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GENETIC DIVERSITY ANALYSIS AND IDENTIFICATION OF DIVERSE GENOTYPES OF CHICKPEA USING MICROSATELLITE MARKERS

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ABSTRACT
Genetic diversity of 41 Indian chickpea genotypes was evaluated using 31 polymorphic microsatellite markers. The average percent polymorphism reported in the present study was 68.27% and numbers of alleles were ranged from 2 to 7, with an average of 2.71 alleles per locus. Cluster tree analysis categorised all the selected chickpea genotypes into two major clusters at similarity coefficient of 0.60. Genetic similarity content was ranged from 0.41 to 0.94 and minimum genetic similarity of 0.41 was observed in between Pusa 244 & H 04-11; and K 850 & H 04-11 chickpea genotypes. A low genetic similarity of 0.51 was reported between ICCV 4958 and HC 5 genotypes. The present study suggested that these diverse genotypes could be used in chickpea breeding program to broaden the genetic base of chickpea and to increase the grain yield potential with resistance and tolerance against biotic and abiotic stresses.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is self-pollinating, legume crop with a diploid set of chromosomes (2n = 2x = 16) and genome size of 738 Mb (Varshney *et al*., 2013). It is an important source of high-value protein (23%) (Jukanti *et al*., 2012) and β-carotene (Abbo *et al*., 2005) for millions of people. After soybean, chickpea is the world's second most important food legume crop. Among chickpea growing countries, India is a major producer of chickpea and contributes to 70% of the world's total production. Chickpea productivity revealed interesting trend from last four decades like productivity consistently increased in India and Mexico while it declined in Turkey, Pakistan, and Iran (Upadhyaya *et al*., 2012). Chickpea productivity is still low in India as compared to other chickpea producing countries like Mexico, Australia and Ethiopia (Sewak *et al.,* 2012). This is mainly due to the narrow genetic base and sexual incompatibility with its wild relatives, which carry the sources for resistance and tolerance to various abiotic and biotic stresses (Choudhary *et al*., 2012). In order to utilize the full genetic potential of chickpea, we need to assess the extent and the pattern of real diversity available in the existing cultivated genotypes.

The diversity of chickpea can be assessed by different methods ranging from the conventional methods like morphological characters, biochemical and molecular methods, with comparative advantages and disadvantages (Jannatabadi *et al*., 2013; Ghaffari *et al*., 2014; Johnson *et al*., 2015). However, molecular markers based methods are believed to be more reliable and repeatable as compared to morphological and biochemical methods. Out of various types of molecular markers, simple sequence repeats (SSRs) are informative molecular markers used for genetic diversity studies because of their simplicity, high levels of polymorphism, high reproducibility, and co-dominant inherit-ance patterns (Roder *et al*., 1998). Using SSR markers, genetic diversity of chickpea from various parts of the world has been revealed by various workers (Winter *et al*., 1999; Upadhyaya *et al*., 2008; Choudhary *et al*., 2012; Shukala *et al*., 2014; Hajibarat *et al*., 2015). Hence, the objective of the present study was to analyse the level of genetic diversity and relationships within the popular Indian chickpea genotypes using SSR markers. The study can supply information about evolutionary relationships and will therefore provide opportunities for breeders and molecular biologists to use diverse accessions for varied applications in chickpea genomics and breeding program.

MATERIALS AND METHODS

Plant Materials

Forty-one chickpea (*Cicer arietinum* L.) genotypes grown across the India were analyzed in the present study (Table 1).

The seeds of all the selected chickpea genotypes were procured from Pulses Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar, Haryana. The plants were grown in pots using standard agronomic practices in Department of Molecular Biology, Biotechnology and Bioinformatics, CCSHAU, Hisar.

DNA extraction and microsatellite marker analysis

Total genomic DNA of 41 chickpea genotypes was isolated from young leaves using a CTAB method (Saghai-Maroof *et al.*, 1984) with minor modifications. A set of 41 SSR primer pairs developed by Lichtenzveig *et al*. (2005) were used to amplify the genomic DNA of all the selected chickpea genotypes. PCR conditions were standardized and PCR reaction was performed in 15 μl reaction volume with final concentrations of 50 ng DNA, 1.5 mM $MgCl_2$, 100 μM of dNTPs, 1X PCR buffer, 50 pM of each forward and reverse primer, 1U of *Taq* DNA polymerase in a PTC-100 programmable thermal cycler (MJ research and Biometra Personal). PCR amplification was carried out with 3 min initial denaturation at 94ºC, followed by 30 cycles of denaturation at 94ºC for 1 min, annealing at 58ºC for 1 min and extension at 72ºC for 1 min. The amplification finished with the final extension of 10 min at 72ºC. PCR products were analysed using 2.0 % agarose gel electrophoresis after staining with ethidium bromide. PCR amplification products were photographed using Bio-Rad gel documentation system.

Data analysis

Based on the presence (taken as 1) or absence (taken as 0) of an allele of each chickpea genotype, microsatellite/SSR amplification profiles were visually scored. The 0/1 matrix was generated to calculate the similarity matrix using 'simqual' subprogram of numerical taxonomy and multivariate analysis system program NTSYS -pc software (Rohlf, 1998). Based on the similarity matrix, dendrogram showing the genetic relationships between genotypes was constructed using unweighted pair group method with arithmetic average (UPGMA) sub-programme of NTSYS-pc.

RESULTS AND DISCUSSION

In the present study, a total of 41 chickpea genotypes and 41 SSR primers were used for the genetic diversity study. Out of 41 primers, 31 (75.6%) primers were found to be polymorphic, 3 primers (H1C09, H1E06 and H3H031) were monomorphic and 7 primers (H1A06, H1H24, H1J24, H1J12, H1L17, H1O09 and H3H022) did not exhibit any amplification for any of the genotypes. The percent polymorphism was ranged from 50-100% with an average of 68.27% for chickpea genotypes. The banding profile and polymorphism generated by one of the primers (H3G11) is shown in Fig. 1. The size of the alleles obtained ranged from 100 to 1300 bp whereas, the number of alleles ranged from 2 to 7 with an average of 2.71 alleles per primer. Singh *et al*. (2008) analysed 21 chickpea cultivars using 18 SSR markers and detected 2 to 5 alleles. Rizvi *et al*. (2014) obtained similar results by using 68 Indian chickpea varieties. Contrary to this, Shukla *et al*. (2014) found an average of 8.61 alleles per locus among the different cultivars of chickpea grown in India. Different workers reported the different level of polymorphism in chickpea because it depends on the type of genotype, sampling strategy and marker used (He *et al.*, 2011; Kong *et al.*, 2011). The primer H1F14 generated a maximum of 7 alleles followed by primer H4G02 which generated 6 alleles (Table 2).

Cluster analysis

The average linkage between the chickpea genotypes was used for constructing a phylogenetic tree. The relationship among the 41 chickpea genotypes used during the present investigation was represented in Fig. 2. The hierarchical cluster analysis identified two major clusters (A & B) at a similarity coefficient of 0.60. Cluster A comprised of 6 genotypes while Cluster B had remaining 35 genotypes. Both the clusters were further divided into sub clusters. However, one genotype KAK-2 (ak) is out grouped from cluster A with the similarity coefficient of 0.698 and rest of the genotypes were further divided into sub clusters. Clusters B was divided into two sub clusters (B_1 and B_2) at a similarity coefficient of 0.672. Sub cluster B_1 comprised eight genotypes in which RSG 963 (l) was out grouped at similarity coefficient of 0.779 and remaining seven genotypes were further divided into sub-sub clusters. The remaining 27 genotypes of cluster B fall in sub cluster B_2. Sub cluster B_2 again divided into two sub-sub clusters (C_1 & C_2) with similarity coefficient of 0.764. One sub-sub cluster C_1 comprised of 12 genotypes and remaining 15 genotypes were

Table 1: List of chickpea genotypes used in the present study

Chickpea genotypes	Code	Chickpea genotypes	Code	Chickpea genotypes	Code
ICCV4958	a	GNG146	o	RAU52	ac
IPC 98-12	b	RS-10	p	RSG-143-1	ad
PDG 84-16	c	Pusa244	q	RSG 888	ae
BG 276	d	Vijay	r	RSG 11	af
H-208	e	ICCV96029	s	Pusa 267	ag
ICCV96030	f	JG218	t	HC-1	ah
HC-3	g	Phule G-5	u	Pusa362	ai
PG96006	h	K-850	v	KAK2	aj
E100Ym	i	GCP101	w	H04-11	ak
GNG 663	j	Virat	x	HC-5	al
C-235	k	ICCV2	y	H04-31	am
RSG 963	l	PDG4	z	H03-56	an
CSJD-844	m	Pusa212	aa	BGM413	ao
RSG 931	n	CSG 8962	ab		

L: 100 bp DNA marker, a-ao: chickpea genotypes

Table 2: Allelic variation of 31 SSR markers employed for genotyping of 41 Indian chickpea genotypes

SrNo.	Primer name	Total number of alleles	Polymorphic alleles	Monomorphic allele	Percent polymorphism
1.	H1A19	2	2	0	100
2.	H1B08	2	1	1	50
3.	H1C19	2	1	1	50
4.	H1E20	2	1	1	50
5.	H1F02	2	2	0	100
6.	H1F14	7	7	0	100
7.	H1H14	2	1	1	50
8.	H1H15	2	1	1	50
9.	H1H18	2	1	1	50
10.	H1J15	3	3	0	100
11.	H1K18	2	1	1	50
12.	H1O01	5	5	0	100
13.	H1O12	2	2	0	100
14.	H1P17	2	1	1	50
15.	H1A04	2	1	1	50
16.	H2B201	2	1	1	50
17.	H2B202	2	1	1	50
18.	H1B203	4	3	1	75
19.	H2I20	2	1	1	50
20.	H2J04	3	3	0	100
21.	H2L101	2	2	0	100
22.	H2L102	2	1	1	50
23.	H3A03	2	1	1	50
24.	H3A052	2	1	1	50
25.	H3B01	3	2	1	66.6
26.	H3B04	2	1	1	50
27.	H3C08	2	1	1	50
28.	H3G11	4	3	1	75
29.	H4G02	6	6	0	100
30.	H4H05	5	5	0	100
31.	H4H07	2	1	1	50
	Mean	2.71	2.03	0.68	68.27

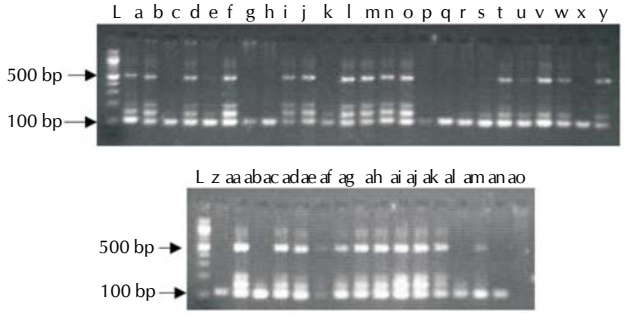

Figure 1: Agarose gel electrophoresis profiles of 41 chickpea genotypes amplified using the SSR primer, H3G11

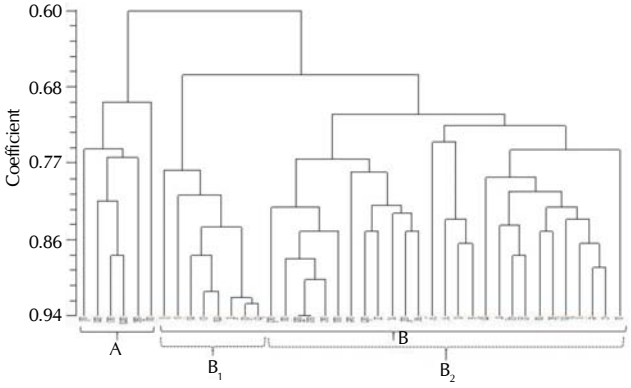

Figure 2: Dendrogram for 41 Indian chickpea genotypes derived from UPGMA cluster analysis using 31 SSR markers

in other sub-sub cluster C_2. Agrawal and Srivastava (2010) also identified two major clusters of 68 chickpea genotypes at a similarity coefficient of 0.71 and genotype, RS-10 & Vijay were reported to be in single major cluster as reported in the present study.

Similarity matrix

Similarity matrix generated by Jaccard's coefficient has shown the extent of relatedness between the genotypes. The overall range of similarity (0.41 to 0.94) found among the chickpea genotypes of India is low compared to that found by Irula *et al*. (2002) where the similarity between 14 chickpea species ranged from 0.30 to 0.98. The large variability in diversity is due to the use of exotic and wild germplasm in the study. Lower the similarity between the two chickpea genotypes better is the scope to include them in a hybridization programs to get transgressive segregants (Agrawal and Srivastava, 2010). The similarity matrix generated among the 41 genotypes (data not shown) showed the maximum similarity between Pusa 267 and HC-1 (94%). It was also substantiated by the

dendrogram (Fig. 2) where Pusa 267 and HC- 1 were found be placed very close in a single sub-cluster. The minimum genetic similarity of 0.41 was observed in between Pusa 244 & H 04-11; and K 850 & H 04-11. A low genetic similarity of 0.51 and 0.52 was also found between ICCV 4958 (drought tolerant) and HC 5 (wilt resistance and root rot resistance); and, RS- 10 (drought tolerant) and RSG 963 (pod borer resistance), respectively. Effective use of Pusa 244 × H 04-11, K 850 × H 04-11, HC-5 × ICCV 4958 and RS- 10 × RSG 963 crosses of chickpea may give rise to better grain yielding cultivars with resistance against biotic and abiotic stresses. Further, these crosses can be used to broaden the genetic base of chickpea to develop improved plant types. Agrawal and Srivastava (2010), Jayalakshmi *et al*. (2014) and Parhe *et al*. (2014) also identified genetically diverse chickpea genotypes, which can be included in future chickpea hybridization programme.

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REFERENCES

Abbo, S., Molina, C., Jungmann, R., Grusak, M.A., Berkovitch, Z., Reifen, R., Kahl, G., Winter, P. and Reifen, R. 2005. Quantitative trait loci governing carotenoid concentration and weight in seed of chickpea (*Cicer aritenium* L.). *Theor. Appl.Genet*. **111:** 185-195.

Agrawal, P. K. and Srivastava, A. 2010. Assessment of genetic diversity among chickpea cultivars of India using RAPD markers. *Indian J. Genet*. **70(3):** 264-270.

Choudhary, P., Khanna, S. M., Jain, P. K., Bharadwaj, C., Kumar, J., Lakhera, P. C. and Srinivasan, R. 2012. Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. *Genet. Mol. Res*. **11(2):** 891-905.

Ghaffari, P., Talebi, R. and Keshavarzi, F. 2014. Genetic diversity and geographical differentiation of Iranian landrace, cultivars, and exotic chickpea lines as revealed by morphological and microsatellite markers. *Physiol. Mol. Biol. Plants.* **20(2):** 225-233.

Hajibarat, Z., Saidi, A., Hajibarat, Z. and Telabi, R. 2015. Characterization of genetic diversity in chickpea using SSR markers, Start Codon Targeted Polymorphism (SCoT) and Conserved DNA-Derived Polymorphism (CDDP). *Physiol. Mol. Biol. Plants*. **21(3):** 365-373.

He, Q., Li, X. W., Liang, G. L., Ji, K., Guo, Q. G., Yuan, W. M., Zhou, G. Z., Chen, K. S., van de Weg, W. E. and Gao, Z. S. 2011. Genetic diversity and identity of Chinese loquat cultivars/accessions (*Eriobotrya japonica*) using apple SSR markers. *Plant Mol. Biol. Rep.* **29:** 197-208.

Irula, 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.

Jannatabadi, A. A., Talebi, R., Armin, M., Jamalabadi, J. and Baghebani, N. 2013. Genetic diversity of Iranian landrace chickpea (*Cicer arietinum* L.) accessions from different geographical origins as revealed by morphological and sequence tagged microsatellite markers. *J. Plant Biochem. Biotechnol*. **23(2):** 225-229.

Jayalakshmi, V., Ronald, G. R. and Lakshmanna, K. 2014. Diversity analysis of chickpea germplasm in scarce rainfall zone of Andhra Pradesh, India. *Legume Res.* **37(6):** 682-684.

Johnson, P. L., Sharma, R. N. and Nanda, H. C. 2015. Genetic diversity and association analysis for yield traits chickpea (*Cicer arietinum* L.) under rice based cropping system. *The Bioscan.* **10(2):** 879-884.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L. and Chibbar, R. N. 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.). *A review. Br. J. Nutr.* **108:** S12-S26.

Kong, Q., Li, X., Xiang, C., Wang, H., Song, J. and Zhi, H. 2011. Genetic diversity of radish (*Raphanus sativus* L.) germplasm resources revealed by AFLP and RAPD markers. *Plant Mol. Biol. Rep.* **29:** 217-223.

Lichtenzveig, J., Scheuring, C., Dodge, J., Abbo, S. and Zhang, H. B. 2005. Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea (*Cicer arietinum* L.). *Theor. Appl. Genet.* **110:** 492-510.

Parhe, S. D., Harer, P. N. and Nagawade, D. R. 2014. Investigation of genetic divergence in chickpea (*Cicer arietinum* L.) genotypes. *The Bioscan.* **9(2):** 879-882.

Rizvi, H., Babu, B. K. and Agrawal, P. K. 2014. Molecular analysis of kabuli and desi type of Indian chickpea (*Cicer arietinum* L.) cultivars using STMS markers. *J. Plant Biochem. Biotechnol.* **23(1):** 52-60.

Roder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M., Lorey P. and Ganal, M. W. 1998. A microsatellite map of wheat. *Genetics*. **149:** 2007-2023.

Rohlf, F. J. 1998. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.0, Exeter Software, New York.

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.

Sewak, S., Iquebal, M. A., Singh, N. P., Solanki, R. K. and Sarika. 2012. Genetic diversity studies in chickpea *(Cicer arietinum)* germplasm. *J. Food Legumes*. **25(1):** 31-36.

Shukla, N., Shukla, R. S. and Chavan, A. 2014. Stability and molecular characterization to screen out heat tolerant genotypes of chickpea (*Cicer arietinum* L.) *The Bioscan.* **9(2):** 845-851.

Singh, R., Singhal, V. and Randhawa, G. J. 2008. Molecular analysis of chickpea (*Cicer arietinum* L.) cultivars using AFLP and STMS markers. *J. Plant Biochem. Biotechnol.* **17:** 167-171.

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

Upadhyaya, H. D., Kashiwagi, J., Varshney, R.K., Gaur, P.M., Saxena, K. B., Krishnamurthy, L., Gowda, C. L. L., Pundir, R. P. S., Chaturvedi, S. K., Basu, P. S. and Singh, I. P. 2012. Phenotyping chickpeas and pigeonpeas for adaptation to drought. *Front. Physiol.* **3(169):** 1-10.

Varshney, R. K., Song, C., Saxena, R.K., Azam, S., Yu, S. *et al.* 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat. Biotechnol*. **31:** 240-246.

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