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Prioritization of Microsatellite Markers Linked with Drought Tolerance Associated Traits in Chickpea (*Cicer arietinum* L.)

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Abstract

Background: Being a vital source of high-quality dietary protein, chickpea is an unavoidable legume. The present investigation was performed to study the applicability of the microsatellite markers linked with drought tolerance in Indian chickpea genotypes collected from different genetic background.

Methods: In *Rabi* 2021-22, forty chickpea genotypes including national check for drought tolerance, elite cultivars, released varieties and advanced breeding lines were screened employing forty microsatellite markers linked with drought tolerance associated traits.

Result: Among forty drought tolerance related microsatellite markers, twenty-six were found to be polymorphic and produced a total of 66 alleles, with a mean of 2.5 alleles per locus. Model-based population structure analysis clearly distinguished the drought tolerant genotypes including ICC4958, JG74, JAKI9218, JG16, JG6, JG14 and JG11. The principle coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) further confirmed these results. Findings of the present investigation would have a greater potential for further utilization in breeding of drought specific chickpea cultivar(s).

Key words: Chickpea, Drought tolerance, Microsatellite markers, Polymorphic information content, Population structure.

Introduction

Legumes are very essential in human diet as they are not only complementing nutrients in the cereal diet but also improve the taste and texture of staple dish (Varol *et al*., 2020; Yadav *et al*., 2023). Chickpea (*Cicer arietinum* L., 2n = 16) is a nutrition-rich economical source of high-quality protein comprised of globulin and albumins as compared to animal protein and is hence vital for nutritional security in developing nations, especially in India (Gupta *et al*., 2021; Ningwal *et al*., 2023). “Desi” and “Kabuli” are two main types (Sahu *et al*., 2020) of cultivated chickpeas in the world. In which “Desi” chickpea covers about 80-85% of the total chickpea cultivation area in the world, primarily grown in South Asia, East Africa and Australia. In India, chickpea has contributed to the ‘Pulse Revolution’, making the country near self-sufficient in pulses from 2014-15 to 2020-21 with a remarkable increase in chickpea production and productivity. Chickpea production rose to an all-time high of 12.61 mt during 2020-21 from a level of 7.59 mt in 2014-15, with an increase of nearly 66% in production and >26% in productivity during six years (Dixit, 2021).

Abiotic stresses, especially drought and heat reduce yield of different crops (Mishra *et al*., 2021; Sharma *et al*., 2021; Asati *et al*., 2022) including chickpea over 70% (Varshney *et al*., 2019). The application of molecular markers may shorten the crop breeding cycle and ultimately leads to crop improvement particularly in complex traits controlled by polygenes (Mishra *et al*., 2022; Solanki *et al*., 2022; Yadav *et al*., 2023). Several quantitative trait loci (QTLs) for drought-tolerance associated traits have been reported in chickpea. The QTL-hotspot genomic region present on CaLG04 explained >50% phenotypic variation for drought tolerance (Varshney *et al*., 2014). Additionally, genome-wide microsatellite markers associated with drought tolerance related traits were also reported (Li *et al*., 2018; Varshney *et al*., 2019).

Marker-assisted backcross breeding has been successfully employed for developing superior chickpea lines with improved yield and drought tolerance. Drought tolerant variety Pusa Chickpea 10216 has also been released for commercial cultivation in India (Bharadwaj *et al*., 2021).

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In the light of above facts, employing drought tolerance linked microsatellite markers, the present study was aimed to assess the pattern and level of genetic diversity and developing population structure among national check for drought tolerance, elite cultivars, released varieties and advanced breeding lines.

MATERIALS AND METHODS

Plant materials

Forty chickpea genotypes were obtained from the All India Coordinated Research Project (AICRP) on Chickpea, Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (Table 1). The genotypes were grown in the Net house of Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur during *Rabi* 2021-22.

DNA extraction and quantification

Genomic DNA from young leaves of grown chickpea genotypes was isolated using the cetyl triethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). Two grams of leaf samples were ground in liquid nitrogen into a fine powder using sterilized pestle and mortar. About 100 mg of leaf powder was transferred to 2 ml micro-centrifuge tubes containing 1 ml of extraction buffer (0.1M Tris-HCl (pH 7.6), 0.005M EDTA and 0.2M Tris-HCl, 0.05M EDTA, 2M NaCl and 2% CTAB). Sample tubes were incubated at 65°C for one hour then centrifuged at 10000 rpm for 15 minutes with chloroform-isoamyl alcohol (24:1) mixture for protein separation. Supernatant was transferred to fresh tube and chilled isopropanol was used for the precipitation of DNA. Precipitated DNA was pelleted down through centrifugation and the DNA pellet was dried and dissolved in 50 µl of Milli-Q (MQ) water. The quantity and quality of all DNA samples were checked using agarose gel (0.8%). The working DNA sample was obtained by diluting to a final DNA concentration of 40-50 ng per microlitre (µL).

Polymerase chain reaction (PCR) and gel electrophoresis

Forty genome wide distributed drought tolerance linked microsatellite markers were selected based on polymorphic information content (PIC), allelic richness and heterozygosity from previous studies (Table 2). Primers were synthesized from Integrated DNA Technologies (Asia Pacific, Singapore). The PCR reactions were performed with a total volume of 10 µl containing 40-50 ng of DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 mM each of the forward and reverse primers and 1U *Taq* DNA polymerase in a Sure Cycler 8800 (Agilent Technologies) after optimizing the annealing conditions specific for each primer pair. The PCR was performed with an initial denaturation step of 5 min at 94°C, tracked by 35 cycles of 30s denaturation at 94°C, annealing at 55-60°C (depending on the primer) for 50s, initial extension at 72°C for 50s. The final extension was accomplished at 72°C for seven min. The PCR products were resolved on 2.8% agarose gel in 1 × TAE buffer with a 6 × DNA loading dye. Electrophoresis was carried out on horizontal electrophoresis with 65V for two and half hours using a standard 100 bp DNA ladder (Promega, Madison, USA). Gel pictures were snapped on a Vilber Laurmet, QUANTUM-ST5 gel electrophoresis system.

Analysis of genotypic data through a model-based approach

Scoring each polymorphic SSR marker was done with the help of Gel Analyzer V19.1 software. The allelic data scores were used for computing locus-based diversity indices with the help of GenAlEx V6.51b2 software and Power Marker V3.25 software. The Population structure for 40 genotypes was constructed using STRUCTURE version 2.3.4 software. An analysis of molecular variance (AMOVA) and principal coordinates analysis (PCoA) within and among populations were performed using GenAlEx V6.51b2.

RESULTS AND DISCUSSION

Modern chickpea improvement program largely necessitates the identification of highly diverse chickpea germplasm, highly polymorphic trait-specific molecular markers, which can be effectively utilized for the development of improved varieties. Thus, the characterization of chickpea germplasm has the great potential for playing a vital role in future international breeding programs (Mir *et al*., 2022), especially in India (Asati *et al*., 2022; Yadav *et al*., 2023), which is the largest chickpea-producing nation (Dixit, 2021). Consequently, for identification of new sources of germplasm having valuable genes/QTLs to improve yield and enhance resistance/tolerance to various abiotic and biotic stresses, investigation of structure and nature of genetic diversity and phylogenetic relationship within and among cultivated chickpea varieties, breeding lines and its wild relatives is obviously needed (Mir *et al*., 2022). For Indian chickpea genotypes, molecular diversity and population structure studies using drought tolerance related traits linked microsatellite markers are very limited, hence this study was initiated to analyze the nature and structure of genetic diversity and phylogenetic relatedness among 40 chickpea genotypes.

Polymorphism among chickpea genotypes

The SSRs have been widely utilized for genetic diversity analysis, germplasm grouping and population structure analysis in numerous crops, including chickpea (Choudhary *et al*., 2012; Mishra *et al*., 2020; Rathore *et al*., 2022). The present investigation determined the suitability of selected trait linked microsatellite markers for genetic diversity analysis of chickpea genotypes. Out of 40 applied markers, only 26 showed consistent polymorphic banding patterns with genotypes, indicating their suitability for genetic diversity analysis. The remaining fourteen that produced monomorphic bands among genotypes revealing one allele at each locus were not considered for diversity analysis.

Overall, 66 alleles were identified by 26 polymorphic markers from the 40 tested genotypes with a mean of 2.5

alleles per locus (Table 3). The allelic richness (Na) per locus ranged between two (most of the markers) to four (TR 19 and NCPGR 127). The numbers of effective alleles (Ne) ranged from 1.22 (ICCeM058) to 3.57 (NCPGR 127), with an overall mean of 1.92 effective alleles per locus. The most frequent major allele frequency (0.90) was found for the marker ICCeM0058 and the lowest (0.40) for marker NCPGR127, with an average of 0.66. The highest gene diversity (0.72) and PIC value (0.67) were found for the marker NCPGR127, whilst the lowest gene diversity (0.18) and PIC value (0.16) was investigated for the marker ICCeM0058, with an average of 0.44 and 0.37, respectively. The microsatellite marker analysis result indicated moderate allelic richness per locus and relatively moderate to high PIC, Ho and He values. A high level of genetic diversity indicated the existence of higher molecular variation among the 40 tested chickpea genotypes, in agreement with the previous studies (Mir *et al*., 2022). During present study, the most of the loci (20) exhibited a low level of observed heterozygosity in comparison to the expected heterozygosity

Table 1: Details of the forty chickpea genotypes used for present study with their parentage and source.

Genotype name	Status	Pedigree	Source
ICC4958	National check for drought tolerance	JGC 4958	JNKVV, Jabalpur
JAKI9218	Released variety	(ICCC 37 × GW5/7) × ICCV 107	JNKVV, Jabalpur
JG11		(Phule G-5 × Narsinghpur bold) × ICCC 37	
JG16		ICCC 44 × ICCV 10	
JG63		Single Plant selection from JG 62	
JG74		A composite from genetic stock	
JG6		(ICCV10XK850) × (H208XRS11)	
JG14		(GW5/7XP326) × ICCL83149	
JG17		BDNG 9-3 × Narshingpur Bold	
JG24		(JG 74 × ICC 4958)-21	
JG28		[(JM - 1 × IPC 9239) × JG 7] -14-11	
JG32		[(JM - 1 × IPC 4958) × JG 315] -2	
JG33		[(JM - 1 × IPC 9239) × JG 322] -30-3	
JG36		JG 12 × JG 16	
JG42		[(JM 1 ×IPC 9239) JG7] 14-11-2011-42	
JG226		JG 74 × JG315	
JG205		JG 315 × ICC 96029	
ICCV15102		ICCV03112 × ICCV10	ICRISAT, Patancheru
ICCV15115		ICCV10 × ICCV 96970	
ICCV15118		ICCV 05530 × ICCV 88510	
ICCV19616		JAKI 9218/ICCV 05103	
ICCV181664		ICC 4958 TM/JG 130	
JG2003-14-16	Advanced breeding line	[(JM1 × ICC4929) × ICC4958]-2-14-16	JNKVV, Jabalpur
JG2016-44		(ICC 96029 × ICC11551) 44	
JG2016-45		(JG 74 × ICC11551) 45	
JG2016-1411		JG 14 × JG 11	
JG2016-1614		JG 16 × JG 14	
JG2016-9605		JG 74 × ICC 96029	
JG2016-9651		JG 130 × ICC 96029	
JG2016-74315		[{(JG 74 × WR 315) × JG 74} -2010 -1- 3 -5-11-15-10-2]	
JG2016-634958		JG 63 × ICC 4958	
JG2016-921814		JAKI 9218 × JG 14	
JG2017-48		(JG 315 × ICC 96029)48	
JG2018-51		JG63 × ICC1205	
JG2022-74		JG12 ×JG74	
JG2016-36		JG12 ×JG16-1	
JG2022-75		JG12 ×ICC4958	
JG2021-6301		JG12 × ICCV06301	
JG2021-1424		JG14 ×JG24	
JG2021-1617		JG16 × JG17	

Table 2: Details of the drought tolerance related markers used in the study.

Primer name	Type	Repeat motif	LG	Linked QTL/gene	Linked trait
STMS11	STMS	(GA) 20	04	QTLSW1	QTL hotspot for drought tolerance
TAA170	STMS	(TTA) 33	04	QR3sdw01	Root length, pods and seeds per pod, harvest index, yield
NCPGR142	STMS	(CT) 24	04	QTL	Drought tolerance
NCPGR127	STMS	(GA) 18	04	QR3pht03, QR3pod01, QR3100sdw03	Pods per plant, 100 seed weight, root length, days to 50% flowering, days to maturity, biomass
NCPGR21	STMS	(CT) 15	04	QR3pht03, QR3sdw01, QR3pod01, QR3100sdw03	Root length, pods per plant, seeds per pod, harvest index and yield
ICCM0249	SSR	(T) 12N (TAA) 29	04	QR4100sdw02	100 seed weight, QTL hotspot for drought tolerance
GA24	STMS	(GA) 19	04	QTL	QTL hotspot for drought tolerance
GA11	STMS	(CT) 21	02	QTL	Pod filling, single seed weight
ICCeM006	EST-SSR	(TTC) 6	NA	QTL	Drought stress response
TA21	STMS	(TAA) 51	07	QTL	Stomatal conductance, drought tolerance, days to maturity
TA28	STMS	(TAA) 37CAA (TAA) 30	07	QTL	Pods per plant, number of branches per plant
TA72	STMS	(ATT) 36	04	QTLar2	Seed yield
H5AO8	SSR	(TTA) 118CCA (TTA) 8TT (TTA) 3	02	Q1-1	Harvest index, canopy temperature
ICCeM0040	EST-SSR	(TTC) 9	01	QTL	Drought stress response
ICCeM0055	EST-SSR	(TC) 8TA (TC) 20	01	QTL	Drought stress response
ICCeM0058	EST-SSR	(AT) 16	04	QTL	Drought stress response
ICCeM005	EST-SSR	(AAATGA) 5	02	QTL	Drought stress response
TA80	STMS	(TTA) 23	06	Sfl/sfl	Single-/double-podding locus *sfl*
TA110	STMS	(TTA) 22	02	Foc1,3	Fusarium wilt resistance,Seed size
H3DO5	SSR	(TAA) 41	03	Dt1	Indeterminate stem growth
TR19	STMS	(TAA) 27	02	Foc4	Fusarium wilt, Ascochyta blight resistance, seed colour
ICCeM0035	EST-SSR	(CT) 16	02	QTL	Drought stress response
TA34	STMS	(AAT) 34	03	*Prostrate*	Growth habit, plant height
TA106	STMS	(TAA) 26	06	QTL	Root surface area, seed yield
TA8	STMS	(TAA) 44	01	Q1-1	Harvest index
TA14	STMS	(TAA) 22ATGA (TAA) 4T (A) 3TGAT (AAT) 5 ATT (A) 3TGATAATA AAT(GAT) 4 (TAA) 5	06	QTL	Days to 50% flowering, seed yield
TA25	STMS	(TAA) 45	08	QTL	Botrytis grey mould resistance
NCPGR184	STMS	(AT) 6 (GT) 16	01	QTL	100-seed weight, harvest index

Table 2: Continue....

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NCPGR164	STMS	(CT) 6ca (CT) 14	08	*QR3df01, QR3dm01*	Days to 50% flowering, biomass
NCPGR136	STMS	(GT) 7gc (GT) ac (GT) gc (GT) gg (GT) 10	01	QTL	Yield
NCPGR49	STMS	(CT) 17c (CT) 3ttctt (CT) 6tt (CT) 7	03	QTL	Plant height
ICCM0009b	SSR	(A)	11	01	QTL 100-seed weight
H1I16	SSR	(GA) 20	06	QTL	Root surface area
CaM2187	SSR	(AT) 14n (AT) 9	08	QTL	Plant height
CaM1918	SSR	(AAT) 5n (ATT) 6	08	*QR3df01, QR3dm01*	Days to 50% flowering, days to maturity
CaM1760	SSR	(TA) 9	06	*QR3pht01*	Plant height
CaM0399	SSR	(TC) 10n (TC) 7	06	*QR3pht01*	Plant height
CaM0393	SSR	(ATA) 17	01	QTL	Harvest index
CaM0046	SSR	(AT) 8	01	QTL	Drought tolerance
TA27	STMS	(TAA) 21	02	Foc-3, Foc-4	Fusarium wilt resistance

and high fixation index (Choudhary *et al*., 2012). It indicated high levels of expected inbreeding among tested chickpea genotypes because chickpea is a self-pollinated crop and only 0 to 1.58% of outcrossing is reported (Ghaffari *et al*., 2014). The rest of the six loci had a high level of observed heterozygosity compared to the expected heterozygosity with a low associated fixation index. It implied that higher mutation rates or inbreeding depression could be associated with these loci (Choudhary *et al*., 2012).

Population structure

Model-based clustering method of the STRUCTURE software is valuable in showing the presence of population structure in tested genotypes, identifying different genetic populations, assigning individuals to populations and identifying pure and admixed individuals (Varshney *et al*., 2019). A population structure model without admixture was investigated using all 40 genotypes and 26 polymorphic markers for maximum likelihood by correlating allele frequency with a 5000-burn period with a run length of 50000 to 10 Markov Chain Monte Carlo (MCMC) with varying K from 1 to 15 with five iterations. The highest peak was observed at K = 4 with 30.78 Delta K value in Delta K analysis, indicating the presence of four populations in tested genotypes (Fig 1). At K=4, all genotypes were stratified into four populations *viz*., P1, P2, P3 and P4, representing 22., 22.5, 37.5 and 17.5% of genotypes used in structure analysis, respectively (Fig 2A, presents the inferred population structure). Population group 2 had clearly distingued the drought tolerant genotypes *viz*., ICC4958, JG74, JAKI9218, JG16, JG6, JG14 and JG11 from other studied chickpea genotypes. These distinguished drought tolerant chickpea genotypes had been already established as drought tolerant chickpea genotypes. The ICC4958 is a national check for drought tolerance in India, JG74 is an early maturing chickpea cultivar and established as most suitable chickpea cultivar for rainfed conditions of central region of India (Mannur *et al*., 2019), whilst JAKI9218 and JG16 are established as drought tolerant chickpea varieties. JG14 is identified as a heat tolerant chickpea variety (Dixit *et al*., 2019) and JG11 is established as a drought tolerant chickpea variety (Dixit, 2021).

In principal coordinate analysis (PCA) the first two Eigen vectors classified the studied genotypes into four major groups and distinguished the drought tolerant chickpea genotypes from investigated genotypes, which were comparable to observation obtained from STRUCTURE analysis (Fig 2B). The genotypes were divided into four structured populations, revealing that tested chickpea genotypes evolved from four population types demonstrating varying degrees of introgression from four types of ancestors into tested genotypes. It might be due to the resulting genotypes from independent evolutionary mechanisms including genetic drift, migration, mutation, selection and germplasm exchange that split them into discrete gene pools.

Analysis of molecular variance (AMOVA)

The four population groups generated from the structural analysis were subjected to AMOVA to estimate the percentage of genetic variation within as well as among individuals and populations (Table 4). The total genetic variance of genotypes was contributed by 7% variation within individuals, 77% among individuals and 16% among populations (Fig 3). Pairwise inbreeding coefficient within subpopulations (Fst) value (0.155) demonstrated significant variation among all the four pairs of populations, suggesting that all four groups were different from each other. The results acquired from AMOVA analysis agreed with the

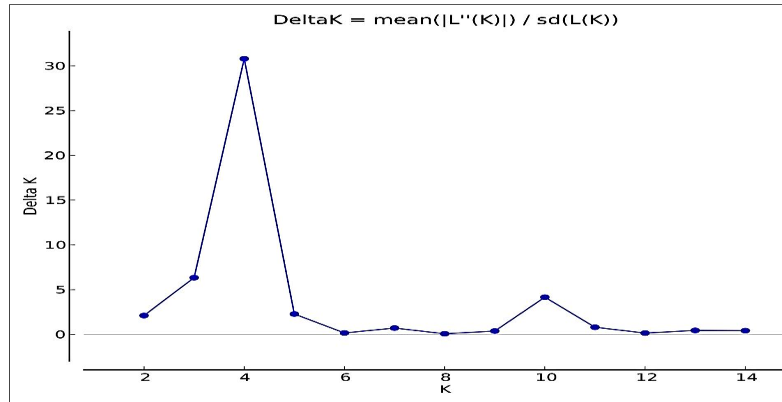

Fig 1: Delta K analysis for assuming structured populations.

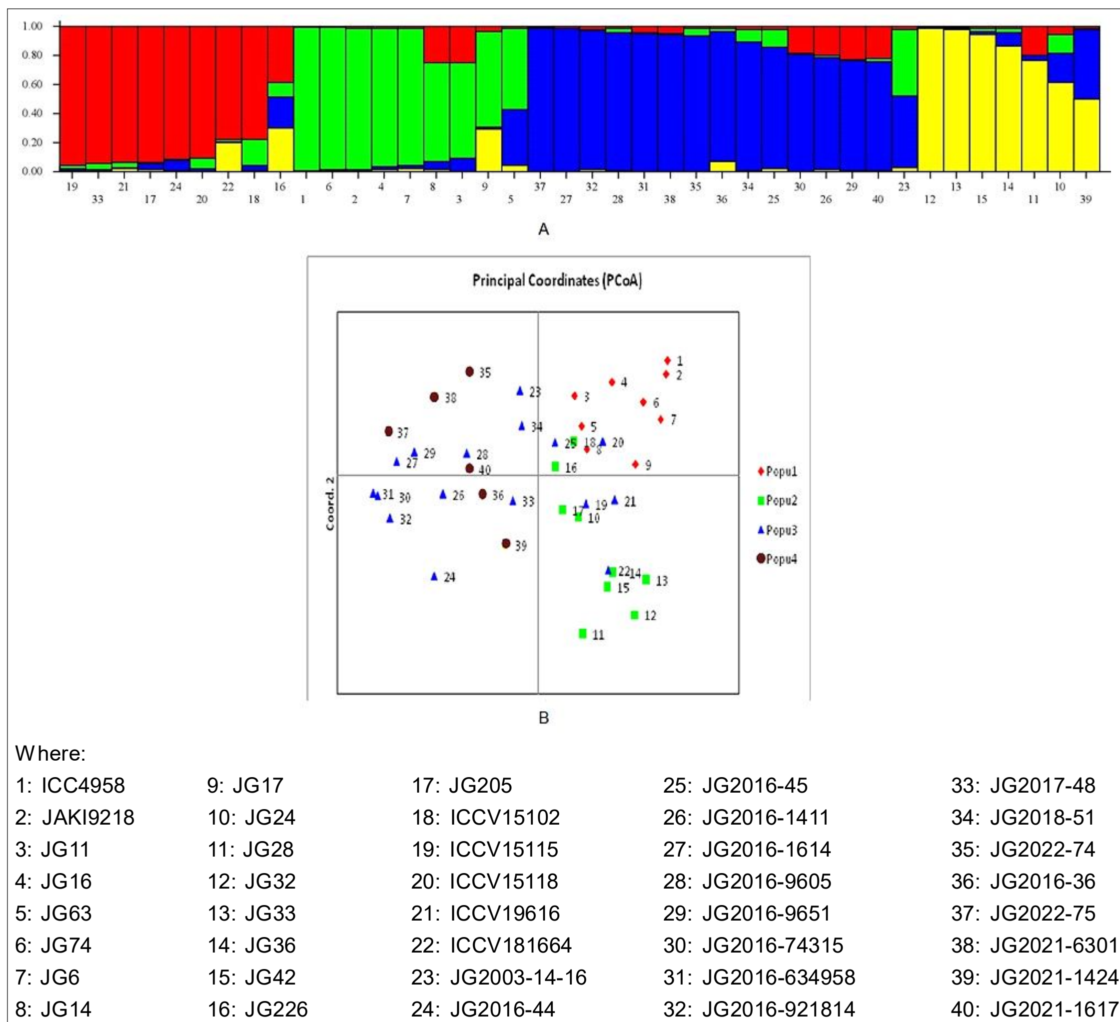

Fig 2: (A): Population structure, (B): Principal coordinate analysis of 40 chickpea genotypes.

Table 3: Estimated genetic diversity parameters of 26 polymorphic microsatellite loci across 40 chickpea genotypes.

Locus	Na	Ne	MAF	I	Ho	He	F	Fis	GD	PIC
TA21	2	1.72	0.70	0.61	0.00	0.42	1.00	1.00	0.42	0.33
TA27	2	1.69	0.71	0.60	0.07	0.41	0.81	0.67	0.40	0.32
TA28	3	1.77	0.73	0.77	0.00	0.43	1.00	1.00	0.43	0.39
TA72	2	1.28	0.88	0.37	0.00	0.21	1.00	1.00	0.21	0.19
H5AO8	2	1.40	0.83	0.46	0.00	0.28	1.00	1.00	0.28	0.24
ICCeM0040	2	1.98	0.54	0.69	0.12	0.49	0.74	0.75	0.49	0.37
ICCeM0058	2	1.22	0.90	0.32	0.20	0.18	-0.11	-0.25	0.18	0.16
TA80	3	2.10	0.63	0.87	0.00	0.52	1.00	1.00	0.52	0.45
H3DO5	2	1.95	0.58	0.68	0.00	0.48	1.00	1.00	0.48	0.36
TR19	4	3.11	0.43	1.21	0.17	0.67	0.74	0.70	0.67	0.61
TA34	2	2.00	0.50	0.69	0.00	0.50	1.00	1.00	0.50	0.37
TA106	3	1.59	0.78	0.68	0.00	0.37	1.00	1.00	0.37	0.34
TA8	3	1.96	0.68	0.85	0.00	0.49	1.00	1.00	0.49	0.44
TA14	2	1.95	0.58	0.68	0.00	0.48	1.00	1.00	0.48	0.36
TA25	3	1.57	0.78	0.65	0.00	0.36	1.00	1.00	0.36	0.32
NCPGR184	3	2.34	0.53	0.93	0.00	0.57	1.00	1.00	0.57	0.48
NCPGR164	3	2.00	0.63	0.80	0.00	0.50	1.00	1.00	0.50	0.41
NCPGR136	2	1.75	0.69	0.62	0.32	0.43	0.24	0.06	0.42	0.33
H1I16	3	1.58	0.76	0.59	0.02	0.36	0.93	0.94	0.36	0.30
CaM2187	2	1.72	0.70	0.61	0.00	0.42	1.00	1.00	0.42	0.33
CaM1918	2	1.34	0.85	0.42	0.00	0.25	1.00	1.00	0.25	0.22
CaM0046	2	1.98	0.55	0.68	0.00	0.49	1.00	1.00	0.49	0.37
TAA170	3	2.05	0.63	0.84	0.00	0.51	1.00	1.00	0.51	0.43
NCPGR127	4	3.57	0.40	1.33	0.00	0.72	1.00	1.00	0.72	0.67
NCPGR21	3	2.27	0.60	0.95	0.00	0.56	1.00	1.00	0.56	0.49
ICCM00249	2	1.99	0.53	0.69	0.00	0.49	1.00	1.00	0.49	0.37
Mean	2.5	1.92	0.66	0.71	0.03	0.44	0.89	0.88	0.44	0.37
SD	0.6	0.51	0.13	0.22	0.08	0.12	0.26	0.31	0.12	0.11
SE	0.1	0.10	0.03	0.04	0.01	0.02	0.05	0.06	0.02	0.02

Na= Numbers of alleles; Ne= Numbers of effective alleles; MAF= Major allele frequency; I= Shannon information index; Ho= Observed heterozygosity; He= Expected heterozygosity; F= Fixation index; Fis= Inbreeding depression; GD= Gene diversity; PIC= Polymorphic information content; SD= Standard deviation; SE= Standard error.

Table 4: Summary AMOVA table of populations assigned from structure.

Source	Df	SS	MS	Est. Var.	%
Among Pops	3	85.662	28.554	0.966	15%
Among Indiv	36	363.726	10.103	4.820	77%
Within Indiv	40	18.500	0.463	0.463	7%

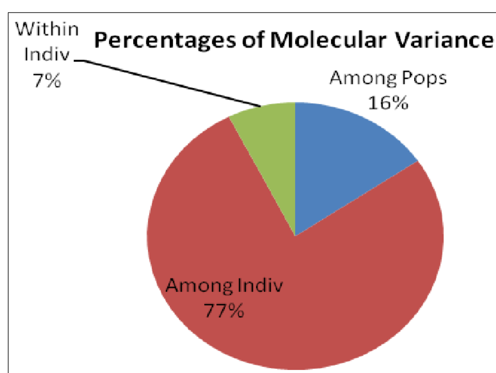

Fig 3: AMOVA of structured populations.

observations found through the basic summary statistics, structure analysis and principal coordinate analysis, confirming both the presence of a statistically moderate genetic diversity among genotypes and a high level of population structure among genotypes.

The AMOVA results demonstrated that most variation among genotypes might be credited to variation among individual genotypes compared to variation among and within the population. It suggested that individual variation of genotypes might be more valuable for chickpea improvement programs. The PCoA result depicted that the chickpea genotypes were equally spread in all four quadrants regardless of their population structure. Population one was

exclusively grouped in quadrant I, showing their distinctness from the rest of the populations evenly spread in all four quadrants because of their highly diverse nature. Similar results have also been reported in various earlier studies using microsatellite markers (Mir *et al*., 2022).

CONCLUSION

Trait linked microsatellite markers based on the molecular characterization of 40 chickpea genotypes showed presence of substantial genetic variation and population structure among genotypes. Summary statistics concluded that a moderate genetic variation was present among the genotypes. Marker NCPGR127 having the highest Na, Ne, He, Ht, gene diversity and PIC values followed by TR19. These markers might be used for selecting drought tolerant genotypes through marker assisted selection (MAS) approach. The population structure analysis identified four major population groups among studied genotypes clearly distinguished the drought tolerant genotypes *viz*., ICC4958, JG74, JAKI9218, JG16, JG6, JG14 and JG11 from other studied chickpea genotypes. The results obtained from the present investigation could thus be efficiently employed in the MAS programme to breed drought tolerant cultivar (s).

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REFERENCES

Asati, R., Tripathi, M.K., Tiwari, S., Yadav, R.K., Tripathi, N. (2022). Molecular breeding and drought tolerance in chickpea. Life. 12(11): 1846. https://doi.org/10.3390/life12111846.

Bharadwaj, C., Tripathi, S., Soren, K.R., Thudi, M., Singh, R.K., Sheoran, S. *et al.* (2021). Introgression of "QTLhotspot" region enhances drought tolerance and grain yield in three elite chickpea cultivars. Plant Genome. 14(1): e20076. https://doi.org/10.1002/tpg2.20076.

Choudhary, P., Khanna, S.M., Jain, P.K. (2012). Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. Genetics of Molecular Research. 11: 891-905.

Dixit, G.P. (2021). Sustaining Chickpea growth in India: Breeder's Perspective. Journal of Food Legumes. 34: 73-75.

Dixit, G.P., Srivastava, A.K., Singh, N.P. (2019). Marching towards self-sufficiency in chickpea. Current Science. 116: 239-242.

Doyle, J.J., Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin. 19: 11-15.

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

Gupta, N., Tiwari, S., Tripathi, M.K., Bhagyawant, S.S. (2021). Antinutritional and protein-based profiling of diverse desi and wild chickpea accessions. Current Journal of Applied Science and Technology. 40(6): 7-18.

Li, Y., Ruperao, P., Batley, J., Edwards, D., Khan, T., Colmer, T.D., Sutton, T. (2018). Investigating drought tolerance in chickpea using genome-wide association mapping and genomic selection based on whole-genome resequencing data. Frontiers in Plant Science. 9: 190. doi: 10.3389/fpls.2018.00190.

Mannur, D.M., Babbar, A., Thudi, M., Sabbavarapu, M.M., Roorkiwal, M. *et al.* (2019). Super Annigeri 1 and improved JG 74: Two Fusarium wilt-resistant introgression lines developed using marker-assisted backcrossing approach in chickpea (*Cicer arietinum* L.). Molecular Breeding. 39: 1-13.

Mir, A.H., Bhat, M.A., Fayaz, H., Wani, A.A., Dar, S.A., Maqbool, S., Mir, R.R. (2022). SSR markers in revealing extent of genetic diversity and phylogenetic relationships among chickpea core collection accessions for Western Himalayas. Molecular Biology Reports. 49: 11469-11479.

Mishra, N., Tripathi, M.K., Tiwari, S., Tripathi, N., Trivedi, H.K. (2020). Morphological and molecular screening of soybean genotypes against yellow mosaic virus disease. Legume Research. 45(10): 1309-1316. DOI: 10.18805/LR-4240.

Mishra, N., Tripathi, M.K., Tiwari, S., Tripathi, N., Gupta, N., Sharma, A. (2021). Morphological and physiological performance of Indian soybean [*Glycine max* (L.) Merrill] genotypes in respect to drought. Legume Research. 1-9. DOI: 10.18805/LR-4550.

Mishra, N., Tripathi, M.K., Tripathi, N., Tiwari, S., Gupta, N., Sharma, A. (2022). Screening of soybean genotypes against drought on the basis of gene-linked microsatellite markers. In Book: Innovations in Science and Technology. 3: 49-61.

Ningwal, R., Tripathi, M.K., Tiwari, S., Asati, R., Yadav, R.K., Tripathi, N., Yasin, M. (2023). Identification of Polymorphic ssr marken and diversity analysis in a set of desi chickpea genotypes. Biological Forum-An International Journal. 15(3): 45-51.

Rathore, M.S., Tiwari, S., Tripathi, M.K., Gupta, N., Yadav, S., Singh, S., Tomar, R.S. (2022). Genetic diversity analysis of groundnut germplasm lines in respect to early and late leaf spot diseases and biochemical traits. Legume Research. 1-6. DOI: 10.18805/LR-4833.

Sahu, V.K., Tiwari, S., Gupta, N., Tripathi, M.K., Yasin, M. (2020). Evaluation of physiological and biochemical contents in desi and Kabuli chickpea. Legume Research. 45(10): 1197-1208. DOI: 10.18805/LR-4265.

Sharma, A., Tripathi, M.K., Tiwari, S., Gupta, N., Tripathi, N., Mishra, N. (2021). Evaluation of soybean (*Glycine max* L.) genotypes on the basis of biochemical contents and anti-oxidant enzyme activities. Legume Research. DOI: 10.18805/LR-467.

Solanki, R.S., Babbar, A. and Tripathi, N. (2022). Genetic diversity analysis in *kabuli* chickpea (*Cicer arietinum* L.) genotypes based on quantitative traits and molecular markers. Bangladesh Journal of Botany. 51: 581-587.

Varol, I.S., Kardes, Y.M., Irik, H.A., Kirnak, H., Kaplan, M. (2020). Supplementary irrigations at different physiological growth stages of chickpea (*Cicer arietinum* L.) change grain nutritional composition. Food Chemistry. 303: 125402. doi: 10.1016/j.foodchem.2019.125402.

Varshney, R.K., Thudi, M., Nayak, S.N., Gaur, P.M., Kashiwagi, J., Krishnamurthy, L. *et al.* (2014). Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). Theoretical and Applied Genetics. 127: 445-462.

Varshney, R.K., Thudi, M., Roorkiwal, M., He, W., Upadhyaya, H.D., Yang, W. *et al.* (2019). Resequencing of 429 chickpea accessions from 45 countries provides insights into genome diversity, domestication and agronomic traits. Nature Genetics. 51: 857-864.

Yadav, R.K., Tripathi, M.K., Tiwari, S., Tripathi, N., Asati, R., Patel, V., Sikarwar, R.S., Payasi, D.K. (2023). Breeding and genomic approaches towards development of fusarium wilt resistance in chickpea. Life. 13: 988. https://doi.org/10.3390/life13040988.