Applied Biological Research 19 (3): 248-256; (2017)
DOI: 10.5958/0974-4517.2017.00037.4

ASSESSMENT OF GENETIC DIVERSITY AMONG CHICKPEA (*Cicer arietinum* L.) GENOTYPES USING SEED STORAGE PROTEIN PROFILING AND RAPD MARKERS

H.J. Maisuria[1], R.M. Patel[2] and K.P. Suthar[3*]

[1]*Department of Agriculture Biotechnology, Anand Agricultural University, Anand - 388 110, Gujarat (India)*
[2]*ASPEE SHAKILAM Biotechnology Institute, Navsari Agricultural University (NAU), Surat - 395 007, Gujarat (India)*
[3]*Department of Plant Molecular Biology and Biotechnology, ASPEE College of Horticulture and Forestry, NAU, Navsari - 396 450 Gujarat (India)*
*e-mail: *kpsbiotech@rediffmail.com*

(Received 2 February, 2017; accepted 22 August, 2017)

ABSTRACT

Seed storage protein profiling and RAPD were used in present study for the analysis of genetic diversity among 15 chickpea genotypes. SDS-PAGE of seed storage protein differentiated 15 genotypes into three major clusters. RAPD analysis was done using highly polymorphic ten primers which amplified 104 bands with 2.70 average polymorphic bands. RAPD analysis reported 24.4 average per cent polymorphism and highest in OPH-03. OPH-19 reported highly informative primer based on PIC and MI value. Similarity coefficients of RAPD data were ranged from 0.55 to 0.95. Dendrogram generated three major clusters which distinguished Fusarium wilt susceptible and resistant genotypes in cluster I and cluster II, respectively, whereas cluster III included both resistant and susceptible genotypes. RAPD marker was found more informative than seed storage protein profiling for diversity analysis. Further, no specific banding was detected that could discriminate resistant from susceptible genotypes critically.

Key words: *Cicer arietinum*, Fusarium wilt, genetic diversity, RAPD, SDS-PAGE, seed storage protein

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important food legume crop after dry bean and pea (Anon, 2015). It is known as 'poor man's meat' that serves as a crucial source of protein for human diet (Barman, 2012). The productivity of chickpea is far below its potential because of less improved variety for cultivation and various biotic and abiotic stresses (Winter *et al.,* 2000). The most destructive is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) (Haware and Nene, 1980) which inflicts high yield loss up to 90% annually (Chaube and Pundhir, 2009).

Morphological traits and biochemica0l markers have been used to establish genetic relationships among the *Cicer* species. The seed storage proteins are non-enzymatic and the sole source of nitrogen and sulphur during germination (Aarif *et al.,* 2015). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage protein is widely used for establishment and characterization of phylogenetic relationship of crop and their wild relatives because of its simplicity and cost effectiveness (Ghafoor *et al.,* 2002; Javid *et al.,* 2004; Iqbal *et al.,* 2005; Nisar *et al.,* 2007). However, these markers are developmental stage-specific, environmentally-influenced and available in limited number. So, there is need to exploit molecular markers for diversity analysis that overcome limitations of conventional markers (Chawla, 2012).

Genetic diversity can easily be studied through DNA based markers in the plant genome. Random amplified polymorphic DNA (RAPD) is one of the most frequently used polymerase chain reaction (PCR) based marker for the analysis of genetic diversity owing to its simplicity, fast analysis and cheaper cost (Williams *et al.,* 1990). Earlier studies have reported the very lower amount of polymorphism within chickpea genotypes (Agrawal and Srivastava**,** 2010; Suthar *et al.*, 2012; Bhagyawant *et al.*, 2015). The polymorphism of chickpea is not sufficiently discriminated by only use of either seed storage protein or RAPD markers. Also, the combined analysis of biochemical and DNA based marker is very less reported for diversity assessment (Devi *et al*., 2014). Hence, present work was planned to assess the genetic diversity in cultivated chickpea accessions using seed storage protein profiling and RAPD. Further, the chickpea genotypes differing in their reaction to wilt reaction was chosen to find any resistance/susceptibility specific polypeptide or DNA amplicon if found.

MATERIALS AND METHODS

Plant material

Fifteen chickpea genotypes differing their reaction to Fusarium wilt disease *viz.,* 6 susceptible genotypes (Cadu-54, L-550, C-104, Shanho, JG-62 and GG-4), 1 intermediate/susceptible genotype (Chaffa), 1 intermediate/resistant genotype (Annigeri), 5 resistant genotypes (K-850, WR-315, BG-212, Surutato-77 and JG-74) and 2 tolerant genotypes (GG-1 and GG-2) were used in present study. These genotypes were obtained from ICRISAT, Hyderabad (India) and Pulse and Castor Research Station, NAU, Navsari (India).

Seed storage protein profiling

Seed storage protein was extracted by homogenizing 200 mg defatted seed powder in 1 mL 50 mM phosphate buffer (pH 7.8) containing 0.1% β-mercapto-ethanol using mortar and pestle. The fine paste was collected in a centrifuge tube and centrifuged at 12,000 rpm for 15 min at room temperature. The extraction was repeated twice and the supernatant containing protein was collected and stored in a refrigerator at 4ºC (Hameed *et al.,* 2009). Protein concentration was estimated by a method as described by Bradford (1976). The clear supernatant containing 200 µg protein mixed with a sample buffer (62.5 mM tris-HCl pH 6.8, 2.5% SDS, 0.002% bromophenol blue, 0.7135 M (5%) β-mercapto-ethanol and 10 % glycerol) and boiled for 2-3 min in boiling water. SDS-PAGE was performed as per the guidelines of Laemmli (1970) where electrophoresis was carried out on 12% resolving gel at a constant current of 15 mA for the first 50 min followed by 25 mA until the completion of electrophoresis. After electrophoresis, staining of the gel was done using 0.20% coomassie brilliant blue R-250 in methanol: acetic acid: millipore water (40:10:50) for 4 h. Distaining was done in the same solution without dye until clear bands on gel were visible.

DNA isolation

Genomic DNA was extracted from defatted seed powder by DNA extraction method described by Chakraborty *et al.* (2006) where phenol: chloroform: isoamyl alcohol (25:24:1) treatment was given thrice due to the presence of higher protein in the chickpea seed. The absorbance ratio 260/280 on Nanodrop ND 2000 (Wilmington, USA) nearly to 1.8 was used for obtaining quality DNA. Further, it was confirmed by clear sharp intact DNA band on 0.8% agarose gel.

RAPD analysis

After screening with 100 decamer primers (OPH, OPI, OPJ, OPK, and OPL series) only 10 primers giving polymorphic bands were selected for RAPD analysis. The 25 µL PCR reaction mixture contained 10 X *Taq* buffer (2.5 µL), 50 ng μL^{-1} genomic DNA (1.0 µL), 25 mM $MgCl_2$ (1.5 µL), 10

mM each dNTP pre-mix (0.5 µL) and 5 U μL^{-1} *Taq* polymerase (0.2 µL). The temperature profile consisted of an initial denaturation step of DNA at 94°C for 5 min, followed by 35 cycles: 94°C for 45 sec., 38°C for 1 min and 72°C for 2 min. After final cycle, the samples were incubated at 72°C for 10 min to ensure complete extension followed by a hold at 4°C. Amplified products were separated on 1.5% agarose gel containing 0.5 µg mL^{-1} ethidium bromide (Et-Br) at 60 V with standard DNA ladder. The separated bands were visualized under UV light and photographed under Bio-Rad Gel documentation system (California, USA).

Dendrogram construction

The binary data was prepared using the presence or absence of seed storage protein band and RAPD amplified fragment band positions as either 1 or 0, respectively. The data was analyzed using NTYSYS-PC (Numerical Taxonomy and Multivariate Analysis) system version 2.02i by Exeter software (Rohlf, 2004). Jaccard's similarity coefficient was calculated using SIMQUALK program. A dendrogram was produced using the mean of the unweighted pair group method with arithmetic averages (UPGMA) analysis (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Seed storage proteins have been used as a biochemical marker for the assessment of genetic diversity within and between species, genome relationship and genetic improvement in crop plants (Abou-El-enain and Ahmed, 2012; Aarif *et al.*, 2015; Singh *et al.*, 2015). In several crop plants, seed storage protein patterns have successfully been used to resolve the breeding, taxonomic and evolutionary problems due to its stability in almost all conditions (Aarif *et al.*, 2015). Seed storage protein profiling was done with the dual objective of genetic characterization of genotypes as well as to identify possible protein marker to discriminate resistance or susceptibility if any. The banding pattern of seed storage protein resolved total 24 bands (Fig. 1) with relative mobility (Rm) values in the range of 0.04 to 0.97. The total number of bands present in genotypes ranged from 15 to 20 with a maximum in genotypes Annigeri, K-850 and GG-4 and minimum in Shanho. Similarity coefficient based on seed storage protein profiling ranged from 0.63 to 0.96 which showed very low diversity in chickpea genotypes. Minimum genetic similarity (0.63) was observed between genotypes Shanho and JG-62; and maximum genetic similarity in genotypes BG-212: JG-74: GG-1and Surutato-77:GG-2 (Table 1). Dendrogram using NTYSYS-PC software for seed storage protein data generated three main clusters (Fig. 2). Cluster I consisted of highly susceptible genotypes (Cadu-54, L-550, C-104 and Shanho). Cluster II showed three sub-clusters IIA, IIB and IIC. Cluster IIA consisted of one intermediate/susceptible (Chaffa) and intermediate/resistance (Annigeri) genotypes. Cluster IIB had two resistant (K-850 and Surutato-77), one

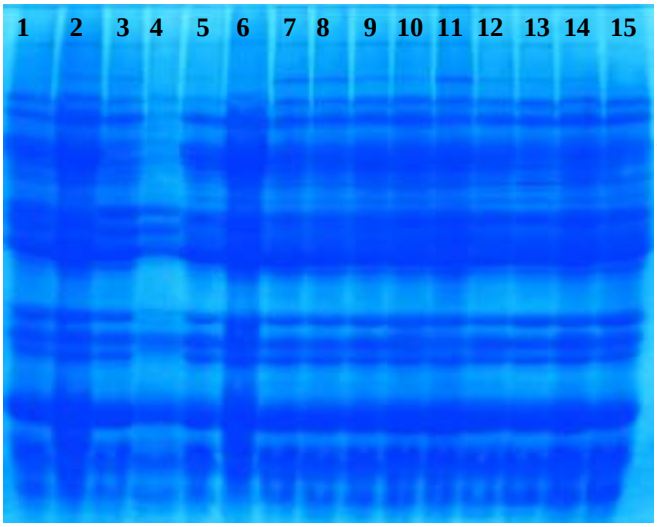

Fig. 1: Seed storage protein profiling of chickpea genotypes resolved by SDS PAGE analysis; Lane 1: Cadu-54; 2: L-550; 3: C-104; 4: Shanho, 5: JG-62; 6: Chaffa; 7: Annigeri; 8: K-850; 9: WR-315; 10: BG-212; 11: Surutato-77; 12: JG-74; 13: GG-1; 14: GG -2 and 15: GG-4

Table 1: Jaccard's similarity coefficient between the chickpea genotypes based on seed storage protein profiling data

Genotypes	Cadu-54	L-550	C-104	Shanho	JG-62	Chaffa	Annigeri	K-850	WR-315	BG-212	Surutato-77	JG-74	GG-1	GG-2	GG-4
Cadu-54	1.00														
L-550	0.96	1.00													
C-104	0.88	0.92	1.00												
Shanho	0.83	0.88	0.88	1.00											
JG-62	0.71	0.67	0.75	0.63	1.00										
Chaffa	0.79	0.83	0.92	0.79	0.83	1.00									
Annigeri	0.75	0.79	0.88	0.83	0.79	0.96	1.00								
K-850	0.83	0.88	0.88	0.83	0.71	0.88	0.92	1.00							
WR-315	0.79	0.83	0.83	0.88	0.75	0.92	0.96	0.88	1.00						
BG-212	0.83	0.88	0.79	0.83	0.71	0.88	0.92	0.92	0.96	1.00					
Surutato-77	0.79	0.83	0.83	0.79	0.75	0.92	0.96	0.96	0.92	0.96	1.00				
JG-74	0.83	0.88	0.79	0.83	0.71	0.88	0.92	0.92	0.96	1.00	0.96	1.00			
GG-1	0.83	0.88	0.79	0.83	0.71	0.88	0.92	0.92	0.96	1.00	0.96	1.00	1.00		
GG-2	0.79	0.83	0.83	0.79	0.75	0.92	0.96	0.96	0.92	0.96	1.00	0.96	0.96	1.00	
GG-4	0.75	0.79	0.79	0.75	0.71	0.88	0.92	0.92	0.88	0.92	0.96	0.92	0.92	0.96	1.00

tolerant (GG-2) and one susceptible (GG-4) genotypes. Cluster IIC consisted of three resistant (WR-315, BG-212 and JG-74) and one tolerant (GG-1) genotypes. Cluster III consisted of only one susceptible genotype (JG-62). The SDS-PAGE analysis of seed storage protein does not resolve any susceptibility or resistance specific polypeptide band. Genetic diversity based on SDS-PAGE of seed storage protein among four chickpea genotypes showed that though genotypes showed the very high difference in protein content, the polypeptide fragment banding pattern was similar in each genotype and no extra band was observed (Singh *et al.*, 2015). The less polymorphism reported in chickpea genotypes might be due to extensive breeding and self-pollinated nature of the crop. Similar low diversity was observed in twenty-two intraspecific Kabuli chickpea using seed storage protein profiling where similarity coefficient was in the range of 0.60 and 1.00. (Aarif *et al.*, 2015). The low diversity in these genotypes might be due to the similar gene pool of common ancestral origin.

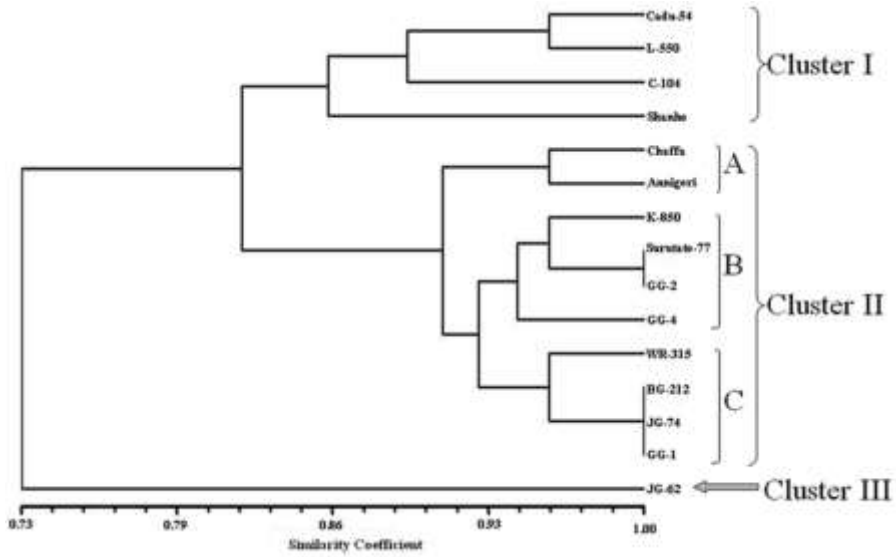

Fig. 2: Dendrogram depicting the genetic relationship among the chickpea genotypes based on seed storage protein profiling

Table 2: Percent polymorphisms revealed by RAPD markers among chickpea genotypes

Primers name	Amplicon size (bp)	Bands (No.)	Polymorphic bands (No.)	Monomorphic bands (No.)	Polymorphism (%)	PIC value	MI
OPH-03	300-1000	07	3	4	42.85	0.58	24.85
OPH-18	350-800	10	2	8	20.00	0.73	14.60
OPH-19	200-600	10	4	6	40.00	0.79	31.60
OPI-17	300- 650	12	5	7	41.66	0.68	28.32
OPI-18	300-1000	11	2	9	18.18	0.71	12.90
OPJ-02	100-600	14	4	10	07.00	0.27	01.89
OPJ-20	200-700	11	2	9	18.18	0.22	03.99
OPK-04	150-550	09	1	8	11.11	0.30	03.33
OPL-13	100-750	10	1	9	10.00	0.23	23.00
OPL-20	300-800	10	3	7	30.00	0.69	20.70
Total		104	27	77	244.00	5.20	165.18
Mean		10.4	2.7	7.7	24.4	0.52	16.52

PIC: Polymorphic information content (PIC = 1-$\sum Pi^2$), MI: Marker index (MI = PIC* % polymorphism)

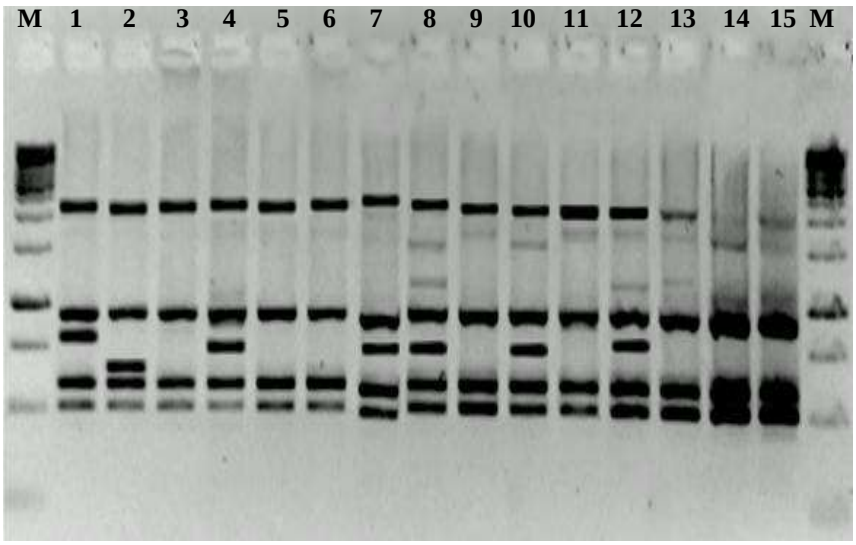

Fig. 3: RAPD profile of chickpea genotypes produced by OPH-19; Lanes: M: 100 bp DNA ladder; 1: Cadu-54; 2: L-550; 3: C-104; 4: Shanho, 5: JG-62; 6: Chaffa; 7: Annigeri; 8: K-850; 9: WR-315; 10: BG-212; 11: Surutato-77; 12: JG-74; 13: GG-1; 14: GG -2 and 15:GG-4

RAPD markers represent an efficient and economical way to generate molecular data and have successfully been used in various taxonomic and phylo-genetic studies (Agrawal and Srivastava, 2010). Genetic diversity is normally measured as the average sequence divergence between any two individuals for a given loci (Rasool, 2013). Initially, a total twenty primers of each OPH, OPI, OPJ, OPK and OPL series were screened, out of which 10 primers showed clear polymorphic bands and were used for diversity analysis among chickpea genotypes differing their reaction to Fusarium wilt disease (Table 2). Ten random primers, used for RAPD analysis produced total 104 amplicons. Per cent polymorphism generated by RAPD primers ranged from 7.00 to 42.85 with an average value of 24.4. The number of polymorphic amplicons per primer varied from 1 (OPL-13 and OPK-04) to 5 (OPI-17) with an average of 2.7. The average polymorphic information content (PIC) of RAPD primers was 0.52 ranging from 0.22 to 0.79 for primer OPJ-20 and OPH-19, respectively. On the basis of per cent polymorphism, PIC value and marker index (MI) value, the primer OPH-19 was more informative for chickpea (Fig. 3). Similarity coefficient based on RAPD data ranged from 0.55 to 0.95 for all the genotypes with minimum genetic similarity between JG-62 and GG-2 and maximum similarity (0.95) between genotypes Cadu-54 and L-550, and between GG-2 and GG-4 (Table 3). It revealed that chickpea genotypes are not much diverse from one another. The reason for low diversity appears obligatory cleistogamic nature and monotonous genome (Abou-El-enain and Ahmad, 2012). Therefore, many other markers are needed to analyze DNA polymorphism.

Table 3: Jaccard's similarity coefficient among chickpea genotypes based on the RAPD data

Genotypes	Cadu-54	L-550	C-104	Shanho	JG-62	Chaffa	Annigeri	K-850	WR-315	BG-212	Surutato-77	JG-74	GG-1	GG-2	GG-4
Cadu-54	1.00														
L-550	0.95	1.00													
C-104	0.84	0.84	1.00												
Shanho	0.90	0.90	0.79	1.00											
JG-62	0.85	0.85	0.82	0.84	1.00										
Chaffa	0.85	0.87	0.77	0.80	0.83	1.00									
Annigeri	0.78	0.80	0.75	0.72	0.81	0.83	1.00								
K-850	0.73	0.73	0.68	0.68	0.72	0.71	0.79	1.00							
WR-315	0.65	0.66	0.65	0.59	0.64	0.67	0.76	0.86	1.00						
BG-212	0.69	0.69	0.66	0.67	0.70	0.70	0.80	0.84	0.84	1.00					
Surutato-77	0.68	0.68	0.58	0.68	0.64	0.69	0.74	0.78	0.74	0.85	1.00				
JG-74	0.68	0.68	0.61	0.65	0.64	0.72	0.76	0.78	0.79	0.87	0.91	1.00			
GG-1	0.66	0.66	0.56	0.65	0.62	0.69	0.72	0.72	0.72	0.82	0.86	0.93	1.00		
GG-2	0.65	0.63	0.56	0.61	0.55	0.67	0.69	0.71	0.74	0.80	0.84	0.91	0.93	1.00	
GG-4	0.66	0.65	0.58	0.61	0.59	0.66	0.72	0.74	0.74	0.84	0.86	0.91	0.93	0.95	1.00

Dendrogram based on NTYSYS-pc of RAPD data formed three major clusters. Cluster I consisted five susceptible (Cadu-54, L-550, C-104, Shanho and JG-62) and one intermediate/ susceptible (Chaffa) genotypes; cluster II consisted one intermediate/resistant (Annigeri) and three resistant (K-850, WR-315, and BG-212) genotypes; cluster III consisted two resistant (Surutato-77 and JG-74), two tolerant (GG-1 and GG-2) and also one susceptible (GG-4) genotypes (Fig. 4). The dendrogram constructed from RAPD data showed good discrimination in diverse genotypes for Fusarium wilt resistant and susceptible with some exceptions. However, no specific banding pattern was observed in RAPD which was particularly linked to either resistance or susceptibility. Genetic diversity among wilt resistant and susceptible chickpea genotypes has been studied by Datta and Lal (2011) using 19 RAPD and 22 simple sequence repeat (SSR) primers for analysis. RAPD primers amplified a total of 121 amplicons with average polymorphism of 87%, average PIC of 49 and

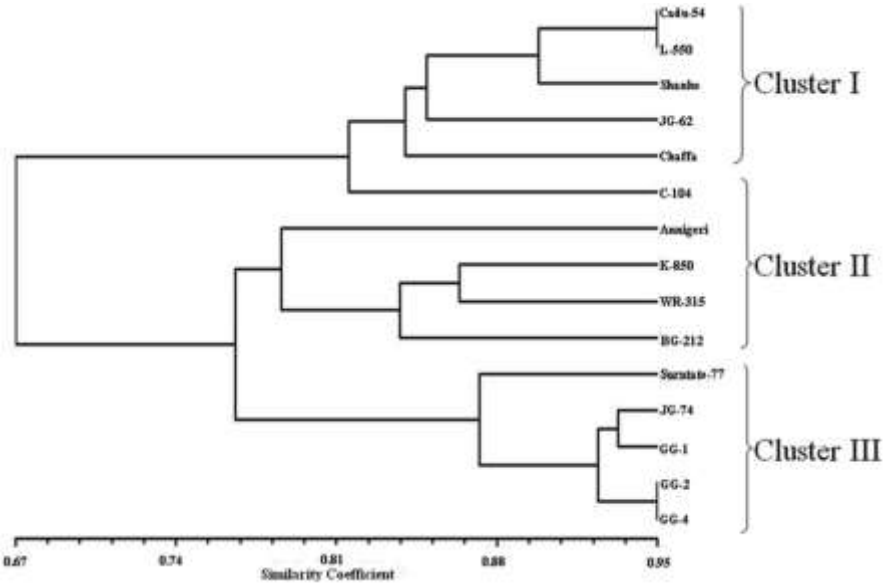

Fig. 4: Dendrogram depicting the genetic relationship among the chickpea genotypes based on RAPD data

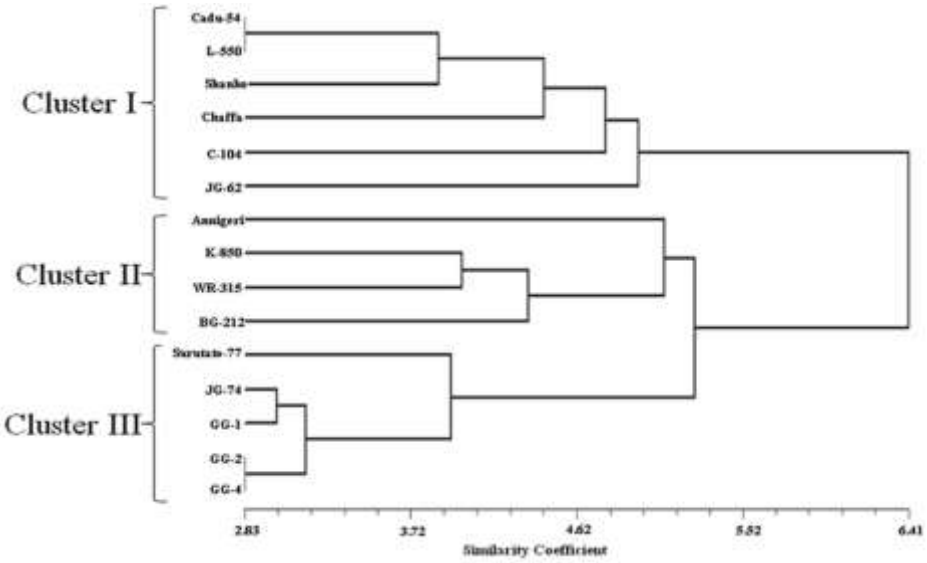

Fig. 5: Dendrogram depicting the genetic relationship among the chickpea genotypes based on seed storage protein profiling and RAPD data

similarity index ranging from 0.30 to 0.96. They concluded that with some ambiguity RAPD based dendrogram could separate the resistant chickpea genotypes from susceptible ones into two discrete groups. Suthar *et al.* (2012) observed 76.3% polymorphism for RAPD marker and the genetic similarity among 24 chickpea genotypes was in the range of 0.56 to 0.94 so represent moderate genetic diversity among the genotypes. Ahmad *et al.* (2014) while working on marker assisted selection (MAS) of chickpea for Fusarium wilt used 5 RAPD and 15 SSR markers for assessment of diverse 24 indigenous and 46 exotic chickpea accessions. The dendrogram developed from the coefficient of similarity divided the genotypes into two main groups with 1st cluster having 48 genotypes resistant to Fusarium wilt and remaining 22 genotypes grouped in 2nd cluster were susceptible.

Dendrogram using combined data of SDS-PAGE and RAPD generated three major clusters (Fig. 5). Cluster I had five susceptible (Cadu-54, L-550, Shanho, C-104 and JG-62) and one intermediate/susceptible (Chaffa) genotypes while Cluster II had three resistant (K-850, WR-315 and BG-212) and one intermediate/resistant (Annigeri) genotypes. Cluster III had two resistant (Surutato-77 and JG-74), two tolerant (GG-1 and GG-2) and one susceptible (GG-4) genotypes. The combined dendrogram did not separate intermediate/resistant and intermediate/susceptible genotypes from resistant and susceptible genotypes, respectively. Further, the combined analysis using seed storage protein profiling and RAPD marker data reported the very low level of genetic diversity among chickpea genotypes.

Conclusion: The present study on SDS-PAGE of seed storage protein profiling showed minor fluctuation to discriminate resistant and susceptible genotypes. There was no correlation of seed storage protein profile with genetics for disease, because of the conservative trait of seed storage protein. So, SDS-PAGE is not effective to assess genetic diversity in cultivated chickpea. However, dendrogram based on RAPD data was able to discriminate resistant genotypes from susceptible ones with some exceptions. The clustering might be due to similar genetic background or similarity in disease-related allelic structure of genotypes. Using this information, diverse genotypes are drawn which have a better role as parental material in hybrid production. Screening of disease resistant chickpea genotypes using MAS will facilitate gene pyramiding and molecular breeding.

Acknowledgements: The authors are thankful to International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad (India) and Pulse and Castor Research Station, Navsari Agricultural University, Navsari (India) for providing genotype seed material for the present study.

REFERENCES

Aarif, M., Rastogi, N.K., Verulkar, S.B., Saxena, R.R., Xalxo, M.S., Chandrakar, P.K. and Johnson, P.L. 2015. Intraspecific diversity of Kabuli chickpea genotypes based on seed storage protein profiling. *Journal of Food Legumes*, **28**: 31-33.

Abou-El-enain, M.M. and Ahmed, S.M. 2012. Intraspecific diversity of Egyptian and foreign new lines of chickpea based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isozyme markers. *African Journal of Biochemistry Research*, **6**(10):135-139.

Agrawal, P.K. and Srivastava, A. 2010. Assessment of genetic diversity among chickpea cultivars of India using RAPD markers. *Indian Journal of Genetics and Plant Breeding*, **70**: 264-270.

Ahmad, Z., Mumtaz, A.S., Ghafoor, A., Ali, A. and Nisar, M. 2014. Marker-assisted selection (MAS) for chickpea *Fusarium oxysporum* wilt resistant genotypes using PCR based molecular markers. *Molecular Biology Reports*, **41**: 6755-6762.

Anonymous. 2015. *FAO Statistical Databases.* Food and Agriculture Organization of the United Nations, Rome, Italy (*http://www.fao.org/faostat/en/#data/QC*)

Barman, P. 2012. *Study on Association of Molecular Markers with Fusarium Wilt Resistance in Chickpea (Cicer arietinum L.)*. Ph.D. Thesis, Department of Biotechnology. Gauhati University, Gauhati, Assam, India.

Bhagyawant, S.S., Gupta, N., Gautam, A., Chaturvedi, S.K. and Shrivastava, N. 2015. Molecular diversity assessment in chickpea through RAPD and ISSR Markers. *World Journal of Agricultural Research*, **3**(6): 192-197.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*,**72**: 248-254.

Chakraborty, D., Sarkar, A., Gupta, S. and Das, S. 2006. Small and large scale genomic DNA isolation protocol for chickpea (*Cicer arietinum* L.), suitable for the molecular marker and transgenic analyses. *African Journal of Biotechnology*, **5**(8): 585-589.

Chaube, H.S. and Pundhir, V.S. 2009. Vascular disease. pp. 461-467. **In**: *Crop Diseases and their Management* (2nd edn.), PHI Learning Private Limited, New Delhi, India.

Chawla, H.S. 2012. Introduction to plant biotechnology. pp. 356-397. **In**: *Molecular Marker and Marker Assisted Selection* (3rd edn.). Oxford & IBH Publishing Co., New Delhi, India.

Datta, J. and Lal, N. 2011. Characterization of genetic diversity in *Cicer arietinum* L. and *Cajanus cajan* L. Millspaugh using random amplified polymorphic DNA and simple sequence repeat markers. *Genomics and Quantitative Genetics*, **3**: 30-41.

Devi, R., Nandeesha, P., Kaashyap, M., Kumara, S. and Datta, S. 2014. Phylogenetic analysis of pulse crops using RAPD and seed storage protein markers. *Indian Journal of Agricultural Biochemistry*, **27**: 81-84.

Ghafoor, A., Ahmad, Z., Qureshi, A.S. and Bashir, M. 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.

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

Haware, M.P. and Nene, Y.L. 1980. Influence of wilt at different stages on the yield loss in chickpea. *Tropical Grain Legume Bulletin*, **19**: 38-40.

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

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

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

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 Journal of Botany*, **39**: 1575-1581.

Rasool, S. 2013. Genetic diversity as revealed by RAPD analysis among chickpea genotypes. *Pakistan Journal of Botany*, **45**: 829-834.

Rohlf, F.J. 2004. *Numerical Taxonomy and Multivariate Analysis System.* Version 2.02i: 3-9. Exeter software, Applied Biostatics, New York, USA.

Singh, P.K., Panwar, N. and Bhagyawant, S.S. 2015. Evaluation of genotypic variation using SDS-PAGE. *European Journal of Biotechnology and Bioscience*, **3**(7): 39-40.

Sneath, P.H.A. and Sokal, R.R. 1973. *The Principles and Practice of Numerical Classification. Numerical Taxonomy*. W.F. Freeman & Co., San Francisco, USA.

Suthar, K.P., Bhatnagar, R., Shukla, Y.M., Patel, N.J., Suthar, V.P. and Patel, J.P. 2012. Assessment of genetic diversity among chickpea genotypes using RAPD, protein profiling and isozyme markers. *Indian Journal of Agricultural Biochemistry*, **25**: 25-30.

Williams, S.J.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphism amplified by arbitrary primers useful as a genetic maker. *Nucleic Acids Research*, **18**: 6531-6535.

Winter, P., Benko-Iseppon, A.M., Huttel, B., Ratnaparkhe, M., Tullu, A., Sonnante, G., Pfa, V.T., Tekeoglu, M., Santra, D., Sant, V.J., Rajesh, P.N., Kahl, G. and Muehlbauer, F.J. 2000. A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* and *C. reticulatum* cross: Localization of resistance genes for Fusarium wilt races 4 and 5. *Theoretical and Applied Genetics*, **101**: 1155-1163.