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GENETIC DIVERSITY AND GEOGRAPHIC RELATIONSHIP AMONG LOCAL AND EXOTIC CHICKPEA GERMPLASM

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Abstract

The chickpea (*Cicer arietinum* L.) germplasm, comprising of 118 accessions were evaluated for total seed protein, using SDS-PAGE. Both local and exotic germplasm were used for elaborating genetic diversity and geographic relationships of the accessions. Seed protein profile revealed that variations in major bands were there in accessions 52352, 52530, 52607, 52672, 52670 and 53484. Where as variation in minor bands were visible in most of the accessions. Cluster analysis revealed that the recorded genetic diversity was actually due to the analyses of different gene pools, which might be due to varying degree of out breeding. Geographic relationship based on total seed protein profiles provided clues of introduction of the same germplasm in different areas and transgression of genes into different landraces.

Introduction

The knowledge of genetic diversity is a useful tool in gene-bank management and breeding experiments like tagging of germplasm, identification and/or elimination of duplicates in the gene stock and establishment of core collections. Another practical application of the knowledge of genetic diversity is its use in sorting of populations for genome mapping experiments (Kaga *et al*., 1996). Characterization of germplasm using biochemical fingerprinting has got special attention due to its increased used in crop improvement and the selection of desirable genotypes for breeding crops. The use of genetic markers and protein profiling is also successfully used to resolve the taxonomic and evolutionary problems of several crop plants (Ladizinsky & Hymowitz, 1979; Murphy *et al*., 1990; Khan, 1990; Nakajima, 1994; Das and Mukarjee, 1995; Ghafoor *et al*., 2002). Genetic diversity of seed storage proteins has been reported for many crops; *Lima* bean (Lioi *et al*., 1999), *Phaseolus vulgaris* (Ferreira *et al*., 2000) and Chickpea (Ghafoor *et al.,* 2003). Ahmad and Slinkard (1992) reported phylogenetic relationship among Cicer species based on SDS-PAGE data and suggested *Cicer reticulatum* is the wild progenitor of cultivated chickpea. The basic criterion of phylogenetic relationship is the gene homology, which in many cases cannot be measured directly because of reproductive barriers between species. The seed storage protein analyses helps in identification and characterization of diversity in crop varieties, cultivars and their wild varieties but also elaborate genetic transgression and phylogenetic relationship of the accessions. It is also known that variation in protein bands elaborate the relationship among the collection from various geographical regions (Satija, 2002; Ghafoor *et al*., 2003 and Asghar *et al*., 2003).

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Keeping in view the importance of chickpea and seed protein profiling, the present experiments were conducted to: (1) conduct Cluster Analysis for sorting out the accessions obtained from the same locality, (2) elaborate the geographical relationship of accessions obtained from different sources.

Materials and Methods

Sum 118 accessions of *Cicer arietinum* L. both local and exotic were evaluated with SDS-PAGE markers. Single seed of each accession was ground to fine powder with mortar and pestle for the extraction of proteins. 400µl of protein extrication buffer (PEB) was added to 0.01 g of seed flour and vortexed (Automatic lab Mixer DH-10) thoroughly to homogenize. For purification the homogenated samples were centrifuged at 15,000 rpm for 10 minutes at room temperature. The extracted crude proteins were recovered as clear supernatant, which was transferred into 1.5 ml Eppendorf tubes and stored at 2º C until it were run on the polyacrylamide gel.

The electrophoretic procedure was carried out using Slab type SDS-PAGE (Model: AE-6530M, Japan), with 12.25% polyacrylamide gel, resolving gel (3.0M Tris-HCl) pH9, 0.4% SDS and 4.5% stacking gel (0.4M Tris-HCl pH 7.0, 0.4% SDS). Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125 % SDS) was added to the top pool of the apparatus. A 15 µl of the supernatant was loaded with the micropipette into the wells of the gel. Apparatus was connected with uninterrupted electric supply (100 V) until the bromophenol blue (BPB) was reached to the bottom of gel plate.

The gels were then stained for an hour with the solution containing 0.2% Commassie Brilliant Blue dissolved in 10% glacial acetic acid, 40% methanol and water in the ratio of 10:40:50. Gels were destained in a solution containing 5% acetic acid and 20% methanol. The excessive CBB was removed by Kimwipe tissue paper from the destaining solution. The destained gels were analyzed either on direct photographic method or by drying the gels on sheets using gel-drying processor for about 2-4 hours. The data was recorded on the basis of presence and absences of protein bands i.e. 1 for the presence and 0 for the absence of bands. The intensity of band was considered as major and minor bands i.e. the high intensity glowing bands as major and low intensity glowing bands as minor. Cluster analysis was carried out using software STATISTICA (www.statsoft.com).

Results

Thirty-three bands were recorded for various accessions, out of which 22 were polymorphic in nature. Panoramically the protein profile differed from one another either in their major or minor bands (Fig. 1). The number of bands is marked with thin arrows for minor and thick solid arrows for major bands. Among polymorphic bands, 17 were minor and 5 were major. Minor polymorphic bands were B1, B2, B3, B7, B9, B11, B13, B15, B16, B17, B18, B24, B25, B26, B27, B28 and B29; whereas major polymorphic bands were B5, B14, B23, B30 and B31. The presence and absence of protein bands for each cluster is clear from Table 1. The cluster 7, 8, 10 and 11 comprised of the accessions with maximum bands, whereas cluster 2, 4 and 13 were with minimum bands. Cluster analysis sorted the accessions into two major lineages linkage distance 3.4 (2 L-3.4) and if tree is observed critically at linkage distance of 2.0, the two lineages were further divided into 20 clusters (20 C-2.0), Lineage first consists of single cluster, with one accession (52607) that was colleted from Faisalabad, Punjab while lineage second

comprises of 19 clusters (Fig. 2). Among the lineage second; cluster 2, 3, 4, 9, 13, 16, 17, 18, and 19 consists of single accession in each case, while cluster 5, 8 and 11 consist of 2 accessions each, cluster 6, 7 and 12 comprises of 4 accessions each, cluster 10 has 8 accessions, cluster14 consists of 47 accessions, cluster 15 have 32 and last cluster 20 consists of 3 accessions (Table 2).

Cluster 1, consisting of accession 52607 (Punjab Faisalabad), showed the lowest degree of Euclidian distance and highest degree of linkage distance. This accession showed utmost level of variation and individuality in their banding outline from both local and exotic accessions. Second lineage consists of both local and exotic accessions, Cluster 2, 3 and 4, encircle both Pakistani and USA accessions having the highest degree of variation within lineage second, (lowest degree of Euclidian distance and highest degree of linkage distance). Cluster 6, 9, 16 and 17 also showed the lowest variation within the lineage second having high degree of Euclidian distance. Cluster 5 and 8, consisting of 5 accessions obtained from USA, showed close relation to the local accessions, having nearly the same Euclidean distance. Cluster 7, 11, 12 and 20 consists of local accessions; cluster 15 sorting only exotic (USA) accessions, which indicated that SDS-PAGE could group the accession on the basis of geographic origin.

Discussion

Protein electrophoresis is a powerful tool for population genetics (Parker *et al*., 1998) and the SDS-PAGE technology is particularly considered as a reliable way because storage proteins are largely independent of environmental fluctuations (Javid *et al*., 2004; Iqbal *et al*., 2005). Biochemical markers assess accurate genetic diversity index (Akhtar, 2001; Rabbani *et al.*, 2001). Our findings revealed that considerable intra-specific variation was available in the analyzed accessions. The variation in major bands was available in accessions 52352 (Punjab, Bhakkar), 52530 (Punjab, Bhakkar), 52607 (Punjab, Faisalabad), 52872 (Punjab, Attock), 52970 (Punjab, Layyah), 53070 (USA) and 53484 (USA), whereas majority of the accessions showed diversity in banding pattern, for minor bands. Our results do not conform the findings of Mehrani (2002) and Ghafoor *et al*. (2003), who reported low intra–specific diversity for seed protein in pea and chickpea. The contradiction is apparently due to use of diverse genepools both from the local and exotic sources.

Our conclusions based upon the analyses of results are, partial geographic relationship is established among some accessions e.g. accessions in cluster No. 14, other germlines clustered distantly like cluster No. 15, and other had local affinities e.g. cluster No. 20. All these information are available in Table 2 and its phylogenetic relationship is given in Figure 2. The results also show that all the germplasm originated from the same stock (Figure 2). It further clarifies that the parameter taken for elaborating the genotypic relationship is very stable and is least affected by latitudinal or altitudinal change, and/or environmental stresses. Sorting of the accessions from different locality in the same cluster might be due to introduction and genetic transgression.

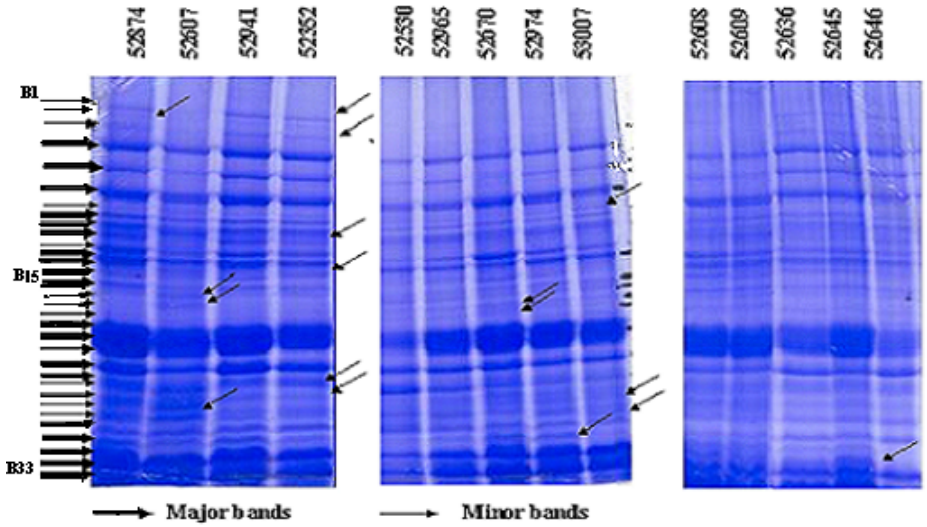

Fig. 1. Electrophoregram of 14selected accessions of chickpea, showing polymorphic protein bands

Table 1. Number of bands present/absent within 20 clusters of chickpea germplasm

Clusters	Bands present	Bands absent
Cluster 1	26	7
Cluster 2	17	16
Cluster 3	21	12
Cluster 4	15	18
Cluster 5	27	6
Cluster 6	27	6
Cluster 7	30	3
Cluster 8	31	2
Cluster 9	21	12
Cluster 10	30	3
Cluster 11	31	2
Cluster 12	22	11
Cluster 13	18	15
Cluster 14	20	13
Cluster 15	20	13
Cluster 16	28	5
Cluster 17	21	12
Cluster 18	26	7
Cluster 19	29	4
Cluster 20	29	4

Table 2. Cluster analysis based on SDS-PAGE

Lineage1	Cluster I	52607 (Punjab, Faisalabad)
Lineage2	19 Clusters	
	Cluster 2	52970 (Punjab, Layyah)
	Cluster 3	52848 (Punjab, Chakwal)
	Cluster 4	53070 (USA)
	Cluster 5	53242 (USA), 53248 (USA)
	Cluster 6	52610 (Punjab, Faisalabad), 53280 (USA), 53256 (USA), 53066 (USA)
	Cluster 7	52646 (Punjab, Faisalabad), 52645 (Punjab, Faisalabad), 52636 (Punjab, Faisalabad), 52651 (Punjab, Faisalabad)
	Cluster 8	52609 (Punjab, Faisalabad), 52608 (Punjab, Faisalabad)
	Cluster 9	583029 (USA)
	Cluster 10	53043 (USA), 53024 (USA), 52974 (Punjab, Layyah), 5300 (USA), 52965 (Punjab, Bhakkar), 52956 (Punjab, Khushab), 52944 (Punjab, Mianwali), 52943 (Punjab, Khushab)
	Cluster 11	52816 (Punjab, Bhakkar), 52809 (Punjab Bhakkar)
	Cluster 12	52872 (Punjab, Layyah), 52941 (Punjab, Khushab), 52874 (Punjab, Layyah), 52805 (Punjab, Jhang)
	Cluster 13	52530 (Punjab, Bhakkar)
	Cluster 14	53535 (Syria), 53534 (Syria), Nroo-91, M-200, Piadar, Parbat, NCS-200, Bettel-98, Dasht, 53536 (Syria), 53533 (Syria), 53532 (Syria), 53530 (Syria), 53526 (Syria), 53525 (Syria), 53524 (Syria), 53523 (Syria), 53522 (Syria), 53521 (Syria), 53520 (Syria), 53517 (Syria), 53490 (USA), 53501 (USA), 53500 (USA), 53498 (USA), 53497 (USA), 53496 (USA), 53495 (USA), 53494 (USA), 53493 (USA), 53492 (USA), 53491 (USA), 53466 (USA), 53467 (USA), 53470 (USA), 53510 (USA), 53509 (USA), Punjab, 53506 (USA), 53508 (USA), 53505 (USA), 53504 (USA), 53503 (USA), 53469 (USA), 53468 (USA), 53464 (USA), 53463 (USA)
	Cluster 15	53489 (USA), 56484 (USA), 53483 (USA), 53482 (USA), 53481 (USA), 53488 (USA), 53487 (USA), 53486 (USA), 53485 (USA), 53479 (USA), 53478 (USA), 53476 (USA), 53475 (USA), 53477 (USA), 53473 (USA), 53474 (USA), 53472 (USA), 53471 (USA), 53454 (USA), 53453 (USA), 53452 (USA), 53451 (USA), 53459 (USA), 53458 (USA), 53457 (USA), 53455 (USA), 53443 (USA), 53448 (USA), 53447 (USA), 53445 (USA), 53446 (USA), 53442 (USA)
	Cluster 16	52161 (Punjab, Unknown)
	Cluster 17	52352 (Punjab, Khushab)
	Cluster 18	52540 (Punjab, Bhakkar)
	Cluster 19	52535 (Punjab, Bhakkar)
	Cluster 20	52326 (Sind, Thatta), 52323 (Punjab, Faisalabad), 52048 (Sind, Jacobabad)

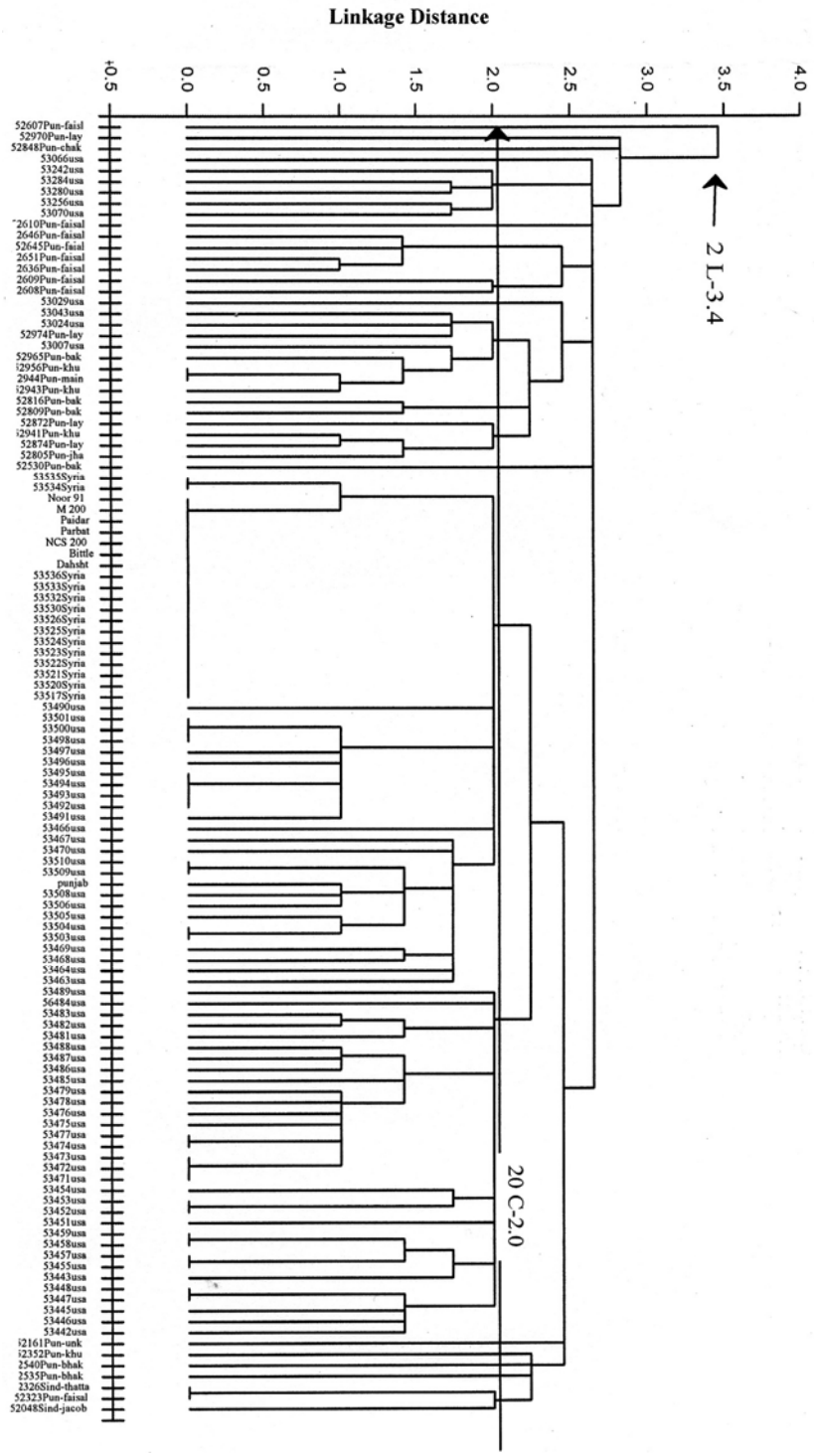

Fig. 2. Phylogenetic tree based on SDS-PAGE analysis showing average linkage in chickpea germplasm.

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.

Akhtar, M. 2001.*Phylogenetic relationship among Vigna species based on agronomic and biochemical analysis*. M. Phil. Thesis, Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan, 99pp.

Asghar, R., T. Siddique and M. Afzal. 2003. Inter and intra-specific variation in SDS-PAGE electrophoregrams of total seed protein in chickpea (*Cicer arietinum* L.) germplasm. *Pak. J. Bio. Scie.,* 6(24):1991-1995.

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

Ferreira, J.J., E A. Lvarrez, M.A. Roca and R. Giraldez. 2000. Determination of the out crossing rate of *Phaseolus vulgaris* L. using seed protein markers. *Euphytica,* 113: 259-263.

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.). *Pakistan Journal of Botany.* 35(4): 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. Wileczek based on morphological traits and SDS-PAGE. *Euphytica,* 123: 378-2002.

Iqbal, S.H., A. Ghafoor and N. Ayub. 2005. Relationship between SDS-PAGE markers and *Ascochyta blight* in chickpea. *Pakistan Journal of Botany,* 37:87-96.

Javid, A., A. Ghafoor and R. Anwar. 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pakistan Journal of Botany,* 36: 87-96.

Kaga, A., N. Tomooka, K. Hosaka and O. Kamijima. 1996. Species relationship in the subgenus *Ceratotropis* (genus *Vigna*) as revealed by RAPD analysis. *Euphytcia,* 88: 17-24.

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

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

Lioi, L.F., Sparvoli and R. Bollini. 1999. Variation and genomic polymorphism of lectin-related protein in Lima Bean (*Phaseolus lunatus* L.) seed. *Genetic Resources and Crops Evaluation,* 46: 157-182.

Mehrani, P. 2002. *Genetic diversity in local and exotic pea (Pisaum sativum L.) germplasm for morphological traits and SDS-PAGE markers.* M. Phil. Thesis, Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan, 104pp.

Murphy, R.W., J.W. Sites, D.G. Buth and C.H. Haufler. 1990. Protein isozymes eletrophoresis. In: *Molecular systematic.* D.H. Hillis and C. Moritz (Eds): *Sinnauer Association, Sunderland*, MA pp. 45-126.

Nakajima, K. 1994. Biotechnology for crop improvement in Japan. In: *Biotechnology application in agriculture in Asia and Pacific*, 87-107. Published by Asian Productivity Organization.

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.

Rabbani, M.A., A.A. Qureshi, M. Afzal, R. Anwar and S. Komatsu. 2001. Characterization of mustard [*Brassica junncea* (L.) Cezern. & Cross] germplasm by SDS-PAGE of total seed protein. *Pakistan Journal of Botany*, 33(3): 173-179.

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,* 29:2, 122-135; 21

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