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COMPARATIVE SEED STORAGE PROTEIN PROFILING OF KABULI CHICKPEA GENOTYPES

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Abstract

Seed storage protein profiles of 8 kabuli (white seeded) chickpea mutants/genotypes were analysed by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total soluble proteins were resolved on 10% SDS polyacrylamide gels. Low variability in tested kabuli germplasm was observed. On the bases of seed protein banding patterns, 5 genotypes could be identified clearly and biochemical fingerprints of these varieties are reported. Dendrogram based on electrophoretic data clustered the genotypes in three groups. Three genotypes viz., Pb-1, CM-94/99 and PKV Kabuli 2 showed 100% homology therefore could not be separated on the bases of seed storage proteins. One exotic genotype ILC-195 and two mutants CM-2000 and CM-98/99 were comparatively divergent from other genotypes. Identified protein markers i.e., KSSP-100, KSSP-93 and KSSP-64 can be used for identification of CM-98/99, ILC-195 and CM-2000 respectively. Moreover CM-315/99 and Noor-91 can also be identified by combination of identified protein markers. In conclusion, electrophoresis (SDS-PAGE) of seed storage proteins can economically be used to assess genetic variation and relation in germplasm. The specific bands of seed storage protein profiles may be used as markers for identification of the mutants/ genotypes.

Introduction

Information about genetic diversity of germplasm is a useful tool in gene bank management and in planning experiments, as it facilitates efficient sampling and utilization of germplasm by identifying and/or eliminating duplicates in the gene stock and helps in the establishment of core collection (Ghafoor & Ahmad, 2005). Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up DUS (distinctness uniformity stability) test for candidate cultivar (Chowdhury *et al*., 2002). One practical application of knowledge of genetic diversity is in the design of populations for genome mapping experiments (Kaga *et al*., 1996).

Characterization of germplasm using biochemical fingerprinting has got special attention. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Murphy *et al*., 1990; Khan, 1990; Das & Mukarjee, 1995; Ghafoor *et al*., 2002).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. Seed storage proteins have been used as genetic markers in four major areas: 1) Analysis of genetic diversity within and between species, 2) Plant domestication in relation to genetic resources conservation and breeding, 3) Genome relationship and 4) A tool in crop improvement (Ghafoor *et al*., 2002). As seed storage proteins are largely independent of environmental fluctuations, their profiling using SDS-PAGE technology is particularly considered as a consistent tool for economic characterization of germplasm (Javid *et al*., 2004; Iqbal *et al*., 2005).

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In Pakistan, the desi chickpea is grown on nearly 85% area and kabuli type is grown on only 15% area. The area under kabuli chickpea is further decreasing due to lack of bold seeded high yielding varieties and susceptibility to diseases. Only three varieties of kabuli chickpea i.e., Noor-91 (released in 1991), Pb-1 (released in 1930) and CM2000 (released in 2000) have been released till to date and have now become susceptible to patho-types of fungus *Ascochyta rabiei* and wilt/ root rot diseases. The exotic kabuli material having long photoperiod and cooler mediterranean climate have very low adaptability in our environment. Moreover, two thirds of the world germplasm accessions at ICRISAT are desi type (80.67%) and nearly one third (18.87%) are kabuli type (Pundir *et al*., 1988). The genetic variability of kabuli chickpea for several economically important traits is very limited. Induced mutation may be expected to enhance and widen the genetic variability of kabuli chickpea.

Analyses of seed storage proteins provide aid for identification and characterization of diversity in crop varieties, cultivars and their wild varieties and phylogenetic relationship of the accessions (Nisar *et al*., 2007). 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). Phylogenetic relationship among *Cicer* species based on SDS-PAGE data has suggested that *Cicer reticulatum* is the wild progenitor of cultivated chickpea (Ahmad & Slinkard, 1992). Polymorphism in seed storage proteins has been associated with geographical origin of germplasm. (Satija, 2002; Ghafoor *et al*., 2003). Analysis of seed storage protein profiles by SDS-PAGE in M_3 lines of cowpea (*V. unguiculata*) has provided evidence that induced mutation could create additional variability to supplement existing germplasm and that SDS-PAGE is a useful tool for discriminating and estimating genetic similarities among selections (Osanyinpeju & Odeigah, 1998).

Keeping in view the importance of protein profiling, present study was conducted using 8 high yielding and locally wider adaptable kabuli advance lines/ mutants, 1) to reveal out the polymorphisms in seed storage proteins, 2) to find out protein markers with potential to differentiate between parents and mutants and 3) to identify protein based markers for genotype identification.

Materials and Methods

For assessment of variation of seed storage protein, soluble proteins were extracted from mature seeds of 8 chickpea kabuli genotypes/mutants (Pb-1, CM94/99, CM98/99, CM315/99, ILC-195, CM-2000, Noor 91 and PKV Kabuli 2) and analyzed by SDS-PAGE (Table 1).

Preparation of protein samples: For extraction of soluble proteins, seeds were grounded in 50 m*M* phosphate buffer (pH 7.8) and centrifuged in micro-centrifuge machine (Eppendorf 5415c) 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 0.5*M* 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.

Table 1. Kabuli chickpea genotypes used in study along with origin and parentage.

Genotype	Pedigree	Origin	100 grain wt (g)	Seed size
Pb-1	Local collection from Thal areas	Pakistan	16.5	Small
Noor-91	Selection from Flip 81-293 C (ILC191 x ILC 49)	ICARDA Syria	28.2	Bold
CM-2000	Mutant of ILC-195, Gamma rays, 150 Gy.	Pakistan	26.2	Bold
CM-94/99	Mutant of Pb-1, 0.2 % EMS	Pakistan	24.1	Bold
CM-98/99	Mutant of Pb-1, 0.2 % EMS	Pakistan	33.4	Extra-bold
CM-315/99	Mutant of Pb-1, 0.2 % EMS	Pakistan	24.4	Bold
PKV Kabuli 2	ICCX-870026-BP-BP-14P-BP-BP (ICCV2 x Surutato 77) x ICC7344.	India	40.0	Extra-bold
ILC-195	Krasvadar territory 1286	USSR	28.2	Bold

Protein profiling using SDS-PAGE: Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970). Equal quantities of proteins (150 micro grams) from each sample along with protein molecular weight marker (SM0441, Fermentas) 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 De-staining 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.

Results

Seed storage proteins were resolved in to 20 to 22 detectable peptides on SDS-PAGE (Fig. 1). Polymorphism was observed in three variable regions i.e., high, medium and low molecular weight. Molecular weights of peptides ranged from 108 kDa to 25 kDa (Table 2). These proteins were indicated as Kabuli Seed Storage Proteins (KSSP) followed by their molecular weight.

Performance of seed protein profiling for exploring genetic diversity showed that genotype ILC-195 CM-2000 and CM-98/99 were comparatively more divergent from other kabuli chickpea germplasm. Maximum variability was found in ILC-195 and CM-2000.

Dendrogram based on electrophoretic data clustered the genotypes in 3 groups at 80% homology (Fig. 2). Three genotypes i.e., Pb-1, CM-94/99 and PKV Kabuli 2 with maximum homology in seed storage proteins were grouped together in cluster 1. These genotypes showed 100% homology and therefore could not be separated on the bases of seed storage proteins. Genotype CM-98/99 and Noor-91 showed 96% homology with each other and CM-98/99 was 90% similar with these genotypes. These three genotypes grouped into 2nd cluster, which was 77% homologous to other two clusters. Two genotypes ILC-195, and CM-2000 were grouped into 3rd cluster with 87% homology.

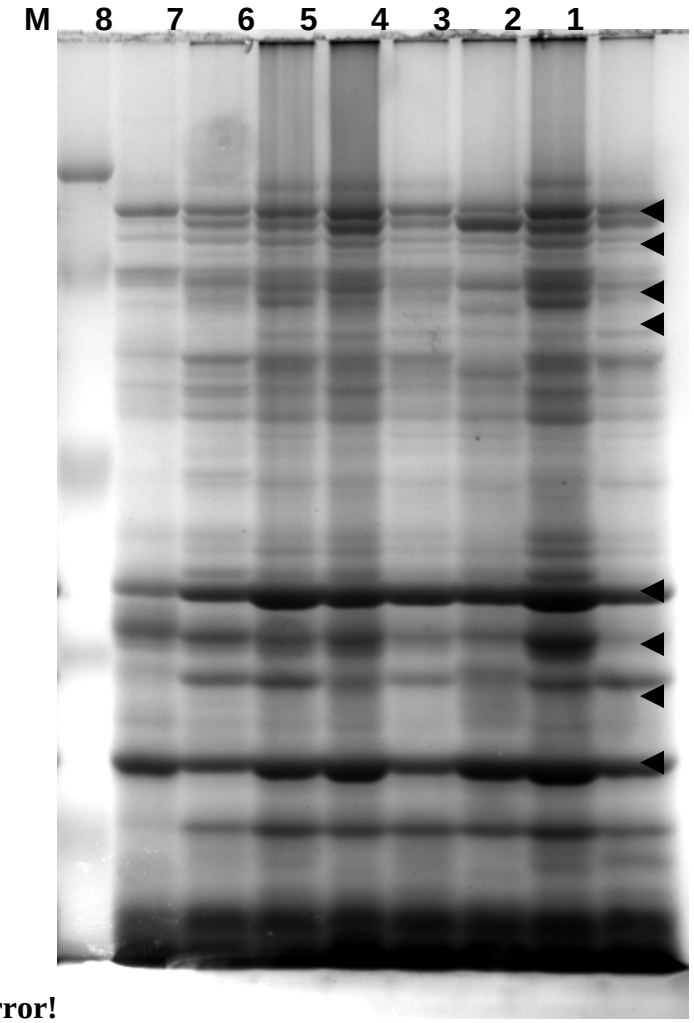

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Fig. 1. Seed storage protein profiles of kabuli chickpea genotypes using SDS-PAGE. **1**: Pb-1, **2**: Noor-91, **3**: CM-2000, **4**: CM-94/99, **5**: CM-98/99, **6**: CM-315/99, **7**: PKV Kabuli 2, **8**: ILC-195 **M**: Protein molecular weight marker SM0441 (Fermentas).

Protein profiles of CM-98/99 showed the presence of protein marker (Kabuli Seed Storage Protein with mol. wt. of 100 kDa) KSSP-100 instead of KSSP-102 (Table 2). This protein marker differentiates CM-98/99 from other genotypes and thus can be used for genotype identification.

In ILC-195, two-protein markers viz., KSSP-78, KSSP-33 differentiate it from other genotypes but these markers co-exists in its mutant CM-2000. Thus this protein marker provides evidence for ILC-195 to be a parent of CM-2000. However 3rd protein marker KSSP-93 was absent only in ILC-195 and differentiates it from all other genotypes including its mutant. This protein marker can be used for identification of ILC-195 and also for its demarcation from its mutant CM-2000.

Table 2. Molecular weights of resolved peptides indicated as kabuli Seed Storage Proteins (KSSP) followed by their molecular weight

Marker	ILC-195	PKV Kabuli 2	CM-315/99	CM-98/99	CM-94/99	CM-2000	Noor-91	Pb-1
	Molecular weight (kDa)							
KSSP-108	108	108	108	108	108	108	108	108
KSSP-102	------	**102**	**102**	------	**102**	**102**	**102**	**102**
KSSP-100	------	------	------	**100**	-------	------	------	------
KSSP-96	96	96	96	96	96	96	96	96
KSSP-93	------	**93**	**93**	**93**	**93**	**93**	**93**	**93**
KSSP-87	**87**	**87**	**87**	**87**	**87**	------	**87**	**87**
KSSP-83	83	83	83	83	83	83	83	83
KSSP-79	------	**79**	**79**	**79**	**79**	------	**79**	**79**
KSSP-78	**78**	------	------	------	------	**78**	------	------
KSSP-72	72	72	72	72	72	72	72	72
KSSP-67	**67**	**67**	**67**	**67**	**67**	------	**67**	**67**
KSSP-64	------	------	------	------	------	**64**	------	------
KSSP-61	61	61	61	61	61	61	61	61
KSSP-56	56	56	56	56	56	56	56	56
KSSP-45	45	45	45	45	45	45	45	45
KSSP-41	41	41	41	41	41	41	41	41
KSSP-39	39	39	39	39	39	39	39	39
KSSP-38	38	38	38	38	38	38	38	38
KSSP-36a	**36.8**	36.8	-------	------	36.8	36.8	------	36.8
KSSP-36b	-------	------	**36.0**	36.0	------	------	36.0	------
KSSP-34a	**34.7**	34.7	------	------	34.7	34.7	------	34.7
KSSP-34b	-------	-------	**34.0**	34.0	-------	------	34.0	------
KSSP-33	**33.3**	-------	------	------	-----	33.3	-----	-----
KSSP-32a	------	**32.6**	32.6	-------	32.6	------	------	32.9
KSSP-32b	------	------	------	**32**	-------	-----	32	------
KSSP-31	31	31	31	31	31	31	31	31
KSSP-30	30	-------	30	30	-------	30	30	------
KSSP-29		**29.0**			**29.0**			**29.0**
KSSP-26	26	26	26	26	26	26	26	26
KSSP-25	25	25	25	25	25	25	25	25

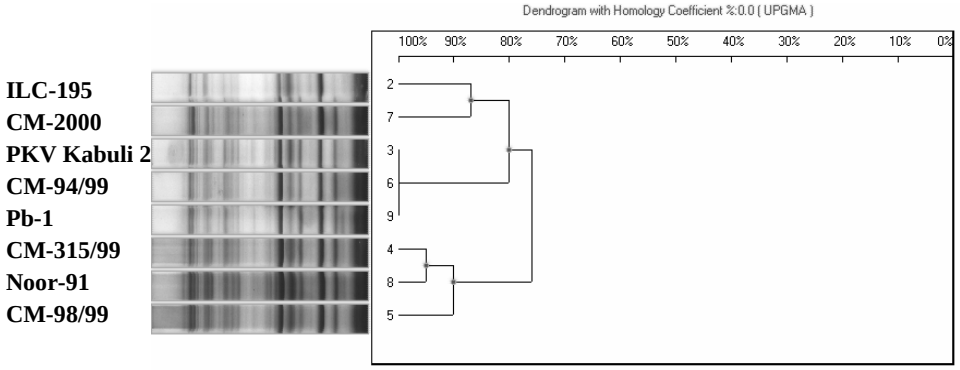

Fig. 2. Kabuli chickpea genotypes grouped in to different clusters based on polymorphism in seed storage proteins.

Genotype CM-2000 was closest to ILC-195, which is its parent as it shares protein markers KSSP-78 and KSSP-33 as mentioned above. However a unique protein marker KSSP-64 was present in CM-2000. This marker not only discriminates CM-2000 from its parent ILC-195 but also from all other genotypes. KSSP-64 thus can be used for identification of CM-2000. This also provides evidence that CM-2000 is a mutant of ILC-195 with detectable changes in seed storage proteins and is not as such induction after adaptability trails. Actually CM-2000 is an EMS induced mutant of ILC-195.

Two more genotypes i.e., CM-315/99 and Noor-91 can also be identified using combination of protein markers. Two protein markers, KSSP-36b and KSSP-34b were expressed only in CM-315/99, Noor-91 and CM-98/99. As mentioned above, CM-98/99 can be differentiated from other genotypes by protein marker KSSP-100. Remaining two genotypes can be identified by two markers. Protein marker KSSP-32a was present in CM-315/99 while its variant 32b was present in Noor-91. Combination of these protein markers thus can be used for identification of CM-315/99 and Noor-91.

Collectively, seed storage protein profiling using SDS-PAGE has the potential to make a distinction between parents and mutants. Clustering based on seed storage protein profiles provides information about the phylogenetic relationship of genotypes. As the genotypes, ILC-195 CM-2000 and CM-98/99 have at least one or more unique seed storage protein marker that can separate them from one another and also from other kabuli genotypes used in the study. Two other genotypes CM-315/99 and Noor-91 can be identified using combination of protein markers.

Discussion

Protein electrophoresis is a powerful tool for population genetics (Parker *et al*., 1998). As storage proteins are not affected by environmental fluctuations, their profiling using SDS-PAGE technology is particularly considered as a reliable tool for economic characterization of germplasm (Javid *et al*., 2004; Iqbal *et al*., 2005).

Comparisons of seed storage proteins have been found to provide no biological basis for separating closely related small and large seeded lentils (Ladizinsky, 1979). Similar was true for chickpea genotypes evaluated in the present study as no difference in seed storage proteins of bold and small seeded genotypes was observed.

Cluster analysis based on SDS-PAGE has proved to be a powerful tool for differentiating *Vigna radiata* and *Vigna mungo*, whereas a low level of inter-specific genetic diversity was observed and no clear differentiation was recorded either for agronomic characteristics or origin as various clusters consisted mixed genotypes from different origins (Ghafoor *et al*., 2002). Similarly in present study no clear differentiation was recorded for origin of genotypes based on seed storage proteins.

Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species (Jha & Ohri, 1996; Mennella *et al*., 1999). However, only few studies indicated that cultivar identification was not possible with the SDS-PAGE method (Ahmad & Slinkard, 1992; De Vries, 1996). The SDS-PAGE is considered to be a practical and reliable method for species identification (Gepts, 1989). In present study identified protein markers, can be used for identification of three genotypes i.e., ILC-195, CM-2000 and CM-98/99. Further these protein markers showed the potential for differentiating the induced mutants from their parents. Two other genotypes CM-315/99 and Noor-91 can be identified using combination of identified protein markers.

In conclusion, 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. Moreover seed storage protein based markers can be used for identification of genotypes i.e., ILC-195, CM-2000, CM-98/99, CM-315/99 and Noor-91 in future. It is suggested that genotypes with similar banding patterns should be further characterized by 2-D electrophoresis.

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