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Research Article

Genetic diversity analysis in chickpea grown under heat stress conditions of Madhya Pradesh

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Abstract
The genetic diversity study was conducted in 100 promising chickpea genotypes sown under late planting using Mahalanobis's D^2 Statistics. Further the diversity is also confirmed by using SSR molecular markers. Based on D^2 values, 100 genotypes were grouped into sixteen clusters. The cluster I consisted of maximum 29 genotypes, followed by Cluster II, cluster V and cluster VI, which had 26, 13 and 12 genotypes, respectively. Maximum intra-cluster distance (164.10) was observed in cluster V, followed by cluster VI (150.23), cluster III (123.98) and cluster II (102.52). However, maximum inter cluster distance was noticed between cluster IX and cluster XV (853.43), followed by cluster X and cluster XV (749.13) and cluster VII and cluster XV (742.58). Molecular analysis grouped 100 genotypes into three major clusters. First major cluster consisted four genotypes (ICCV 93025, ICCV 93024, ICCV 03401 and ICCV 95315) and second cluster consisted two genotypes (ICCV 03405 and ICCV 01306). Third and last major cluster group contained 94 genotypes. The PIC value among the primers ranged from 0.3245 (TA 78) to 0.4375 (TA 135) and the highest gene diversity were found in TA 135 (0.5378). Among all eleven polymorphic SSR primers, only TA78 (0.0300), H5BO4 (0.0900), ICCAM0123a (0.0500), TA135 (0.0600) and H1G16 (0.1500) were shown heterozygosity. The genotypes conferring diversity at molecular studies (unaffected by environmental variations) can be used in future hybridization programmes and also helpful for plant breeders to screen effective heat tolerance genotypes for the development of high yielding improved lines for diverse environments.

Key words
Chickpea, D^2 statistics, genetic variability, SSR

Introduction:

Chickpea is one of the most important *Rabi* pulse crop in India. India is the largest chickpea producer accounting a share of about 67% in global chickpea production with about 9.01 m ha area, 7.58 m t production. The total area in Madhya Pradesh reached 3.04 M ha with 3.29 M t of production and productivity of 1082 kg/ha (FAOSTAT, 2013-14). Madhya Pradesh is contributing 40% of India's total chickpea production. In India, chickpea is usually grown in winter-season but during the past three decades, there is significant shift in the growing environment from the cooler, long-season environments of northern India to the warmer, short-season environments of central and southern India. Chickpea area under late-sown conditions is increasing particularly in northern and central India due to inclusion of chickpea in new cropping systems and intense sequential cropping practices leading to a prolonged exposure of chickpea to high temperature. Heat stress is a serious constraint to chickpea production in northern and central India. Reproductive stages (flowering and podding) in chickpea are susceptible to changes in external environment and heat stress (Summerfield *et al.,* 1984; Wang *et al.,* 2006; Krishnamurthy *et al.,* 2011). D^2 analysis which has given by Mahalanobis (1936) is a very potent technique of measuring genetic divergence. Now it is reliably and extensively used in plants for measuring genetic divergence (Tripathi *et al*., 2013, Shinde *et al*., 2013, Shekhawat *et al*., 2014). The aim of forming clusters and finding the intra and inter cluster divergence is to provide the base of selecting parents for a planned breeding programme.

The knowledge of genetic diversity has a significant impact on the improvement of crop plants and this information has been successfully used for efficient germplasm management, fingerprinting and genotype selection. Genetic diversity can be estimated using phenotypic identification or molecular markers. However, morphological traits have a number of limitations including low polymorphism, low heritability, late expression, and may be controlled by epistatic and pleiotropic gene effects (Eivazi *et al.,* 2008). Molecular markers are useful and complement to morphological characterization of accessions because they are plentiful, independent of plant tissue or environmental effects, and allow cultivar identification very early in plant development (Manifesto *et al.,* 2001). Simple sequence repeats (SSRs) are common and informative molecular markers used for genetic diversity studies because of their simplicity, high levels of polymorphism, high reproducibility, and co-dominant inheritance patterns (Powell *et al.,* 1996).
Keeping in view the above facts, present investigation was undertaken to work out genetic

divergence among 100 genotypes using morphological and molecular markers to help the breeders in selecting promising and genetically diverse parents for desired improvement in chickpea for late sown conditions.

Materials and method

Experimental materials: The experimental material comprising 100 genotypes of chickpea (74 Desi and 26 Kabuli, Received from ICRISAT, Hyderabad) were grown during Rabi 2012-13 under late planting conditions (sown on 26th January, 2013) in a Randomized Complete Block Design with three replication at the Seed Breeding Farm, Department of Plant Breeding and Genetics, JNKVV, Jabalpur . Data were recorded on five randomly tagged plants for days to flower initiation, days to 50% flowering, days to pod initiation, days to maturity, plant height (cm), number of primary branches, number of secondary branches, total number of pods per plant, number of effective pods per plant, 100 seed weight (g), seeds per pod, seed yield per plant (g) and harvest index (%). The D^2 analysis was carried out, according to the standard method of Mahalanobis (1936). The clustering of genotypes was done by Tochers' method, as described by Rao (1952). The intra and inter cluster distances were computed as suggested by Singh and Chaudhary (1977).

*Determination of DNA Isolation and Concentration:*Samples from the youngest leaves (200 mg) were collected from one month old chickpea genotypes for extraction of genomic DNA performed using the modified CTAB method (Saghai-Maroof *et al.,* 1984). Concentrations of DNAs were determined on the spectrophotometer (Nanodrop, model ND-100) after isolation. DNAs were diluted to 50 ng μl^{-1} for equal amounts of the samples from prepared concentration by running on the 0.8% agarose gel (1xTAE buffer) using known λ/HindIII DNA ladder.

*DNA amplifications using the SSR molecular marker:*Eleven SSR primers (Table 1) synthesized by IDT (Promega, USA) and used for DNA amplifications using the PCR reaction mixture containing 2 µl DNA (50 ng μl^{-1}) and 20 µl reaction mixture [2 µl x10 PCR buffer solution (Promega, USA), 1.4 µl of 25 mM $MgCl_2$ (Promega, USA), 0.2 µl 25 mM dNTP (Promega, USA), 0.2 µl 500 U Taq DNA Polymerase (Promega, USA), 0.5 µl 25 pmol μl^{-1} primer (from each of the forward and reverse primers) and 15.7 µl nuclease free water] were performed in 35 cycles on the Eppendorf PCR device. PCR profile was optimized for amplification by using primers of unique sequence with higher GC ratio at high stringency. The PCR reaction was carried out with a initial denaturation at 94°C for 4 min, final denaturation at 94°C for 30 sec, annealing at above 50-52°C of temperature melting (Tm) for 30 sec, and extension at 72°C for 30 sec, a final extension at 72°C for 5 min and 4°C as holding temperature. The amplified PCR products were size separated by 2.5% (w/v) agarose gel with 1X TAE stained with ethidium bromide and visualized under UV transilluminator and then photographed using Syngene gel documentation system. In order to determine approximated size of bands, 100bp ladder (Promega, USA) was run along with the amplified PCR products. The scoring of SSR amplicons was done for each genotype.

Scoring and cluster analysis for molecular data: For each SSR locus, sizes of the alleles were estimated for all the thirty eight genotypes and scored in the form of a binary matrix where ‘1’ represented the presence of a band and ‘0’ denoted its absence. Pair-wise genetic similarity (GS) was calculated among 38 chickpea genotypes using Jacard’s similarity coefficient. The values of GS may range from ‘1’ (identical profiles for all marker in the two genotypes) to ‘0’ (no common bands). The binary data generated for all the variety for the polymorphic markers was entered in the Power Marker version 3.25 software. The similarity matrix was used to generate dendrogram for cluster analysis. Polymorphic information content (PIC) for each SSR locus was calculated based on number of bands per primer using the formula PIC = 1-Σfi2, where fi is the frequency of the ith allele in the genotype (Smith *et al.* 1997). A high PIC value establishes the power of SSR as a tool for genetic diversity quantification.

Result and discussion

Analysis of variance for characters under study indicated significant differences among all characters. Among the 13 characters studied, 100-seed weight (24.34%), harvest Index (21.84%), number of pods per plant (18.40%) and seed yield per plant (15.25%) contributed maximum in the manifestation of genetic divergence (Table 2). These four traits accounted for 79.83 per cent of total variability. Jethava *et al.* (2000) reported that seed yield per plant, number of pods per plant and 100-seed weight contributed maximum towards genetic diversity. Parashi *et al.,* (2013) reported that seed yield per plant, number of pods per plant contributed maximum to genetic diversity. Prakash and Shekhawat (2012) also reported that 100- seed weight and pods per plant contributed maximum to genetic diversity.

On the basis of Mahalanobis D^2 statistics and Tocher’s method, 100 genotypes used in the present investigation were grouped into 16 clusters. The cluster I was the largest and consisted of 29 genotypes, followed by cluster II (26 genotypes), cluster V (13 genotypes), cluster VI (12genotypes) and cluster III (9 genotypes), whereas cluster IV, VII, VIII, IX, X, XI, XII, XIII,

XIV, XV and cluster XVI had only one genotype in each (Table 3). Jeena and Arora (2002) found twenty eight genotypes in cluster I, remaining 11 clusters were mono-genotypic. The D^2 analysis established the presence of broad diversity among the inbreds by the formation of 16 clusters. Jethava *et al.* (2000) , Raval and Dobariya (2004), Parameshwarappa *et al.*,(2011), Parashi *et al.,* (2013) obtained 16, 15, (20, 16, 25 in E1, E2 and E3) and 13 clusters of genotypes, from 70, 52, 103 and 33 genotypes respectively, indicating presence of broad genetic diversity in their materials.

Cluster XV showed highest mean performance for days to flower initiation, days to 50% flowering, days to pod initiation, days to maturity and plant height; cluster XIV for number of primary branches, number of secondary branches, total number of pods per plant and seed yield per plant; cluster XI for number of primary branches and number of seeds per pod and cluster VII for harvest index and number of primary branches. Cluster IX recorded minimum performance for days to flower initiation, days to 50% flowering, days to maturity, plant height and number of primary branches. On the basis of cluster means, the genotypes of cluster XV, XIV, XI, VII and IX were found superior and may be further utilized in breeding programme for yield advancement in chickpea**.**

The intra cluster distance ranged from 0.00 to 164.10 (Table 4) .The cluster V showed maximum intra cluster D^2 value (164.10), followed by cluster VI (150.23), cluster III (123.98), cluster II (102.52) and cluster I (75.35) revealing the inclusion of diversion of diverse genotypes in these clusters. The inter cluster D^2 values ranged from 67.02 to 853.43. Maximum inter cluster distance *i.e.,* 853.43 was reported between cluster IX (ICCV 93014) and XV (ICCV 08102), which indicated that the genotypes included in these different clusters may give high heterotic response and thereby better segregants (Lal *et al.,* 2001). Minimum inter cluster D^2 value was observed between cluster IX and VIII indicating the close relationship among the genotypes included in these two clusters.

Genome analysis tools provide access to thousands of polymorphisms and monitor genetic diversity (Glaszmann *et al*., 2010). Various molecular markers such as restriction fragment length polymorphism (RFLP) (Udupa *et al*., 1993) and random amplified polymorphic DNA (RAPD) (Iruela *et al*., 2002) found to be unsuccessful to detect genetic diversity within chickpea. Among the all markers, simple sequence repeats (SSRs) widely used to detect genetic variation within chickpea species (Udupa *et al*., 1999) and construct genomic map (Flandez-Galvez *et al*., 2003) due to most abundance and co-dominant nature with the highest information content of SSRs in the genome (Gupta and Varshney, 2000). A total of eleven SSR markers were used for estimating genetic diversity in 100 chickpea genotypes.

The polymorphic information content among the primers ranged from 0.3245 (TA 78) to 0.4375 (TA 135) (Table 5). The mean value of polymorphic information content was 0.3629. The marker TA 135 showed highest polymorphic information content (0.4375) as well as highest gene diversity (0.5378). Tiwari *et al*. (2014) found PIC values between 0.049 and 0.550 with mean value of 0.254 and genetic diversity from 0.0512 to 0.622 with a mean value of 0.316. Upadhyaya *et al.,* (2008) found values 0.905 of PIC for TA135, respectively and also found 02.70 heterozygosity for TA135, respectively whereas, in this investigation TA135 show 0.0600 heterozygosity.

Major allele frequency of 11 SSR markers ranged from 0.5400 (TA 135) to 0.7150 (TA 78). The mean value of major allele frequency among the primers was 0.6309. The highest gene diversity was found with TA 135 (0.5378), followed by primer H1B17 (0.5022), ICCM0127 (0.4950), H1G16 (0.4916), TA125 (0.4550), Cam0656 (0.4550) and ICCM0123a (0.4520). Minimum diversity was shown by marker Cam1536 (0.4352). Zakia *et al.* (2012) found 0.69 genetic diversity for primer TA135.

Among all eleven SSR primers only TA78 (0.0300), H5BO4 (0.0900), ICCAM0123a (0.0500), TA135 (0.0600) and H1G16 (0.1500) have shown heterozygosity (Figure 1, 2 & 3). The heterozygosity in self-pollinating species such as chickpea mainly results from the low level (0 to 1.58%) of out crossing as has been reported earlier (Gowda, 1981) and the number of gene-targeted molecular markers such as SSR of chickpea are limited and show a low level of polymorphism in chickpea (Jafari *et al*., 2013).

Reif *et al*. (2005) observed that modified Euclidian distance is best suited for the estimation of genetic distances under the evolutionary forces that influence the genotypes under consideration is not available and no specific mutation model can be attributed to the allelic variation observed at the SSR loci.

Based on electrophoretic banding pattern, molecular diversity among 100 genotypes of chickpea was estimated and generated genetic distance-based dendrogram for cluster analysis using neighbor-joining method implemented in Power Marker version 3.25. Cluster analysis revealed that all the genotypes were grouped into three major groups. First major group consisted four genotypes (ICCV 93025, ICCV 93024, ICCV 03401 and ICCV 95315). Second major group

consisted two genotypes (ICCV 03405 and ICCV 01306). Third and last major cluster group contained 94 genotypes. These major groups were further subdivided into several sub groups.

The results of present study revealed that as these 100 genotypes, which are morphologically very much distinct from each other are very much similar at molecular level (on the basis of diversity analysis at both levels). This may be due to the genotypic effect (same origin) or the interaction between environments. From this study, it is clearly evident that as molecular markers are independent to environmental fluctuations and also stage or tissue specific, hence more emphasis should be given to the results obtained with molecular studies.

The identified promising lines *viz*., ICCV 93025, ICCV 93024, ICCV 03401, ICCV 95315, ICCV 03405 and ICCV 01306 could also be potentially utilized by plant breeders for developing elite chickpea lines/cultivars with higher yield and enhanced adaptation to late sown conditions.

References

Eivazi, A.R.; Naghavi, M.R.; Hajheidari, M.; Pirseyedi, S.M., Ghaffari, M.R.; Mohammadi, S.A.; Majidi, I.; Salekdeh, G.H.; Mardi, M. 2008. Assessing wheat (*Triticum aestivum* L.) genetic diversity using quality traits, amplified fragment length polymorphisms, simple sequence repeats and proteome analysis. *Annals of Applied Biology,* 152: 81–91.

Flandez-Galvez, H., Ford, R., Pang, E.C.K. and Taylor, P.W.J. 2003. An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged microsatellite site and resistance gene analog markers. *Theor. Appl. Genet*., 106: 1447–1456.

Glaszmann, J.C., Kilian, B., Upadhyaya, H.D. and Varshney, R.K. 2010. Accessing genetic diversity for crop improvement. *Curr. Opin. in Pl. Biol.,* 13 : 1–7.

Gowda, C.L.L. 1981. Natural outcrossing in chickpea. *Int. Chickpea Newslr.,* 5: 6.

Gupta, P.K. and Varshney, R.K. 2000. The development and use of microsatellite markers for genetics and plant breeding with emphasis on bread wheat. *Euphytica,* 113: 163-185.

Iruela, M., Rubio, J., Cubero, J.I., Gil, J. and Milan, T. 2002. Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. *Theor. Appl. Genet.,* 104: 643–651.

Jafari, N., Behroozi, R., Bagheri, A. and Moshtaghi, N. 2013. Determination of Genetic diversity of cultivated chickpea (*Cicer arietinum* L.) using *Medicago truncatula* EST-SSRs. *J. Pl. Mol. Breed.,* 1: 1-16.

Jeena, A.S. and Arora, P.P. 2002. Mahalanobis D^2 Technique in chickpea. *Agric. Sci. Digest.,* 22 (3): 209 – 210.

Jeethava, A. S., Yasufzal, A. S., Poshiya, V. K. and Vaddoria, M. A. 2000. Divergence analysis in chickpea. *Gujrat Agric. Univ, Res. J.,* 22 (1): 23-28.

Krishnamurthy, L., Gaur, P. M., Basu, P.S., Chaturvedi, S. K., Tripathi, S., Vandez, V., Rathore, A., Varshney, R. K. and. Gowda, C. L. L. 2011. Large genetic variation for heat tolerance in the reference collection of chickpea (*Cicer arietinum* L.) germplasm. *Plant Genetic Res.,* 9: 59-61.

Lal, D., Krishna, R. and Gurpreet, S. 2001. Genetic divergence in chickpea. *Ind. J. Pulses Res.,* 14 (1): 63-64.

Mahalanobis, P. C. 1936. On generalized distance in statistics. *Proceed. National Institute of Sci.,* 2: 49-55.

Manifesto, M.M.; Schlatter, A.R.; Hopp, H.E.; Suarez, E.Y. and Dubcovsky, J. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.,* 41: 682–690.

Parameshwarappa, S. G., Salimath, P. M., Upadhyaya, H. D., Patil, S. S. and Kajjidoni, S.T. 2011. Genetic Divergence under three environments in a minicore collection of chickpea *(Cicer arietinum L.), Indian J. Plant Genet. Res.,* 24 (2): 177-185.

Parashi, V.S., Lad, D.B., Mahse, L.B., Kute, N.S. and Sonawane, C.J. 2013. Genetic diversity studies in chickpea (*Cicer arietinum* L.), *Bioinfolet,* 10 (1): 337-341.

Powell, W.; Morgante, M.; Andre, C.; Hanafey, M.; Vogel, J.; Tingey, S. and Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding,* 2: 225–238.

Prakash, V. and Shekhawat, U. S. 2012. Genetic divergence in yield and yield contributing traits in chickpea. *J. Progressive Agric.,* 3 (1): 50- 51.

Rao, C. R. 1952. Advanced Statistical Methods in Biometric Research. John Wiley and Sons, Inc New York. Pp 357-363.

Raval, L. J. and Dobariya, K. 2004. Assessment of genetic divergence in chickpea (*Cicer arietinum* L.). *Ann. Agric. Res.,* 25 (1): 30-34.

Reif, J.C., Melchinger, A.E. and Frisch, M. 2005. Genetical and mathematical properties of similarity and dissimilarity coefficients applied in plant breeding and seed bank management. *Crop Sci.,* 4: 1-7.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. 1984. Ribosomal DNA spacer-length in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Nat. Acad. Sci. U. S. A.,* 81: 8014- 8018.

Shekhawat, N., Jadeja, G. C., Singh, J. and Ramesh. 2014. Genetic diversity analysis in relation to seed yield and its component traits in Indian mustard (*Brassica juncea* L.). *The Bioscan,* 9 (2): 713-717.

Shinde, D., Chavan, S. and Jadhav, B. D. 2013. Study on genetic divergence in sweet sorghum [*Sorghum bicolor* (L.) Moench]. *The Bioscan,* 8 (1): 135-138.

Singh, R. K. and Chaudhary, B. D. 1977. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi. pp 266.

Spurthi, N.N., Hongyan, Z., Varghese, N., Datta, S., Choi, H.K., Horres, R., Jüngling, R., Singh, J., Kishor, P.B.K., Sivaramakrishnan,

S., Dave, A., Hoisington, D.A., Kahl, G., Winter, P., Cook, D.R. and Varshney, R.K. 2010. Integration of novel SSR and gene based SNP marker loci in the chickpea genetic map and establishment of new anchor points with *Medicago truncatula* genome. *Theor Appl Genet.,* 120 (7): 1415–1441.

Summerfield, R.J., Hadley, P., Roberts, E.H., Minchin, F.R. and Rawsthorne, S. 1984. Sensitivities of chickpeas (*Cicer arietinum* L.) to hot temperatures during the reproductive period. *Experimental Agric.,* 20:77-93.

Tiwari, A., Babbar, A., Prakash, V. and Pandey, S. 2014. Assessment of genetic diversity in chickpea (*Cicer arietinum* L.) genotypes using SSR markers under heat stress condition. *Prog. Res.,* 9 (1): 183-189.

Tripathi, A., Bisen, R., Ahirwal, R.P., Paroha, S., Sahu, R. and Ranganatha, A. R. G. 2013. Study on genetic divergence in sesame (*Sesamum indicum* L.) germplasm based on morphological and quality traits. *The Bioscan,* 8 (4): 1387-1391.

Udupa, S.M., Robertson, L.D., Weigand, F., Baum, M. and Hahl, G. 1999. Allelic variation at (TAA)*n* microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. *Mol. and General Genet.,* 261: 354–363.

Udupa, S.M., Sharma, A., Sharma, R.P. and Pai, R.A. 1993. Narrow genetic variability in *Cicer arietinum* as revealed by RFLP analysis. *J. Pl. Biochem. and Biotech.,* 2 : 83-86.

Upadhyaya, H.D., Dwivedi, S.L., Baum, M. and Varshney, R.K. 2008. Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biol.,* 8: 106.

Wang, J., Gan, Y.T., Clarke, F. and McDonald, C.L. 2006. Response of chickpea yield to high temperature stress during reproductive development. *Crop Sci.,* 46: 2171-2178.

Winter, P., Pfaff, T., Udupa, S. M., Hüttel, B., Sharma, P. C., Sahi, S., Arreguin-Espinoza, R., Weigand, F., Muehlbauer, F. J. and Kahl, G. 1999. Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Mol Genet.,* 262: 90-101.

Zakia, A., Mumtaz, A. S., Nisar, M. and Khan, N. 2012. Diversity analysis of chickpea (*Cicer arietinum* L.) germplasm and its implications for conservation and crop breeding. *Agric. Sci.,* 3 (5):723-731.

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Table 1. SSR primer used for genetic diversity in chickpea genotypes

S.№.	Marker	LG	Forward sequence	Reverse sequence
1.	H1G16	LG-1	GTTTGCTTTCAACACCGAGA	CCCATGAAGGCCTGAATTAT
2.	TA135	LG-4	TGGTTGGAAATTGATGTTTT	GTGGTGTGAGCATAATTCAA
3.	Cam0620	LG-3	ATTCCCAAACATTGGCAAAA	GTTTGGGGTTACTCATGGGA
4.	TA78	LG-5	CGGTAAATAAGTTTCCCTCC	CATCGTGAATATTGAAGGGT
5.	Cam1536	LG-7	TGATTTGGAGCATCGTCAAC	TTGGAGTAGTTTTTGGGGGA
6.	ICCM0123a	LG-7	GGATGGTCTGCTGGAATCAT	AAAGACAACAAAAAGACAATCATG
7.	Cam0656	LG-7	TCACTCTCTCGCAAAACCCT	GAGTGAAACCGAGAGCGAAC
8.	TA125	LG-7	TTGAAATTGAACTGTAACAGAACATAA A	TAGATAGGTGATCACAAGAAGAGA
9.	H5B04	LG-8	CATAATTTTAAAAGAGGCACGTTAAAT	ATATAAGCAAAAATAAAGATGAGT
10.	ICCM0127	LG-6	TGTTGAACGAATTTACTCATCG	GGTGGGCTCCTATTGTTTGA
11.	H1B17	LG-6	ATTCGAGGTGGTACCTCTAGTGA	GAGGAACCGACGATGTATCTATT

Table 2: Proportional contribution of seed yield and its attributing characters to divergence among 100 genotypes of chickpea

Source	Times ranked 1st	Contribution %
Days to flower initiation	150	3.03%
Days to 50% flowering	11	0.22%
Days to pod initiation	187	3.78%
Days to maturity	215	4.34%
Plant height (cm)	146	2.95%
Number of primary branches	0	0.00%
Number of secondary branches	32	0.65%
Total number of pods per Plant	911	18.40%
Number of effective pods per Plant	219	4.42%
Number of seeds per pod	38	0.77%
100-seed weight (g)	1205	24.34%
Harvest index (%)	1081	21.84%
Seed yield per plant (g)	755	15.25%

Table 3. Distribution of chickpea genotypes on the basis of D^2 statistic

Cluster	No. of genotypes	Genotypes
1	29	ICCV 91005, ICCV 96027, JG14, ICCV 6103, ICCV 91006, ICCV 11111, ICCV 91902, ICCV 10115, ICCV 11109, ICCL 84207, ICCV 97318, ICCV 90015, ICCV 91008, ICCL 84228, ICCL 84242, ICCV 91020, ICCV 97024, ICCL 83233, ICCL 86235, ICCV 91021, ICCV 11104, ICCV10114, ICCL 84233, ICCV 10116, JG16, ICCV 10117, ICCV 10104, ICCV 00105, ICCV 10118
2	26	ICCV 96317, ICCV 09314, ICCV 03405, ICCV 05307, ICCV 03410, ICCV 06301, KAK2, ICCV 08104, ICCV 01302, ICCV 03401, ICCV 01306, ICCV 97022, ICCV 05310, ICCV 04306, ICCL 87206, JGK2, ICCV93054, ICCV 93024, ICCV 05113, ICCV 06102, ICCV 05112, ICCV 07118, ICCV 11118, ICCV 11311, ICCV 05303
3	9	ICCV 96001, JAKI 9218, ICCV 91024, JG130, ICCV 04304, ICCV 04307, Vaibhav, ICCV 08108, ICCL 86201
4	1	ICCV 93025
5	13	ICCL 82216, ICCV 11302, ICCV 05107, ICCV 04312, ICCV 04303, ICCV 95332, ICCV 91011, NbeG3, ICCV 93316, ICCV 96316, ICCV 06108, ICCV 90037, ICCL 85207
6	12	ICCV 07111, ICCV 08109, ICCV 07104, ICCV 90005, ICCV 07112, ICCV 93053, ICCV 93005, ICCV 91026, ICCV 91009, ICCV 03211, ICCV 09308, ICCV 03209,
7	1	ICCV 11113
8	1	ICCL 85201
9	1	ICCV 93014
10	1	ICCL89214
11	1	ICCV 03208
12	1	ICCV 00401
13	1	ICCV 95315
14	1	ICCL 84251
15	1	ICCV 08102
16	1	ICCV 91007

Table 4. Average inter- and- intra cluster distances for 100 genotypes of chickpea

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII	Cluster XIII	Cluster XIV	Cluster XV	Cluster XVI
Cluster I	75.35															
Cluster II	196.60	102.52														
Cluster III	247.33	229.07	123.98													
Cluster IV	105.19	138.78	305.46	0.00												
Cluster V	238.10	205.61	323.45	112.90	164.10											
Cluster VI	190.52	340.77	234.63	224.27	287.52	150.23										
Cluster VII	108.01	188.52	383.66	72.84	240.67	318.64	0.00									
Cluster VIII	129.47	163.25	286.06	182.13	352.52	366.02	128.74	0.00								
Cluster IX	150.66	149.78	349.82	135.92	280.89	403.53	106.91	67.02	0.00							
Cluster X	105.86	287.90	330.63	220.81	402.96	298.75	118.35	108.43	118.77	0.00						
Cluster XI	178.61	193.78	212.66	272.95	377.04	350.94	211.38	100.12	108.26	102.71	0.00					
Cluster XII	246.26	159.99	182.37	236.64	243.94	296.74	333.02	271.67	337.77	431.38	323.25	0.00				
Cluster XIII	382.54	236.42	172.30	328.42	296.34	326.43	524.52	432.21	508.59	621.96	456.46	99.93	0.00			
Cluster XIV	373.20	441.89	443.55	232.56	226.70	268.02	455.94	624.90	524.18	610.03	618.66	426.52	416.27	0.00		
Cluster XV	496.47	536.45	265.41	524.50	503.52	285.02	742.58	683.09	853.43	749.13	677.33	319.86	157.08	457.56	0.00	
Cluster XVI	295.59	373.14	433.18	176.29	239.59	262.11	405.98	506.75	507.49	571.14	663.96	271.86	299.86	219.92	354.91	0.00

Table 5: Different parameters used for molecular diversity analysis in chickpea

Marker	Major Allele Frquency	Gene Diversity	Heterozygosity	PIC
TA125	0.6500	0.4550	0.0000	0.3515
TA78	0.7150	0.4076	0.0300	0.3245
H5BO4	0.6650	0.4456	0.0900	0.3463
H1B17	0.6000	0.5022	0.0000	0.4027
Cam0620	0.6700	0.4422	0.0000	0.3444
ICCM0127	0.5500	0.4950	0.0000	0.3725
Cam0656	0.6500	0.4550	0.0000	0.3515
ICCAM0123a	0.6550	0.4520	0.0500	0.3498
Cam1536	0.6800	0.4352	0.0000	0.3405
TA135	0.5400	0.5378	0.0600	0.4375
H1G16	0.5650	0.4916	0.1500	0.3707
Mean	0.6309	0.4654	0.0345	0.3629

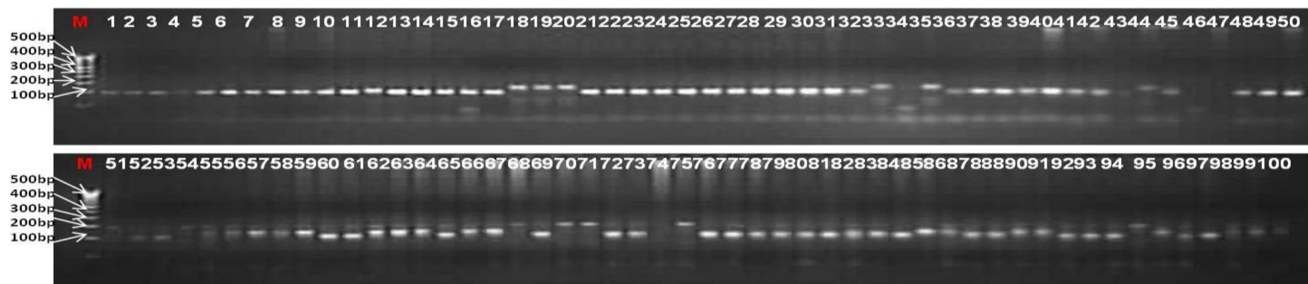

Fig.1. The banding pattern of TA 78 SSR markers among genotypes on 2.5 %

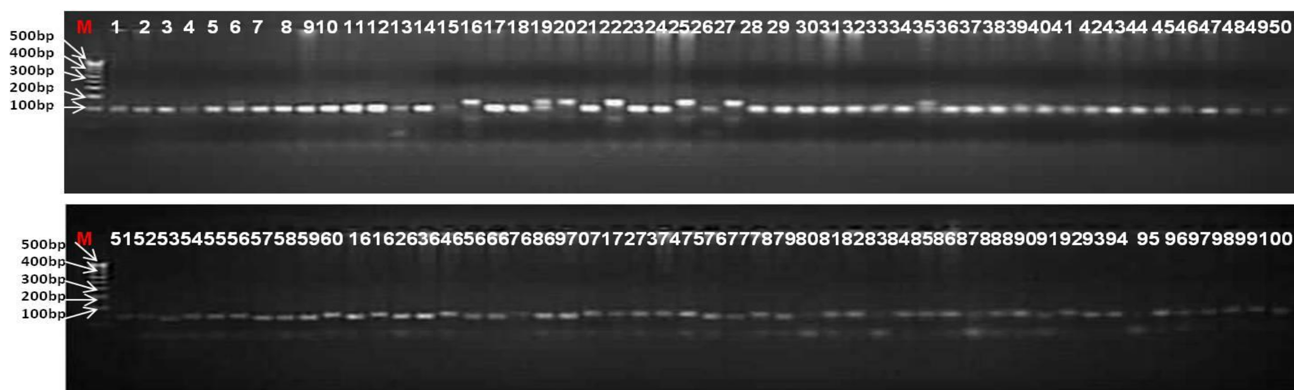

Fig.2. The banding pattern of H5BO4 SSR markers among genotypes on 2.5 %

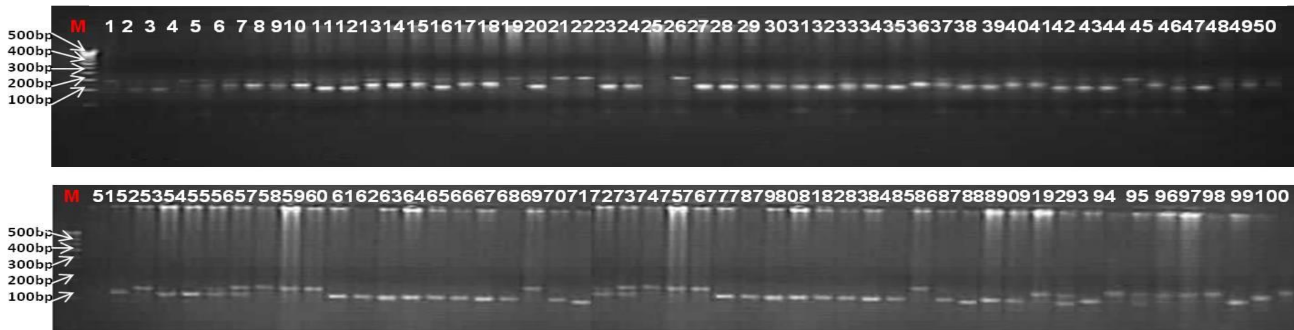

Fig.3. The banding pattern of TA 135 SSR markers among genotypes on 2.5 %

M – 100bp Ladder
Lane 1 – ICCL82216
Lane 2 – ICCL83233
Lane 3 – ICCL84207
Lane 4 – ICCL84248
Lane 5 – ICCL84233
Lane 6 – ICCL84242
Lane 7 – ICCL84251
Lane 8 – ICCL85201
Lane 9 – ICCL85207
Lane 10 –ICCL86201
Lane 11 –ICCL86235
Lane 12 –ICCL87206
Lane 13 –ICCL87207
Lane 14 –ICCL89214
Lane 15 –ICCV 90005
Lane 16 –ICCV 90015
Lane 17 –ICCV 90037
Lane 18 –ICCV 91005
Lane 19 –ICCV 91006
Lane 20 –ICCV 91007

Lane 21–ICCV 91008
Lane 22–ICCV 91009
Lane 23–ICCV 91011
Lane 24–ICCV 91020
Lane 25–ICCV 91021
Lane 26–ICCV 91024
Lane 27–ICCV 91026
Lane 28–ICCV 91902
Lane 29–ICCV 93005
Lane 30–ICCV 93014
Lane 31–ICCV 93024
Lane 32–ICCV 93025
Lane 33–ICCV 93053
Lane 34–ICCV 93054
Lane 35–ICCV 93316
Lane 36–ICCV 95315
Lane 37–ICCV 96001
Lane 38–ICCV 96027
Lane 39–ICCV 96316
Lane 40–ICCV 96317

Lane 41 –ICCV 97022
Lane 42 –ICCV 97318
Lane 43 –ICCV 00401
Lane 44 –ICCV 01302
Lane 45 –ICCV 01306
Lane 46– ICCV 03401
Lane 47– ICCV 03405
Lane 48– ICCV 03410
Lane 49 –ICCV 04303
Lane 50 –ICCV 04304
Lane 51 –ICCV 04306
Lane 52 –ICCV 04307
Lane 53 –ICCV 04312
Lane 54 –ICCV 05303
Lane 55 –ICCV 05307
Lane 56 –ICCV 05310
Lane 57 –ICCV 06301
Lane 58 –ICCV 09308
Lane 59 –ICCV 09314
Lane 60 –ICCV 95332

Lane61 – ICCV 00105
Lane62 –ICCV 03208
Lane63 –ICCV 03209
Lane64 –ICCV 03211
Lane65 –ICCV 05107
Lane66 –ICCV 05112
Lane67 –ICCV 05113
Lane68 –ICCV 06102
Lane69 –ICCV 06103
Lane70 –ICCV 06108
Lane71 –ICCV 07104
Lane72 –ICCV 07111
Lane73 –ICCV 07112
Lane74 –ICCV 07118
Lane75 –ICCV 08102
Lane76 –ICCV 08104
Lane77 –ICCV 08108
Lane78 –ICCV 08109
Lane79 –ICCV 10104
Lane80 –ICCV 10114

Lane 81 –ICCV 10115
Lane 82 –ICCV 10116
Lane 83 –ICCV 10117
Lane 84 –ICCV 10118
Lane 85 –ICCV 97024
Lane 86 –ICCV 11104
Lane 87 –ICCV 11109
Lane 88 –ICCV 11111
Lane 89 –ICCV 11113
Lane 90 –ICCV 11118
Lane 91 –ICCV 11302
Lane 92 –ICCV 11311
Lane 93 –JG14
Lane 94 –JG16
Lane 95 –JG130
Lane 96 –JAKI9218
Lane 97 –NBeG3
Lane 98 –Vaibhav
Lane 99 –JGK2
Lane 100 –KAK2