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COMPARATIVE SEED STORAGE PROTEIN PROFILING OF MUNG BEAN GENOTYPES

AMJAD HAMEED[1]*, MEMUNA QURESHI[2], MUHAMMAD NAWAZ[2] AND NAYYER IQBAL[1]

[1]*Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Faisalabad, Pakistan.*
[2]*Department of Bioinformatics and Biotechnology, GC University, Faisalabad, Pakistan.*
**Corresponding author e-mail: amjad46pk@yahoo.com*

Abstract

Seed storage protein profiling of mung bean genotypes was performed by SDS-PAGE. Total soluble seed proteins from mung bean seeds were resolved on 12% gels in 22 protein polypeptide bands with molecular weights ranging from 16 to 103 kDa. Very low level of variability was observed in tested genotypes. Dendrogram based on electrophoretic data grouped the 13 genotypes into three clusters at 93% homology. A total of 5 genotypes (NM 13-1, NM 2006, NM 92, Chakwal 97 and Mung 88) were grouped together in the cluster-1. The 2nd cluster consisted of one genotype i.e. AZRI 2006 which was most divergent in the tested germplasm. Seed storage peptide i.e., MSSP-19 differentiated the genotype, AZRI 2006 from rest of tested genotypes and thus can be used for its identification. Remaining seven genotypes grouped together in the 3rd cluster. Based on seed storage protein marker i.e. MSSP-82, NM 2006, can also be differentiated from one of its parent genotype i.e., NM 92. Uniprot and NCBI protein databases were searched for already reported and characterized seed storage proteins in mung bean. Based on information retrieved from the databases and search engines, the resolved protein peptides mainly belong to 8S and 7S vicilin, 2S and 11S globulin and 2S albumin. In conclusion, electrophoresis (SDS-PAGE) of seed storage proteins provided an economic way to assess genetic variation in mung bean germplasm.

Introduction

Mungbean is one of the important kharif pulses of Pakistan. Mungbean improvement in Pakistan was mainly confined to the collection and evaluation of local and exotic germplasm before 1970s. There was only one mungbean variety VAR 6601 which was under cultivation at that time (Bashir *et al.,* 1988). Thereafter, five mungbean cultivars, viz., NM 28, NM 20-21, NM 121-25, NM 13-1 and M 19-19 were developed through mutation breeding at Nuclear Institute of Agriculture and Biology (NIAB) and released in 1980s.

Biochemical fingerprinting to characterize the germplasm is considered as a reliable tool of intellectual property protection of crop varieties and germplasm. Genetic markers are used for rapid identification and protection of crop varieties. Electrophoresis of seed proteins in barley and wheat and of isozymes in maize, soybean and sunflower have been used as additional characters for establishing distinctness of varieties (Bhat *et al.,* 2001). Among the biochemical techniques, sodium dodycyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used technique due to its validity and simplicity for describing genetic structure of plant germplasm. Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of several plants (Pervaiz *et al*., 2011; Shah *et al*., 2011; Emre, 2011). Analysis of seed protein can also provide a better understanding of genetic affinity of the germplasm (Shah *et al*., 2011). This method can also be used as a promising tool for differentiating the cultivars of a particular plant species. SDS-PAGE is also considered to be a practical and reliable method for species identification because seed storage proteins are largely independent of environmental fluctuation. Other important aspect of investigation in polyacrylamide gel electrophoresis (PAGE) is to establish genetic similarity index based on bands. Analysis of seed storage proteins profiles by SDS-PAGE has revealed that variation in seed proteins of both single and composite samples of *Vicia faba* with the molecular weights greater than 45kDa and electrophoretic pattern of the bulk extracted sample were characteristic of each cultivar, therefore it was recommended to be used for the cultivar identification (Sammour *et al.,* 1992). Genetic diversity of seed storage proteins has been reported for Lima bean (Lioi *et al.,* 1999), *Phaseolus vulgaris* (Ferreira *et al.,* 2000) and chickpea (Ghafoor *et al.,* 2003).

Electrophoretic markers provide an indirect method for genome probing. Polyacrylamide gel techniques allow us to: 1) identify variation among the taxa of each species; 2) screen the purity of the ever expanding number of cultivars; 3) verify whether or not two or more morphologically identical accession in the collection were also electrophoretically identical; and 4) exploit the important traits of landraces and wild relatives for increasing crop production and stabilizing yield (Sammour *et al.,* 1991). Polymorphism in seed storage proteins has been associated with geographical origin (Ghafoor *et al.,* 2002). Seed storage protein analysis represents a valid alternative and/or improved approach to varietals identification. Diverse genotypes of *Vigna mungo* and *V. radiata* resembling to *V. mungo* for seed characters have been studied to determine the extent of genetic variation based on morphological characters and SDS-PAGE, specific bands were suggested to be used for identifying *Vigna radiata* from mixed germplasm with *Vigna mungo*. The SDS-PAGE proved to be a powerful tool for differentiating *Vigna radiata* and *Vigna mungo*; whereas a low level inter-specific genetic diversity was observed and no clear differentiation was observed both for agronomic characteristics and for geographical origin (Ghafoor *et al.,* 2002).

The present study was conducted with objectives 1) seed storage protein profiling of mung bean genotypes using SDS-PAGE. 2) To assess genetic diversity in mungbean germplasm based on electrophoretic data and 3) possible identification of resolved mungbean seed storage proteins using Uniprot and NCBI protein databases.

Materials and Methods

Thirteen mungbean varieties released since 1983 were used for seed storage protein profiling. Details about these genotypes are given in Table 1.

Seed protein extraction: For extraction of soluble proteins, seeds were grounded in 50 mM phosphate buffer (pH 7.8) and centrifuged in micro-centrifuge machine (Sigma 1-14) for 10 min at 14,000rpm. The supernatant was separated and used for protein profiling. Protein concentration of extracts was measured by dye binding assay as described by Bradford (1976). Supernatant was mixed (4:1) with cracking solution (10 ml containing 1g SDS, 0.01g bromphenol blue, 2ml Mercaptoethanol, 1.5ml 0.5M tris, pH 6.8, 5g sucrose and 6.5 ml water) on vortex mixer and heated in a boiling water bath for five minutes to denature the proteins.

Seed protein profiling: Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970). Equal quantities of samples along with protein molecular weight marker (108, 78.6, 50.6, 35.9, 27.1, 19.2 kDa bands) were loaded into 10% gels. Electrophoresis was performed at constant voltage (100 volts). At end of electrophoresis, gels were fixed in solution containing 10% Acetic acid and 40% Ethanol for 15 min., with constant agitation on a shaker. After fixing gel was washed with distilled water for 15 min., with changing the water after every 5 min. Gels were then stained with coomassie blue G-250 dye and Destaining in water overnight.

Gel documentation and analysis: Finally gels were photographed using UVIpro platinum gel documentation system (UVItec UK). Computerized gel analysis was performed using UVI pro Platinum 1.1 Version 12.9 for windows (copyright® 2004-2006). Cluster analysis was performed using software UVI BANDMAP version 11.3 by UVItec UK.

Peptide search in databases: Uniprot database (www.uniprot.org) and National Center for Biotechnology Information protein database (www.ncbi.nlm.nih.gov) were searched for the mungbean seed storage protein by using different queries, with their molecular weights that have been reported earlier in order to identify the peptides which were found in this study.

Table 1. Mung bean varieties used along with year of approval, parentage and some salient characteristics.

Sr #	Name of variety	Year of approval	Parentage radiation dose	Salient Characteristics			
				Crop duration (days)	Reaction of MYMV	1000 seed wt (g)	Grain yield kgh^{-1}
1.	NM 28	1983	PK-17,20KR	75	MOD.TOL	30	1050
2.	NM 13-1	1986	VR-6601,10KR	65	Resistant	33	1150
3.	NM 19-19	1986	PK-22,40KR	70	Resistant	31	1300
4.	NM 20-21	1986	PK-22,40KR	65	Resistant	31	1250
5.	NM 121-25	1986	RC71-27,20KR	75	Tolerant	32	1200
6.	NM 51	1990	VR-6601X VC1973A,10KR	75	Resistant	45	1550
7.	NM 54	1990	VR-6601X VC-1973A,10KR	70	Tolerant	54	1500
8.	NM 92	1996	NM-36X VC-27688	65	Resistant	52	1700
9.	NM 98	1998	NM-20-21X VC-1482E	75	Resistant	38	1750
10.	NM 2006	2006	VC15600X NM-92	75	Resistant	59	1900
11.	AZRI 2006	2006	LNo.1 X NM-96		Tolerant		1800
12.	Chakwal 97	2000			Resistant		1500
13.	Mung 88	1990	RARI Bahawalpur				

Results

In the present study, seed storage proteins were resolved into 20-22 detectable peptides using SDS-PAGE. Molecular weights of the resolved peptides ranged from 103 KDa to 16 KDa (Table 2). These proteins were indicated as Mung Seed Storage Proteins (MSSP) followed by their molecular weights.

Seed storage protein profiles of mung bean genotypes along with the dendrogram are presented in Fig. 1. Banding patterns of genotypes included in the study are presented as a histogram in Fig. 2. Dendrogram based on electrophoretic data grouped the 13 genotypes into three clusters at 93% homology (Fig. 1). Present results depicted a low genetic diversity in the tested germplasm based on seed storage proteins. Among the tested genotypes AZRI 2006 was most divergent as compared to others. Number and names of genotypes in different clusters are presented in the Table 3. A total of 5 genotypes were grouped together in the cluster-1. Among these five genotypes, NM 13-1 and NM 2006 showed similar protein profiles. Other three genotypes, NM 92, Chakwal 97 and Mung 88 were also similar in seed protein profiles when compared with each other. These two subsets of genotypes in this cluster showed 97% homology with each other.

Table 2. Molecular weights of resolved peptides indicated as mung bean seed storage proteins (MSSP) followed by their molecular weight (kDa).

Marker	NM 13-1	NM 2006	NM 92	NM 98	NM 28	NM 121-25	NM 20-21	NM 54	NM 51	NM 19-19	AZRI 2006	Chakwal 97	Mung 88
MSSP-103	103	103	103	----	----	----	----	----	----	----	103	103	103
MSSP-101	----	----	----	101	101	101	101	101	101	101	----	----	----
MSP-97	97	97	97	97	97	97	97	97	97	97	97	97	97
MSSP-91	91	91	91	91	91	91	91	91	91	91	91	91	91
MSSP-82	----	----	82	82	----	82	82	82	82	----	82	82	82
MSSP-79	79	79	79	79	79	79	79	79	79	79	79	79	79
MSSP-74	74	74	74	74	74	74	74	74	74	74	74	74	74
MSSP-67	67	67	67	67	67	67	67	67	67	67	67	67	67
MSSP-56	56	56	56	56	56	56	56	56	56	56	56	56	56
MSSP-49	49	49	49	49	49	49	49	49	49	49	49	49	49
MSSP-40	40	40	40	40	40	40	40	40	40	40	40	40	40
MSSP-35	35	35	35	35	35	35	35	35	35	35	35	35	35
MSSP-32	32	32	32	32	32	32	32	32	32	32	32	32	32
MSSP-30	30	30	30	30	30	30	30	30	30	30	30	30	30
MSSP-28	28	28	28	28	28	28	28	28	28	28	28	28	28
MSSP-24	24	24	24	24	24	24	24	24	24	24	24	24	24
MSSP-22	22	22	22	22	22	22	22	22	22	22	22	22	22
MSSP-21	21	21	21	21	21	21	21	21	21	21	21	21	21
MSSP-19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9
MSSP-19	19	19	19	19	19	19	19	19	19	19	----	19	19
MSSP-17	17	17	17	17	17	17	17	17	17	17	17	17	17
MSSP-16	16	16	16	16	16	16	16	16	16	16	16	16	16

Note: ---- Indicates absence of peptide

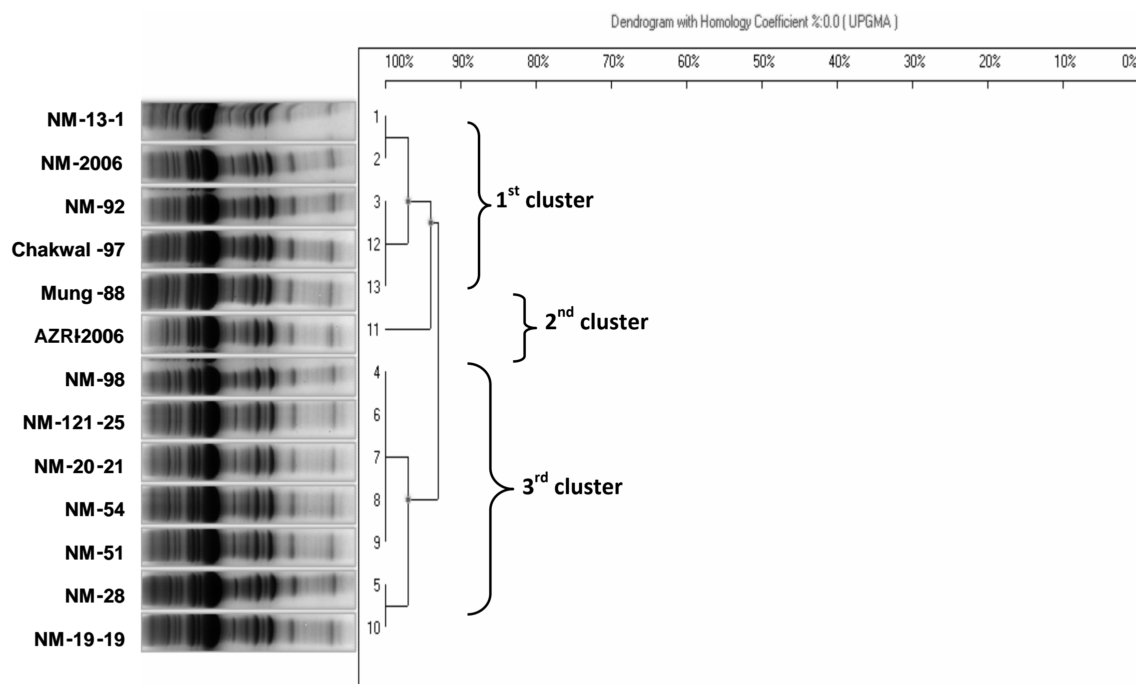

Fig. 1. Mung bean genotypes grouped into different clusters based on polymorphism in Seed Storage Proteins.

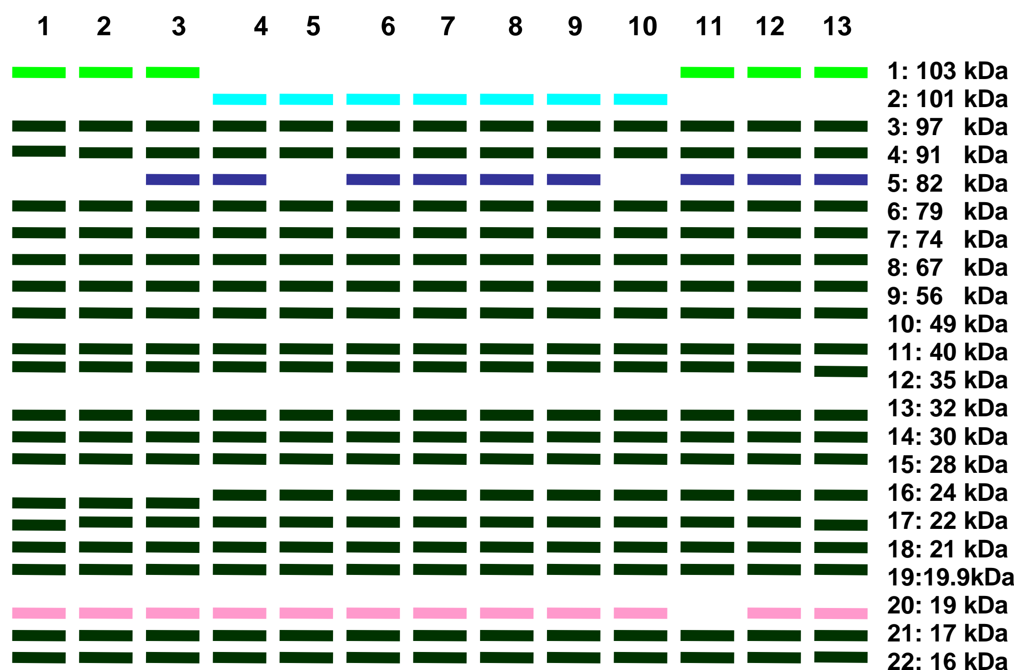

Fig. 2. Histogram of mung bean genotypes based on identified protein markers.

Table 3. Distribution of mung bean genotypes in three clusters based on electrophoretic data.

Cluster	Number of genotypes	Names of genotypes
I	5	NM 13-1, NM 2006, NM92, Chakwal-97 and mung 88
II	1	AZRI-2006
III	7	NM 121-25, NM 20-21, NM 54, NM 51, NM 28, NM 19-19, NM 98

The 2nd cluster consisted of one genotype AZRI 2006 which was most divergent in the tested germplasm. This genotype showed 94% homology with genotypes of the 1st cluster. Remaining seven genotypes grouped together in the 3rd cluster. Within 3rd cluster, five genotypes i.e. NM 98, NM 121-25, NM 20-21, NM 54 and NM 51 showed 100% homology in seed storage proteins. Other two genotypes NM 28 and NM 19-19 were grouped together in the 3rd cluster showing similar protein profiles. These two subsets of genotypes in this 3rd cluster were 97% homologous with each other. Genotypes grouped in 3rd cluster showed 93% homology with the other two clusters.

In seed storage protein profiles of seven genotypes NM 98, NM 28, NM 121-25, NM 20-21, NM 54, NM 51 and NM 19-19 a protein peptide of 101 kDa (MSSP-101) was present instead of a peptide of 103 kDa (MSSP-103) present in the other genotypes (Table 2). This protein peptide thus differentiates these seven genotypes from other genotypes. Moreover, genotype NM 20-21 and its mutant NM 98 share this protein (MSSP-101) providing evidence for same genetic background. Among these seven genotypes, two genotypes NM 28 and NM 19-19 can be differentiated from other genotypes due to the absence of 82 kDa (MSSP-82) protein peptide. However, no protein peptide further differentiated these two genotypes i.e. NM 28 and NM 19-19 from each other. Similarly, among remaining six genotypes that were differentiated from other seven genotypes by the presence of peptide MSSP-103, two genotypes can be further discriminated. A protein peptide MSSP-82 was absent in 2 genotypes viz., NM 2006 and NM 13-1 and thus differentiated them from other four genotypes. However, no protein peptide further discriminated between these two genotypes. One important point to be mentioned here is that this protein peptide i.e., MSSP-82 absent in NM 2006 was present in its parent NM 92. Therefore, based on this seed storage protein marker i.e., MSSP-82, NM 2006, a hybrid can be differentiated from its parent genotype NM 92.

Interestingly, a protein peptide of 19kDa was specifically absent in a genotype i.e., AZRI-2006 while it was present in all other genotypes. This seed storage peptide (MSSP-19) differentiated the genotype AZRI-2006 from rest of tested genotypes and thus can be used for its identification.

Peptide identification using database search and available literature: Resolved mung bean seed storage proteins were searched in different protein databases [NCBI (www.ncbi.nlm.nih.gov/) and Uniprot (www.uniprot.org/)] and reported literature for known peptides with similar molecular weights. A total of 17 entries were found for the search items (mungbean, V*igna radiate,* seed storage proteins). The results of databases search are presented in Table 4.

Table 4. Searched peptides with known molecular weights.

Sr #	Acc#	Protein name	Mol. wt	Mol. function	Reference
1.	Q43680	Mung bean seed albumin	30.233kDa	unknown	Liu *et al*., (1993)
2.	Q8W435	Peptidyl-prolyl cis-trans isomerase	18.207kDa	peptidyl-prolyl cis-trans isomerase activity	Kaga *et al*., (1998)
3.	A8WEL5	MIPS	56.503KDa	Binding activity	Wongkaew *et al*., (2009)
4.	Q8W436	BPng110	23.060kDa	unknown	Kaga *et al*., (1998)
5.	Q8W437	PBng143	25.277kDa	unknown	Kaga *et al*., (1998)
7.	Q01390	Sucrose synthase SS1	91KDa	Sucrose synthase activity	Arai *et al.,* (1992)
8.	Q01912	1-aminocyclopropane-1-carboxylate synthase	41.477kDa	1-aminocyclopropane-1-carboxylate synthase activity	Botella *et al*., (1992)
9.	B1NPN8	8S globulin alpha subunit	51.942kDa	nutrient reservoir activity	Ding *et al*., (2008)
10.	P32294	AUX22B	22.050kDa	protein dimerization activity	Yamamoto *et al*., (1992)
11.	O24541	AUX22C	21.273kDa	protein dimerization activity	Hashimoto *et al*., (1997)
12.	O24543	AUX22E	22.695kDa	protein dimerization activity	Hashimoto *et al*., (1997)
13.	P37116	NADPH--cytochrome P450 reductase	76.506kDa	FMN binding	Shet *et al*., (1993)
14.	P21616	Pyrophosphate-energized vacuolar membrane proton pump	79.980kDa	Inorganic diphosphatase activity	Hung *et al*., (1995)
15.	Q4KXC5	Starch branching enzyme	84.333kDa	1,4-alpha-glucan branching enzyme activity	Shih Y*et al*., (2004)
16.	P37115	Trans-cinnamate 4-monooxygenase	57.888kDa	electron carrier activity	Mizutani *et al*., (1993)
17.	O22552	V-type proton ATPase 16 kDa proteolipid subunit	16.528kDa	hydrogen ion transmembrane transporter activity	Hung *et al*., (1997)

Table 5. Identification of peptides in database.

Sr#	Known protein marker	Characterized in literature with known family	Reference
1.	MSSP-103		
2.	MSSP-101		
3.	MSSP-97		
4.	MSSP-91		
5.	MSSP-82		
6.	MSSP-79	79kDa (α-vignin of 16.5S Globulins)	Freitas *et al*., (2004)
7.	MSSP-74	16.5S Globulins	Freitas *et al*., (2004)
8.	MSSP-67	(7S vicilin)	Krishna & Bhati *et al*., (1985)
9.	MSSP-56	56kDa (7S vicilin)	
10.	MSSP-49	(7S vicilin)	
11.	MSSP- 40	(7S vicilin)	
12.	MSSP-35		
13.	MSSP-32	8S vicilin	Gangwar *et al*., (2009); Mendoza *et al*., (2001)
14.	MSSP-30	30kDa (2S albumin) 16.5S globulin	Freitas *et al*., (2004); Tripathy *et al*., (2010)
15.	MSSP-28	28kDa 7S globulins	Mendoza *et al*., (2001)
16.	MSSP-24	24kDa 11S globulin	Mendoza *et al*., (2001)
17.	MSSP-22	22kDa(16.5S globulin)	
18.	MSSP-21	21kDa	
19.	MSSP-19	19kDa	
20.	MSSP-19.9		
21.	MSSP-17	11S globulins	Malviya *et al.,* (2008); Gangwar *et al*., (2009)
22.	MSSP-16	7S globulins	Mendoza *et al*., (2001)

According to available literature accessed by different search engines (Google scholar, Pubmed, Pubmed central), mungbean seed storage proteins mainly consists of globulin proteins, legumins and vicilins. Based on available information in the literature we were able to identify several seed storage proteins those we resolved by SDS-PAGE. Information about the identified peptides based on similarity in their approximate molecular weights with previously reported peptides in the mung bean seeds is presented in Table 5.

Discussion

Profiling of seed storage proteins based on SDS-PAGE can be employed for various purposes, such as varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species, generating pertinent information to complement evaluation and passport data (Sammour, 1991; Malviya *et al.,* 2008). In mungbeans, SDS-PAGE cannot be used to identify genotypes on the basis of intraspecific variation because similar banding patterns were observed in some accessions that differed on the basis of characterization and evaluation; therefore, this technique might be more suitable to identify inter rather than intraspecific variation in *Vigna* spp., (Ghafoor *et al.,* 2002). Present results also depicted a low genetic diversity in the tested germplasm based on electrophoresis of seed storage proteins as dendrogram based on electrophoretic data grouped the tested genotypes into three clusters at 93% homology.

Seed protein electrophoresis technique is a reliable yet, relatively inexpensive way of developing genetic markers for the identification and genetic analysis of several important agriculture commodities (Bushuk & Zillman, 1978; Wrigley *et al.,* 1982; Kreis *et al.,* 1983; Ferguson & Grabe, 1986). Electrophoresis (SDS-PAGE) of seed storage proteins can be economically used to assess genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes (Hameed *et al.,* 2009). Present results showed that one mungbean hybrid (NM 2006) can be differentiated from one of parents (NM 92) on the basis of seed storage protein profiles. Furthermore; one genotype i.e. AZRI-2006 can be differentiated from rest of tested germplasm. Therefore, SDS-PAGE can be an economic method of choice for varietal identification and mutant differentiation in mungbean.

The seed storage proteins are nonenzymatic and have the sole purpose of providing proteins (nitrogen and sulphur source) required during germination and establishment of a new plant. Legumes seed storage proteins are mostly (7-11S globulins), which tend to be deficient in sulphur containing amino acids. Uppal & Matta (1996) working with black gram or urad bean (*Vigna mungo*) and Krishna & Bhatia (1985) with pigeon pea (*Cajanus cajan*) found the major globulin fraction as 7S vicilin, a trimeric protein composed of one large (70–72 kDa) and two small (40–57 kDa) subunits not linked by disulphide bridges. In the present study, proteins in this molecular weight range seem to be the small and large subunits of 7S vicilin. Mungbean seed storage proteins can be characterized on the basis of physiochemical and structural properties on seed protein fractions into 8S and 11S globulins (Tang *et al.,* 2010). Seed storage protein in mung bean (*Vigna radiata*) can be classified on the bases of Vicilin type (8S) and basic 7S globulins and legumin type (11S) globulins. Malviya *et al.,* (2008) identified 11S and 2S globulins as seed storage protein having molecular weights of 17 kDa, and 14 kDa in green gram. In the present study, we also detected a peptide of 17 kDa that can be a globulin subunit. Previously, Mendoza *et al.,* (2001) isolated protein fractions from mungbean [*Vigna radiata* (L.) Wilczek] that were globulin types with 360 kDa, legumin with 200 kDa and vicilin with 135kDa for basic 7S subunit on native gel. While SDS-PAGE revealed that 11S was composed of two bands of 40 kDa and 24 kDa, 8S was composed of 60 kDa, 48 kDa, 32 kDa, and 26 kDa bands, and basic 7S was composed of 28 kDa and 16 kDa bands. In the present study, peptides with molecular weights 28 kDa and 16 kDa were also detected on SDS-PAGE that may be 7S subunit, while peptide with molecular weight 24kDa may be 11S globulin and 32 kDa peptide can be 8S vicilin subunit according to earlier report.

In conclusion, SDS-PAGE profiling of seed storage proteins proved to be an economical and simple technique for analysis of genetic variation in mungbean germplasm. Variability in seed proteins was mainly in basic globulins, vicilin and legumin. Narrow genetic variability in mungbean germplasm based on seed storage proteins pointed towards the need to exploit the large germplasm collection with diverse morphoagronomic traits.

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