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GENOTYPIC VARIABILITY AND MUTANT IDENTIFICATION IN *CICER ARIETINUM* L. BY SEED STORAGE PROTEIN PROFILING

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Abstract

A collection of thirty-four chickpea genotypes, including five kabuli and twenty-nine desi, were analyzed by SDS-PAGE for seed storage protein profiling. Total soluble seed proteins were resolved on 12% gels. A low level of variability was observed in desi as compared to kabuli genotypes. Dendrogram based on electrophoretic data clustered the thirty-four genotypes in four major groups. As large number of desi genotypes illustrated identical profiles, therefore could not be differentiated on the basis of seed storage protein profiles. One kabuli genotype ILC-195 found to be the most divergent showing 86% similarity with all other genotypes. ILC-195 can be distinguished from its mutant i.e., CM-2000 and other kabuli genotypes on the basis of three peptides i.e. SSP-66, SSP-43 and SSP-39. Some proteins peptides were found to be genotype specific like SSP-26 for ICCV-92311. Uniprot and NCBI protein databases were searched for already reported and characterized seed storage proteins in chickpea. Among 33 observed peptides, only six seed storages proteins from chickpea source were available in databases. On the basis of molecular weight similarity, identified peptides were SSP-64 as Serine/Threonine dehydratase, SSP-56 as Alpha-amylase inhibitor, SSP-50 as Provicillin, SSP-39 as seed imbibition protein, SSP-35 as Isoflavane reductase and SSP-19 as lipid transport protein. Highest variability was observed in vicillin subunits and β subunits of legumins and its polymorphic forms. In conclusion, seed storage profiling can be economically used to asses the genetic variation, phylogenetic relationship and as markers to differentiate mutants from their parents.

Introduction

In worldwide food legume crop production, chickpea (*C. arietinum* L.) ranks third. However, cultivated chickpea has narrow genetic diversity with a high level of morphological variation (Shah *et al*., 2011; 2012). Non availability of sequenced genome of chickpea and very limited EST resources are two hindrances in understanding the actual genetic architecture of this plant (Jain & Chattopadhyay 2010). Use of seed storage proteins as markers is well established and has been used to check 1) genome relationship 2) domestication of plant with respect to conservation of genetic resources and 3) genetic diversity analysis within and between species (Ghafoor *et al.,* 2002). Protein profiling is an essential part of proteomics and requires new technologies that accurately, reproducibly, and comprehensively identify and quantify the proteins contained in biological samples (Smolka *et al.,* 2002). Seed protein profiles and other genetic markers have been very effectively and widely utilized for determining the evolutionary and taxonomic aspects in several crop plants (Khan, 1990; Murphy *et al.,* 1990; Das & Mukarjee, 1995; Ghafoor *et al.,* 2002).

The most undemanding, economical and widely applied biochemical technique for investigation of genetic makeup of germplasm is sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Because seed proteins are not influenced by environmental changes, their profiling using SDS-PAGE is considered as a reliable tool for economical germplasm characterization (Javid *et al.,* 2004; Iqbal *et al.,* 2005; Hameed *et al.,* 2009).

Seed protein profiling abet for characterization and analysis of available diversity in crop cultivars, varieties, wild relatives and phylogenetic relationships (Nisar *et al.,* 2007). Seed storage proteins based genetic diversity has been reported for *Phaseolus vulgaris* (Freitas *et al.,* 2000), lima bean (Lioi *et al.,* 1999), and *C. arietinum* (Ghafoor *et al.,* 2003; Hameed *et al.,* 2009). Studies based on seed protein profiling by SDS-PAGE for establishing phylogenetic relationship among *Cicer* species have suggested *Cicer reticulatum* as wild progenitor of cultivated chickpea (Ahmad & Slinkard, 1992). Moreover, there are reports that provide evidence for association of polymorphism in seed storage proteins with the geographical origin of germplasm (Satija *et al.,* 2002; Ghafoor *et al.,* 2003).

With above said background, current study was performed with the objectives 1) seed storage protein profiling of selected chickpea genotypes/mutants/lines using SDS-PAGE, 2) To assess the genetic diversity in chickpea germplasm based on electrophoretic data and 3) Possible identification of resolved chickpea seed storage proteins using Uniprot and NCBI protein databases.

Materials and Methods

Five kabuli (white seeded) and twenty-nine desi (brown seeded) genotypes of chickpea were used for seed storage protein profiling. Details i.e., seed size; origin and parentage of these genotypes are given in the Table 1.

Seed protein extraction: For extraction of chickpea seed proteins, samples were grounded in 50 mM phosphate buffer (pH 7.8) followed by centrifugation for 10 min at 14,000rpm in micro-centrifuge machine (Sigma 1-14). For protein profiling, the supernatant was pipette out and used for analysis. Total soluble protein concentration in extracts was quantified by dye binding assay reported by Bradford (1976). To denature the proteins for gel electrophoresis, protein samples were mixed with cracking solution (10 ml containing, 0.01g bromphenol blue, 1g SDS, 1.5ml 0.5M tris pH 6.8, 2ml Mercaptoethanol, 05g sucrose and water) (4:1 ratio) and then placed in boiling water for 5 minutes.

Table 1. Seed size, origin, character and parentage of different genotypes used in the study.

#	Genotype	Seed size	Character	Origin	Parentage
	Kabuli (white seeded)				
01.	Pb-1	Small	BS	Local collection	-----
02.	CM-2008	Medium	WR	NIAB, Pakistan	Pb-1, EMS, 0.2%
03.	ILC-195	Bold	BT	ICARDA, Syria	ICARDA, Syria
04.	CM-2000	Bold	ICS	NIAB Pakistan	ILC-195,Gamma rays, 150Gy
05.	ICCV-92311	V. Bold	WR, SE	India	ICRISAT accession
	Desi (brown seeded)				
06.	ICCV-95230	V. Small	WS	India	ICRISAT accession
07.	PUSA-329	Small	WR, BR	India	ICRISAT accession
08.	CH-24/00	Medium	WS, BR	NIAB Pakistan	ICCV-95227 × Pb-91
09.	CM-2100/96	Bold	WR, PBR	NIAB Pakistan	6153, Gamma rays, 200Gy
10.	Pb-2008	Bold	WR	AARI Pakistan	
11.	6153	Bold	BS	Indo-Pak	-----
12.	K-850	Bold	WR	Indo-Pak	-----
13.	CM-72	Small	BS	NIAB Pakistan	6153, Gamma rays, 150Gy
14.	C-44	Bold	WS	AARI Pakistan	Thal collection
15.	Pb-91	Bold	BT	AARI Pakistan	NEC 138-2 × RC32
16.	C-727	Small	BS	AARI Pakistan	Pb-1 × F8
17.	Paidar-91	Small	BT	AARI Pakistan	C-235 × ILC-191
18.	CM-88	Small	WS	NIAB Pakistan	C-727, Gamma rays, 100Gy
19.	CM-98	Bold	WS	NIAB Pakistan	K-850, Gamma rays, 300Gy
20.	Bittle-98	Bold	WS	AARI Pakistan	(C-44×C-87) × C-44
21.	Pb-2000	Bold	WS	AARI Pakistan	C-44 × C-87
22.	CH-58/99	Bold	BR	NIAB Pakistan	CM-72 × Pb-91
23.	CH-23/00	Medium	WS	NIAB Pakistan	ICCV-95227 × Pb-91
24.	Vanhar-2000	Bold		AARI Pakistan	
25.	P1-13	Bold	BR	NIAB Pakistan	Pb-91×Paidar-91
26.	CM-3444/92	Bold	Dwarf	NIAB Pakistan	Pb-91, Gamma rays, 150Gy
27.	CM-2234/97	Bold	EM	NIAB Pakistan	6153, Gamma rays, 150Gy
28.	CM-1359/89	Bold	EM	NIAB Pakistan	C-44, Gamma rays,350Gy
29.	CM-72/02	Bold	EM	NIAB Pakistan	Pb-2000,Gamma rays,300Gy
30.	CM-210/01	Bold	BR, PBR	NIAB Pakistan	Pb-2000,Gamma rays,350Gy
31.	CM-1057/97	Small	WR	NIAB Pakistan	PUSA-329,Gamma rays,250Gy
32.	CM-97479	Small	WR	NIAB Pakistan	PUSA-329,Gamma rays,250Gy
33.	CM-97325	Small	WR	NIAB Pakistan	PUSA-329,Gamma rays,250Gy
34.	CM-97477	Small	WR	NIAB Pakistan	PUSA-329,Gamma rays,250Gy

Note: EM = Early maturation, WR = Wilt resistant, WS =Wilt susceptible, BS =Blight susceptible, BT =Blight tolerant, BR = Blight resistant, PBR = Pod borer resistant, ICS = Iron chloroses susceptible, SE= Supper early

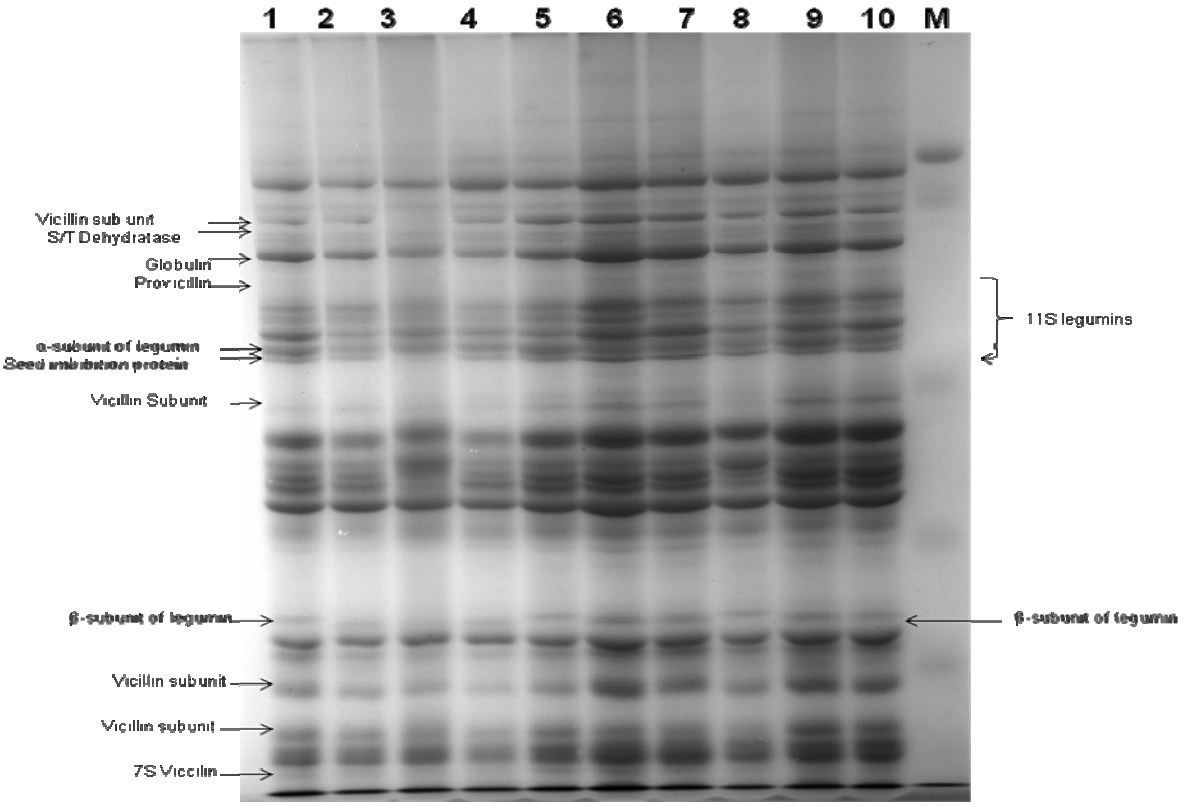
Seed protein profiling: The SDS-polyacrylamide gels for proteins profiling of chickpea samples were prepared and run according to the method described by Laemmli (1970). Equal amount of denatured protein samples were loaded into 12% gels along with protein molecular weight marker (RMR002 BIO BASIC INC.). The process of gel electrophoresis was carried out keeping the constant voltage (100 volts) through out the run. On completion of electrophoresis, gels were removed and then fixed with a solution containing 40% Ethanol and 10% Acetic acid for 15 min on a on a rocking shaker. Gel fixing was followed by washing with distilled water for 15 min. while water was replaced with fresh one after every 5 min. Protein bands in gels were stained with coomassie blue G-250 dye. Gels were destained in distilled water overnight for more clear background.

Gel documentation and analysis: for permanent record and analysis, photograph of gels were made with the help of "UVIproplatinum" gel documentation system (UVItec UK). Computerized gel analysis i.e. protein molecular weight determination was performed using UVI pro Platinum 1.1 Version 12.9 for windows (copyright® 2004-2006). For cluster analysis and dendrogram construction, a special software UVI BANDMAP version 11.3 by UVItec UK was used.

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 chickpea 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.

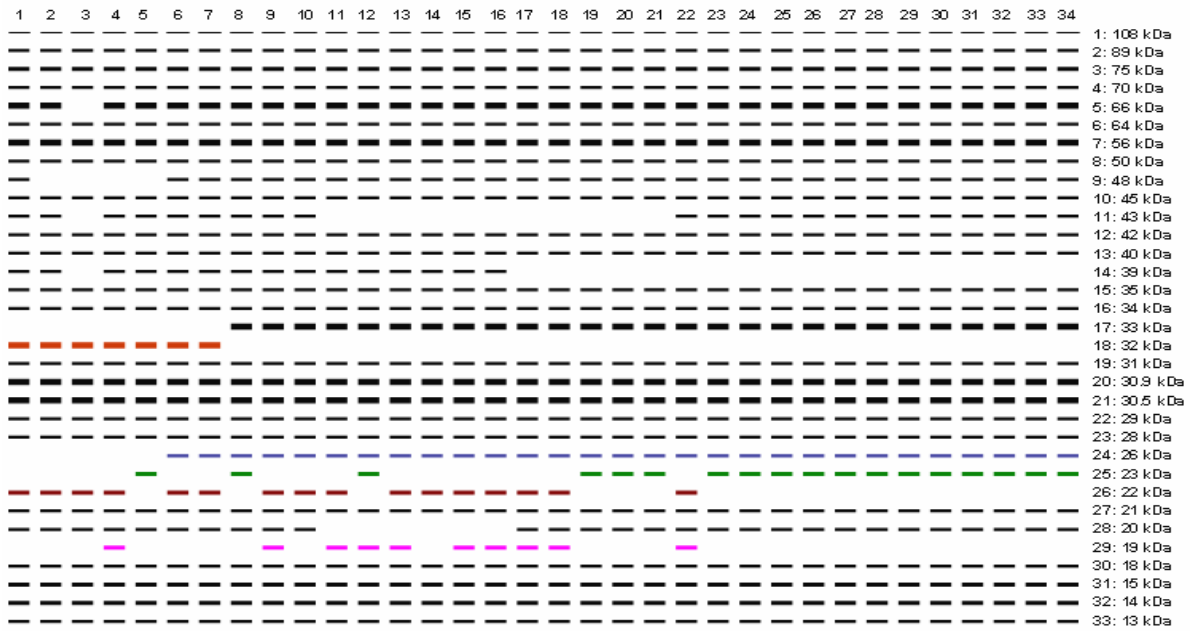
Results

Seed storage proteins of chickpea genotypes were resolved in 32-34 detectable protein bands by SDS-PAGE. Mainly polymorphism in the peptides was detected in four variable regions. Molecular weights of peptides ranged from 108KDa to 13KDa. Resolved proteins were labeled as Seed Storage Proteins (SSP) followed by their molecular weights in KDa. A representative diagram showing the seed storage protein profiles of ten genotypes is presented in Fig. 1. Over all banding patterns of all 34 genotypes is presented as a cumulative histogram in Fig. 2.



Lane 1: Pb-1, Lane 2: CM-2008, Lane 3: ILC-195, Lane 4: CM-2000, Lane 5: ICCV-92311, Lane 6: ICCV-95230, Lane 7: PUSA-329, Lane 8: CH-24/00, Lane 9: CM-2100/96, Lane 10: Pb-2008, M: Protein Molecular weight Marker (RMR002 BIO BASIC INC).

Fig. 1. Representative electrophorogram of chickpea genotypes with possible identification.



1: Pb-1, 2: CM-2008, 3: ILC-195, 4: CM-2000, 5: ICCV-92311, 6: ICCV-95230, 7: PUSA-329, 8: CH-24/00, 9: CM-2100/96, 10: Pb2008, 11: 6153, 12: K-850, 13: CM-72, 14: C-44, 15: Pb-91, 16: C-727, 17: Paidar-91, 18: CM-88, 19: CM-98, 20: Bittle-98, 21: Pb-2000, 22: CH-58/99, 23: CH-23/00, 24: Vanhar-2000, 25: P1-13, 26: CM-3444/92, 27: CM-2234/97, 28: CM-1359/89, 29: CM-72/02, 30: CM-210/01, 31: CM-1057, 32: CM-97479, 33: CM-97325, 34: CM-97477.

Fig. 2. Histogram showing protein variability in chickpea seed proteins.

Dendrogram based on protein profiles grouped the 34 genotypes in 4 clusters at 87% homology (Fig. 3). Seed storage protein profiles showed that the ILC-195 was the most divergent genotype as compared to others. The 1st cluster consisted of the six genotypes. Within this cluster two desi genotypes, ICCV-95230 and PUSA-329, showed the 100% homology in the seed storage protein profiles. Other two kabuli genotypes, Pb-1 and CM-2000, showed 98% homology with each other and also with ICCV-95230 and PUSA-329. collectively all these four genotypes showed 94% homology with other two kabuli genotypes i-e CM-2008 and ICCV-92311 in this cluster. These two genotypes, CM-2008 and ICCV-92311 showed 95% similarity with each other and were most divergent in 1st cluster.

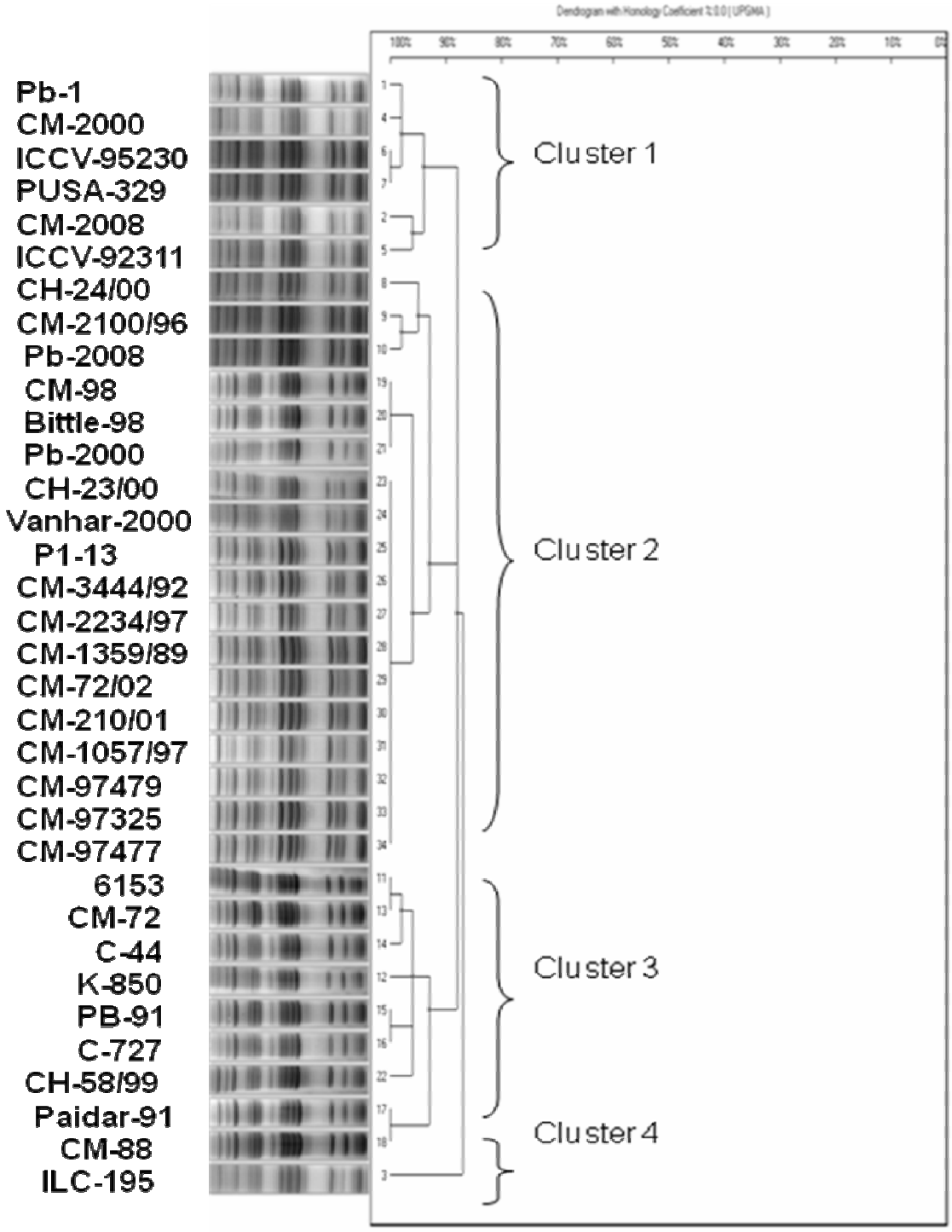


Fig. 3. Dendrogram based on the electrophoretic data of 34 chickpea genotypes.

A total of 18 genotypes were grouped together in the 2nd cluster. Within 2nd cluster, twelve desi genotypes i.e. CH-23/00, Vanhar-2000, P1-13, CM-3444/92, CM-2234/97, CM-1359/89, CM-72/02, CM-210/01, CM-1057/97, CM-97479, CM-97325 and CM-97477, showed 100% homology in seed storage proteins therefore could not be differentiated on these grounds. Three genotypes CM-98, Bittle-98 and Pb-2000 grouped together in this cluster with 100% similarity among them and 97% homology with above said 12 genotypes in this cluster. Other three genotypes i.e. CM-2100/96, Pb-2008 and CH-24/00 were slightly distinct (94% homology) from other genotypes in this cluster. In this subset of genotypes, CM-2100/96 and Pb-2008 showed 98% homology with each other and 96% similarity with CH-24/00.

Nine genotypes were grouped together forming the 3rd cluster. In this cluster, two genotypes, 6153 and CM-72 showed 100% homology with each other and 97% homology with C-44. Other two genotypes Pb-91 and C-727 showed 100% similarity with each other and 95% homology with K-850 and CH-58/99. Remaining two genotypes Paidar-91 and CM-88 were 100% homologous to each other while showed 94% homology with other genotypes in this cluster.

The 4th cluster consisted of only one genotype i.e., ILC-195, which was the most divergent among all the 34 genotypes and showed 86% homology with all other genotypes in 3 clusters.

Protein Profiles of ILC-195 showed that this genotype was distinct from all others genotypes as the protein markers SSP-66, was specifically absent in ILC-195. While comparing kabuli genotypes, SSP-43 and SSP-39 were present in all other kabuli genotypes with exception of ILC-195. Therefore, on the basis of absence of these peptides ILC-195 can be differentiated from other kabuli genotypes. As SSP-70 was present in all other 33 genotypes including CM-2000, which is the mutant of ILC-195 lacking this peptide (Fig. 2). Therefore, by this difference in peptide mutant i.e., CM-2000 can be differentiated from its parent ILC-195. Moreover, CM-2000 can be differentiated by presence of SSP-19, which was absent in all kabuli genotypes (Fig. 3).

In Pb-1 (kabuli genotype) SSP-48 was present which was absent in all other kabuli genotypes. However, this peptide was present in all desi genotypes. Another protein SSP-23 was present in only one kabuli genotype i.e., ICCV-92311. Being genotype specific, this seed storage protein can be used as distinguishing marker for this genotype.

ICCV-95230 and PUSA-329 were the only two desi genotypes that showed more similarity to kabuli genotypes as compared to all other desi genotypes by the presence of protein marker SSP-32 that was absent in all other desi genotypes. These two genotypes share these peptides with the kabuli genotypes.

Three genotypes i.e., CM-2100/96, CM-72 and CM-2234/97 included in this study were mutants derived from 6153. No doubt these 4 genotypes shared many proteins in the profile; the mutant CM-2100/96 showed 2 unique bands i.e., SSP-43 and SSP-20 which were not present in its parent. Similarly, in CM-2234/97 a protein peptide i.e. SSP-39 was absent while it was present in its parent genotype 6153. In contrast, SSP-23 was present in CM-2234/97 while it was absent in its parent 6153 and other two derived mutants. However, CM-72 did not show any difference in the protein banding pattern as compared to its parent. Above said differences in the protein profiles can be used for differentiation of 6153 from its mutants.

Similarly, Pb-2000, Bittle-98 (hybrids) and CM-1359/89 (mutant) were evolved from C-44. The protein profile of C-44 differentiated it from its hybrids/mutant genotypes. A protein peptide SSP-43 was absent in C-44 but present in one of its mutant i.e., CM-1359/89. Likewise, SSP-23 was absent in C-44 but present in above mentioned three genotypes evolved from it. Mutagenesis of germplasm was found to cause change in protein profile in the resulting hybrids/mutants. For example, SSP-39 was present only in parent C-44 but absent in all genotypes evolved from it. CM-72/02 and CM-210/01 which were mutants of the Pb-2000 (hybrid of C-44 x C-87), can be differentiated from Pb 2000 because of the presence of SSP-43, which was absent in Pb-2000.

Genotype C-727 (parent) and its mutant CM-88 can be differentiated from each other by two protein markers that are SSP-39 and SSP-20. The peptide SSP-39 was present in C-727 but absent in CM-88 while SSP-20 was absent in C-727 but present in CM-88. Moreover, CM-98 a mutant of the K-850 can be distinguished from its parent by the presence of SSP-20 and the absence of SSP-39.

Three protein markers, SSP-26, SSP-23 and SSP-20 were present in CM-3444/92 while absent in its parent i.e., Pb-91. While three protein markers SSP-39, SSP-22 and SSP-19 were present in Pb-91 but absent in CM-3444/92. P1-13 is a hybrid of Pb-91 and Paidar 91. A peptide SSP-43 was absent in both parents but present in P1-13. SSP-39 was present in Pb-91 but absent in Paidar 91 while their hybrid i.e., P1-13 did not show the presence of this peptide. On the other hand SSP-20 was present in one parent i.e., Paidar 91 and absent in other parent i.e., Pb-91 while this peptide was also present in their hybrid P1-13.

Identification of the peptides using database search and available literature: Along with assessment of genetic variation in germplasm and characterization of mutants/hybrids, resolved seed storage proteins were searched in protein databases and in previously reported literature for their possible similarity based on their approximate molecular weights. Uniprot and NCBI protein databases were searched to find chickpea seed storage proteins subunits that have been reported and present in the databases. A total of six entries were available for searched items (*Cicer arietinum* and seed protein, chickpea and seed storage proteins, legumes and seed proteins) in these databases. Information about these proteins available in the databases is given in Table 2. According to available literature accessed by different search engines (Google scholar, Pubmed, Pubmed central), chickpea seed storage proteins were mainly globulin protein, 7S vicilins and 11S legumins (major protein fractions) and 2S albumins (minor protein fraction). 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 weight with previously reported peptides in the chickpea seeds is presented in the Table 3 and Fig. 1.

Table 2. Information about chickpea seed storage proteins available in Uniprot and NCBI protein databases.

M.W (kDa)	Protein	Function	Reference
65.153	Serine/Threonine dehydratase	L-threonine ammonia-lyase activity, pyridoxal phosphate binding	John *et al.,* 1995
56.251	Globulin Family	Alpha-amylase inhibitor, seed storage protein	Hao *et al.,* 2009
50.600	Provicillin	Nutrient reservoir activity	Yasothornsrikul *et al.,*1990
39.465	Seed imbibition protein	Imbibition protein	Cervantes, 1996
35.408	Isoflavane reductase	Oxidoreductase	Tiemann *et al.,* 1991
19.576	LTP family	Peptidase activity	Singh *et al.,* 2008

Table 3. Information about possible families of resolved peptides based on their similarity in approximate molecular weight with reported peptides.

S #	M. wt. (KDa)	Possible protein family	Reference
1.	108.000		
2.	87.550		
3.	75.729		
4.	70.988	7S Vicillin Subunit	Chang *et al.,* 2009
5.	65.153	Serine/Threonine dehydratase	John *et al.,* 1995
6.	62.809		
7.	56.480	Globulin family(α-amylase inhibitor)	Hao *et al.,* 2009
8.	51.421	7S Vicillin Subunit Provicillin	Chang *et al.,* 2009 Yasothornsrikul *et al.,* 1990
9.	46.835	11S legumin	Sánchez-Vioque *et al.,* 1999
10.	44.544	11S legumin	Sánchez-Vioque *et al.,* 1999
11.	42.779	11S legumin	Sánchez-Vioque *et al.,* 1999
12.	41.077	α-subunit of legumin	Chang *et al.,* 2009
13.	39.465	α-subunit of legumin	Chang *et al.,* 2009
		Seed imbibition protein	Cervantes, 1996
14.	35.408	Vicillin subunit Isoflavane reductase	Chang *et al.,* 2009 Tiemann *et al.,* 1991
15.	34.691		
16.	33.919	Vicillin subunit	Chang *et al.,* 2009
17.	32.815		
18.	32.176		
19.	30.866		
20.	30.171		
21.	29.792		
22.	28.957		
23.	27.896		
24.	27.100		
25.	26.025		
26.	23.500	β-subunit of legumin	Chang *et al.,* 2009
27.	22.595	β-subunit of legumin	Chang *et al.,* 2009
28.	21.003		
29.	20.075		
30.	19.576	LTP family (Peptidase activity)	Singh *et al.,* 2008
31.	17.840	Vicillin Subunit	Chang *et al.,* 2009
32.	15.370	Vicillin Subunit	Chang *et al.,* 2009
33.	14.916		
34.	13.131	7S Vicillin	Gueguen, 1991

Discussion

Gel electrophoresis of seed protein is an authentic tool for population genetics studies (Parker *et al.,* 1998). Main advantage of seed protein profiling is that these are not affected or altered by environmental changes, that's why can be used as a consistent tool for germplasm characterization in most economical way (Javid *et al.,* 2004; Iqbal *et al.,* 2005; Hameed *et al.,* 2009).

Previously, comparisons of seed storage proteins for separating closely related small and large seeded lentils was found to provide no difference in proteins as a biological basis (Ladizinsky, 1979). Moreover, comparative seed protein profiling of kabuli chickpea genotypes did not show any significant differences on the basis of bold and small seeded chickpea (Hameed *et al.,* 2009). Similar findings were observed in this study that seed storage protein profiling using SDS-PAGE of both kabuli and desi chickpea genotypes did not show any difference in protein profiles based on seed size.

Cluster analysis based on protein profiles by SDS-PAGE has proved to be excellent to differentiate *Vigna mungo* and *Vigna radiate.* However, a low inter-specific genetic diversity was observed with no clear differentiation either for origin or agronomic characteristics and various clusters consisted mixed genotypes from different origins (Ghafoor *et al.,* 2002). Previously no clear discrimination was evident for origin of kabuli chickpea genotypes based

on seed protein profiles (Hameed *et al.,* 2009). However, according to our present results protein profiles of ILC-195 showed that this genotype was distinct from all others genotypes included in the study. Interestingly, ILC-195 was only genotype from Syrian origin included in the study and thus protein profiles were able to differentiate it. A distinct protein profile of this genotype seems to be due to its distinct Syrian origin. This observation provides evidence that seed storage protein profiling by SDS-PAGE can discriminate chickpea genotypes based on their origin, however, it needs confirmation by using more number of genotypes from diverse backgrounds.

Seed protein profiling has been used as an economical and reliable technique for distinguishing cultivars of particular crop species (Jha & Ohri, 1996; Mennella *et al.,* 1999; Pervaiz *et al.,* 2011; Irfan, 2011). Moreover, profiling by SDS-PAGE is considered to be a useful and reliable method for identification of species (Gepts, 1989). On the other hand, few studies also pointed out that cultivar identification was not possible with this method (Ahmad & Slinkard, 1992; De Vries, 1996). However, it has been reported that seed storage protein profiling can be economically used to evaluate genetic variation, relationship in germplasm and to differentiate mutants from their parent genotypes (Hameed *et al.,* 2009). In the present study, similar findings were observed that most of mutants/hybrids can be differentiated from their parents on the basis of seed protein profiling by SDS-PAGE. ILC-195, the parent, can be distinguished from its mutant genotype i.e. CM-2000. Similarly, CM-2100/96, CM-72 and CM-2234/97, three mutants of 6153 can be differentiated from parent on the basis of seed storage profiles of these genotypes. Moreover, Pb-2000, CM-1359/89 and Bittle-98, can be differentiated from parent genotype i.e. C-44. Genotype C-727 the parent of mutant CM-88 can be distinguished based on seed storage profiles. The same was true for mutant CM-3444/92 and its parent i.e., Pb-91. Similarly, P1-13 a hybrid of Pb-91 and Paidar showed different protein banding pattern compared to its both parents.

Seed storage proteins of chickpea consist of two major types; low molecular weight albumins and globulin. Globulins were further classified as 7S vicillin subunits and α and β subunits of legumins (Chang *et al.,* 2009). According to their molecular weight in kDa, in the present study, most of subunits have been identified. Chang *et al.,* (2009) identified the vicillin subunits having molecular weight of 70.2, 50.7, 35.0, 33.6, 18.9 and 15.5 kDa as chickpea seed proteins in their study by using SDS-PAGE. In the present study, we found the peptides of the molecular weight of 70.988, 50.416, 35.408, 33.919, 17.840, 15.370 kDa that can be recognized as Vicillin subunits. Similarly, Chang *et al.,* (2009) found α subunits of legumins with molecular weights of 40.6 and 39.5 kDa. In the present study, subunits having molecular weight of 41.077 and 39.465 kDa were resolved and these can be α subunits of legumins. According to Chang *et al.,* (2009) subunits having molecular weights of 22.5 and 23.5 kDa were the β subunits of legumins. In the present study peptides of 23.500 and 22.595 kDa can be determined as β subunits of legumins. Based on SDS-PAGE, Sanchez-Vioque *et al.,* (1999) reported the major protein fractions of chickpea with 46.5 to 39.8 and 25.3 to 24.3 kDa subunits and related them with 11S legumins. In the present study, we also found five peptides of 46.835, 44.544, 42.779, 41.077 and 39.465 kDa within this range. These above mentioned peptides seem to be the11S legumin subunits. Gueguen (1991) reported a peptide of 13 kDa as 7S vicillin subunit. In the present study, we also found a peptide of 13.131 kDa that can be 7S vicillin.

Previously, lipid transport protein family, with MW of 19.576 kDa has been examined for its peptidase activity in chickpea (Singh *et al.,* 2008). In the present study, we also found a peptide with the molecular weight of 19.865 kDa (SSP-19) in the seed storage protein profiles of CM-2000, CM-2100/96, 6153, K-850, CM-72, Pb-91, C-727, Paidar-91, CM-88 and CH-58/99 genotypes. Most probably this protein with M.Wt of 19.865 kDa seems to be lipid transport protein. Isoflavane reductase enzyme with MW of 35.408 has been reported for its oxidoreductase activity in chickpea (Tiemann *et al.,* 1991). Similarly in the present study, we detect a peptide with molecular weight of 35.408 kDa (SSP-35) in the seed storage protein profiles of all tested genotypes. This detected peptide with M.Wt of 35.900 kDa most probably can be Isoflavane reductase enzyme. Cervantes, (1996) reported a seed imbibitions protein with MW of 39.465 kDa in chickpea. In the present study, we also found a peptide having molecular weight of 39.190 kDa (SSP-39) in the protein profiles of Pb-1, CM-94/99, CM-2000, ICCV-92311, ICCV-95230, PUSA-329, CH-24/00, CM-2100/96, 9800, 6153, K-850, CM-72, C-44, Pb-91 and C-727 genotypes. This peptide, having MW of 39.190 kDa seems to be seed imbibitions protein. Provicillin, from the vicillin family of globulin with MW of 50.6 kDa has been reported as nutrient reservoir activity in chickpea seeds (Yasothornsrikul *et al.,* 1990). In the present study, we also detect a peptide with MW of 50.416 kDa (SSP-50) in all tested genotypes. This detected peptide can be provicillin. Alpha-amylase inhibitor a protein from globulin family with MW of 56.251 kDa has been reported in chickpea by Hao *et al.,* (2009). In the present study, we also found a peptide having molecular weight of 56.480 kDa (SSP-56) in all the tested chickpea genotypes. This peptide seems to be the alpha-amylase inhibitor. Serine/threonine dehydratase enzyme with MW of 65.153 kDa has been examined for its L-threonine ammonia-lyase activity and pyridoxal phosphate binding activity in chickpea (John *et al.,* 1995). In the present study the peptide with MW of 64.125 (SSP-64) was detected in all tested genotypes. This detected peptide seems to be serine/threonine dehydratase protein.

In conclusion, seed storage protein profiling based on SDS-PAGE can efficiently be used to asses the genetic variability among the chickpea germplasm. This economic technique can be used to differentiate mutants/hybrids from their parent genotypes. In tested chickpea germplasm variability was mainly observed in vicillin subunits and β subunits of legumins and its polymorphic forms.

References

Ahmad, F. and A.E. Slinkard. 1992. Genetic relationships in the genus *Cicer* L., as revealed by polyacrylamide gel electrophoresis of seed storage proteins. *Theor. Appl. Genet.,* 84: 688-692.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the

principle of protein-dye binding. *Ann. Biochem.,* 72: 248-254.

Cervantes, E. 1996. EMBL/GenBank/DDBJ databases.

Chang, Y.W., I. Alli, A.T. Molina, Y. Konishi and J.I. Boye. 2009. Isolation and characterization of Chickpea (*Cicer arietinum* L.) Seed Protein Fractions. *Food Bioprocess Technol.,* Doi: 10.1007/s11947-009-0303-y

Das, S. and K.K. Mukarjee. 1995. Comparative study on seed proteins of *Ipomoea*. *Seed Sci. & Technol*., 23: 501-509.

De Vries, I.M. 1996. Characterisation and identification of *Lactuca sativa* cultivars and wild relatives with SDS electrophoresis (*Lactuca sect. Lactuca*, *Compositae*). *GRACE*, 43: 193-202.

Freitas, R.L., R.B. Ferreira and A.R. Teixeira. 2000. Use of a single method in the extraction of the seed storage globulins from several legume species. Application to analyse structural comparisons within the major classes of globulins. *Int. J. Food Sci. Nutr*., 51(5): 341-52.

Gepts, P. 1989. Genetic diversity of seed storage proteins in plants. In: *Plant Population Genetics, Breeding and Genetic Resources.* (Eds.): A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir. pp. 64-82. Sinauer Associates Inc., Sunderland, Massachusetts.

Ghafoor, A., F.N. Gulbaaz, M. Afzal, M. Ashraf and M. Arshad. 2003. Inter-relationship between SDS-PAGE markers and agronomic traits in chickpea (*Cicer arietinum* L.). *Pak. J. Bot*., 35: 613-624.

Ghafoor, A., Z. Ahmad, A.S. Qureshi and 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: 367-378.

Gueguen, J. 1991. Pea and fababean proteins. In: *Development in food proteins*. (Ed.): B.J.F. Hudson. pp. 35-78. Elsevier: London, U.K.

Hameed, A., T.M. Shah, B.M. Atta, N. Iqbal, M.A. Haq and H. Ali. 2009. Comparative seed storage protein profiling of Kabuli chickpea genotypes. *Pak. J. Bot*., 41(2): 703-710.

Hao, X., J. Li, Q. Shi, J. Zhang, X. He and H. Ma. 2009. Characterization of a novel legumin alpha-amylase inhibitor from chickpea (*Cicer arietinum* L.) seeds. *Biosci. Biotechnol. Biochem.,* 73: 1200-1202.

Iqbal, S.H., A. Ghafoor and N. Ayub. 2005. Relationship between SDS-PAGE markers and *Ascochyta* blight in chickpea. *Pak. J. Bot*., 37: 87-96.

Irfan, E. 2011. Determination of genetic diversity in the *Vicia* L. (*section vicia*) by using SDS-PAGE. *Pak. J. Bot.*, 43(3): 1429-1432.

Jain, D. and D. Chattopadhyay. 2010. Analysis of gene expression in response to water deficit of chickpea (*Cicer arietinum* L.) varieties differing in drought tolerance. *BMC Plant Biol.,* 10: 24. doi:10.1186/1471-2229-10-24.

Javid, A., A. Ghafoor and R. Anwar. 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pak. J. Bot*., 36(1): 25-29.

Jha, S.S. and D. Ohri. 1996. Phylogenetic relationships of *Cajanus cajan* (L.) Millsp. (pigeonpea) and its wild relatives based on seed protein profiles. *GRACE,* 43: 275-281.

John, S.J., V. Srivastava and S. Guha-Mukherjee. 1995. Cloning and sequencing of chickpea cDNA coding for threonine deaminase. *Plant Physiol.,* 107: 1023-1024.

Khan, M.K. 1990. Production and utility of chickpea (*Cicer arietinum* L.) in Pakistan. *Progressive Farming*, 10: 28-33.

Ladizinsky, G. 1979. Species relationships in the genus *Lens* as indicated by seed protein electrophoresis. *Bot. Gaz*., 140: 449-451.

Laemmli, U.K. 1970. Cleavage of structure proteins assembly of the head of bacteriophage T4. *Nature*, 22: 680-685.

Lioi, L., F. Sparvoli and R. Bollini. 1999. Variation and genomic polymorphism of lectin-related protein in Lima Bean (*Phaseolus lunatus* L.) seed. *Genet. Resour. Crop Ev.,* 46: 157-182.

Mennella, G., S.V. Onofaro, A. Tonini and V. Magnifico. 1999. Seed storage protein characterization of *Solanum* species and of cultivars and androgenetic lines of *S. melongena* L., by SDS-PAGE and AE-HPLC. *Seed Sci. Technol.,* 27: 23-35.

Murphy, R.W., J.W. Sites, D.G. Buth and C.H. Haufler. 1990. Protein I: Isozyme Electrophoresis. In: *Molecular Systematics.* (Eds.): D.H. Hillis and C. Moritz, pp. 45-126. Sinauer Assoc., Sunderland, MA.

Nisar, M., A. Ghafoor, M.R. Khan, H. Ahmad, A.S. Qureshi, H. Ali. 2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. *Pak. J. Bot*., 39: 1575-1581.

Parker, P.G., A.A. Snow, M.D. Schug, G.C. Booton and P.A. Fuerst. 1998. What molecules can tell us about populations: choosing and using a molecular markers. *Ecology*, 79: 361-382.

Pervaiz, Z.H., T. Sadia, M.A. Rabbani, M.S. Masood and S.A. Malik. 2011. Diversity in major seed storage proteins of rice landraces of Pakistan. *Pak. J. Bot*., 43(3): 1607-1612.

Sánchez-Vioque, R., A. Clemente, J. Vioque, J. Bautista and F. Millán. 1999. Protein isolate from chickpea (*Cicer arietinum* L.): Chemical composition, functional properties and protein characterization. *Food Chemistry,* 64: 237-243.

Satija, D.R., B. Adrash, S.K. Gupta and A. Bala. 2002. Genetic diversity in relation to protein and protein fractions in chickpea (*Cicer arietinum* L.). *Crop Improvement,* 29: 122-135.

Singh, A., I.K. Singh and P.K. Verma. 2008. Differential transcript accumulation in *Cicer arietinum* L., in response to a chewing insect Helicoverpa armigera and defence regulators correlate with reduced insect performance. *J. Exp. Bot.,* 59: 2379-2392.

Shah, T.M., B. M. Atta, M. A. Haq and J. I. Mirza. 2012. Radio sensitivity of various chickpea genotypes in M_1 generation II-Field studies. *Pak. J. Bot.* 44(2): 631-634.

Shah, T.M., B. M. Atta, M. A. Haq and J. I. Mirza. 2011. Induced genetic variability in chickpea (*Cicer arietinum* L.). IV Spectrum of morphological mutations. *Pak. J. Bot.* 43(4): 2039-2043.

Smolka, M., H. Zhou and R. Aebersold. 2002. Quantitative protein profiling using two-dimensional gel electrophoresis, isotope-coded affinity tag labeling, and mass spectrometry. *Molecular & Cellular Proteomics*, 1: 19-29.

Tiemann, K., D. Inze, M. Van-Montagu and W. Barz. 1991. Pterocarpan phytoalexin biosynthesis in elicitor-challenged chickpea (*Cicer arietinum* L.) Cell cultures. Purification, characterization and cDNA cloning of NADPH: isoflavone oxidoreductase. *Eur. J. Biochem.,* 200: 751-757.

Yasothornsrikul, S., L. Wen, S.L. Mehta, R. Kansal, M. Subbaratnam and G.R. Reeck. 1990. Nucleotide sequence of a cDNA encoded for provicilin precursor of chickpea**.** EMBL/GenBank/DDBJ databases.

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