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GENETIC DIVERSITY ANALYSIS IN *KABULI* CHICKPEA (*CICER ARIETINUM* L.) GENOTYPES BASED ON QUANTITATIVE TRAITS AND MOLECULAR MARKERS

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Abstract

Chickpea (*Cicer arietinum* L.) provides high quality protein for human and animal consumption as well as offers economic benefits to farmers because of the high market value for its grains. Fifty indigenous and exotic *kabuli* chickpea lines varying in seed yield and plant type were subjected to 20 SSR markers analysis for assessment of genetic diversity. Among twenty SSR primers, eleven of them were able to produce polymorphic bands. Quantitative traits based clustering pattern was found to be similar to molecular clustering for most of the *kabuli* genotypes such as JGK 1, JGK 2, JGK 3, JGK 32-1 and some of the ICRISAT (ICCVs) and ICARDA (FLIPs) lines. This indicated the association of used molecular markers with targeted quantitative traits in the present study.

Introduction

Chickpea (*Cicer arietinum* L.) is a native of southern Europe and also an important food crop in Asia, Africa and Central America. It provides high quality protein for human and animal consumption as well as offers economic benefits to farmers because of the high market value for its grains (Yegrem 2021). Chickpea not only meets its own nitrogen requirement but also leaves residual nitrogen for succeeding crop and maintaining soil fertility, improves physical and chemical properties of the soil, and decreases pests, diseases and weeds of rainy season (*kharif*) crops (Dotaniya *et al.* 2022). Early maturing, disease resistant and extra large seeded *kabuli* chickpea varieties may be helpful in generating additional income for farmers, diversifying cereal based cropping systems, bringing additional area under chickpea in general and of *kabuli* in particular in irrigated areas of the country (Babbar and Thakur 2012, Dhuria and Babbar 2016, Tiwari and Babbar 2017).

Low genetic diversity in the cultivated chickpea is one of the causes for narrow genetic base leading to lower yield gains in chickpea (Bharadwaj *et al.* 2010, Shivwanshi and Babbar 2018). So, there is a need to develop genotypes for specific environment. Diversity assessment can be performed using morphological, biochemical and molecular markers. Morphological markers are phenotypic traits, and equating phenotype and genotype can be problematic because same morphologies might have different genetic make-ups. Molecular markers, on the other hand, can capture changes at the DNA level, and so more accurately reflect genuine genetic variation and links among accessions and their shared ancestry than phenotypic markers (Glaszmann *et al.* 2010, Luo *et al.* 2020).

Molecular markers being stable and informative have been used for characterization of crop plants diversity. These are vital for marker assisted breeding programs targeting chickpea yield enhancement (Bharadwaj *et al.* 2010) and to provide information on allelic variation in the breeding breeding material (Tiwari *et al.* 2019). Microsatellites being abundant, highly polymorphic, co-dominant, multi-allelic and uniform in distribution across the genome are considered important in plant breeding (Kachare *et al.* 2019, Tiwari *et al.* 2019). Unique allelic profiles generated using

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the scored loci differentiates the genotypes based on the genetic data (Konsam *et al.* 2014). Simple Sequence Repeat (SSR) markers are one of the best choices for studying the genetic variability (Kachare *et al.* 2019). Thus, simple sequence repeat (SSR) markers were used in this study to understand the genetic relationships among chickpea genotypes for use in future breeding programs. Therefore, the present study was aimed to assess the genetic diversity of *kabuli* chickpea germplasm by SSR markers.

Materials and Methods

The present investigation was conducted at Seed Breeding Farm, Department of Plant Breeding and Genetics, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur India during 2017-18 and 2018-19. Experimental material consisted of indigenous and exotic 50 *kabuli* chickpea lines obtained from AICRP on Chickpea, JNKVV, Jabalpur, ICRISAT, Patancheru and ICARDA, Morocco. The experiment was conducted under Randomized Complete Block Design, used recommended agronomic practices for phenotypic evaluation and normal growth of crop. Seeds were line sown with a spacing of 45 cm row to row and 10 cm between plants. Observations were recorded on single plant basis on five randomly selected plants from each plot: Days to flower initiation (DFI), Days to 50% flowering (DF), Days to pod initiations (DPI), Days to maturity (DM), Plant height (cm) (PH), Number of primary branches per plant (NPB), Number of secondary branches per plant (NSB), Total number of pods per plant (NPP), Number of effective pods per plant (TNPP), Number of seeds per pod (NSP), 100-Seed weight (g) (100SW), Biological yield per plant (g) (BY), Harvest index (%) (HI), Seed yield per plant (g) (SY). Means across three replications were calculated for each trait and used for data analysis.

DNA was extracted from the fresh young leaves of 50 *kabuli* chickpea genotypes using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Saghai-Maroof *et al.* 1984). The quality of DNA was checked in 0.8 per cent agarose gel electrophoresis. A total of 20 chickpea SSR primers were selected in order to have a random coverage of markers distributed throughout the genome of chickpea. PCR reactions were carried out in 20 µl with different ingredients and parameters as standardized by Kachare *et al.* (2019). The PCR products were then run on agarose gel at 65 volts for 2 hrs and resolved by ethidium bromide staining procedure. The amplified fragments were visualized and photographed under UV light using a gel documentation system (Syngene, UK). Out of 20 SSR markers, eleven of them produced polymorphic bands were used for the analysis (Table 1). DNA fragment sizes on agarose gel were estimated by comparisons with 100 bp ladder run on the same gel. The amplified fragments were scored '1' for presence and '0' for absence to create a binary data matrix. The binary matrix was used to estimate Jaccard's genetic similarity coefficient (Jaccard 1908) for SSR. Dendrogram was constructed by using unweighted pair group method with arithmetic averages (UPGMA) using Power Marker version 3.25. Major allele frequency, average number of allele per locus, gene diversity, heterozygosity and polymorphism information content were calculated using Power Maker V 3.25 (Liu and Muse 2005).

Results and Discussion

Amplified products were observed for 50 genotypes of *kabuli* chickpea using 20 SSR primers. Major allele frequency of 20 SSR markers ranged from 0.5400 (TA180) to 1.000 (H5A04, TA 76, TR 20, H5B04, GAA 50, TA 58, Cam 0656, TA 34, TA 135). The mean value of major allele frequency among the primers was 0.8990 while highest gene diversity was found by marker TA 180 (0.4968).Gene diversity showed a mean value of 0.1413. The highest gene diversity were found in TA 180 (0.4968) followed by primer Cam0620 (0.4872), GA16 (0.4488), H1P092 (3880), ICCM123a (0.2408), Cam0443 (0.2408), TA37 (0.1800), TA125 (0.1128), TA59

(0.0768), TA76 (0.768) and TR19 (0.0768). Minimum diversity was showed by 9 markers i.e., 0.000 in H5A04, TA 76, TR 20, H5B04, GAA 50, TA 58, Cam 0656, TA 34 and TA 135.

The polymorphic information content (PIC) among the locus ranged from 0.000 (H5A04, TA76, TR20, H5B04, GAA 50, TA 58, Cam 0656, TA 34, TA 135) to 0.3734 (TA180). The mean value of polymorphic information content was 0.1177. TA180 (0.3734) showed highest polymorphic information content and also showed highest gene diversity by marker TA180 (0.4968) (Table 1). Gene diversity is defined as the probability that two randomly chosen alleles from the population are different. Low level of genetic diversity with an average PIC value 0.1342 was obtained in the present study. The low genetic diversity and PIC values indicate that the analysis was carried out within the primary gene pool, comprising of genotypes which are more closely related to each other compared to the secondary and tertiary gene pool (Choudhary *et al*. (2012).

Table 1. Details of molecular markers used in the study.

Marker	Allele size (bp)	Major allele frequency	Allele no.	Gene diversity	PIC
GA 16	230-300	0.66	2	0.4488	0.3481
H1P092	190-215	0.76	4	0.3880	0.3481
TA78	240-250	0.96	2	0.0768	0.0739
TA180	200-210	0.54	2	0.4968	0.3734
TA37	270-280	0.90	2	0.1800	0.1638
H5A04	200	1.00	1	0.0000	0.0000
TA 76	150	1.00	1	0.0000	0.0000
Cam0620	140-150	0.58	2	0.4872	0.3685
TR 20	160	1.00	1	0.0000	0.0000
Cam0443	250-350	0.86	2	0.2408	0.2118
ICCM0123a	250-270	0.86	2	0.2408	0.2118
TA 125	200-250	0.94	2	0.1128	0.1064
H5B04	250	1.00	1	0.0000	0.0000
TR 19	230-240	0.96	2	0.0768	0.0739
GAA 50	250	1.00	1	0.0000	0.0000
TA 58	250	1.00	1	0.0000	0.0000
Cam 0656	200	1.00	1	0.0000	0.0000
TA 59	205-300	0.96	2	0.0768	0.0739
TA 34	160	1.00	1	0.0000	0.0000
TA 135	250	1.00	1	0.0000	0.0000
Mean		0.8990	1.6500	0.1413	0.1177

The dendrogram constructed based on UPGMA between genotypes showed that 50 genotypes initially formed only two groups (group A and B) shown in Fig. 1 in which group A contained single chickpea genotype (ICCV 171312) and group B contained 49 genotypes. Group B was further divided into two clusters (cluster B1 and B2). Cluster B1 comprised 6 genotypes namely JGK3, JGK2, JGK5, JGK32-1, FLIP93-93C and FLIP88-85C. Cluster B2 encompassed 43 genotypes and further divided into two sub groups (subgroup B2A and B2B). Subgroup B2A comprised 3 genotypes viz., JGK1, FLIP11-164C and FLIP11-180C. Subgroup B2B encompassed 40 genotypes and further bifurcated into two parts (part C and D). Part C had 19 chickpea

genotypes *viz*,. ICCV 171313, ICCV 171314, ICCV 171315, FLIP11-65C, ICCV 171309, ICCV 14509, ICCV 06303, ICCV 6301, FLIP 08-986, FLIP 09-348C, FLIP 11-64C, FLIP 11-78C, ICCV 14511, ICCV 14308, ICCV 14313, ICCV 14314, ICCV 14501, ICCV 14508 and ICCV 14510. Among these genotypes ICCV 171313, ICCV 171314 and ICCV 171315 showed high genetic similarity and clustered together. Part D encompassed remaining 21 genotypes namely FLIP 11-220C, FLIP 11-232C, ILC 482, FLIP 11-51C, FLIP 11-53C, FLIP11-156C, FLIP11-93C, FLIP11-183C, FLIP11-84C, FLIP11-87C, FLIP11-91C, ICCV 171301, ICCV 171305, FLIP 11-211C, FLIP 11-195C, FLIP 11-197C, ICCV 14513, ICCV 171306, ICCV 171308, ICCV 14500 and ICCV 14512 (Fig. 1).

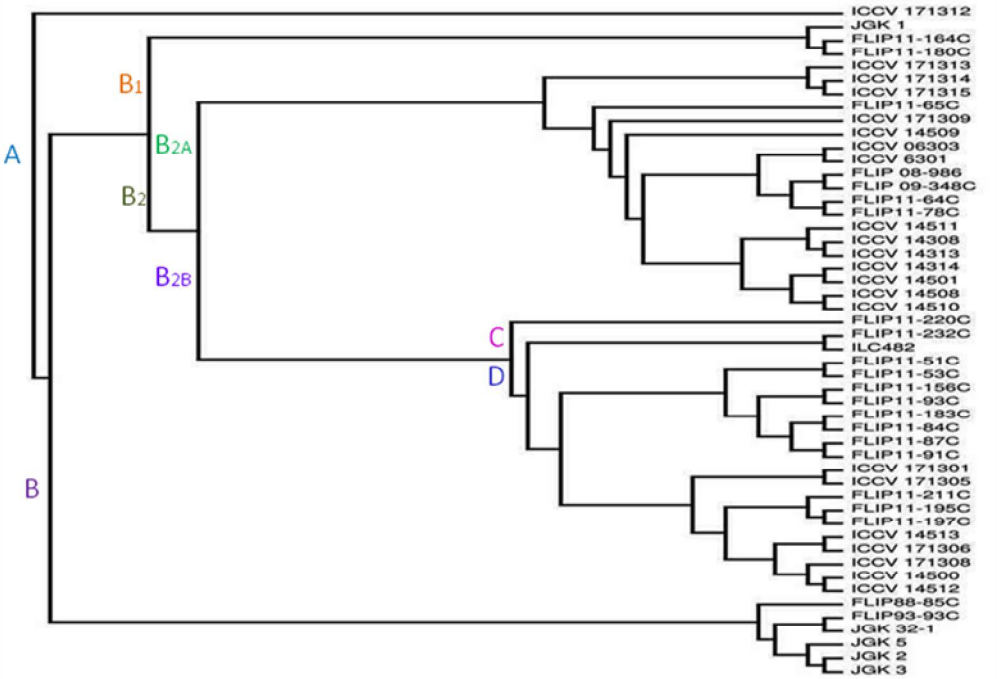

Fig. 1. UPGMA dendrogram of 50 genotypes of *kabuli* chickpea based on the 20 SSR primers.

The total numbers of alleles amplified were 33 with a mean value of 1.65. The highest number of alleles was amplified by marker H1P092 and unique alleles were amplified by three markers *viz.*, GA16, Cam0443 and ICCM123a. Allele size was found to be highest in Cam0443 (350bp) whereas lowest in Cam0620 (140bp). Marker GA16 amplified a total of 2 alleles out of which one was unique allele amplified in the genotype H12-22 with allele size of 300bp. Marker Cam0443 amplified a total of 2 alleles out of which one was unique allele in IPC 2010-14 genotypes with an allele size of 350bp. Similarly; ICCM123a amplified three alleles, out of which one was unique allele in genotypes NDG 15-5 with an allele of 270bp.

The overall clustering pattern did not strictly follow the grouping of accessions according to their geographic origins. But some of the genotypes groups according to their origin such as JGK 1, JGK 2, JGK 3 etc. Similarly, most of the ICCV and FLIP genotypes grouped together. This might be due to the extensive germplasm exchange among farms from different geographical regions. In contrast, some accessions from the same geographical origins remained distinct and sub clustered among them. Overall it is agreed that *C. arietinum* is far less variable than its wild annual relatives. These results indicate that despite extensive breeding efforts, the varieties under current cultivation are closely related among themselves. This is probably due to the use of few

key varieties for hybridization. Hence, it is essential to increase the number of accessions in the primary gene pool to maximize the genetic diversity available for introgression into cultivated species *C. arietinum*.

The plant breeder always fascinated by great amount of diversity in crop plants. Genetic divergence which is due to genetic factors is the basis for heritable improvement. The present study was also aimed at analyzing the genetic divergence among the 50 indigenous and elite lines of *kabuli* chickpea to identify the superior and divergent parental lines for formulating the crossing programme. Mahalanobis (D^2) statistics is a powerful tool widely used by breeders in quantifying the degree of divergence at genotypic level. Contribution of individual traits towards the total divergence was found maximum for number of effective pods per plant, biological yield, 100-seed weight, harvest index and seed yield per plant in E I,EII and pooled analysis in the *kabuli* chickpea lines. These traits responsible for expressing maximum diversity between the clusters should be considered prime during selection. This effect has been widely studied (Gediya *et al.* 2018).

Based on the genetic distance 50 indigenous and exotic *kabuli* chickpea lines were grouped into 8 clusters in EI, 13 clusters in EII and 11 clusters in pooled analysis (Table 2). Clustering pattern of genotypes confirmed the quantum of diversity present in the material under study and assumed that the genotypes within cluster have smaller D^2 values among themselves than those from genotypes belonging to different clusters. Genotypes *viz*., FLIP11-91C, FLIP93-93C, ICCV 6301, FLIP11-51C, ICCV171315, FLIP11-87C, ICCV14501, ICCV14313, FLIP11-180C, ICCV171309, ILC482, FLIP08-986, ICCV6301, FLIP09-348C, ICCV14511and ICCV171308 were grouped independently in different clusters. Similar findings were reported by Naveed *et al.* (2015) and Gediya *et al.* (2018). The clustering pattern showed that genotypes from different source were clubbed into one group and also genotypes of same source forming different cluster indicated no relationship between geographical and genetic divergence. High heterotic combinations may be obtained when genotypes of these distinctly placed clusters considered in hybridization programme. Two clusters in EI, five clusters in EII and four clusters in Pooled analysis were monogenotypic in nature. These results indicate existence of some homology between closely situated clusters. Similar findings were reported earlier by Gaikwad *et al.* (2014).These results showed that crossing between the genotypes of the clusters separated by maximum inter-cluster distance can give desirable transgressive segregates.

On the basis of cluster mean values of *kabuli* chickpea lines, in E I cluster IV (FLIP93-93C) had highest mean value for days to 50% flowering followed by days to pod initiation, number of primary branches per plant and number of seeds per pod, cluster VIII (ICCV 171308, ICCV 171313) observed highest mean value for total number of pods per plant, number of effective pods per plant and seed yield, cluster V (ICCV 171306, ICCV 171309) had days to flower initiation, day to maturity and biological yield. In EII cluster X (ICCV 14501) had high mean value for days to flower initiation followed by days to 50% flowering, day to pod initiation and number of secondary branches per plant. Similarly in pooled analysis cluster VIII (ICCV 171315) had highest cluster mean value for days to flower initiation, days to maturity, biological yield and seed yield per plant, cluster V had highest cluster mean value for days to 50% flowering, day to pod initiation, plant height and 100-seed weight.

Results revealed highly significant difference among all the genotypes for all the quantitative traits studied indicating the presence of sufficient variability in the material under study for selection of these traits. On the basis of studied traits superior genotypes were selected and used in hybridization programme as a donor parent. Early maturing genotypes of this study may be utilized in breeding programme for introgression of earliness in different cultivars. Short duration cultivars fit in crop rotation and tolerant to pest due to escape /avoid its attack in plant reproductive phase due to less days taken for its maturity. Intercrossing of genotypes involved in these clusters could be practiced for inducing variability in the respective characters and their rationale improvement for increasing seed yield.

Table 2. Distribution of genotypes in different clusters using D^2 in *kabuli* chickpea EI, EII and Pooled analysis.

	EI		EII		Pooled analysis	
Cluster	No. of genotypes	Genotypes included in the cluster	No. of genotypes	Genotypes comprised in the cluster	No. of genotypes	Genotypes grouped in the cluster
I	33	ICCV 14308, ICCV 14501, ICCV 14511, ICCV 14509, ICCV 14508, ICCV 14313, ICCV 14314, ICCV 14510, ICCV 14500, ICCV 14513, ICCV 14512, ICCV 171314, ICCV 06303, FLIP 09-348C, FLIP 08-986, FLIP11-51C, FLIP11-53C, FLIP11-64C, FLIP11-65C, FLIP11-8C,FLIP11-84C, FLIP11-87C, FLIP11-156C, FLIP11-164C,FLIP11-180C, FLIP11-195C, FLIP11-197C, FLIP11-232C, ILC482, FLIP88-85C, JGK 32-1, JGK 3, JGK 5	13	ICCV 14314, ICCV 171312, ICCV 14510, ICCV 171313, FLIP11-156C, ICCV 06303, ICCV 14513, FLIP11-211C, FLIP11-53C, ICCV 14512, ILC482, FLIP11-78C, FLIP88-85C	18	ICCV 14308, ICCV 14501, ICCV 14509, ICCV 14508, ICCV 14313, ICCV 14314, ICCV 14510,ICCV 14500,ICCV 14513, ICCV 14512, ICCV 171314, ICCV 06303, FLIP11-53C, FLIP11-65C, FLIP11-156C, FLIP11-195C, FLIP11-197C
II	1	FLIP11-91C	16	FLIP11-93C, FLIP11-183C, FLIP11-197C, ICCV 171301, FLIP11-232C, FLIP11-195C, ICCV 6301, FLIP 08-986, ICCV 171306, FLIP11-64C, ICCV, 171308, FLIP11-164C, FLIP11-91C, FLIP93-93C, FLIP11-65C, FLIP11-84C	10	FLIP11-84C, FLIP88-85C, FLIP11-87C, FLIP11-232C, FLIP11-78C, FLIP11-164C, FLIP11-64C, FLIP11-91C, FLIP11-51C, FLIP93-93C
III	8	ICCV 171301, ICCV 171305, ICCV 171315, ICCV171312, FLIP11-183C, FLIP11-211C, FLIP11-93C, FLIP11-220C	6	ICCV 14509, ICCV 14508, ICCV 14511, FLIP11-220C, ICCV 14500, ICCV 171305	5	FLIP11-211C, FLIP11-220C, FLIP11-93C, FLIP11-183C, ICCV 171301
IV	1	FLIP93-93C	4	ICCV 14308, JGK 1, JGK 2, JGK 3	8	ICCV 171305, ICCV 171312, ICCV 171315, FLIP11-180C, JGK 32-1, JGK 2, JGK 3, JGK 1
V	2	ICCV 171306, ICCV 171309	1	FLIP11-51C	1	ILC482
VI	1	ICCV 6301	3	FLIP 09-348C, JGK 32-1, JGK 5	1	FLIP 08-986
VII	2	JGK 1, JGK 2,	1	ICCV 171314	1	ICCV 6301
VIII	2	ICCV 171308, ICCV 171313	1	ICCV 171315	3	ICCV 171306, ICCV 171309, ICCV 171313
IX	-	-	1	FLIP11-87C	1	FLIP 09-348C
X	-	-	1	ICCV 14501	1	ICCV 14511
XI	-	-	1	ICCV 14313	1	ICCV 171308
XII	-	-	1	FLIP11-180C	-	-
XII	-	-	1	ICCV 171309	-	-

The genetic distance had a definite role to play in efficient selection of parents for hybridization programme. These diverse genotypes may be used in chickpea improvement programme.

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