

STABILITY AND MOLECULAR CHARACTERIZATION TO SCREEN OUT HEAT TOLERANT GENOTYPES OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

Thirty diverse genotypes sown in three different dates were screened using twenty SSR primers. Field observations were recorded for 14 phenological and morphological characters. Among which days to 50% flowering has less susceptibility for these genotypes against environmental fluctuations. The component $G \times E$ interaction were found significant for harvest index, total seeds per pod and effective pods per plant, hundred seed weight, biological yield and seed yield. The highest gene diversity was found in TA 146 (0.898), followed by primer TR 19 (0.884) and ICCM 0249 (0.877). Based on a dendrogram all the 30 genotypes were grouped into two clusters A and B. The genotype JG 19 showed highest gene similarities (96.77%) with JG 21, similarly MP-JG 99-115 showed 96.77% resemblance with JG 17 and JG 14-11 which are observed in same cluster. The genotypes, ICCV 07102, JG 21 and JG 22 were found suitable for all the dates of sowing while genotypes JG 16, JG 21, GG 2, ICC 4958, and JG 22 were suitable for late and very late sowing.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual legume and the only cultivated species within genus *Cicer*. Its considerable nutritive value makes it a valuable source for both food and feed it also play an important role in maintaining soil fertility, particularly in dry, rainfed areas (Katerji *et al.*, 2001). The genotypic and environmental interaction are usually present under all condition in pure lines, hybrid, synthetics or any other material used for breeding which complicate the breeding work and forbid the progress of the crop improvement programme (Eberhart and Russell, 1966). A significant $G \times E$ interaction for a quantitative trait such as seed yield can seriously limit the efforts on selecting superior genotypes for both new crop production and improved cultivar development (Kumar *et al.*, 2013 and Kang and Gorman, 1989). Thus, it is imperative to study the performance of a crop in more than one environment to identify genotypes which give high productivity over a wide range of environments. Such genotypes will be very useful for utilizing their potentials for the development of high yielding and stable varieties. Area under chickpea is being increased in MP due to expansion of irrigation facilities and farmers would like prefer the high yielding early genotypes tolerant to heat.

Molecular markers have been shown to play a crucial role in crop improvement programmes. Such markers serve as efficient and powerful tools for marker-assisted selection of agronomically important traits. An insight into genetic base of

chickpea varieties would provide valuable guidance to the breeders in planning future crossing programmes and directing the goal oriented efforts towards increasing the genetic base of chickpea varieties. However, cultivated chickpea has low level of genetic polymorphism. Now the availability of large number of microsatellite markers is offering immense scope in assessing the diversity and utilizing the diverse lines in map construction. It is important to characterize the genetic diversity in plant species since they serve as a resource base for as yet unidentified genetic information. Therefore the objective of present study was to determine the influence of $G \times E$ interaction for their phenological and morphological parameters as well as yield attributing traits grown under normal and heat stress environments Also to analyze all the chickpea genotypes for their molecular diversity regarding further utilization these genotypes in breeding programme.

MATERIALS AND METHODS

The present research was carried out during Rabi 2010-11, under AICRP on Chickpea (lead center) in the experimental field of seed breeding farm where as molecular work was carried out in Biotechnology centre, JNKVV, Jabalpur (M.P). Jabalpur is situated at 23.9°N latitude and 79.58°E longitude at an altitude of 411.87m above the mean sea level. This region has subtropical, semi-arid climate. The main features are hot and dry summer and cold winter with occasional

showers. The average rainfall is about 1400 mm, which is received mostly during July to September. The temperatures vary from 4.0°C minimum in January to 42°C maximum in May. The experimental material comprised of 30 genotypes which were grown in a RCBD with three replications on three different dates (Table 1 and 2). Each Plot size was 4.0 m x 0.90m = 3.6m² consisting of 2 rows of 4m length, the row to row distance was 45 cm and plant to plant spacing was 10 cm. The experiment was conducted with recommended agronomic practices. The statistical analysis was processed by Windostat Version 8.6 from indostat services.

Field observations are recorded on single plant basis on five selected plants from each plot of each replication for 14 phenological and morphological characters which are days to flower initiation, days to 50% flowering, days to pod initiation and days to maturity plant height, primary branches, secondary branches, total number of pods per plant, effective pods per plant, seeds per pod, 100-seed weight, biological yield, harvest index and seed yield per plant. The data were statistically analyzed in accordance with method described by Eberhart and Russell (1966). For molecular analysis, DNA from 100 mg of fresh young leaf tissue was collected in the winter season of 2010-2011 and was immediately frozen in liquid nitrogen and stored at -80°C. Isolation of DNA was carried out using modified CTAB method. Twenty SSR primers were screened, out of which only 18 were polymorphic (Table 4). Thermo Hybrid Thermal Cycler, USA was used to carry out amplifications in 10 µL volumes which had 20-25 ng plant genomic DNA, 10 X ~Tris buffer (15 mM MgCl₂ and Gelatine), 10 mM dNTP mix, 1.0 µL primer and 0.3 µL of 3U/µL Taq. PCR analysis was taken up by having preparation of 4 min at 90°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s at 50°C and 45 s elongation at 72°C and finally extension at 72°C for 5 min were performed (Visalakshi Chandra *et al.*, 2013).

Band patterns for each of the microsatellites markers were recorded for each genotype by assigning a letter to each band. Alleles were numbered as A, B, C, D, etc sequentially from the smallest to the largest sized band. Only clear and detectable bands were scored for data analysis. The PCR products from SSR analyses were scored quantitatively as presence or absence of amplicons. DNA bands were scored '1' for its presence and '0' for its absence. The scores were analyzed for estimating the polymorphism per cent and also to identify the relative efficiency of different repeats in revealing polymorphism. The data was analyzed in NTSYS-PC software (version 2.21b). For Clustering, UPGMA was used based on the similarity matrix generated on combined data. Polymorphic information content for each SSR primer pair was calculated.

RESULTS AND DISCUSSION

Analysis of variance revealed significant variance due to genotype against pooled deviation for all the characters except flower initiation, days to 50% flowering, plant height, primary branches and secondary branches, indicating the presence of genetic variability for the traits under investigation. The component genotype × environment interaction and G × E

linear were found significant for harvest index, total seeds per pod, effective pods per plant, hundred seed weight, biological yield and seed yield indicated that the genotypes interacted considerably to environmental condition and major portion of G × E interaction was attributed to linear component in respect of these traits. Non-linear component (pooled deviation) was also found to be significant for most of the characters except flower initiation, primary branch and seeds per pod. The differences among the environment were significant for all the characters except seeds per pod and harvest index (Table 3). The G × E interaction showed the significant difference for total number of pods per plant, effective pods per plant, 100-seed weight, biological yield and harvest index. Being significant G × E interactions resulted in making decision easily about performance of each genotype in terms of each characteristic; Duzemir (2011), Bozoglu and Gulumsir (2000) and Mart (2000) also observed similar findings. The variation due to environment (linear) was found significant for total number of pod per plant, effective pod per plant, 100-seed weight, biological yield and harvest index. However, pooled deviation was found to be significant for all the characters under study except seeds per pod.

Regression coefficient and mean square deviation from regression for days to flower initiation ranges from (0.618 to 1.557 and -0.831 to 43.99), days to 50% flowering (0.681 to 1.320 and -0.589 to 53.289), days to pod initiation (0.377 to 1.390 and -0.712 to 61.160), days to maturity (0.661 to 1.354 and -0.826 to 38.683), plant height (0.050 to 2.625 and -0.995 to 281.599), number of primary branches (-0.570 to 2.590 and -0.030 to 0.123), secondary branches (-0.500 to 1.709 and -0.420 to 129.472), total number of pods per plant (-0.673 to 5.089 and -1.153 to 335.215), effective pods per plant (-0.599 to 5.504 and -1.854 to 318.073), seeds per pod -0.812 to 5.985 and -0.023 to 0.109), 100 seed weight (-14.906 to 5.957 and -0.979 to 146.961), biological yield (-0.659 to 2.959 and -1.398 to 77.388), harvest index (-31.108 to 28.109 and -1.779 to 479.024) and seed yield per plant (-0.55 to 4.440 and -1.38 to 88.85g) (Table 4A and 4B).

When the overall mean, regression coefficient and mean square deviation from regression are taken into consideration, genotypes ICC 9942 and ICC 16181 were found to be stable for days to 50% flowering, with mean values greater than population mean and regression coefficient lesser than one with deviation from regression. It means a day to 50% flowering has less susceptibility for these genotypes against change of environmental condition in the expression of this character. Days to flower initiation is depends on photoperiod and temperature range in chickpea (Saxena and Singh, 1995). Looking to the above parameters the genotypes ICCL 81248, ICCV 07118, JG 16 and JG 22 for days to pod initiation; ICC 16181, ICCV 07110, ICCV 07117, JG 16, Vishal, JG 2003-14-16, MP JG 2003-115 and JG 14-11 for days to pod maturity; JG 2003-14-16 for plant height; JG 1-14 and JG 1307 for secondary branches; ICCV 07118 for seeds per pod; JG 18 for 100-seed weight; ICCV 06301 for seed yield per plant, were found to be stable respectively. It indicated that these genotypes should be given due consideration at the time of formulation of breeding programme specially for late sown and very late sown conditions.

Table 1: Description of chickpea genotypes used in the experiment

S.N	Genotype	Type	Developed from	Parentage
1	GG 2	Desi	J.N.U. Junagarh	JG 1258 × BDN 9-3
2	ICC3325	Desi	ICRISAT, Hyderabad	P 3971
3	ICC4958	Desi	ICRISAT, Hyderabad	JGC 1
4	ICC8474	Desi	ICRISAT, Hyderabad	JM 502
5	ICC9942	Desi	ICRISAT, Hyderabad	RPSP 33
6	ICC16181	Desi	ICRISAT Hyderabad	-
7	ICCL16216	Kabuli	ICRISAT, Hyderabad	P 481 × (P 1630 × JG 62)
8	ICC81248	Desi	ICRISAT, Hyderabad	-
9	ICCV06301	Kabuli	ICRISAT, Hyderabad	ICCV 92311 × ICCV 95423
10	ICCV06302	Kabuli	ICRISAT, Hyderabad	ICCV 92325 × ICCV 95423
11	ICCV07102	Desi	ICRISAT, Hyderabad	ICCV 10 × ICCL 87322
12	ICCV07105	Desi	ICRISAT, Hyderabad	ICCV 10 × ICCL 87322
13	ICCV07109	Desi	ICRISAT, Hyderabad	ICC 4958 × Annigeri
14	ICCV07110	Desi	ICRISAT, Hyderabad	ICC 4958 × Annigeri
15	ICCV07117	Desi	ICRISAT, Hyderabad	[ICCV10 × (P1679-2 × K4) × NARC 9005
16	ICCV07118	Desi	ICRISAT, Hyderabad	ICCV 2 × PDG 84-16
17	JG 16	Desi	JNKVV, Jabalpur	ICCC 42 × ICCV 10
18	JG 130	Desi	JNKVV, Jabalpur	(PG 5 × Narsingpur bold) × JG-74
19	VISHAL	Desi	M.P.K.V, Rahuri	K 850 × ICCL 80074
20	JG1-14	Desi	JNKVV, Jabalpur	[(JM-1 × ICC4929) × IPC 92-39]-14
21	JG 2003-14-16	Desi	JNKVV, Jabalpur	[(JM1 × ICC 4929) × ICC 4958]-2-14-16
22	JG1307	Desi	JNKVV, Jabalpur	JG 130 × JG 7
23	MP JG 2003-115	Desi	JNKVV, Jabalpur	[(JGM1 × ICC 4929) × ICC 4958] - 115
24	JG 14-11	Desi	JNKVV, Jabalpur	IPC 92-39 × JG 74
25	MP JG 99-115	Desi	JNKVV, Jabalpur	(JGM-1 × ICC 4929) × IPC 92-39
26	JG 17	Desi	JNKVV, Jabalpur	BDNG 9-3 × Narshingpur Bold
27	JG 18	Desi	JNKVV, Jabalpur	JG 74 × ICC 96029
28	JG 19	Desi	JNKVV, Jabalpur	IPC 9239 × JG 74
29	JG 21	Desi	JNKVV, Jabalpur	(JG 7 × IPC 4958)- 2-9-2
30	JG 22	Desi	JNKVV, Jabalpur	ICCC 37 × K 1189

Table 2: Sowing season and timing of experimental material

	Sowing season	Sowing time
E1 (normal planting)	November,2010	Last week of November
E2 (late planting)	December,2010	Last week of December
E3 (very late planting)	January,2011	Last week of January

Most of the genotypes showed varying degree of stability for different characters. Genotypes like ICC 8474, ICCV 06302, ICCV 07102, ICCV 07110, JG 16, JG 130, Vishal, JG 2003-14-16, JG 21 and JG 22 with regression coefficient ($\hat{\alpha}$) less than one and mean value less than their population mean exhibited above average stability for most of the character except 100-seed weight. Genotypes ICCL 81248, ICCV 06301, ICCV 07117, JG 1-14 and, JG 1307 with regression coefficient ($\hat{\alpha}$) greater than one and mean value less than their population mean exhibited below average stability performance for all character except primary branch and days to flower initiation. Hence, these genotypes should be grown under poor environmental conditions. Similarly, genotypes GG 2, ICC 3325, ICC 16216, ICCV 07105, ICCV 07118, MP JG 2003-115, JG 14-11, MP JG 99-115, JG 17, JG 18 and JG 19 showed specific adaptation since they had regression coefficient ($\hat{\alpha}$) greater than one and mean value more than their population mean for majority of the characters. Therefore, these genotypes may be exploited under optimal favorable environmental condition. Genotypes, GG 2, ICC 16216 (seed yield per plant), ICCV 07105, JG 1-14 and JG 14-11 (days to maturity), ICC

9942 and ICC 16181 (secondary branches), ICCV 07117, (100-seed weight), ICCL 81248 and ICCV 06301 (primary branches), JG 130 (harvest index) and JG 18 (biological yield) with regression coefficient ($\hat{\alpha}$) less than one, mean value more than their population mean and deviation from regression (s^2_{di}) minimum were less influenced by the environment and showed high stability. Hence, these genotypes may be grown under wide range of environments particularly in tolerance to terminal heat and found best genotypes out of thirty under present investigation.

Thirty SSR primer pairs could amplify 1-4 loci/primer pair. Out of these 18 SSR primer pairs were polymorphic while 12 were monomorphic. They could generate 155 amplicons giving on an average 8.61 amplicons/primer pair. The polymorphic information content ranged from 0.51 to 0.87 and genetic similarity between cultivars ranged from 0.69 to 0.97. The mean value of polymorphic information content was 0.662 (Table 5). TA 146 showed highest polymorphic information content (0.891) as well as highest gene diversity (0.898). Further 155 out of 167 amplicons were polymorphic (92.81%) indicating considerable variability in the material under study. This study revealed that all the 30 promising lines grouped into 2 major clusters (Fig. 1). Bhardwaj *et al.* (2010) also grouped different chickpea lines into two clusters in their study using molecular markers. First major group consisted nine lines which were divided in two sub group viz. seven lines GG 2, ICC 4958, ICC 8474, ICCV 07110, ICCV 07109, CCV 06302, ICCV 06301 in sub group 'A' and two lines ICC 9942

Table 3: Stability analysis of variance of pooled data for different morpho-physiological traits in chickpea

	Df	FI	F _{50%}	PS	DM	Pl.ht	FB	SB	TNIPP	EPPP	SPP	HSW	BY(%)	HI	SYPP
Genotypes	29	8.29	6.79	37.37***	20.52***	123.57	0.06	4.46	276.39**	314.04***	0.06*	92.07***	92.07***	426.56***	40.104**
Env. + (Var * Env)	60	39.13***	62.09***	95.50***	123.33***	36.45	0.08**	9.00*	329.84***	227.04**	0.03	35.68*	35.68*	197.55***	39.698***
Environments	2	997.73***	1765.71***	25556.11***	3534.62***	534.68***	1.15***	143.18***	3618.39***	2210.47***	0.08	66.00*	66.00*	29.95	433.265***
Genotype * Env	58	6.08	3.35	10.65	5.70	19.27	0.05	4.37	216.44*	158.65*	0.03	34.64*	34.64*	203.33***	26.127*
Environments(Lin)	1	1995.47***	3531.41***	5112.22***	7069.23***	1069.36***	2.30***	286.36***	7236.78***	4420.94***	0.17*	132.00*	132.00*	59.90	866.530***
Genotype * Env. (Lin)	29	3.64	2.58	10.93	5.74	13.76	0.06	3.52	320.45**	224.08**	0.04	50.68**	50.68**	354.64***	38.760**
Pooled deviation	30	8.22	3.98***	10.03***	5.47***	23.95***	0.03	5.05***	108.68***	90.11***	0.03	17.98***	17.98***	50.29***	13.045***
Pooled error	174	0.73	0.55	0.72	0.77	1.07	0.03	0.43	1.75	1.92	0.02	1.03	1.03	1.73	1.420
Total	89	29.08	44.07	76.56	89.83	64.84	0.07	7.52	312.42	255.39	0.04	54.06	54.06	272.17	39.831

*Significant at p<0.05, **Significant at p<0.01

and JG 1-14 in sub group 'B'. Second major group were divided two sub groups 'C' and 'D'. First sub group 'C' further divided into two another subgroups C1 and C2. Sub group C1 consisted of five lines namely, ICC 3325, ICC 10181, ICCL 16216, ICC 81248 and JG 130 whereas, subgroup C2 contained 12 lines namely, ICCV 07102, ICCV 07105, ICCV 07118, JG 14-11, MPJG 99-115, JG 17, JG 18, JG 19, JG 21, MPJG-03-11, JG 2003-14-1 and JG 16. Subgroup 'D' contained only four lines viz., ICCV 07117, Vishal, JG 1307 and JG 22. Molecular study grouped all 'JG' lines (except 'JG 1-14' and 'JG 130') in same cluster and most of the ICC lines grouped in other clusters which were obtained from ICRISAT, Hyderabad. JG 19 (IPC 9239 x JG 74) showed highest gene similarities (96.77%) with JG 21 (JG 7 x IPC 4958- 2-9-2) and similarly MPJG 99-115 showed 96.77% resemblance with JG 17 and JG14-11 and they are in same cluster. Many genotypes which were derived even from diverse parents were clustered together because of selections during the advancement of generations. ICCV 07110 and ICC 16216 showed lowest gene similarity (69.35%) with MPJG 03-115 and JG 1-14, respectively. In this study, *Kabuli* and *Desi* lines did not grouped into two broad categories. This indicates that the *Kabuli* and *Desi* lines have not evolved in wide isolation and only few genes are involved in their differentiation; similar to the observations made earlier (Garje *et al.* 2013, Joshi *et al.* 2013 and Irula *et al.* 2002). In this study GG 2 makes a different sub cluster, indicating it is quite different to rest of the lines.

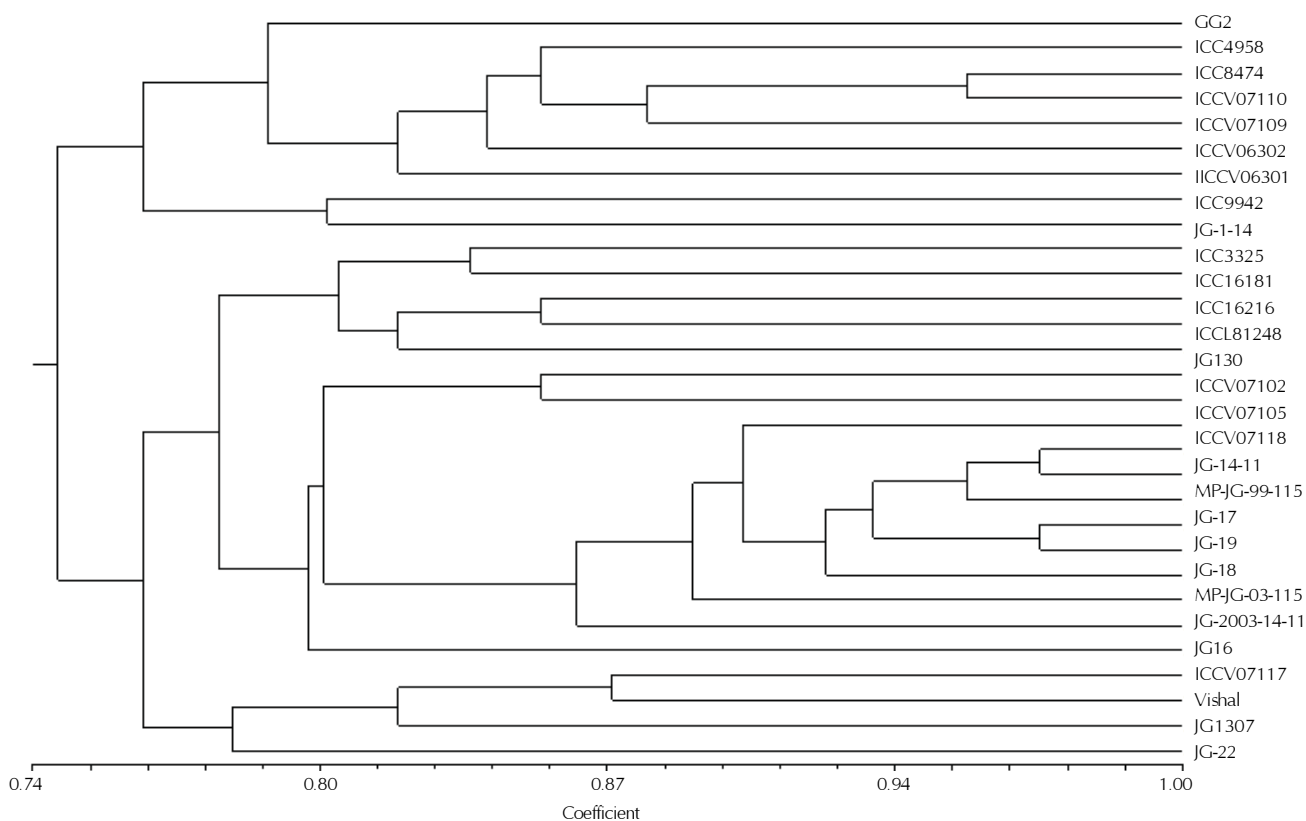
The highest gene diversity was found in TA 146 (0.898), followed by primer TR 19 (0.884) and ICCM 0249 (0.877). While carrying out SSR profiling, due consideration was given to stratified sampling of polymorphic SSR loci covering bin location on various chromosomes which has been indicated by the linkage group location (Table 5), as per the linkage groups reported by Winter *et al.* (2000), Tekeoglu *et al.* (2002), Collard *et al.* (2003) and Udupa and Baum (2003). The SSR polymorphism were assayed using a DNA pooling strategy, although it is not supposed to do as all the genotypes under study are pure lines. The power and potential of SSR markers for a wide range of applications in genetic and breeding of chickpea has been well demonstrated by Flandez-Galvez *et al.* (2003), but still substantial numbers of chickpea microsatellites are not available in public domain. Microsatellite genotypic data from a number of loci have potential to provide unique allelic profiles or DNA fingerprints for establishing genotypes identity as well as in development of molecular linkage map of chickpea. In this study thirty cultivars could easily be studied for their diversity using the 18 informative STMS primers. The 18 primers generated 3-18 alleles with 8.61 alleles/primer pair. Positive correlation was found between PIC values and allele numbers in this study. Marker TA 146 had both the highest PIC value (0.89) and the highest number of alleles (18) followed by marker ICCM0249 with a PIC value of 0.86. Among them TA 146, TR 19 and TA 2 exhibited higher polymorphism pointing towards the scope for further utilization of these markers for chickpea germplasm characterization.

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Table 5: Different parameters used for molecular diversity analysis

Marker	Major Allele Frequency	Allele No.	Gene Diversity	Heterozygosity	PIC
ICCM0249	0.2000	11.0000	0.8778	0.0000	0.8656
ICCM0293	0.5000	10.0000	0.7133	0.0000	0.6940
TA-2	0.3167	12.0000	0.8428	0.0333	0.8288
ICCM0127	0.3667	7.0000	0.7600	0.0000	0.7260
ICCM0065a	0.4667	6.0000	0.6756	0.0000	0.6259
TA-3a	1.0000	1.0000	0.0000	0.0000	0.0000
TA-72	0.2833	10.0000	0.8311	0.0667	0.8111
TR-20	0.3333	9.0000	0.7628	0.0667	0.7277
TS-54	0.4333	11.0000	0.7733	0.0000	0.7575
TA-146	0.2333	18.0000	0.8983	0.8000	0.8912
TR-58	0.3667	5.0000	0.7422	0.0000	0.6992
TA-96	0.4000	9.0000	0.7867	0.0000	0.7673
TA-59	0.6000	6.0000	0.5778	0.0000	0.5321
GA-16	0.4333	7.0000	0.7422	0.0000	0.7125
TA-37	0.3667	5.0000	0.7578	0.0000	0.7208
TA-110	0.3833	8.0000	0.7861	0.0333	0.7630
TA-27	1.0000	1.0000	0.0000	0.0000	0.0000
GA-20	0.3333	6.0000	0.7628	0.0333	0.7261
TR-19	0.1667	12.0000	0.8844	0.0000	0.8734
TS-82	0.5000	3.0000	0.5978	0.0000	0.5169
Mean	0.4342	7.8500	0.6886	0.0517	0.6620

**Figure 1: Cluster dendrogram showing the genetic relationships between 30 genotypes of chickpea based on the alleles detected by 20 microsatellite markers**

molecular work.

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