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Inter and Intra-specific Variation in SDS-PAGE Electrophoregrams of Total Seed Protein in Chickpea (*Cicer arietinum* L.) Germplasm

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Abstract: Genetic variation in germplasm has an important role in identification of varieties. Electrophoretic patterns of the protein fractions are directly related to the genetic background of the proteins and can be used to certify the genetic makeup. SDS-PAGE is a valid technique increasingly being utilized as an approach for species identification. Each variety or a group of varieties exhibit characteristic protein banding patterns. On the basis of these patterns they can be identified accordingly. Twenty-nine accessions of *Cicer arietinum* (Chickpea) germplasm were analysed for total seed protein profile using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) to ascertain the extent of genetic variation and its geographical distribution. A considerable variation in protein banding pattern was observed which was localised to various geographical regions. Inter-specific variation was more as compared to intra-specific variation.

Key words: Germplasm, SDS-PAGE, chickpea, *Cicer arietinum*, protein, seed

INTRODUCTION

Chick pea or "Channa" (*Cicer arietinum L.)* is an important legume crop. It is valued for its nutritive seed with high protein content 25.3-28.9% after dehulling (Hulse, 1991). In Pakistan among legumes Chickpea is the most important crop cultivated under rain fed areas of Thal desert. The area under cultivation is 10,779,000 ha which is three times of other pulses (Anonymous, 1999). Increase in yield could be attained by the use of germplasm /wild relatives, for new combination of favourable genes already existing (Muchlbauer *et al.,* 1988). Recent advances in molecular biology have allowed population geneticists to make genetic comparisons across species as well as within species. Such molecular information has proven to be an important tool in systematics and in reconstructing phylogenies. (Avise, 1994).

Identification of chickpea cultivar using PAGE of storage seed proteins was done by Singh *et al.* (1991). They had extracted the proteins soluble in sodium chloride from mature seeds of nine chickpea cultivars and analysed by non-denaturing PAGE. Most cultivars had 2-3 major bands and a variable number of minor bands. They also analysed the G1 protein fraction by non-denaturing PAGE and SDS-PAGE. They concluded that the combined use of above three methods were effective of cultivar identification. Genetic relationship in the genus *Cicer* L. was revealed by polyacrylamide gel electrophoresis of seed storage proteins (Ahmad *et al.*, 1992). Total seed storage proteins of the cultivated, *C. arietinum* and eight other wild annual species were separated and compared by SDS-PAGE. The seed protein profile was a conservative and species–specific trait.

Kharkwal (1999) conducted another study to find intra-specific relationships in *Cicer arietinum*. Electrophoretic analyses were conducted on seed protein extracts of four chickpea varieties. The results indicated that the desi and kabuli types were varieties of the same species. Additionally no evidence was obtained to indicate that kabuli types should be classified as *Cicer kabulicum*. All four varieties were highly homologous; the number of bands resolved were 9 in each case and the pattern was also the same with the exception of minor differences in position and the distance of bands.

Phylogenetic differentiation and geographical distribution of genetic variation for total seed protein in two hundred and seventeen accessions of *Setaria italica* from Europe and Asia were investigated by SDS-PAGE (Afzal *et al.*, 1994). Total seed storage protein electrophoregrams were characterized and classified into six types which are distributed in different geographical regions of the world.

Another important study of seed protein profile of forage sorghum (*Sorghum bicolor* L. var. Moench) was done by Chauhan *et al.* (2002). Twelve varieties of forage sorghum were analysed by SDS-PAGE for identification or characterization on the basis of protein bands. All

varieties could be distinguished on the basis of presence/absence of specific band, but HC-171 and HC-308 had same number of bands and could only be differentiated on the basis of intensity/thickness of similar bands. The molecular weight of protein was ranged between 5 KDa to 70 KDa. The total number of bands observed was 22, out of which band numbers 10,12, 21 and 22 were common in all the varieties. Band number 2 was present only HC-136, PC-6, PC-1, CSV-15 and MP-Chari. Band number 8 was present in PC-23, PC-121, PC-9B while number 5 was exhibited by PC-1 and band number 4 by MP-Chari and HC-260. Each variety or a group of varieties exhibited characteristic banding pattern on the basis of which they can be identified or classified accordingly.

The objective of the present study was to determine the geographical distribution of genetic variability by protein banding pattern. In order to achieve this objective the following study was performed: 1) electrophoretic detection and characterization of genetic diversity in *Cicer arietinum* accessions, 2) ascertain inter and intra specific variation in Chickpea germplasm.

MATERIALS AND METHODS

Plant material: The study was conducted at Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad. Twenty-nine accessions of Chickpea were selected on the basis of geographical distribution. These accessions were collected from the gene bank, PGRI. The species and the origin of the accessions are shown in Table 1. The seed colour, seed shape and texture of the testa are given in Table 2.

Preparation of seed sample: For extraction of protein, individual seeds were ground to fine powder with mortar and pestle. To extract protein in 0.01 g of seed flour, 400 µl of the protein extraction buffer (0.05 M Tris-HCl, 0.2% SDS, 5 M Urea and 1% ß-mercaptethanol) was added to the tube and mixed well by vortex. Then centrifuged at 15,000rpm for 5 min at room temperature. The extracted crude proteins were recovered as clear supernatant and stored at -20°C.

Preparation of gel: Seed proteins were analysed through slab type SDS-PAGE followed by Laemmli (1970) using 11.25% polyacrylamide gel. Electrophoresis was carried out at 100v for two and half hours. In order to check reproducibility of the method two separate gels were run under similar electrophoretic conditions. After electrophoresis gels were stained with 0.2% (w/v) Coomassie brilliant blue R250 for about 1 hour than destained over night on a gyratory shaker. After that gel was dried using gel drying processor and analysed.

Table 1: List of accessions used for protein variation

Accession No.	Plant species	Origin
PAK0052505	Cicer arietinum	Local
PAK0052521	Cicer arietinum	Local
PAK0052894	*Cicer macracantha*	Local
PAK0052897	*Cicer macracantha*	Local
PAK0052900	*Cicer macracantha*	Local
PAK0052901	*Cicer macracantha*	Local
PAK0052906	*Cicer macracantha*	Local
PAK0052907	*Cicer macracantha*	Local
PAK0052908	*Cicer macracantha*	Local
PAK0052910	*Cicer macracantha*	Local
PAK0052914	*Cicer macracantha*	Local
PAK0052916	*Cicer macracantha*	Local
PAK0053229	*Cicer arietinum*	Iran
PAK0053239	*Cicer arietinum*	Iran
PAK0053250	*Cicer arietinum*	Italy
PAK0053257	*Cicer arietinum*	Mexico
PAK0053287	*Cicer arietinum*	Jordan
PAK0053300	*Cicer arietinum*	Pakistan
PAK0053362	*Cicer arietinum*	India
PAK0053406	*Cicer arietinum*	Iran
PAK0053436	*Cicer arietinum*	Iran
PAK0054299	*Cicer arietinum*	Iran
PAK0054300	*Cicer arietinum*	Turkey
PAK0054301	*Cicer arietinum*	Turkey
PAK0054302	*Cicer arietinum*	Morocco
PAK0054303	*Cicer arietinum*	Morocoo
PAK0054304	*Cicer arietinum*	Exotic species
PAK0054305	*Cicer arietinum*	Exotic species
PAK0054306	*Cicer arietinum*	Exotic species

Table 2: Seed characters of chickpea germplasm

Accession No.	Seed colour	Seed shape	Testa texture
PAK0052505	Light brown	Pea shape	Smooth
PAK0052521	Off white	Pea shape	Smooth
PAK0052894	Black	Angular	Rough
PAK0052897	Black	Angular	Slightly rough
PAK0052900	Black	Angular	Slightly rough
PAK0052901	Dark brown	Angular	Slightly rough
PAK0052906	Dark brown	Pea shape	Rough
PAK0052907	Brown	Pea shape	Rough
PAK0052908	Brown	Angular	Smooth
PAK0052910	Dark brown	Angular	Rough
PAK0052914	Black	Irregular	Smooth
PAK0052916	Black	Irregular	Smooth
PAK0053229	Greenish yellow	Angular	Rough
PAK0053239	Off white	Brain shape	Rough
PAK0053250	Black	Angular	Rough
PAK0053257	Yellowish brown	Angular	Rough
PAK0053287	Yellowish brown	Pea shape	Smooth
PAK0053300	Yellowish brown	Pea shape	Smooth
PAK0053406	Light brown	Angular	Rough
PAK0053412	Blackish brown	Angular	Rough
PAK0053436	Black	Angular	Rough
PAK0054299	Off white	Pea shape	Slightly rough
PAK0054300	Off white	Brain shape	Smooth
PAK0054301	Off white	Pea shape	Smooth
PAK0054302	Off white	Brain shape	Rough
PAK0054303	Off white	Pea shape	Smooth
PAK0054304	Off white	Pea shape	Rough
PAK0054305	Off white	Pea shape	Smooth
PAK0054306	Off white	Pea shape	Rough

RESULTS AND DISCUSSION

Twenty-nine samples of Chickpea were evaluated for inter and intra-specific variation in seed storage protein. Results were obtained from the analysis of electrophoregrams and zymograms (Fig. 1, 2, 3). Eighteen bands were observed in total and accessions were classified on the basis of presence/absence and thickness/intensity of specific protein band. All accessions were classified into five clusters (Table 3) on the basis of similarity in banding pattern.

Cluster 1 included nine local wild accessions. These accessions were lacking minor band numbers 1, 2, 3, 4, 6, 7 and 9 and major band numbers 11, 13 and 14. Cluster 2 included only one local accession. This accession had less number of protein bands as compared to other wild accessions. Band numbers 5 and 8 were considerably thin. Accessions in this cluster were lacking those bands, which were absent in cluster 1. In addition to that band numbers 15, 16 and 17 were also absent. Cluster 3 included only two accessions. Both of these belonged to Iran. Band number 15 was absent and all other bands were present in these accessions. The major band numbers 6, 7, 10, 11, 12, 17 and 18 were thick. Cluster 4 included eleven accessions, two from Iran, two local, one from each Italy, India, Jordan, Mexico and Pakistan and two were exotic species. Accessions in this cluster were lacking a major band number 15. Cluster 5 included six accessions,

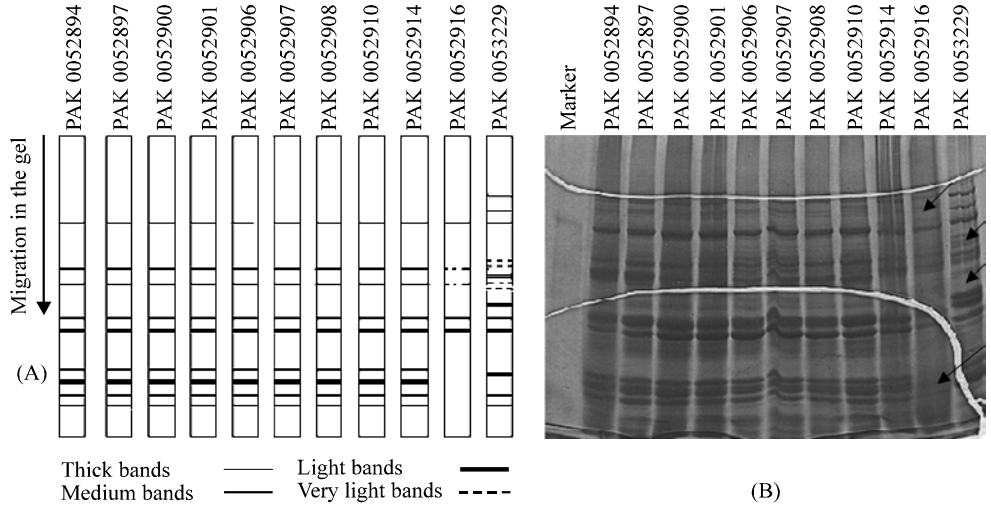

Fig. 1: Zymogram (A) and electrophoregrams (B) showing variation of total seed protein in Chickpea (*Cicer arietinum*) accessions: cluster 1, 2 and 4

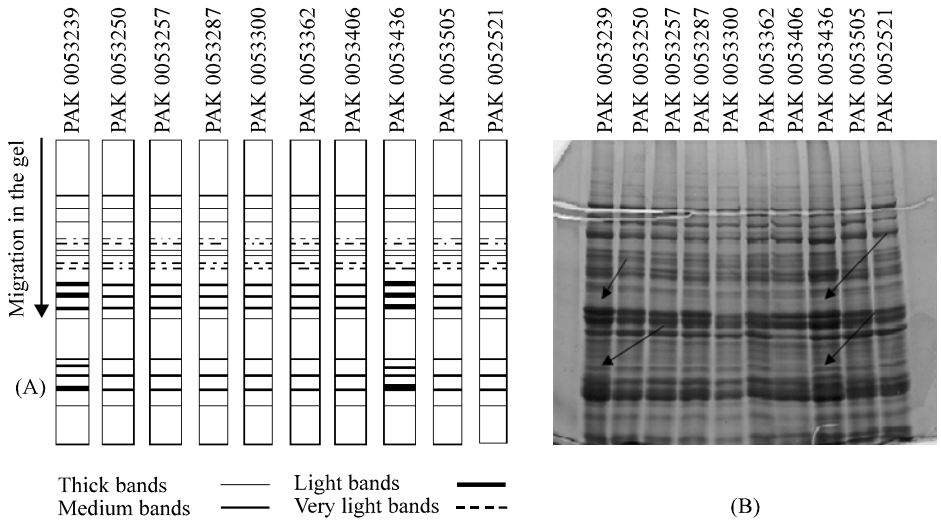

Fig. 2: Zymogram (A) and electrophoregram (B) of total seed protein of chickpea (*Cicer arietinum*) accessions showing variation in cluster 3 and 4

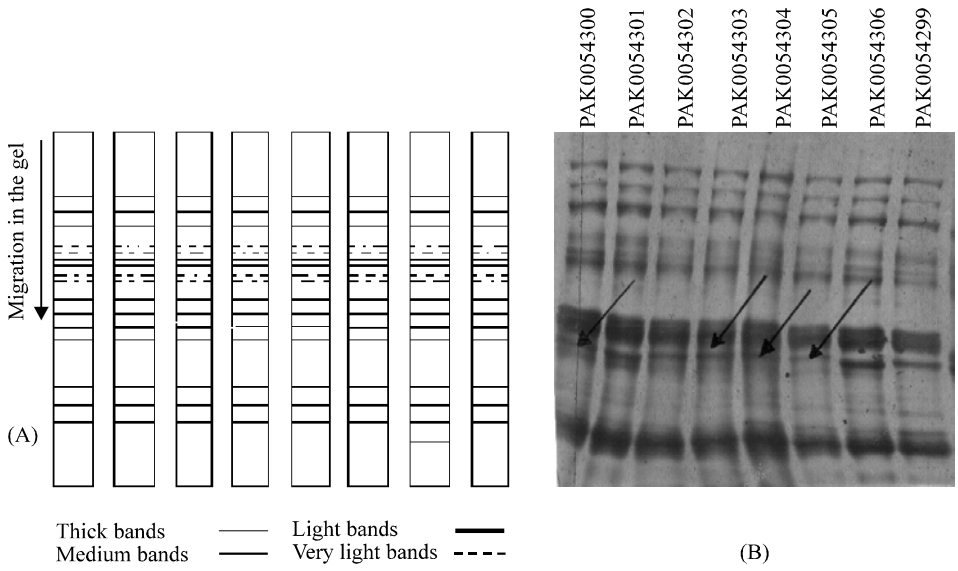

Fig. 3: Zymogram (A) and electrophoregram (B) showing variation in accessions of Chickpea germplasm cluster 4 and 5

Table 3: Grouping of accessions on the basis of similarity in banding patterns

Cluster No.	Accession No.	Origin
1	PAK0052894	Local
1	PAK0052897	Local
1	PAK0052900	Local
1	PAK0052901	Local
1	PAK0052906	Local
1	PAK0052907	Local
1	PAK0052908	Local
1	PAK0052910	Local
1	PAK0052914	Local
2	PAK0052916	Local
3	PAK0053239	Iran
3	PAK0053436	Iran
4	PAK0053229	Iran
4	PAK0053252	Italy
4	PAK0053257	Mexico
4	PAK0053287	Jordan
4	PAK0053300	Pakistan
4	PAK0053362	India
4	PAK0053406	Iran
4	PAK0052505	Local
4	PAK0052521	Local
4	PAK0054305	Exotic species
4	PAK0054306	Exotic species
5	PAK0054299	Iran
5	PAK0054300	Turkey
5	PAK0054301	Turkey
5	PAK0054302	Morocco
5	PAK0054303	Morocco
5	PAK0054304	Exotic species

two from Morocco, two from Turkey, one from Iran and one accession was exotic. All these accessions had major band number 12 considerably thin and lacking band number 15 (Fig. 1, 2, 3, Table 3).

Germplasm is vital source in generating new plant types having desirable traits that helps in increasing crop quality and production as well, thus improves level of human nutrition. Genetically heterogeneous populations produce more and stable yield than genetically homogenous lines.

Inter and intra-specific variation in SDS-PAGE electrophoregrams of total seed protein in wheat, barley and their wild relatives was detected by Masood *et al.* (1994). One hundred and eight accessions of *Triticum*, *Aegilops* and *Hordeum* species collected from Central Asia were evaluated for inter and intra-specific variation. A wide range of genetic variation was observed in the number and electrophoretic mobilities of high molecular weight glutenin and hordein subunits. It was suggested that electrophoresis of total seed protein was a useful and effective method to analyse genetic variability present in plant genetic resources. Our findings in Chickpea agreed with the observations of Masood *et al.* (1994). Similarly Kharkwal conducted another experiment to study intraspecific relationships in *Cicer arietinum* in 1999. He found that electrophoretic analysis conducted on seed protein extracts of four chickpea varieties indicated that the desi and kabuli types were varieties of the same species.

Keeping in view importance of variation in protein banding pattern our results showed that wild accessions contain less number of protein bands as compared to cultivated accessions. Wild accessions lack major band numbers 11, 13 and 14 and minor band numbers 1, 2, 4, 6, 7 and 9. Where as these bands were observed in all the cultivated accessions belonging to different geographical regions. This may be due to the reason that these bands were either added up due to extensive cross breeding or in the process of evolution. Our findings are similar with

the results of Chauhan *et al.* (2002) where they had identify the different varieties of sorghum on the basis of intensity and presence and absence of specific protein bands.

Accessions, which were placed in one cluster, may or may not be similar. Since the appearance of bands on gel can be influenced by several factors which prevent accurate assessment of band homology among genotypes. Proteins coded by different genes may have similar motilities and produce over lapping bands.

It can be concluded from the results that inter specific variation is more as compared to intra specific variation and genetic variability in chickpea germplasm is not associated with its origin as previously observed by Kharkwal (1999) in his study on desi and kabuli chickpea.

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