifungal activities (GUPTA *et al.*, 2018).

Interspecific hybridization is one of the methods for the creation of genetic variability and widening of the genetic base of a crop species by developing interspecific lines that act as a genetic reservoir for novel genes (PANDIYAN *et al.*, 2010). Mungbean, urdbean (*Vigna mungo* L. Hepper) and ricebean (*Vigna umbellata* Thunb.) species exhibit some special traits which can be inter-transferred in each other via hybridization (SHARMA *et al.*, 2013). Among the Asiatic *Vigna* species, urdbean is most promising for interspecific hybridization with mungbean. Mungbean is an early maturing crop; possess long pods with more seeds/pod. Urdbean crop contains nonshattering pods with large seeds and has high resistance to fungal and viral diseases. Ricebean has a high response to irrigation, high yield, and resistance to mungbean yellow mosaic virus and bruchids (SINGH and BAINS, 2006). A number of interspecific lines have been developed by crossing mungbean x urdbean (MU) and mungbean x ricebean (MR) to inttogress genes for productivity and other desirable traits and assess the genetic diversity and variability at this institute. Among MR interspecific lines, SML 1827 has been released for cultivation in Punjab state and its physicochemical properties; viz. weight per seed was higher than two check varieties and at par with three check varieties. To our knowledge this is the first study where interspecific lines have been assessed for protein quality. Electrophoretic analysis of seed proteins has been used as one of the biochemical attributes to differentiate various cultivars/lines of a particular crop species and further to resolve the taxonomy and evolutionary problems of different plants. The present study reports the protein quality parameters, seed storage protein

profiling of mungbean genotypes using SDS-PAGE, and possible identification of the resolved seed storage proteins using NCBI and Uniprot databases.

MATERIALS AND METHODS

Seeds of 20 interspecific mungbean lines (13 MU and 7 MR) and 5 released cultivars (SML 668, SML 832, TMB 37, ML 818 and ML 2056) used in the present investigation were procured from the Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The details of mungbean interspecific lines and their parents have been reported earlier by JAIN and SHARMA (2021). The seeds were hand cleaned and crushed into fine powder by Cemotec 1090 sample mill. The seed flour was stored in air tight plastic containers and used for biochemical analysis.

Biochemical Parameters

Total soluble proteins in seed flour (0.1 g) were extracted twice with 5 ml of 0.1 N NaOH followed each time by centrifugation at 4°C for 15 min at 6000 g. After pooling the supernatants, 2 ml aliquot was taken and an equal volume of chilled 20% TCA was added and left undisturbed for 1 h under refrigerated conditions followed by centrifugation at 4°C for 15 min at 6000 'g'. The residue obtained was dissolved in 10 ml of 0.1 N NaOH and used for protein estimation (LOWRY *et al.,* 1951). Free amino acids were extracted from mungbean flour (0.2 g) by refluxing with 15 ml of 80% ethanol and then with 70% ethanol for 15 min each on a boiling water bath and contents were estimated by the method described in KAUR and SHARMA (2021). Sulphur containing amino acid (methionine and cysteine) and tryptophan were determined by the methods already described in SINGLA *et al.,* (2017).

Protein fractionation

Mungbean storage proteins were isolated and fractionated from the seeds by the given protocol (KAUR and SHARMA, 2021). Subsequently, protein content in each fraction was determined as described above.

Electrophoretic analysis

For total soluble protein extraction, 200 mg of mungbean flour was homogenized with 1 ml of Tris buffer (pH 8.3) in pestle and mortar. Samples were centrifuged at 10,000 g for 10 min and the supernatant thus obtained was used for SDS PAGE analysis. Quantified protein samples were mixed with sample buffer to make the total volume of 30 µl and heated for 3 min in boiling water bath to ensure complete denaturation and optimum SDS-binding.

Protein samples were subjected to SDS-PAGE as described by LAEMMLI (1970). Equal quantities of protein samples were loaded into 12% gel along with protein molecular marker (14-66 kDa BioLit^{Tm}). Electrophoresis was performed at a constant 180V until the dye (bromophenol blue) reached the bottom of the gel. After completion of electrophoresis, the gel was placed in the fixing solution (12.5% trichloroacetic acid) for 1 h, washed with distilled water and kept overnight in the staining solution of Coomassie brilliant blue R-250. The gel was transferred to destaining solution (methanol: glacial acetic acid: water in the ratio of 10:7:83) the following day and washed 3-4 times till clear bands appeared and gel became transparent. Gels were photographed and the molecular weight of different proteins/subunits was determined by using AlphaView software (AlphaImager Gel documentation system from Protein Simple, USA).

Cluster analysis was performed using PyElph1.4 © 2011 Ana Brandusa Pavel and Christian Vasile.

Peptide search in database

Peptides were searched in National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and Uniprot database (www.uniprot.org) for mungbean seed storage proteins on the basis of their molecular weight that has been reported in earlier literature to identify the proteins separated in the present study.

Statistical analysis

The data was statistically analyzed by ANOVA at 1% and 5% significant level. Mungbean genotypes were divided into 3 classes as low, medium and high groups on the basis of equal class distribution method. The correlation between different protein parameters of mungbean genotypes and Multivariate Principal Component Analysis was done using XLSTAT software v.2020.3.

RESULTS AND DISCUSSION

Total soluble proteins and Free amino acids

Total soluble proteins (TSP) in mungbean genotypes ranged from 177.19 (SML 2016) to 230.31 mg/g (SML 1817) (Table 1). Genotypes with TSP >212.59 mg/g were categorized as high protein group and <194.89 mg/g as low protein group. Three interspecific lines (SML 1817, SML 1930 and SML 2033) were placed under group with higher TSP content. Eight MU lines (SML 1808, SML 1820, SML1839, SML 1932, SML 1933, SML 1941, SML 2011, and SML 2034), 3 MR lines (SML 1809, SML 1827 and SML 2031) and check varieties SML 832, ML 818 and TMB 37 recorded TSP content between 19.5-21% and were placed under medium protein group.

Free amino acids (FAA) ranged from 0.84 mg/g in SML 1809 to 2.63 mg/g in SML 1827 (Table 1). Mean FAA content in MR crosses (1.4 fold) and MU crosses (1.25 fold) was higher than that of check varieties. Seventeen out of 20 interspecific lines showed higher FAA content than the mean value for check varieties. SML 1827 and SML 1822 seeds had significantly higher FFA in comparison to check varieties with values >1.99 mg/g. Ten genotypes had medium FAA content (1.36-1.99 mg/g) and 13 showed lower content (< 1.36 mg/g).

Mungbean seeds are an invaluable source of high quality and easily digestible protein and its essential amino acid composition matches satisfactorily with soybean and FAO/WHO reference protein (YI-SHEN *et al.,* 2018). The total protein and FAA content of most of the interspecific lines was comparable to the protein content of 15-30% in earlier reports on mungbean (SHI *et al.,* 2016; SKYLAS *et al.,* 2017; VARMA *et al.,* 2018), 18-22% in soybean (XU *et al.,* 2015) and 20-30% in kidney beans (SHEVKANI *et al.,* 2015). The variation in seed protein content and FAA depends upon cultivar type and environment (SITAL *et al.,* 2011). The lower TSP content in some of the MR lines as compared to checks might be due to differences in the behaviour of these interspecific lines in response to introgression of ricebean genome into mungbean background. However, higher TSP content was observed in SML 1817, SML 1930 and SML 2033 which can be exploited to improve the protein content in mungbean or urdbean.

Table 1. Total soluble proteins, free amino acids, methionine, cysteine and tryptophan content in seeds of different mungbean genotypes

Genotype	Total soluble proteins (mg/g)	Free amino acids (mg/g)	Methionine (mg/g seed)	Cysteine (mg/g seed)	Tryptophan (mg/g seed)
		Check Varieties			
SML 668	193.28±0.68^{a}	0.73±0.03^{l}	8.36±0.24^{ab}	0.12±0.002^{cdef}	0.88±0.027^{efgh}
SML 832	208.75±2.48^{a}	0.81±0.05^{kl}	6.03±0.29^{cdefg}	0.12±0.002^{cdefg}	0.87±0.026^{efgi}
TMB 37	209.77±1.37^{a}	1.35±0.02^{fgh}	9.22±0.09^{a}	0.12±0.002^{cdefg}	0.96±0.029^{bcde}
ML 818	199.38±1.20^{a}	1.17±0.01^{hij}	6.68±1.03^{cd}	0.11±0.007^{efgh}	0.97±0.009^{bcde}
ML 2056	193.36±1.82^{a}	1.60±0.03^{cdef}	6.96±0.33^{bc}	0.11±0.003^{fgh}	0.97±0.023^{bcde}
CD (p<0.05)	NS	0.112	1.138	0.009	0.053
Mean	200.91±1.51	1.13±0.03	7.45±0.39	0.12±0.00	0.93±0.02
		MR interspecific lines			
SML 1809	208.36±3.63^{a}	0.84±0.00^{kl}	3.96±0.12^{ijk}	0.12±0.007^{bcdef}	0.99±0.045^{bcde}
SML 1825	182.11±3.87^{a}	1.01±0.04^{ijk}	4.90±0.36^{efghij}	0.11±0.006^{defg}	0.88±0.003^{efgh}
SML 1827	209.30±0.47^{a}	2.63±0.02^{a}	4.92±0.84^{efghij}	0.13±0.003^{bcde}	0.88±0.012^{efgh}
SML 2015	190.86±2.70^{a}	1.73±0.05^{c}	4.80±0.39^{fghijk}	0.12±0.005^{cdefg}	0.91±0.041^{cdef}
SML 2016	177.19±1.24^{a}	1.57±0.01^{cdef}	4.27±0.79^{hijk}	0.11±0.001^{defg}	0.78±0.012^{fghi}
SML 2031	205.00±1.18^{a}	1.70±0.00^{cd}	5.48±0.46^{cdefghi}	0.15±0.004^{a}	0.67±0.017^{i}
SML 2032	188.67±0.89^{a}	1.58±0.01^{cdef}	4.33±0.48^{ghijk}	0.10±0.007^{hi}	0.77±0.015^{ghi}
CD (p<0.05)	NS	0.081	NS	0.010	0.054
Mean	194.50±2.00	1.58±0.02	4.67±0.49	0.12±0.00	0.84±0.02
		MU interspecific lines			
SML 1808	197.19±1.66^{a}	1.14±0.05^{hij}	5.18±0.69^{defghi}	0.12±0.005^{cdef}	0.92±0.044^{cde}
SML 1817	230.31±2.39^{a}	1.14±0.00^{hij}	3.21±0.05^{k}	0.10±0.003^{ghi}	1.09±0.041^{b}
SML 1820	195.47±3.22^{a}	1.72±0.13^{c}	4.52±0.29^{ghijk}	0.13±0.010^{bcd}	1.00±0.009^{bcde}
SML 1822	194.47±2.13^{a}	2.01±0.06^{b}	4.85±0.42^{fghijk}	0.13±0.006^{abc}	1.03±0.015^{bcd}
SML 1829	193.59±4.34^{a}	1.76±0.03^{c}	5.27±0.16^{defghi}	0.12±0.003^{cdef}	0.90±0.032^{defg}
SML 1839	198.36±2.02^{a}	1.27±0.01^{ghi}	5.99±0.09^{cdefgh}	0.10±0.004^{hi}	1.04±0.034^{bc}
SML 1930	218.91±2.76^{a}	1.21±0.06^{ghi}	4.89±0.88^{efghij}	0.09±0.003^{ij}	0.98±0.023^{bcde}
SML 1932	205.16±2.72^{a}	1.21±0.12^{ghi}	6.43±0.17^{cdef}	0.09±0.003^{ij}	0.76±0.022^{hi}
SML 1933	207.66±0.85^{a}	0.94±0.04^{jkl}	7.10±0.40^{bc}	0.09±0.006^{ij}	0.97±0.063^{bcde}
SML 1941	204.38±1.64^{a}	1.65±0.10^{cde}	3.47±0.09^{jk}	0.08±0.003^{j}	1.06±0.116^{b}
SML 2011	209.61±0.36^{a}	1.44±0.02^{efg}	6.30±0.38^{cdef}	0.11±0.003^{defg}	1.25±0.015^{a}
SML 2033	215.08±2.72^{a}	1.45±0.02^{defg}	6.60±0.40^{cde}	0.15±0.009^{a}	1.32±0.022^{a}
SML 2034	211.17±3.52^{a}	1.35±0.01^{fgh}	6.44±0.37^{cdef}	0.14±0.002^{ab}	0.89±0.043^{efgh}
CD (p<0.05)	14.76	0.198	0.840	0.010	0.094
Mean	201.64±2.33	1.41±0.05	5.40±0.34	0.11±0.00	1.02±0.04
Overall CD (p<0.05)	27.29	0.146	0.947	0.010	0.074
Overall Mean	201.89±2.07	1.40±0.04	5.61±0.39	0.11±0.00	0.95±0.03

Mean values followed with different superscripts are significantly different (p<0.05) using Tukey's test

Sulphur containing amino acid and Tryptophan

Methionine and cysteine content varied from 3.21-7.10 mg/g and 0.08-0.15 mg/g among different interspecific lines, respectively (Table 1). Mean methionine content of MR crosses, MU crosses and check varieties was 4.67, 5.40 and 7.45 mg/g, respectively. Nine interspecific lines exhibited lower methionine content than the mean value for all the interspecific lines. SML 668 and TMB 37 checks showed the higher methionine content (>7.22 mg/g). Six each MR and MU lines had lower methionine content (<5.22mg/g) and rest of the genotypes had methionine content in medium group with values in the range of >5.22 and <7.22 mg/g). Interspecific lines SML 1817 and SML 1941 exhibited approximately half of the methionine content in comparison to check varieties. Cysteine content in SML 2031 and SML 2033 was significantly higher than all other studied genotypes. SML 1941 exhibited cysteine content significantly lower than other interspecific lines and check varieties.

Tryptophan content among mungbean interspecific lines ranged from 0.67 mg/g (SML 2031, MR line) to 1.32 mg/g (SML 2033, MU line) with a mean value of 0.95 mg/g (Table 1). The mean tryptophan content in MR crosses was lower and that of MU crosses was higher than the mean value of the check varieties. Fifteen genotypes had medium tryptophan content (>0.886 and <1.1 mg/g) and eight has lower tryptophan content (<0.886 mg/g). SML 2011 and SML 2033 with tryptophan content higher than 1.1 mg/g were placed in high content group and exhibited significantly higher content in comparison to all the checks.

The mean methionine, cysteine and tryptophan content in interspecific lines in present study was less than the mungbean checks but in similar range to that reported by earlier authors. GANESAN and XU (2018) reported 0.29 g methionine per 100 g and 2.1 mg cysteine per g of mungbean seeds. Methionine and cysteine content in seeds of Australian mungbean lines varied from 2.0-2.7 mg/g and 1.4-1.6 mg/g, respectively (SKYLAS *et al.*, 2017). The mean value of methionine content in mungbean seeds was 1.26 g/16g of nitrogen (VARMA *et al.*, 2018). Mungbean proteins are deficient in lysine, methionine and cysteine, but their availability in mungbean grain is 78%, 83% and 94%, respectively (DAHIYA *et al.*, 2015). Cereals have low lysine content but the deficiency varies among cereals. Oats, rice, and finger millet have only marginal lysine deficiency while sorghum, maize, and other millets have more pronounced lysine deficiency (PRICE and WELCH, 2013). Combining mungbean with marginal lysine deficient and high methionine cereals could form a much balance amino acid diet that could improve the protein quality of the diet. Interspecific lines SML 2011 and SML 2033 exhibited higher amounts of methionine and tryptophan and could be used to contribute to the improvement of the nutritional quality of the diet of people and scope of mungbean x urdbean interspecific hybridization for increasing their content in mungbean.

Protein fractionation studies

Mungbean seed storage proteins were further separated into various fractions and their contents were recorded. Albumin content varied from 32.64 mg/g (SML 1822) to 48.28 mg/g (SML 1941) among the interspecific lines (Figure 1). The overall mean of interspecific lines (40.38 mg/g) was lower than the mean value of check varieties. SML 1808, SML 1933, SML 1941, SML 2011, SML 2015, SML 2016, SML 2031 and SML 2034 had higher albumin content (43.02-48.28 mg/g) and exhibited significantly higher values than the mean value of the check

varieties. Nine interspecific lines had low (32.6-37.81 mg/g) and three had medium albumin content (>37.81 and <43.02 mg/g).

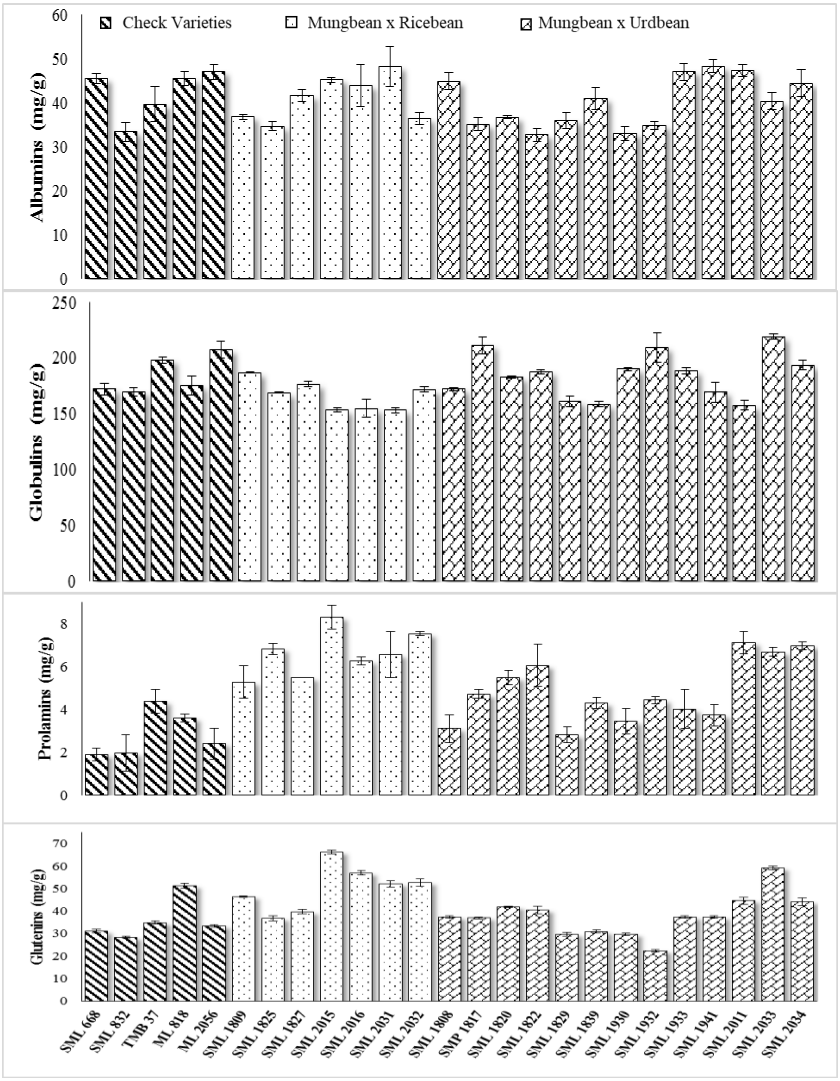


Figure 1. Storage protein fractions in seeds of different mungbean genotypes

Globulin content among mungbean interspecific lines ranged from 153.28 mg/g (SML 2031) to 218.83 mg/g (SML 2033) with overall mean value of 178.26 mg/g. The mean value of globulin content in MR crosses (166.51 mg/g) was lower, while that of MU crosses was at par with the mean value of check varieties. TMB 37 and ML 2056 (checks) and SML 1817, SML 1822, SML 1932 and SML 2033 (MU interspecific lines) had higher globulin content (> 196.98 mg/g), 7 genotypes had medium (175.13-196.98 mg/g) and 12 had lower globulin content (<175.13 mg/g). SML 2015, SML 2016 and SML 2031 exhibited significantly lower globulin content in comparison to that of all the check varieties, whereas SML 1932 and SML 2033 showed the reverse trend.

Prolamin and glutelin content in interspecific lines was lowest in SML 1829 (2.81 mg/g) and SML 1930 (22.22 mg/g) respectively, while SML 2015 showed the highest content for these parameters. Mean values of prolamins in MR crosses and MU crosses were 2.32 and 1.7 fold higher than that of the check varieties. Only interspecific line SML 1829 exhibited lower prolamin content than the mean value of check varieties. The mean glutelin content in seeds of different MR lines was observed to be higher than that of MU lines. Except for SML 1829, SML 1839 and SML 1930, all other interspecific lines exhibited higher glutelin content than the mean value of check varieties.

Higher globulin content is generally considered better for nutritional quality improvement but most of the studied lines showed low contents of sulfur containing amino acids (methionine and cysteine). VARMA *et al.*, (2018) revealed glutelins to be the second major protein fraction next to globulins in mungbean seeds. Storage proteins serve as a source of nitrogen, carbon and sulfur and are thus essential for plant's growth, development and providing resistance against pests, pathogens and desiccation. Absence or low content of storage proteins may affect the seed germination efficiency and post-germinative seedling establishment (WEI *et al*., 2020). Thus, having a knowledge about protein fractions could help in genetic engineering and breeding programs to improve storage protein quality and quantity in mungbean seeds.

PCA analysis and Correlation studies

Principle component analysis is a statistical tool that can be used to find out the inter-relationship among different parameters. The cluster analysis biplot of total soluble proteins, free amino acids, protein fractions, sulphur containing amino acids and tryptophan of mungbean genotypes is shown in Figure 2. The first 4 variables (TSP (29.4%), FAA (19.13%), methionine (15.54%) and cysteine (11.16%) showed 74.8% of total variability. Scatter plot also showed that most of the variance obtained was among the first four eigen values. Methionine and albumin were found to occupy the left side of the plot suggesting correlation between these two variables. TSP, tryptophan and globulin proteins were found to be in the middle part of the plot whereas cysteine, free amino acids, glutenin and prolamin occupied the right side of the plot. The correlation studies revealed a positive correlation between total soluble protein content and globulin fraction at 1% level of significance (Table 2). Albumins were positively correlated to methionine (r=0.457) and globulin to tryptophan (r=0.339) at 1% level of significance. High positive correlation was observed between prolamins and glutelins (r=0.689) at $p<0.01$. Prolamin content was positively correlated to cysteine content (r=0.302) at 1% level of significance and exhibited a negative correlation to methionine (r=-0.283) at 5% level of significance, Glutelins

were also positively correlated to cysteine (r=0.375) at 1% level of significance and exhibited a negative correlation to methionine (r=-0.238) at 5% level of significance. DAHIYA *et al.* (2015) reported a negative correlation between total proteins and methionine suggesting that an increase in methionine content is accompanied by a decrease in total proteins in mungbean. PCA and correlation studies are useful in elaborating the degree and extent of interrelationship among various nutritional parameters in mungbean seeds. These studies provide a basic criterion for developing appropriate selection methods for improving seed quality and economically important protein parameters. PCA was performed to confirm the diversity pattern brought out by cluster analysis. This multivariate technique of classifying mungbean genotypes in accordance with their biochemical parameters could reduce the cost and time in crop improvement.

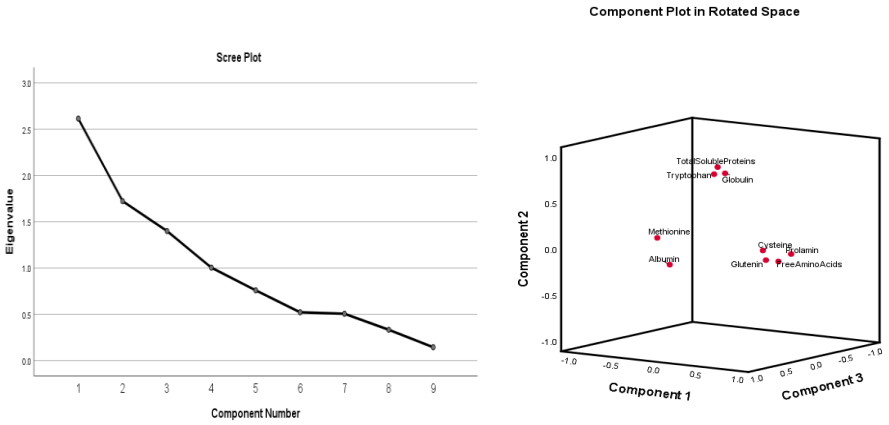


Figure 2. Scatter plot and biplot of total soluble proteins, free amino acids, protein fractions, sulphur containing amino acids and tryptophan of mungbean genotypes.

Table 2. Correlation studies among protein quality parameters and various protein fractions in seeds of different mungbean genotypes

	Albumins	Globulins	Prolamins	Glutelins	Methionine	Cysteine	Tryptophan
TSP	0.097	0.306**	-0.092	-0.059	-0.166	0.152	0.015
Albumins		-0.035	-0.264*	-0.083	0.457**	-0.078	0.042
Globulins			-0.043	-0.096	0.151	0.053	0.339**
Prolamins				0.689**	-0.283*	0.302**	0.01
Glutelins					-0.238*	0.375**	0.086
Methionine						0.15	0.037
Cysteine							-0.023

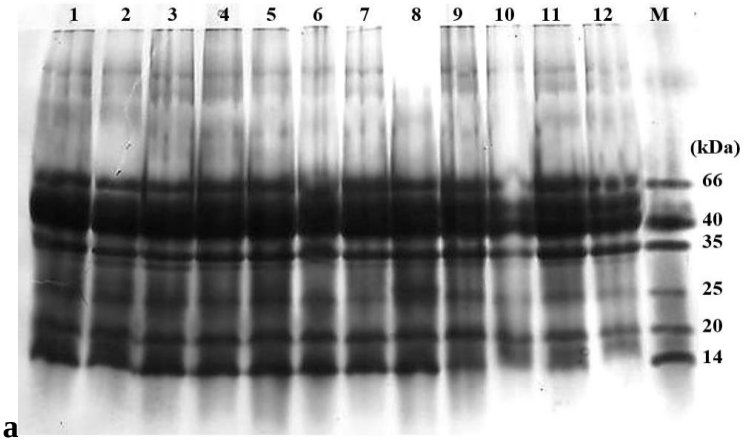
** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

SDS-PAGE profiling

On the gel, the storage protein profile of mungbean seeds from different genotypes revealed the presence of 6-11 bands (Fig 3a andb, Supplementary material; Table 1). Banding patterns of protein in the interspecific lines were almost similar to the check varieties. SML 2011 showed the presence of 6 bands, SML 1827 and SML 2016 showed 8, SML 1808, SML 2033 and SML 2034 showed 9 while SML 2032 showed 11 bands in gel. All other interspecific lines showed the presence of 10 bands on the gel. The protein banding profile showed a number of low and high molecular weight subunits with their molecular weights ranging from 14 kDa (SML 1809) to 160 kDa (SML 1825) with highly prominent subunits of 30-40 kDa. Bands corresponding to approximately molecular weight of 14, 20, 25, 35, 40 and 66 kDa were prominent in all the mungbean genotypes.

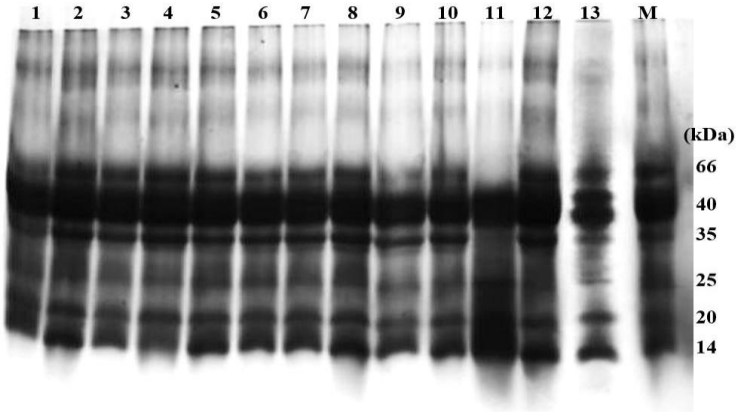
The check varieties exhibited similar banding patterns except for ML 818 that exhibited an extra band of 82.14 kDa. Among MR interspecific lines, bands corresponding to molecular weight 160 and 130 kDa were absent in genotypes SML 1827 and SML 2016, while bandof 100 kDawas absent in SML 1825, SML 2015 & SML 2016 relative to other MR genotypes. However, an extra band of 76.27 kDa was observed in SML 2032. Nine out of 11 bands with molecular weight in the range of 14-152.5 kDa were common in SML 2032 and ML 818. The presence of one extra band in ML 818 and SML 2032 each and their absence in other studied genotypes can be used to differentiate them from rest of the tested lines and for the identification of these lines.



a

1. SML 668, 2. SML 832, 3. TMB 37, 4. ML 818, 5. ML 2056, 6. SML 1809, 7.SML 1825, 8. SML 1827, 9. SML 2015, 10.SML 2016, 11. SML 2031, 12. SML 2032, 13. Marker

Figure 3a. SDS-PAGE profile of mungbean seed storage proteins of the check varieties (1-5, 3a), mungbean x ricebean (6-12, 3a)



b

1. SML 1808, 2. SML 1817, 3. SML 1820, 4. SML 1822, 5. SML 1829, 6. SML 1839, 7. SML 1930, 8. SML 1932, 9. SML 1933, 9. SML 1933, 10. SML 1941, 11. SML 2011, 12. SML 2033, 13. SML 2034, 14. Marker

Figure 3b. SDS-PAGE profile of mungbean seed storage proteins of the check varieties (1-5, 3a), mungbean x urdbean (1-13, 3b) interspecific lines

Similarily, among MU lines, SML 2011 which was an outliner in cluster analyis (Figure 3) showed absence of three bands with molecular weight corresponding to approximately 37, 60 and 100 kDa in comparison to other MU lines indicating the maximum genetic variabilty as compared to other genotypes. SDS-PAGE profiling can be useful in biosystematics analysis, post-translational modifications analysis, varietal identification, phylogenetic studies and differentiate mutants from their parental genotypes (MALVIYA *et al.,* 2008; HAMEED *et al.,* 2012). Electrophoretic analysis of seed protein is reported to be a relatively inexpensive way for developing genetic markers for identification and genetic analysis of various agricultural commodities and also useful to identify interspecific variation in *Vigna* species (GHAFOOR *et al.,* 2002). It is also suitable for testing uniformity, distinctness and stability of varieties for registration and identification for Tris-soluble proteins (SINGH *et al.,* 2015). Overall much difference in protein banding patterns was not observed but there was a difference in the intensity of protein subunit bands in mungbean genotypes with less prominent bands in SML 2016 and SML 2032. Differences in band mobility, width and intensity in legumes have been reported by previous authors (ROY *et al.,* 2001; SARKAR, 2017). The molecular weights of the resolved peptides in 13 mungbean varieties ranged from 16 kDa to 103 kDa (HAMEED *et al.,* 2012). MALVIYA *et al.,* (2008) revealed that green gram consists of 2S (14 kDa) and 11S (17 kDa) globulins as seed storage protein. MENDOZA *et al.* (2001) isolated basic 7S globulins, vicilin type (8S) globulins and legumin type (11S) globulins from mungbean and the SDS-PAGE profile revealed that basic 7S globulin was composed of 2 bands (16 and 28 kDa), 8S was composed of 4 bands (26, 32, 48 and 60 kDa) while 11S was composed of 2 bands (24 and 40 kDa). The present study detected peptides of approximate molecular weights 16 kDa and 28 kDa on SDS-PAGE that may be 7S subunit, while that of 24 kDa and 40 kDa (approx.) may be 11S

globulin, as well as 32 kDa and 60 kDa that may be 8S vicilin subunits on the basis of the report of MENDOZA *et al.* (2001). Recent SDS-PAGE profile of mungbean seed storage proteins by SARKAR (2017) revealed 16-19 peptides ranging from 18.4-116.0 kDa, whereas SASHIKALA *et al.* (2015) reported five major polypeptides of molecular weight 15, 18, 20, 45 and 60 kDa in mungbean seeds.

Cluster analysis

The dendrogram for protein profiles of mungbean genotypes is presented in Figure 4. Based on the electrophoretic data, the 25 genotypes have been grouped into 9 clusters at 95% homology. The obtained results presented high genetic diversity among mungbean genotypes based on seed proteins.

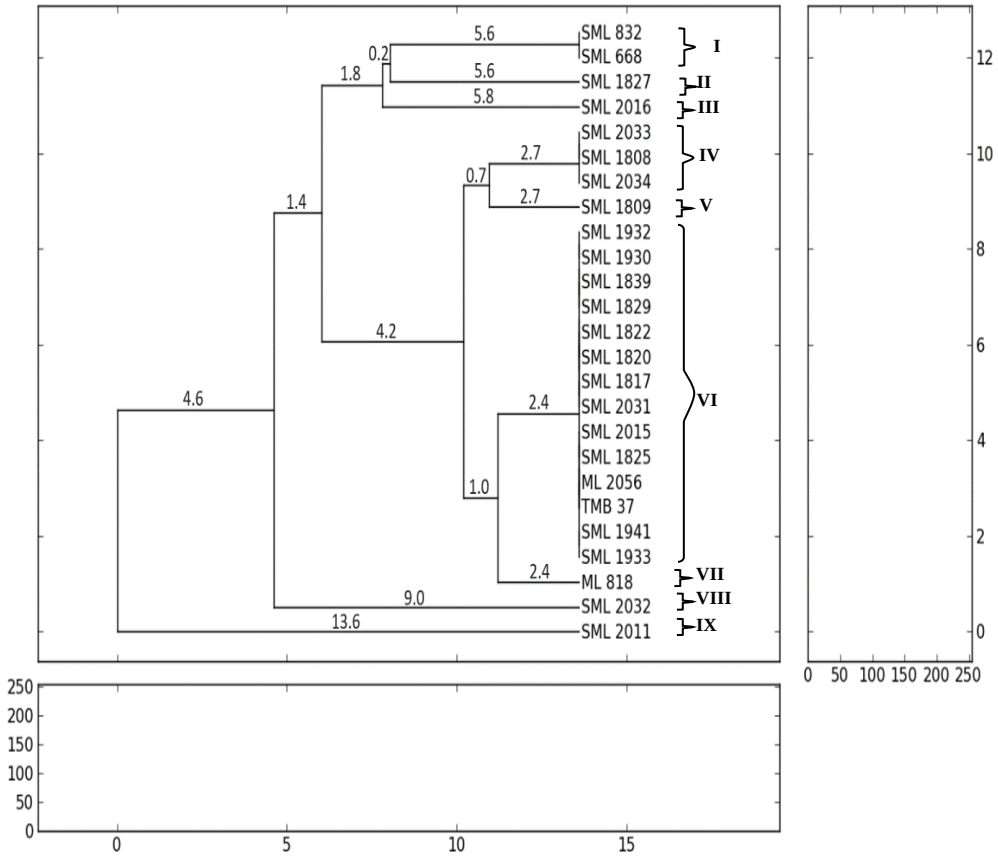


Figure 4. Dendrogram depicting mungbean genotypes grouped into different clusters based on SDS- PAGE profiling of mungbean proteins.

The clusters that were closer in distance showed greater similarity among each other

than those clusters which were distant to each other. In cluster I, the check varieties SML 668 and SML 832 showed similar protein profiles with 100% homology. SML 1827 in cluster II exhibited 59% homology with those of cluster I. SML 2016 (cluster III) exhibited 43% homology to clusters I and II. These two lines showed absence of protein subunits corresponding to molecular weight of 93, 130 and 160 kDa approximately. SML 2033, SML 1808 and SML 2034 formed cluster IV and exhibited 100% homology among each other while 44% homology with clusters I, II and III. SML 1809 (cluster V) exhibited 80% homology with 3MR lines from cluster IV while 44% homology with clusters I, II and III. There were 12 interspecific lines and 2 checks (TMB 37 and ML 2056) in cluster VI that exhibited 100% homology with each other. Cluster VI exhibited 75% homology with cluster IV and V while 82% homology with the check variety ML 818 (cluster VII). Cluster VIII (SML 2032) was found distant from cluster I to VII exhibiting a low homology of 34%. Among all the genotypes, SML 2011 (cluster IX) was found to be dissimilar and an outliner as it exhibited only 6 polypeptide bands on gel. The genetic diversity between MR lines and check varieties was larger than between MR and MU lines. Interspecific lines identified as outliners from these data showed higher contents of sulphur containing amino acids and tryptophan, the essential amino acids deficient in bean seeds. The results of cluster analysis identify the genetic diversity available among various mungbean genotypes and suggest that electrophoretic profiles of mungbean interspecific lines provide valuable information with potential of being used in mungbean genetic improvement. It also helps in understanding that closer the clusters are to each other, more comparable are their protein quality parameters. Thus, interspecific hybridization of mungbean genotypes in distant clusters could help in producing genetically diverse mungbean varieties.

Peptide identification using database search

Different protein databases (NCBI, Uniprot and EMBL) were searched for the reported mungbean proteins of various molecular weights and are assembled in Table 2 (Supplementary material). A total of 42 entries were found in the search (*Vigna radiata*, mungbean, seed proteins). In present studies, MR and MU interspecific lines consisted of major seed storage proteins viz; 2S (30kDa), 7S (28kDa) and 11S (24 kDa) globulins, 8S vicilin (32 kDa), 8S globulin α subunit, 7S vicilin and 16.5 S globulins. A similar search was carried out by HAMEED *et al.* (2012) who reported 17 entries for mungbean seed storage proteins and identified 15 of them in their work. Peptide identification helped in understanding the proteins and their subunits present in the newly developed interspecific lines whose protein information was not yet available. Thus, crossing the interspecific lines lacking a particular protein with those that contain the protein could improve the protein quality in upcoming mungbean lines.

CONCLUSION

SML 1827 (MR line) and SML 2033 (MU line) were rich in total soluble proteins, free amino acids, methionine, tryptophan, albumins and globulins. SML 1825 (MR line) exhibited the lowest content of albumins and glutelins and also exhibited low concentration of other protein quality parameters as compared to all the other mungbean genotypes investigated in the present study. The results of the present study depicted high genetic diversity among the tested mungbean genotypes based on electrophoresis gel and the dendrogram revealed that the

interspecific lines can be differentiated from the check varieties based on the protein profiles. Total soluble proteins, free amino acids, sulphur containing amino acids and protein profiling are important biochemical parameters to study the interspecific variation and genetic improvement of mungbean. Specific protein fractions can be changed through genetic alterations that can result into increased total protein and essential amino acid content in mungbean. On the basis of cluster analysis, crosses between genotypes from farthest clusters are recommended for breeding programmes. This study could provide valuable information to future conventional breeding programs for these newly developed interspecific mungbean lines whose protein quality parameters were not known. Further research on genotypes with similar banding pattern for agronomic, biochemical and technological parameters can be carried out for better management of gene bank.

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REFERENCES

ANISHA, G. and P., PREMA (2008): Reduction of non-digestible oligosaccharides in horse gram and green gram flours using crude α-galactosidase from *Streptomyces griseoloalbus*. Food Chem., *106*(3): 1175-1179.

DAHIYA, P.K., A.R., LINNEMANN, M.A.J.S., VAN BOEKEL, N., KHETARPAUL, R.B., GREWAL, M.J.R., NOUT (2015): Mung Bean: technological and nutritional potential. Critic Rev. Food Sci. Nutr., *55*(5): 670–688.

GANESAN, K. and B., XU (2017): A critical review on phytochemical profile and health promoting effects of mung bean (*Vigna radiata*). Food Sci. Human Wellness, *7*(1): 11–33.

GHAFOOR, A., Z., AHMAD, A.S., QURESHI, M., BASHIR (2002): Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE. Euphytica, *123*(3): 367-378.

GUPTA, N., N., SRIVASTAVA, S.S., BHAGYAWANT (2018): Vicilin - a major storage protein of mungbean exhibits antioxidative potential, antiproliferative effects and ace inhibitory activity. PLoS ONE, *13*(2): e0191265.

HAMEED, A., M., QURESHI, M., NAWAZ, N., IQBAL (2012): Comparative seed storage protein profiling of mungbean genotypes. Pak. J. Bot., *44*(6): 1993–1999.

JAIN, V. and S., SHARMA (2021) Mungbean x ricebean and mungbean x urdbean interspecific lines are nutritionally comparable to mungbean cultivars. J. Food Comp. Anal., *104* : 104171.

KAUR, M. and S., SHARMA (2021) Selenium affects protein accumulation and its distribution in different protein fractions in developing wheat grains. Cereal Res. Comm., 49: 109–117.

LAEMMLI, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, *227*(5259): 680-685.

LOWRY, O.H., N.J., ROSERBROUGH, A.L., FARR, R.J., RANDALL (1951): Protein measurement with folin phenol reagent. J. Biol. Chem., *193*: 265-275.

MALVIYA, N., S., NAYAK, D., YADAV (2008): Characterization of total salt soluble seed storage proteins of grain legumes using SDS-PAGE. Bull. de Ressources Phytogénétiques, *156:* 50-56.

MENDOZA, E.M.T., M., ADACHI, A.E.N. BERNARDO, S., UTSUMI (2001): Mungbean [*Vigna radiata* (L.) Wilczek] globulins: purification and characterization. J. Agric. Food Chem., *49*(3): 1552–1558.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (NCBI). Available at: www.ncbi.nlm.nih.gov. Jan 3, 2020.

PANDIYAN, M., N., SENTHIL, N., RAMAMOORTHI, A.R., MUTHIAH, V. DUNCAN, T. JAYARAJ (2010): Interspecific hybridization of *Vigna radiata* x 13 wild *Vigna* species for developing MYMV donar. Electronic J. Plant Breeding, *1*(4): 600–610.

PRICE, R.K. and R.W., WELCH (2013): Cereal grains. In Encyclopedia of Human Nutrition, 3rd edition. Academic Press, Waltham, Massachusetts, United States (pp. 307-316).

ROY, M., N., MANDAL, P.K., DAS (2001): Seed protein characterization and isozyme diversity for cultivar identification in grasspea (*Lathyrus sativus* L.). Ind. J. Genetics Plant Breeding, *61*(3): 246-249.

SARKAR, M. (2017): Breeding for high productive extra short duration germplasms of mung bean [*Vigna radiata* (L.) Wilczek] and their management for improved storability viability vigour and field performance. Ph.D. thesis, University of Calcutta, Kolkata, India.

SASHIKALA, V.B., Y.N., SREERAMA, V.M., PRATAPE, H.V., NARASIMHA (2015): Effect of thermal processing on protein solubility of green gram (*Phaseolus aureus*) legume cultivars. J. Food Sci. Technol., *52*(3): 1552–1560.

SHARMA, S., R., AHUJA, S., SINGH (2013): Effect of storage period and germination on hard seededness and biochemical constituents of seeds of some *Vigna* species. Indian J. Agric. Biochem., *26*(1): 102–106.

SHEVKANI, K., N., SINGH, A., KAUR, J.C., RANA (2015): Structural and functional characterization of kidney bean and field pea protein isolates: a comparative study. Food Hydrocolloids., *43*: 679-689.

SHI, Z., Y., YAO, Y., ZHU, G., REN (2016): Nutritional composition and antioxidant activity of twenty mungbean cultivars in China. Crop J., *4*(5): 398–406.

SINGH, S. and T.S., BAINS (2006): Wide hybridization in Vigna species. In: Ali M and Kumar S (eds) *Advances in Mungbean and Urdbean*. Kanpur, India: Indian Institute of Pulses Research, pp.169-86.

SINGH, S., P., KUMAR, A., TRIVEDI, A.K., KOSHAL (2015): Seed protein profiling through electrophoresis in mungbean [*V.radiata* (L.) Wilczek]. J. Plant Devt. Sci., *7*(4): 337-340.

SINGLA, P, S., SHARMA, S., SINGH (2017): Amino acid composition, protein fractions and electrophoretic analysis of seed storage proteins in lupins. Indian J. Agric. Biochem., *30:* 33-40.

SITAL, J.S., J.S., MALHOTRA, S., SHARMA, S., SINGH (2011): Comparative studies on biochemical components in mung bean [*Vigna radiata* (L.) Wilczek] varieties cultivated in summer and Kharif seasons. Indian J. Agric. Biochem., *24*(1): 68–72.

SKYLAS, D.J., C.L., BLANCHARD, K.J., QUAIL (2017): Variation in nutritional composition of Australian mungbean varieties. J. Agric. Sci., *9*(5): 45.

UNIPROT DATABASE. Available at: www.uniprot.org. Jan5, 2020.

VARMA, A., S.P., MISHRA, A., TRIPATHI, U.K., SHUKLA (2018): Biochemical composition and storage protein profiling of mungbean (*Vigna radiata* L. wilczek) cultivars. J. Pharmac. Phytochem., *7*(4): 708–713.

WEI, X., W.S., KIM, B., SONG, N.W., OEHRLE, S., LIU, H.B., KRISHNAN (2020) : Soybean mutants lacking abundant seed storage proteins are impaired in mobilization of storage reserves and germination. ACS Omega, 31(14): 8065-8075.

XU, X.P., H., LIU, L.H., TIAN, X.B., DONG, S.H., SHEN, L.Q., QU (2015): Integrated and comparative proteomics of high-oil and high-protein soybean seeds. Food Chem., *172*: 105-116.

YI-SHEN, Z, S., SHUAI, R., FITZGERALD (2018): Mungbean proteins and peptides: nutritional, functional and bioactive properties. Food Nutr. Res., *62*: 1–11.

PARAMETRI KVALITETA PROTEINA I PROFIL REZERVNIH PROTEINA KOD INTERSPECIFIČNIH LINIJA MUNGO PASULJA (*Vigna radiata* L. Wilczek)

V. JAIN i S. SHARMA*

Departman za biohemiju, Pendžab poljoprivredni Univerzitet, Ludhiana, 141004, Indija

Izvod

U ovom istraživanju prikazani su parametri kvaliteta proteina u interspecifičnim linijama iz ukrštanja mung pasulj x pirinač (MR) i mung pasulj x crni mungo pasulj (MU), u odnosu na standarde mung pasulja. Interspecifične linije su sadržale 138,4-230,3 mg/g ukupnih rastvorljivih proteina, 0,84-2,63 mg/g slobodnih amino kiselina, 3,21-7,10 mg/g metionina, 0,08-0,15 mg/g cisteina i 0,67-1,32 mg tritophana/g. Frakcije proteina su bile: 32,6-48,3 (albumini), 153,3-218,8 (globulini), 2,81-8,30 (prolamini) i 22,22-66,23 mg/g glutelini. Među MU linijama, prosečan sadržaj albumina je bio niži, a globulini, prolamini i glutelini su bili značajno viši nego kod standard mung pasulja. Globulini su pokazali pozitivnu korelaciju sa ukupnim rastvorljivim proteinima i triptofanom, dok su albumini imali negativnu korelaciju sa prolaminima i pozitivnu korelaciju sa metioninom. Visoka pozitivna korelacija zabeležena je između prolamina i glutelina, kao i prolamina i cisteina na nivou značajnosti od 1%. I prolamini i glutelini su pokazali negativnu korelaciju sa metioninom na $P<0,05$. Elektroforetskom analizom ukupnih proteina otkriveno je prisustvo 6-11 traka molekulske težine u rasponu od 13-162 kDa. Klaster analiza otkrila je 9 klastera sa 95% homologije sa SML 2011, najudaljenijom interspecifičnom linijom među svim genotipovima. Sve u svemu, SML 1827 i SML 2033 bili su bogati ukupnim rastvorljivim proteinima, slobodnim amino kiselinama, metioninom, triptofanom, albuminima i globulinima. Rezultati sugerišu da elektroforetski profili interspecifičnih linija mung pasulja pružaju vredne informacije sa potencijalom da se koriste u genetskom poboljšanju ove vrste.

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