Madras Agric. J., 99 (4-6): 155-157, June 2012
https://doi.org/10.29321/MAJ.10.100035

Wide and Intra-specific Variation in Total Seed Protein in Chickpea (*Cicer arietinum L*.) Hybrids Through SDS-PAGE

Uday Chand Jha[1*] , Dhan Pal Singh[1] and G. Roopa Lavanya[2]

[1]Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture & Technology, Pantnagar - 263 145 [2]Department of Genetics and Plant Breeding, Allahabad School of Agriculture Sam Higginbottom Institute of Agriculture, Technology and Sciences (Formerly Allahabad Agricultural Institute), Deemed-to-be-University, Allahabad - 211 007

In the present study, 30 accessions of chickpea were analyzed for total seed protein profile using Sodium Dodecyl Sulphate-Poly Acraylamide Gel Electrophoresis (SDS-PAGE) to ascertain the extent of genetic variation. Based on dendrogram of SDS- PAGE, genotypes were divided into nine clusters taking 75% similarity as standard. From the clustering pattern of the 30 genotypes, it was clear that cluster III evolved as large cluster comprising 12 genotypes and clusters I, II, VI, VII, VIII and IX comprised single genotype each. Cluster IV and V comprised two and ten genotypes, respectively. The minimum similarity coefficient was 0.11 hence the genotypes, PG 061 and PG 059 were most dissimilar with PG041. It was observed that four genotypes namely PG 047, PG 052, PG 058 and PG 057 had shown similarity coefficient 1.0. Therefore, these genotypes may be considered genetically similar. SDS-PAGE can be used for characterization of cultivars and species, establishment of phylogenetic relationship among taxa and for genetic studies in many crops.

Key words: Chickpea, SDS-PAGE, total seed protein, similarity coefficient

Pulses are the main source of vegetable protein in human consumption and occupies a wide range of agro climatic conditions and it contributes about 25 per cent to the global production (Anonymous, 2005). Chickpea play an important role in rainfed agriculture by improving physical, chemical and biological properties of soil and is considered an excellent crop for natural resource management, environmental security, crop diversification and consequently for viable agriculture. In the crop breeding programme to develop a suitable variety, breeder has to combine different genotypes with desirable characters into one variety. In order to identify true-recombinations, generally morphological markers are used. However, sometimes morphological markers may be influenced by the environment and are usually dominant or recessive and sometimes it is also difficult to find appropriate morphological marker.

In the recent years, SDS-PAGE markers have been used extensively for characterization of cultivars and species, establishment of phylogenetic relationship among taxa and for genetic studies in many crops. SDS PAGE is a valid technique and is increasingly being used an approach for species identification. Each variety or a group of varieties has specific protein banding patterns and on the basis of these patterns, they can be identified accordingly. Thirty accessions of *Cicer arietinum* (Chickpea) germplasm were analyzed for total seed protein profile using Sodium Dodecyl Sulphate - Polyacraylamide Gel Electrophoresis (SDS- PAGE) to ascertain the extent of genetic variation for total seed protein among the genotypes derived from wide and intervarietal crosses of chickpea based on SDS-PAGE.

Experimental material of the present study consisting 30 genotypes including 25 genotypes selected from segrregating generations of wide and intervarietal crosses (Table 1) and five released varieties of chickpea. Thirty entries were planted in a randomized complete block design with three replications during the crop season 2005-07. Row length was kept at 4 m and the spacing between two rows at 30 cm. Recommended package of practices was followed for raising the crop. Genetic relationship of progenies of a wide cross was also studied on biochemical basis using SDS-PAGE as per the standardized procedure for seed protein in chickpea (Singh *et al.,* 1991). The stacking and separating gels, staining and destaining solutions were prepared according the protocol. Jaccard's similarity coefficient was used for calculating for the genetic similarity between progenies and parents (Jaccard, 1908). If this value is 1.00 then progenies are genetically same. If the value between two progenies is 0.00, then the progenies are genetically different.

*Corresponding author email: u9811981@gmail.com

Table 1. List of experimental material used in the present study

Name of genotypes	Parentage
PG039	BG x KPG-59
PG040	BG x KPG-59
PG041	PG92-4 x Avrodhi
PG042	PG92-4 x Avrodhi
PG043	K850(LM) x Avrodhi
PG044	K850(LM) x KPG-59
PG045	K850(LM) x KPG-59
PG046	K850(LM) x KPG-59
PG047	K850(LM) x KPG-59
PG048	BG362 x PG-186
PG049	BG362 x PG-186
PG050	BG362 x PG-186
PG051	BG362 x PG-186
PG052	BG329 x KPG-59
PG053	BG329 x KPG-59
PG054	BG329 x KPG-59
PG055	BG362 x Avrodhi
PG056	BG362 x Avrodhi
PG057	PG92-97 x *C.reticulatum*
PG058	PG92-97 x *C.reticulatum*
PG059	PG92-97 x *C.reticulatum*
PG060	PG92-97 x *C.reticulatum*
PG061	PG92-97 x *C.reticulatum*
PG062	PG92-97 x *C.reticulatum*
PG063	PG92-97 x *C.reticulatum*
PBG1 (check)	GG578 x NEC206
Pusa256 (check)	(JG62x850-3/27) (L550x208)
PantG186 (check)	ILC613 x PantG114
Avrodhi (check)	T3 x K315
Pusa1053(check)	ICCV3 x FLIP88-120

In the present investigation, proteins were extracted from seeds of 30 chickpea genotypes and then separated on the basis of size and electrical charge in a gel matrix by SDS-PAGE. Distribution of 30 genotypes into different clusters is presented in Table 2. The banding pattern of genotypes is presented in Fig.2 and Fig. 3 and Table 3. All 30 genotypes were divided into nine clusters taking 75% similarity as standard based on electrophoretic banding pattern (with respect to number of bands, their thickness and intensity) of SDS- PAGE. Table 2 showed the cluster number and names of genotypes included in each cluster and dendrogram is represented in Fig.1. Cluster III was evolved as a large cluster comprising 12 genotypes. Clusters IV and V comprised of two and ten genotypes, respectively and clusters I, II, VI, VII, VIII and IX comprised single genotype each.

Table 2. Distribution of 30 chickpea genotypes into different clusters based on SDS – PAGE

Cluster No.	No. of genotypes	Name of genotypes
I	1	PG 056
II	1	PG 055
III	12	(PG 044, PG 047, PG 045, PG 052), (PG 058, PG 057), (PG 048, PG 050, PG 051), (PG 062, PG 040, PG 042).
IV	2	(PG 061, PG 059)
V	10	(PG 043, PG 046, PG 039), (PG060, PG 063), (PG 053, PG 054), PBG1, Pusa1053, Avrodhi.
VI	1	PG 049
VII	1	Pant G 186
VIII	1	Pusa 256
IX	1	PG 041

The genotypes with same parentage were PG 058 and PG 057 (PG 92-97 × *C. reticulatum*), PG 048, PG 049 and PG 050 (BG 362 × Pant G 186), PG 060 and PG 061(PG92-97×*C. reticulatum*), PG 060

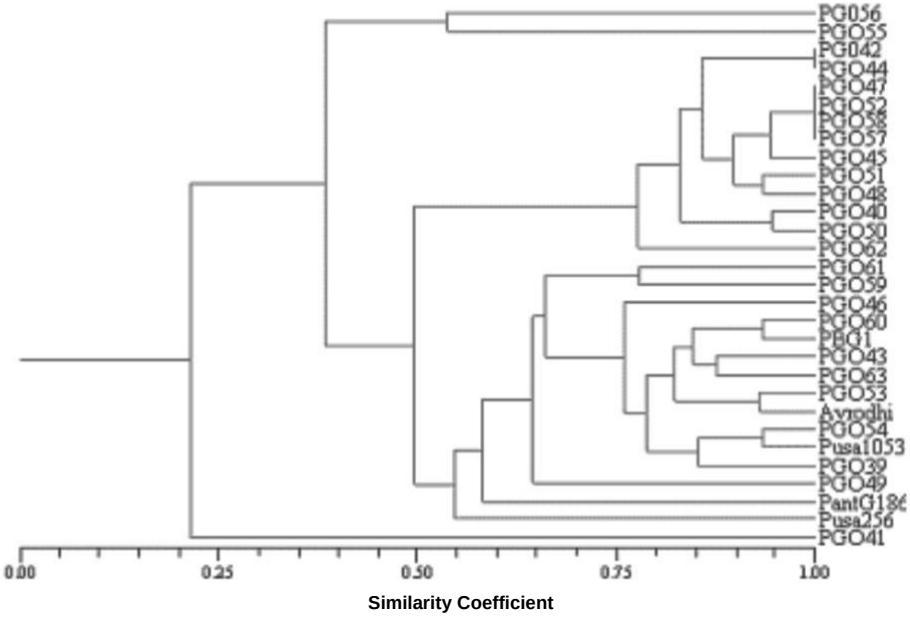

Fig.1 Dendrogram of thirty chickpea genotypes

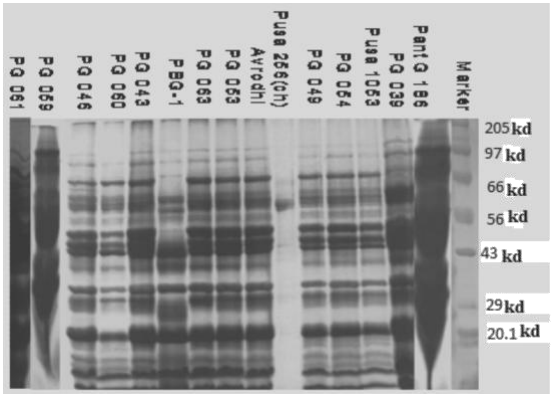

Fig. 2. SDS-PAGE Banding Pattern of Chick Pea type of progenies of a wide cross

and PG 063 (PG 92-97 × *C reticulatum*) and PG 053 and PG 054 (BG 329 × KPG-59) were grouped together in same cluster and in different clusters also. Genotypes PG 043 and PG 046 have one common parent [K 850 (LM)]. PG 039 and PG 046 have also one common parent (KPG-59). The genotypes PG 044, PG 047 and PG 045 had similar

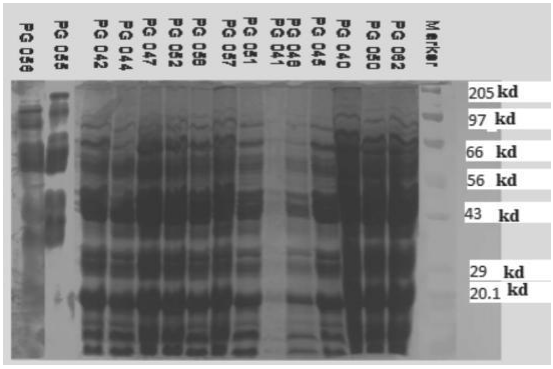

Fig. 3. SDS-PAGE Banding Pattern of Chick Pea type of progenies of a wide cross

parentage (K850 (LM) × KPG-59) and PG 052 had one parent (KPG-59) common with PG 044, PG 047, and PG 045 were grouped together in same cluster *i.e.,* cluster III (Table 2). This may be due to differential recombination of the genes from parents into the different segregating generations. Also this may be due to resolution of limited number of loci for differentiation the genotypes for seed protein electrophoresis (Nisar *et al.,* 2007). The genotypes, PG 055 and PG 056 separated into two different clusters with 75% similarity, inspite of having common parentage (BG362 x Avrodhi).

The minimum similarity coefficient was 0.11 hence the genotypes; PG 061 and PG 059 were most dissimilar with PG041 (Fig. 1). The parents of PG 041 were PG92-4 × Avrodhi and the parents of PG 061 and PG 059 were PG92-97 × *C. reticulatum*. Based on similarity coefficient of genotypes, it was observed that four genotypes namely PG 047, PG 052, PG 058 and PG 057 had shown similarity coefficient 1. Therefore, these genotypes may be considered genetically similar (on the basis of SDS-PAGE). For this reason, all the other 24 genotypes had shown same similarity coefficient with these four genotypes (PG 047, PG 052, PG 057 and PG 058) separately (Hameed *et al.,* 2009).

SDS-PAGE analysis can be used to find out the wild progenitor of different crop species, existence of genetic diversity in germplasm and identification of germplasm and varieties (Ghafoor *et al*, 2002; Ghafoor *et al.,* 2005 and Singh *et al.,* 2004). Protein electrophoresis is also 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 and Iqbal *et al.,* 2005). Therefore, it is concluded that SDS-PAGE analysis is useful tool for a plant breeder for selection and identification of genotypes within a short period of time with greater efficiency.

References

Anonymous, 2005. Hindu: Survey of Indian Agriculture. 54p.

Ghafoor, A., Ahmad, Z., Qureshi, A.S. and Bashir, M. 2002. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) Wilczek based on morphological traits and SDS-PAGE. *Euphytica*, **123**: 367-378.

Ghafoor, A., Ahmad, Z. and Afzal, M. 2005. Use of SDS-PAGE markers for determining quantitative trait loci in blackgram (*Vigna mungo* (L.) Hepper) germplasm. *Pakistan J. Botany*., **37**: 263-269.

Hameed, A., Shah, T.M., Atta, B.M., Iqbal, N., Haq, M.A. and Ali, H. 2009. Comparative seed storage protein profiling of kabuli chickpea genotypes. *Pakisatan J. Botany*, **41**: 703-710.

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

Jaccard, P. 1908. In: An Introduction to Numerical Classification. (Clifford, H.T. and Stephenson, W. eds.). Academic Press, New York. : 54p.

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

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

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

Singh, G., Vig, A.P. and Verma, R. 2004. Varietal identification in paddy (*Oryza sativa* L.) and moong (*Phaseolus mungo* L.) by gel electrophoresis of soluble seed proteins. *J. New Seeds*, **6**: 91-99.

Singh, H.P., Singh, P.V. and Saxena, R.P. 1991. Indentification of chickpea cultivars using SDS- PAGE of storage seed proteins. *Ann. Biol*., **8**: 167-175.

Received: July 14, 2011; Accepted: April 11, 2012