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Research Article

An Assessment of Mungbean [*Vigna radiata* (L.) Wilczek] Genotypes Using Seed Storage Protein Profiles

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ABSTRACT

Twenty genotypes and 8 market samples of mungbean were evaluated on the basis of their seed storage protein (SSP) subunits profile determined by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE). Subunit bands in the range of 14kD to 80kD of molecular weight (MW) were recognized in their protein samples and appeared to show four distinct regions. Region I subunit bands were invariably monomorphic. Eight bands belonging to regions II (42kD-56kD), III (25kD-35kD) and IV (14-22 kD) were polymorphic. From binary data generated from polymorphic bands over the genotypes and market samples, Jaccard's similarity coefficients were computed using NTSYS-pc software and utilized for the dendrogram construction. The dendrogram segregated the 20 genotypes and 8 market samples into 4 clusters. Cluster I included all the genotypes (except RMG 1004) and two market samples. The remaining 6 market samples formed clusters II and III. Genotype RMG 1004 represented cluster IV with maximum divergence.

Keywords: *Mungbean, clustering, dendrogram, electrophoresis, seed storage proteins (SSP).*

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] belongs to the family Leguminosae. It is the third-most important food legume crop of India (Grover, 2011). It is also important for protein nutrition among a large number of people with vegetarian food habits in the country. Mungbean is rich in necessary amino acids particularly lysine, which is lacking in most of the cereal crops (Suresh et al., 2010). Besides its consumption as food in several forms, the green leaves serve as cattle feed. Further, being a nodulated legume, it can fix atmospheric dinitrogen, and hence also used as a green manuring crop. The state of Rajasthan, where it is grown as an important *kharif* pulse crop, stands first by sharing 50.28% of the area under mungbean cultivation and by contributing 48.86% of the production of the country (Kumari, 2024).

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However, its productivity is low (500 kg/ha) in the state, which may be attributed to the unavailability of high-yielding varieties befitting the climatic conditions of Rajasthan as one of the main reasons (Anon, 2011). To achieve improvement in seed yield potential and other traits, the availability of genetic diversity and trait variability within the crop species is most important. Mungbean, being self-pollinated, possesses limited variability (Khan et al., 2000). Therefore, diversity existing among accessions of the crop germplasm, as revealed by their morphogenetic evaluations and SSP profiles, may be documented and exploited in specific crop improvement projects. Numerous plant proteins and their components are encoded at multiple loci throughout the genome and show significant variability with respect to size and charge (Cooke, 1986). Thus, one of the easiest ways to describe heterogeneity in plant genetic resources and to identify cultivars is to study the polymorphism of seed storage proteins using electrophoresis (Ladizinsky & Hymowitz, 1979). In mungbean, using SDS-PAGE, Tripathy et al. (2018) have successfully shown genotypic-specific SSP profiles and also identified a protein band of 48 kD associated with cold-sensitive genotypes. Madhavi et al. (2022) have also reported genotype-specific SSP profiles in mungbean obtained through SDS-PAGE. Recently, the use of seed protein electrophoresis in plant genetics has been extensively reviewed by Pandey et al. (2025). Keeping these in view, the SSP profiles of a set of mungbean genotypes were determined in the present study using SDS-PAGE.

MATERIALS AND METHODS

Seed samples of 20 accessions (comprising both pipeline and released varieties) of mungbean were obtained from the Division of Genetics & Plant Breeding, RARI (SKNAU), Durgapura, Jaipur (Table 1). In addition, 8 samples of mungbean were also procured randomly from different grocery stores of the local market (*Kishanpole* market) of Jaipur. In this study, the genotypes were given alphabetic designations as A to T and market samples as U to Z and AA and BB (Table 1). Cotyledons were isolated from dried seed, ground into a fine powder, and used immediately for the extraction of proteins. For the extraction of proteins, 100 mg of the powder was ground in 1 ml of doubled distilled water in a clean pestle mortar, followed by subsequent grinding by adding 1ml of 1M NaCl to represent both water and salt-soluble proteins (Tripathy et al., 2010; Sharma, 2012; Sharma & Ram Krishna, 2017). The gel electrophoresis (SDS-PAGE) was carried out as described by Laemmli (1970). After transferring 1ml of protein samples into Eppendorf tubes, they were immediately centrifuged for 3 minutes at 10,000 rpm. The resulting supernatant (0.5 ml) was taken into a fresh tube and mixed with cracking buffer (0.2M Tris-HCl buffer, pH 6.8, 10% SDS, 20% glycerol, 10 mM β-Mercaptoethanol, 0.05% bromophenol blue). Immediately, the mixture was denatured at 80 °C (by placing it in a water bath) for 15 minutes. 30 µl of the protein samples were loaded into the wells of the gel and electrophoresed using an electrophoresis unit (BioRAD, Model: Protean II Xi Cell) at a power supply of 30mA DC. Standard marker proteins procured from SRL, Mumbai, India (Range 14 to 80 kD) were used for determining the molecular weights of the dissociated polypeptides. Shortly after staining, the presence (1) and absence (0) of each polymorphic protein subunit band over the 28 samples (*i.e.* genotypes and market samples) were counted on the gels to produce binary data, which was further evaluated using the NTSYS-PC programme [Numerical Taxonomy System, Version 2.1, (Rohlf, 2000)]. The Jaccard's coefficient was determined using the SIMQUAL sub-program. The formula for computing Jaccard's coefficient is $N_{AB}/(N_{AB}+N_A+N_B)$, where N_A and N_B stand for the number of bands in samples A and B, respectively, and N_{AB} is the number of bands present in both samples (Jaccard, 1908). The UPGMA (unweighted pair group method with arithmetic average) dendrogram was built utilizing the similarity matrices (obtained from the programme) to depict the similarity or diversity between the accessions/samples. The Winboot software was employed for bootstrap analysis with 2000 repeats to measure the statistical robustness of cluster branches (Yap & Nelson, 1996). The MW of the marker proteins and their Rf values were plotted on a semi-log graph to create a standard curve. The MW of an unknown protein was extrapolated using the standard plot.

RESULTS AND DISCUSSION

In this investigation, 20 different genotypes of mungbean that represented released varieties and pipeline genotypes suitable for cultivation in semi-arid regions of Rajasthan, and also 8 mungbean seed samples randomly collected from the local market, were used for SSP profiling. The market seed samples were obtained from a local grocery store without any query on their procurement history. However, while the major objective of this study was to examine the differences among the released or pipeline varieties used in this study on the basis of their SSP profiles, the market samples were included to examine their relatedness to these elite varieties, if any.

In pulse legumes, seed storage proteins predominantly represent (63-90%) water-soluble albumins and salt-soluble globulins in their cotyledons; therefore, their simultaneous extraction was done to obtain a relatively more comprehensive SSP profile of the accessions/samples by the procedure of Tripathy et al. (2010) with some modification described by Sharma (2012).

SDS-PAGE is a strong and reliable method for characterizing proteins (Laemmli, 1970). In essence, proteins extracted from the seed samples are broken down into polypeptides using β-mercaptoethanol and SDS. β-Mercaptoethanol breaks the disulfide bonds. In contrast, SDS binds to hydrophobic areas of proteins to disrupt secondary, tertiary, and quaternary structures. SDS also gives the resultant polypeptides a net negative charge. These charged polypeptides are sorted based on their masses during PAGE. Figures 1 and 2 reveal the separation of subunits from extracted protein samples through SDS-PAGE. With the help of marker lane, the molecular weights of sample protein subunit bands were identified. In total, there were 21 subunit bands designated as ‘a’ to ‘u’ (Fig.1). SSP profiling of the genotypes and market seed samples identified four distinct regions of the bands. Region I included 5 subunit bands having MW greater than 66 kD. Region II was darkly stained and relatively thick and revealed 5 subunits near 56 kD and 43 kD of MW. The third region included 3 darkly stained bands of 35 kD and 25 kD. In the fourth region, 8 lightly stained bands between 25- 14 kD were observed.

A perusal of SSP profiles of 20 genotypes and 8 market samples revealed that the subunit bands of region I were invariably monomorphic (designated as “a to e" in Figs. 1 and 2) because these were present in all the individuals, *i.e.* genotypes and market seed samples. A protein subunit band is called polymorphic if it is present in some individuals but not in others. The polymorphic bands belonged to regions II, III and IV. The 5 bands of region II were designated as f, g, h, i and j, whereas those of region III, comprising 3 bands, were given designations as k, l and m. The 8 bands of region IV were designated as n, o, p, q, r, s, t and u. The 8 protein subunit bands with designations g, i, l, o, q, r, s and t were polymorphic. With the help of binary data generated from the presence or absence of the polymorphic bands over the genotypes and market seed samples (Table 2), the Jaccard's similarity coefficient matrix was determined by the NTSYS-pc programme (data not shown). Among genotypes, the similarity coefficient ranged from 0.11 to 1.00 with a mean of 0.75. About 60% of the cases showed absolute similarity. Among the 20 genotypes and 8 market samples, the least genetic similarity (or maximum diversity) value was associated with genotype H only. It was also seen that 15 % of the pairs showed a similarity coefficient value of 0.5, and about 6% cases showed values of 0.33 and 0.17.

A dendrogram was constructed using similarity coefficients (Figure 3). The dendrogram revealed 4 distinct clusters. Genotype RMG 1004, designated as H, was distinctly branched (most diverse as Cluster IV) from the rest. All the genotypes (except H) and two market seed samples, namely, W and AA, were in Cluster I. Six of the remaining market seed samples represented two different clusters (Cluster II and Cluster III) that branched closely in the dendrogram.

The results of this study demonstrated that, on the basis of SSP profiles, all the genotypes (except RMG 1004) and two market seed samples (designated as AA and W) were placed in Cluster-1, hence should be considered similar. Out of the remaining 6 market samples, 4 samples formed Cluster II (designated as U, V, BB and Z) and the other 2 formed Cluster III (designated X and Y). Therefore, members of Cluster II and III are relatively diverse. In case of market seed samples, it may be expected because they might represent the produce from some other released elite varieties than those included in Cluster1 (it may also be from an old variety or even a local variety) or may be mixtures of produce of different varieties (which is readily possible in a grocery stock). The genotype H (RMG1004), however, was found to be the most divergent from all others.

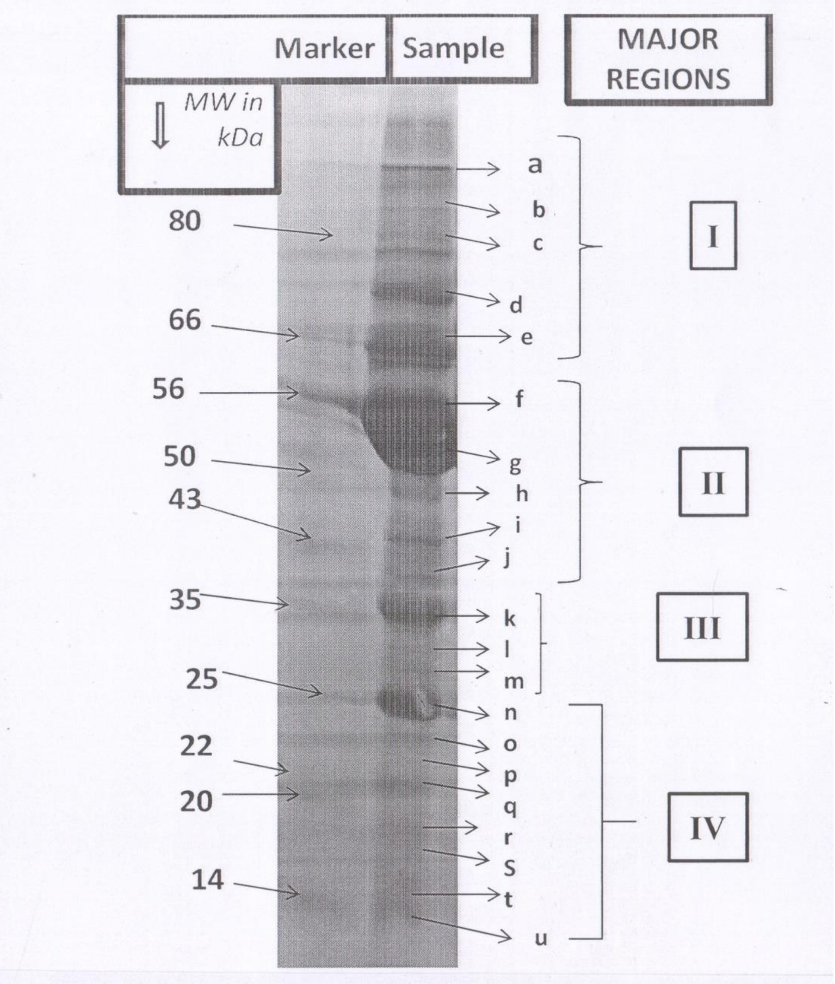

Figure1: Marker protein bands and the bands resolved in the sample during SDS-PAGE of seed storage protein in mungbean.

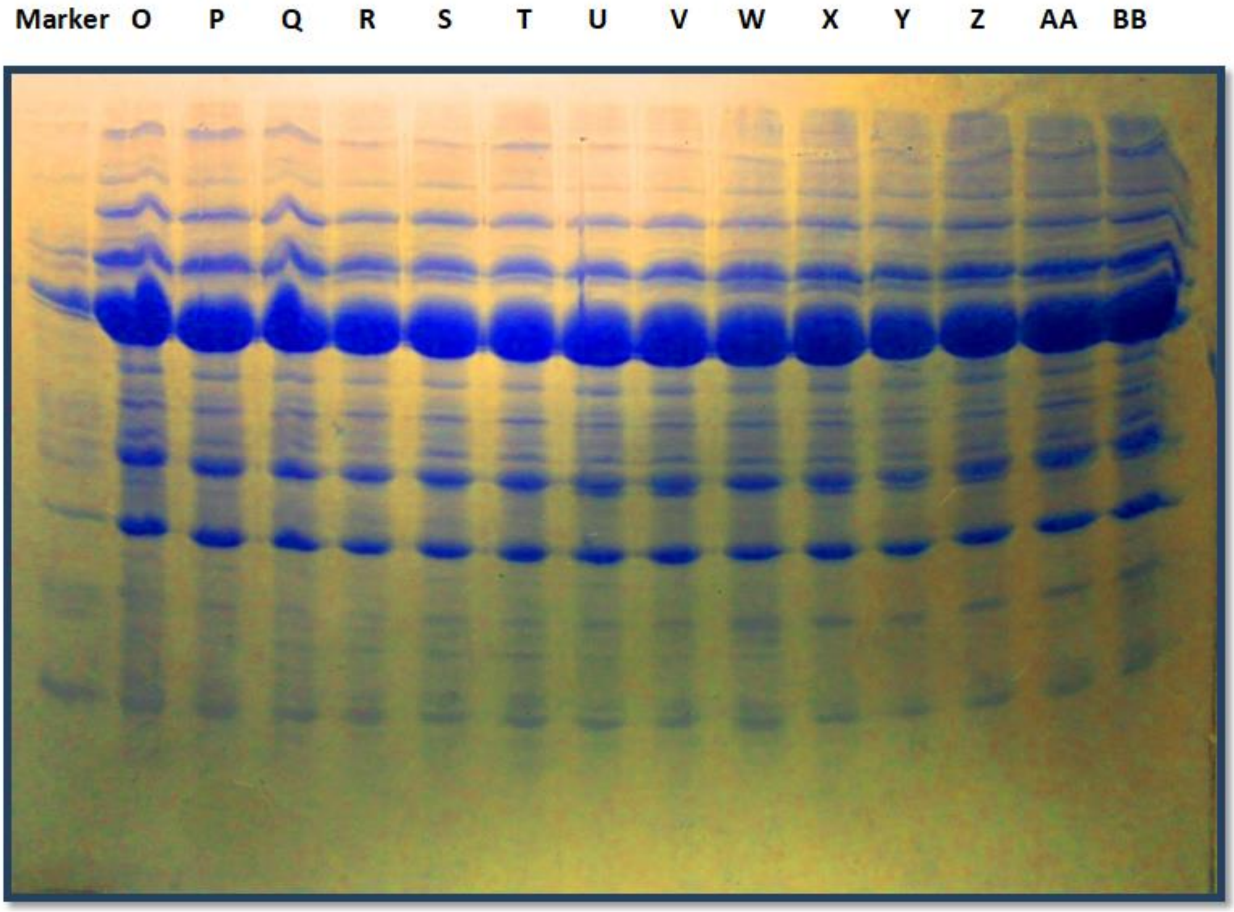

Figure 1: A representative view of the electrophoregram of different mungbean genotypes and market seed samples.

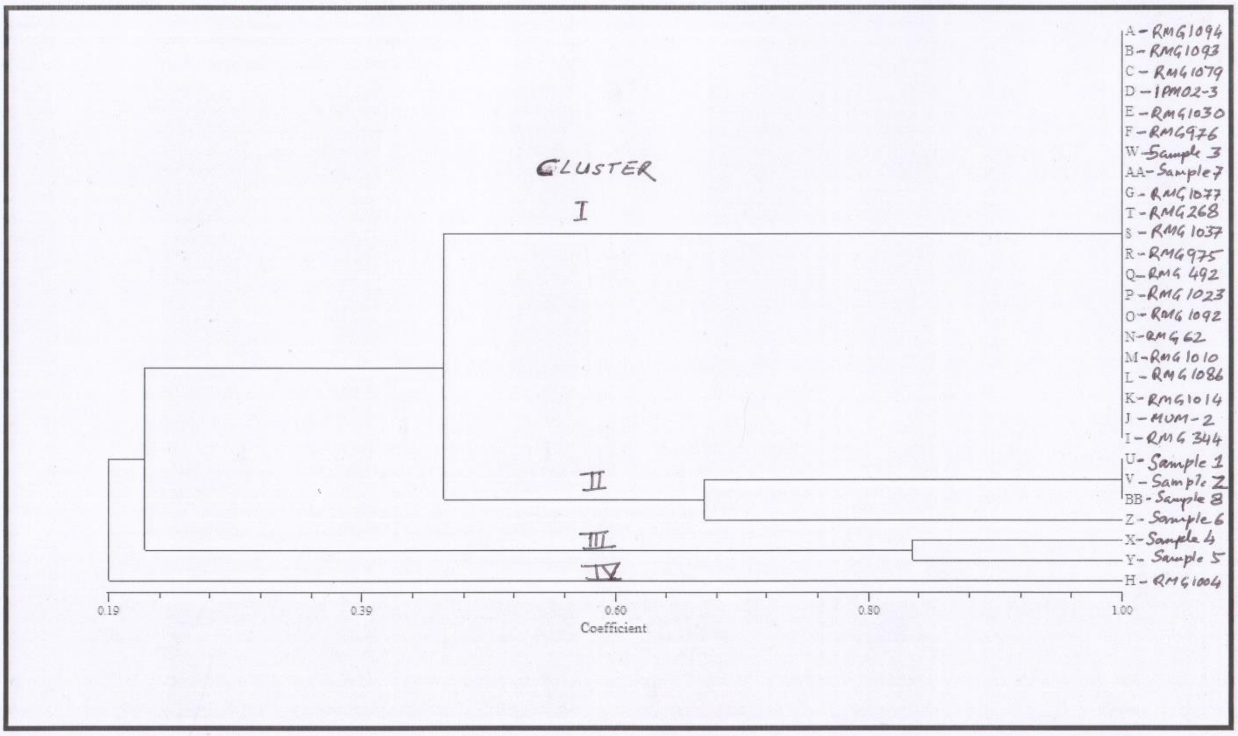

Figure 3: Dendrogram of 28 mungbean samples revealed by UPGMA cluster analysis based on genetic similarity estimates.

Table 1: List of mungbean genotypes/ market seed samples used in the study

SN	Genotype#	Designation	SN	Market seed Sample	Designation
1	RMG - 1094	A	1	Sample 1	U
2	RMG - 1093	B	2	Sample 2	V
3	RMG - 1079	C	3	Sample 3	W
4	**IPM - 02-3**	D	4	Sample 4	X
5	RMG - 1030	E	5	Sample 5	Y
6	RMG - 796	F	6	Sample 6	Z
7	RMG - 1077	G	7	Sample 7	AA
8	RMG - 1004	H	8	Sample 8	BB
9	**RMG - 344**	I			
10	**MUM- 2**	J			
11	RMG - 1014	K			
12	RMG - 1086	L			
13	RMG - 1010	M			
14	**RMG - 62**	N			
15	RMG - 1092	O			
16	RMG - 1023	P			
17	**RMG - 492**	Q			
18	**RMG - 975**	R			
19	RMG - 1037	S			
20	**RMG - 268**	T			
# Those shown in bold are released varieties. Designations are arbitrary and used for the present study only					

Table 2: Binary data obtained from polymorphic protein subunit bands during SDS-PAGE of seed storage proteins of mungbean genotypes and market seed samples

Protein region	Protein subunit band	Protein samples																											
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	BB
II	g	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
II	i	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
III	l	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1
IV	o	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0
IV	q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
IV	r	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
IV	s	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IV	t	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Dummy #	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1
# Since many samples scored only "0" at all the 8 band locations, "1" is added as a dummy band to all the samples to facilitate computation by the NTSYS-pc programme and to avoid numerical bias arising from this addition.																													

CONCLUSION

Based on the results of SSP profiles, it was concluded that;

(1) All the mungbean genotypes studied were similar to each other as they fell in one cluster (Cluster-1) except the RMG 1004.

(2) Genotype RMG1004 was the most diverse of the rest.

(3) Mungbean seed samples obtained from the local market seemed relatively diverse, as these formed 3 different clusters.

Since genotypes included in this study were elite mungbean varieties/promising genotypes suitable for cultivation in Rajasthan, it would have been desirable to have their frequent representation in the retail market, but in our study, 6 out of 8 market samples were from different clusters. A study on the movement of produce from various mungbean growers in Rajasthan may be meaningful as the state accounts for nearly half of the mungbean production in the country. Inclusion of SSP profile-assisted breeding may be helpful in widening the genetic base of the genotype being developed as a variety in the mungbean crop. An extensive cultivation of released varieties of Cluster 1 by the farmers of the state may be desirable.

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Author Contribution: Both authors have contributed equally to the conduct of experiments and the preparation of the final manuscript.

REFERENCES

Cooke, R. J. (1986). Gel electrophoresis: a role in agriculture. *Electrophoresis, 7,* 203-217.

Grover, D. K. (2011). Farm level estimates of harvest and post harvest mungbean losses in Punjab. *Journal of Food Legume, 24,* 225-229.

Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletin de la SociétéVaudoise des Sciences Naturelles, 44,* 223-270.

Khan, S., Mujeeb-Ur-Rehman, Mehraj-Ud-Din-Bhat, & Siddiqui, B. A. (2000). MMS induced biological damage and polygenic variability in green gram (*V. radiata* (L.) Wilczek). *Legume Research, 23,* 126- 129.

Kumari, N., & Malik, D. (2024). Growth and instability in mungbean production in India. *J. Food Legumes*, *37*(2), 224-231.

Ladizinsky, G., & Hymowitz, T. (1979). Seed protein electrophoresis in taxonomic and evolutionary studies. *Theoretical and Applied Genetics, 54,* 145-151.

Laemmli, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature, 227*, 680 – 685.

Madhavi, E., Rao, P. S., & Radhakrishna, K. V. (2022). Characterization and Identification of Mungbean *(Vigna radiata L.* Wilczek*)* Varieties using Chemical Tests and Gel Electrophoresis of Soluble Seed Proteins. *The Andhra Agric. J* 69 (*2*), 217-228.

Pandey, S., Tiwari, S., Bhattacharjee, P., Anand, A., Pazhamala, L. T., Yasin, J. K., & Yadav, R. K. (2025). Seed protein electrophoresis in plant genetics: Commemorating the pioneering contributions of Prof. Chittaranjan Kole and team to the foundation of plant proteomics. *Plant Genome, 18,* e70027. (https://doi.org/10.1002/tpg2.70027).

Rohlf, F. J. (2000). NTSYS-pc: Numerical Taxonomy System. V*er.2.1*. Exeter Software, Setauket, NY, USA, 29-34.

Sharma, D. B. (2012). Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] using protein profile. In: M.Sc.

(Ag.) Thesis submitted to S.K.R.A.U., Bikaner and Campus-Jobner.

Sharma, D. B., & Ram Krishna, K. (2017). Genetic Diversity in Cowpea [*Vigna unguiculata* (L.) Walp.] Accessions using Protein Profiling. *International Journal of Pure and Applied Bioscience, 5*(2), 491-496.

Suresh, S., Jebaraj, S., Juliet, H. S., & Theradimani, M. (2010). Genetic studies in mungbean [*Vigna radiata* (L). Wilczek], *Electronic Journal of Plant Breeding, 1*(6), 1480-1482.

Tripathy, S. K., Behera, S. K., Burnwal, P. K., Das, M., Chakma, B., & Dash, M. (2018). Study of Genetic Diversity and Identification of Soluble Protein Markers for Cold Tolerance in Mungbean. *Int. J. Curr. Microbiol. App. Sci*., *Special Issue-6*, 2086-2093.

Tripathy, S. K., Sardar, S. S., & Mishra, P. K. 2010. Analysis of seed storage protein pattern: a method for studying genetic variation and diversity among *Vigna* genotypes. *Indian. J. Genet., 70*(2), 140-144.

Yap, I. V., & Nelson, R. J. (1996). WinBoot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrogram. IRRI Disc. Ser. No. 14**:** *International Rice Research Institute,* Manila, Philippines.