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RESEARCH ARTICLE

Unveiling genetic diversity and drought tolerance in chickpea genotypes using important indices and microsatellite markers

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Abstract

Chickpea (*Cicer arietinum* L.) is a vital food legume worldwide, rich in proteins, minerals and vitamins. However, its production is significantly impacted by drought stress. Marker-assisted selection offers a promising approach to enhance chickpea resilience to abiotic stresses. This investigation aimed to assess the genetic diversity and putative drought tolerance ability among 78 chickpea genotypes using drought tolerance indices and 19 gene-specific microsatellite markers. A total of 15 microsatellite markers exhibited polymorphism with PIC values ranging from 0.2265 to 0.6744, indicating the presence of substantial genetic variation. Drought tolerance indices, including the relative drought index (RDI), geometric mean productivity, yield index and stress tolerance index, were calculated to evaluate putative genotypic response against drought. Results indicated significant variations in drought tolerance among genotypes, as some genotypes showed higher tolerance based on the indices. Genotype SAGL-162371 showed the highest yield under control (26.02 g/plant) and stress (23.68 g/plant) conditions, along with maximum value for YI (1.8571), STI (2.3941) and GMP (24.8216), while SAGL-22-112 displayed the highest RDI (1.2531). Population structure analysis revealed two subpopulations and AMOVA indicated that 91 % of genetic variation occurred within populations. Cluster analysis, principal coordinate analysis and population structure analysis were employed to assess genetic relationships among the genotypes. These results underscore the presence of substantial molecular and phenotypic diversity, providing valuable genetic resources to develop drought-tolerant chickpea cultivar(s).

Keywords: chickpea; drought tolerance; microsatellite markers; polymorphic information content; population structure; selection indices

Introduction

Chickpea (*Cicer arietinum* L.) or Bengal gram is the most important food legume crop grown across the world (1–2). This crop is used as food or feed since it is a good source of proteins (17-22 %) and rich in 18 different amino acids, vitamins and minerals such as iron, zinc, magnesium and calcium (3–5). This legume crop belongs to the subfamily Faboideae and family Fabaceae (6). It has eight basic sets of chromosomes and a genomic size of about 740 Mbp. Chickpeas are diploid (2n = 2x = 16) and a self-pollinated crop due to cleistogamous flowers. According to a recent report, India produced 117.92 lakh tons of chickpeas on 95.28 lakh ha of land and 12.38 kg/ha productivity (7). Drought limits chickpea production, resulting in 40–60 % mean yield losses globally (8–11). Terminal drought results in a reduction of flower and pod production as well as seed sizes, which is a common occurrence in chickpea plants during the pod filling stage (12–14). Many morpho-physiological and phonological traits are significant in crop adaptation to drought stress during soil drying (15–17). With the start of global warming, it is anticipated that the world will have an even hotter climate with erratic rainfall in the near future (18–21). Due to the complex interplay between genotypes and environment, as well as our limited understanding of the role and function of tolerance mechanisms, selecting for drought tolerance traits is difficult. In order to evaluate genetic variations in drought tolerance, researchers have used a variety of drought tolerance indices. Conventional breeding for drought tolerance is hindered by the polygenic nature of the trait and strong genotype × environment interactions. To address these problems, several selection indices based on mathematical relationships between stress and optimal conditions have been developed, which help assess plant performance under drought conditions.

Marker-assisted selection (MAS) in legumes holds vast potential to overcome the inadequacies of conventional breeding programmes and prominently increase the production and the quality of the crops (22–23). In recent years, molecular markers such as RAPD, RFLP, ISSR and SSR have been extensively used in genetic analyses, investigations of different biotic and abiotic stresses and

breeding studies (24). For allele mining, genetic relationship analysis, genetic mapping, association genetics, gene identification, phylogenetic patterns, population structure, gene cloning and marker-assisted selection in chickpea accessions, simple sequence repeat (SSR) markers are reliable (25, 26). Owing to their benefits, microsatellite or SSR markers were employed to screen the genotypes of chickpeas. The present study aims to evaluate drought tolerance in 78 chickpea accessions through the use of drought indices and gene-linked SSR molecular markers. By incorporating morphological and molecular screening techniques, this research provides a robust background for identifying elite genotypes suitable for future breeding programmes aimed at enhancing drought resilience.

Materials and Methods

Plant materials

78 chickpea genotypes were used in this investigation during the Rabi season of 2021-2022 and 2022-2023. (Table 1). These genotypes were acquired from All India Coordinated Research Project on Chickpea, College of Agriculture, Sehore, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, India and All India Coordinated Research Project on Chickpea, College of Agriculture, Jawaharlal Nehru Agricultural University, Jabalpur, India. Genotypes were grown in a randomised block design in three replications each in a 3 m row length with 30 cm row to row distance and 15 cm plant to plant distance. All genotypes were grown under normal and stressed field conditions to analyse drought-tolerant indices and leaf samples were collected for molecular characterisation. Chickpea genotypes were examined under different environments by manipulating irrigation, such as normal and stress conditions. Irrigation is done as needed under normal conditions, while no irrigation is given after the initial stage under stressed situations.

Drought tolerance indices

Yield index is a ratio of yield under normal and stress conditions. The stress tolerance index (STI) has proven to be a valuable tool for evaluating the potential of genotypes to produce good yield under drought stress conditions. The geometric mean productivity (GMP) accounts for the variability in drought stress severity in different environments over the two years. Yield data for drought indices were recorded after harvesting under both of the years and pooled for the calculation of drought indices under normal and stressed conditions. Drought tolerance indices were calculated using the following formulae:

Yield index (YI) = Ysi/Ys (27) Eqn. 1)

Stress tolerance index (STI) = (Ypi x Ysi)/Yp2 (28) (Eqn. 2)

Geometric mean productivity (GMP) = sqrt (Ypi x Ysi) (28) (Eqn. 3)

Relative drought index (RDI) = (Ysi/Ypi) ÷ (Ys/Yp) (29) (Eqn. 4)

Where, Ysi = Yield of genotypes under stress conditions, Ypi = Yield of genotypes under optimum conditions, Ys = Total mean yield under stress conditions, Yp = Total mean yield under optimum conditions

Table 1. Details of experimental material with their parentage

S. no.	Name of genotypes	Pedigree/parentage	S. no.	Name of genotypes	Pedigree/parentage
1	ICCV-201111	JNKVV, Jabalpur	40	SAGL-162376	JSC 52 × RSG 888
2	JG-36	JG 12 x JG 16	41	SAGL-152314	KAK 2 × VISHAL
3	GCP-101	JNKVV, Jabalpur	42	SAGL-162375	JAKI 9218 × JSC 52
4	ICCV-201105	JNKVV, Jabalpur	43	SAGL-152278	JSC 37 × JSC 36
5	ICCV-201209	JNKVV, Jabalpur	44	SAGL-152242	PG 9425-9 × BG 1108
6	ICCV-201113	JNKVV, Jabalpur	45	SAGL-152238	PG 9425-9 × IPC 9494
7	ICCV-201118	JNKVV, Jabalpur	46	SAGL-162390	JSC 37 × JSC 36
8	ICCV-201212	JNKVV, Jabalpur	47	RVSSG-69	RAK, Sehore
9	ICCV-201217	JNKVV, Jabalpur	48	SAGL-152256	JSC 19 × KAK 2
10	ICCV-201102	JNKVV, Jabalpur	49	SAGL-152208	BG 362 × IPC 9494
11	ICCV-201218	JNKVV, Jabalpur	50	SAGL-152303	JSC 19 × BGD 112
12	ICCV-201108	JNKVV, Jabalpur	51	SAGL-152404	RAK, Sehore
13	CHAFFA	Selection from Niphad (MS)	52	SAGL-152236	KAK 2 × BG 362
14	JG-24	(JG 74 x ICC 4958)-21	53	SAGL-152252	ICC 4958 × BG 1108
15	ICCV-201114	JNKVV, Jabalpur	54	SAGL-152349	KAK 2 × PHULE G5
16	ICCV-201107	JNKVV, Jabalpur	55	SAGL-162389	ICC4812 × JAKI 9218
17	SAGL 22-101	KAK 2 × BG 362	56	SAGL-162371	JSC 52 ×JG 130
18	SAGL 22-102	JG -6 × RVSSG 2	57	SAGL-152342	KAK 2 × JSC 19
19	SAGL 22-103	JG 130 × FG 703	58	SAGL-152334	PG 9425-9 × IPC 9494
20	SAGL 22-104	JSC 33 × JG 11	59	RVSSG-75	RAK, Sehore
21	SAGL 22-105	JAKI 9218 × BGD 112	60	JG-14	(GW5/7 x P327) x ICCL83149
22	SAGL 22-106	RVG 204 × JSC 37	61	JG-11	(Phule G-5 x Narsinghpur bold) x ICCC37
23	SAGL 22-107	RVG 202 × JG 11	62	NBeG-47	RAK, Sehore
24	SAGL 22-108	JAKI 9218 × RSG 888	63	JGG-1	Selection from germplasm
25	SAGL 22-109	JG 11 × JSC 37	64	RVG-205	BGD 112 × JSC 37
26	SAGL 22-111	JG 130 × JSC 37	65	RVG-201	Phule G5 x Bheema
27	SAGL 22-112	RVSSG 74 × GBM 2	66	VISHAL	RAK, Sehore
28	SAGL 22-113	JSC 38 × IPCK 1078	67	JG-63	Single Plant selection from JG 62
29	SAGL 22-114	RVSSG 74 × ICC4958	68	RVSSG-85	RAK, Sehore
30	SAGL 22 -115	SG 9200 × BG 362	69	RVG-210	BG362 × JG 16
31	SAGL-152403	RAK, Sehore	70	SAGL-161032	RAK, Sehore
32	SAGL-152254	BG 362 × ICC 506	71	SAGL-163603	RAK, Sehore
33	SAGL-162370	PG 9425-9 × BG 2064	72	SAGL-161018	JG 130 × BGD 112
34	SAGL-152210	IPC 9494 × ICC 506	73	SAGL-163008	ICC 4812 C RSG 888
35	SAGL-152273	KAK 2 × IPC 9494	74	SAGL-161001	JSC 52 × BGD 112
36	SAGL-152216	JG 16 × VIJAY	75	RVSSG-68	RAK, Sehore
37	RVSSG-64	RAK, Sehore	76	JG-315	Selection from WR 315
38	SAGL-162265	BG 362 × JSC 19	77	JG-74	A composite from genetic stock
39	SAGL-152347	KAK 2 × JSC 19	78	ICC-4958	JGC 4958

DNA extraction and quantification

The cetyl tri-methyl ammonium bromide method was employed to extract the genomic DNA from young and fresh leaves of chickpea genotypes (30). Using a sterile pestle and mortar, about 100 mg of the leaf samples was ground into a fine powder using liquid nitrogen. 500 µL of extraction buffer comprising 0.1 M Tris–HCl (pH 7.6), 0.005 M EDTA, 2 M NaCl and 2 % CTAB was added to the ground leaf powder that had been transferred to two µL microcentrifuge tubes. Sample tubes were heated at 65 ºC for an hour and then they were centrifuged at 10000 rpm for 15 min. The supernatant was transferred to the fresh tube and about an equal volume of a combination of chloroform and isoamyl alcohol (24:1) was added to the sample and mixed well. The sample tube was again centrifuged at 10000 rpm for 15 min. After transferring the supernatant to a new tube, cold isopropanol was added to precipitate the DNA. After being pelleted down by centrifugation. The DNA pellet was collected by centrifugation, washed twice with 70 % ethanol to remove residual salts, air-dried and dissolved in 50 µL of nuclease-free water. The purity of the DNA was assessed on a 0.8 % agarose gel and quantification was done using a Thermo-Fischer nanodrop spectrophotometer. To obtain the working DNA sample, the DNA was diluted until it reached a final concentration of 25 ng per µL.

Polymerase chain reaction (PCR) and gel electrophoresis

A total of 19 drought tolerance-linked microsatellite markers were applied based on the previously described PIC, allelic richness and heterozygosity, as shown in Table 2 (31). Out of these markers, 15 SSRs produced polymorphic fragments. PCR thermal cycles were standardised during the validation of markers. Amplification carried out in a 10 µL reaction volume containing 1 µL Tris-buffer, 0.1 µL of each dNTP, 0.1µL unit *Taq* DNA polymerase, 1.0 µL of primer and 2.0 µL of genomic DNA. Amplification reactions were performed in a Thermal Cycler (BioRad, USA) with the following thermal profile: initial denaturation at 95 ºC for 3 minutes, followed by 35 cycles of 94 ºC for 1 min denaturation, 55 to 62 ºC annealing as per the nature of the marker used and 72 ºC for 1 min primer extension, with a final extension at 72 ºC for 7 min. The amplified PCR products were successively resolved on 3 % agarose gel via electrophoresis at 65 V for 2-3 hr (Bio-Rad, USA). The separated bands were visualised under UV light and photographed by the Gel Documentation System (Syngene, USA).

Molecular analysis

The genetic profile of 78 chickpea genotypes was scored based on the difference in allele size using SSR markers. GenAlEx V6.51b2 (32) and Power Marker V3.25 software were used for calculating summary statistics of scored alleles. MEGA V11.0.9 software was used to visualise the dendrogram tree. STRUCTURE V2.3.4 software was used for constructing a Bayesian model-based population structure of studied genotypes and Structure Harvester Web V0.6.94 was used for visualising the output of the STRUCTURE software (33–36). To summarise the significant variance by population, an analysis of molecular variance (AMOVA) within and among populations was also performed using GenAlEx V6.51b2.

Results and Discussion

Drought tolerance indices

In the current research, drought is the main abiotic stress that affects the productivity of chickpea (37). The primary goals of the chickpea enhancement programme are to identify extremely diverse lines of chickpea germplasm, highly polymorphic molecular markers unique to genes and drought selection indices that may be effectively used in the breeding process to produce superior cultivars (38). Therefore, the characterisation of chickpea germplasm has a strong chance of contributing significantly to upcoming international projects for breeding the legume crops, particularly in India, the country that produces the greatest amount of chickpeas (39, 40). Therefore, it is evidently necessary to study the structure and nature of genetic diversity as well as the phylogenetic connections within and among cultivated chickpea varieties, breeding lines and their wild relatives to identify new sources of germplasm containing useful genes/QTLs to enhance yield and increase resistance/ tolerance to various abiotic and biotic stresses (1). The analysis of the structure and nature of genetic diversity, as well as phylogenetic affiliation among 78 chickpea accessions, along with drought stress indices, was started because there are very few studies on the molecular diversity and population structure of Indian chickpea genotypes using drought tolerance-associated microsatellite markers.

Drought selection indices

Studied four drought tolerance indices such as geometric mean productivity (GMP), yield Index (YI), stress tolerance index (STI) and relative drought index (RDI) was calculated and presented in Table 3

Table 2. List of SSR molecular markers used for screening of chickpea genotypes

S. no.	Name	Forward sequence	Reverse sequence
1	TR19	TCAGTATCACGTGTAATTCGT	CATGAACATCAAGTCTCCA
2	TA14	TGACTTGCTATTTAGGGAACA	TGGCTAAAGACAATTAAAGTT
3	TA125	TTGAAATTGAACTGTAACAGAACATAAA	TAGATAGGTGATCACAAGAAGAGAATG
4	TA110	ACACTATAGGTATAGGCATTTAGGCAA	TTCTTTATAAATATCAGACCGGAAAGA
5	TA8	AAAATTTGCACCCACAAAATATG	CTGAAAA TTATGGCAGGGAAAC
6	TA25	AGTTTAATTGGCTGGTTCTAAGATAAC	AGGATGATCTTTAATAAATCAGAATGA
7	TA80	CGAATTTTTACATCCGTAATG	AATCAATCCATTTTGCATTC
8	TA200	TTTCTCCTCTACTATTATGATCACCAG	TTGAGAGGGTTAGAACTCATTATGTTT
9	TS29	AACATTCATGAACCTACCTCAACTTA	CCATATATGAGTACACTACCTCTCGG
10	TR24	AACAACTTCCTCTTATTTTCCA	CAGTAAAAATCAGCCCAAAC
11	TR56	TTGATTCTCTCACGTGTAATTC	ATTTTGATTACCGTTGTGGT
12	TR26	TCATCGCAGATGATGTAGAA	TTGAACCTCAAGTTCTCTGG
13	TA46	TTTATTGCAATAAAACTCATTTCTTATC	TTCTTTTTGTGTGAAAAAAAAATATAGTGA
14	TA2	AAATGGAAGAAGAATAAAAACGAAAC	TTCCATTCTTTATTATCCATATCACTACA
15	TA72	GAAAGATTTAAAAGATTTTCCACGTTA	TTAGAAGCATATTGTTGGGATAAGAGT
16	TAA170	TATAGAGTGAGAAGAAGCAAAGAG GAG	TATTGCATCAATGTTCTGTAGTGT TT
17	GA20	TATGCACCACACCTCGTACC	TGACGGAATTCGTGATGTGT
18	TA113	TCTGCAAAAACTATTACGTTAATACCA	TTGTGTGTAATGGATTGAGTATCTCTT
19	TA130	TCTTTCTTTGCTTCCAATGT	GTAAATCCCACGAGAAATCAA

Table 3. Mean value of chickpea genotypes based on drought tolerance indices

Genotype	Yield (control)	Yield (stress)	YI	STI	GMP	RDI
ICCV-201111	13.97	11.02	0.8642	0.5983	12.4081	0.9922
JG-36	12.54	10.49	0.8225	0.5110	11.4675	1.0523
GCP-101	14.64	12.01	0.9418	0.6833	13.2605	1.0319
ICCV-201105	19.41	14.11	1.1065	1.0640	16.5475	0.9147
ICCV-201209	14.67	10.77	0.8448	0.6139	12.5692	0.9242
ICCV-201113	15.51	11.13	0.8727	0.6706	13.1368	0.9027
ICCV-201118	16.07	11.43	0.8961	0.7136	13.5513	0.8944
ICCV-201212	22.22	17.39	1.3638	1.5014	19.6562	0.9847
ICCV-201217	14.64	11.09	0.8695	0.6305	12.7379	0.9532
ICCV-201102	14.34	10.65	0.8349	0.5930	12.3530	0.9344
ICCV-201218	14.31	11.62	0.9115	0.6462	12.8952	1.0221
ICCV-201108	14.23	13.07	1.0252	0.7228	13.6386	1.1559
CHAFFA	17.27	14.32	1.1234	0.9613	15.7286	1.0435
JG-24	15.13	12.62	0.9895	0.7419	13.8178	1.0491
ICCV-201114	13.29	11.83	0.9274	0.6107	12.5361	1.1196
ICCV-201107	14.67	14.10	1.1056	0.8037	14.3813	1.2090
SAGL 22-101	14.74	13.83	1.0843	0.7916	14.2727	1.1806
SAGL 22-102	17.04	13.43	1.0533	0.8890	15.1255	0.9920
SAGL 22-103	16.02	10.79	0.8463	0.6715	13.1454	0.8478
SAGL 22-104	21.36	16.56	1.2987	1.3746	18.8080	0.9753
SAGL 22-105	16.63	13.73	1.0765	0.8871	15.1093	1.0383
SAGL 22-106	15.16	12.30	0.9643	0.7245	13.6540	1.0203
SAGL 22-107	18.38	13.21	1.0362	0.9436	15.5830	0.9045
SAGL 22-108	16.15	13.90	1.0899	0.8723	14.9826	1.0825
SAGL 22-109	17.74	15.58	1.2220	1.0742	16.6265	1.1049
SAGL 22-111	11.62	10.96	0.8592	0.4945	11.2810	1.1867
SAGL 22-112	18.92	18.84	1.4776	1.3850	18.8791	1.2531
SAGL 22-113	18.72	14.95	1.1721	1.0871	16.7256	1.0047
SAGL 22-114	15.47	12.88	1.0099	0.7742	14.1154	1.0472
SAGL 22 -115	17.17	17.09	1.3400	1.1400	17.1283	1.2520
SAGL-152403	19.31	17.31	1.3574	1.2985	18.2801	1.1279
SAGL-152254	18.62	13.59	1.0656	0.9832	15.9062	0.9181
SAGL-162370	11.12	9.80	0.7686	0.4234	10.4384	1.1091
SAGL-152210	12.73	12.34	0.9678	0.6103	12.5319	1.2201
SAGL-152273	13.48	7.82	0.6133	0.4096	10.2665	0.7301
SAGL-152216	15.07	11.58	0.9084	0.6780	13.2090	0.9674
RVSSG-64	16.38	12.16	0.9533	0.7738	14.1117	0.9336
SAGL-162265	20.58	19.05	1.4940	1.5235	19.8002	1.1646
SAGL-152347	16.48	10.57	0.8291	0.6770	13.1993	0.8072
SAGL-162376	17.72	13.30	1.0427	0.9153	15.3474	0.9443
SAGL-152314	15.98	10.30	0.8077	0.6394	12.8270	0.8111
SAGL-162375	15.67	11.77	0.9231	0.7166	13.5800	0.9453
SAGL-152278	12.27	11.49	0.9009	0.5475	11.8703	1.1783
SAGL-152242	22.41	18.37	1.4410	1.5998	20.2900	1.0318
SAGL-152238	19.53	17.94	1.4072	1.3614	18.7174	1.1562
SAGL-162390	23.22	23.05	1.8078	2.0800	23.1357	1.2490
RVSSG-69	13.40	10.54	0.8263	0.5484	11.8792	0.9897
SAGL-152256	15.16	10.03	0.7869	0.5912	12.3344	0.8326
SAGL-152208	14.12	9.92	0.7779	0.5440	11.8320	0.8842
SAGL-152303	12.41	10.14	0.7950	0.4887	11.2144	1.0281
SAGL-152404	11.40	8.43	0.6614	0.3737	9.8065	0.9306
SAGL-152236	13.36	10.56	0.8281	0.5481	11.8768	0.9945
SAGL-152252	15.32	14.52	1.1388	0.8644	14.9146	1.1926
SAGL-152349	18.16	13.30	1.0434	0.9386	15.5417	0.9220
SAGL-152347	18.16	12.56	0.9848	0.8862	15.1014	0.8700
SAGL-162371	26.02	23.68	1.8571	2.3941	24.8216	1.1451
SAGL-152342	21.47	16.09	1.2621	1.3424	18.5866	0.9432
SAGL-152334	16.80	9.83	0.7711	0.6417	12.8507	0.7365
RVSSG-75	14.85	10.23	0.8022	0.5901	12.3230	0.8669
JG-14	16.93	11.48	0.9006	0.7554	13.9425	0.8536
JG-11	21.51	20.62	1.6175	1.7236	21.0604	1.2066
NBeG-47	15.54	9.56	0.7499	0.5773	12.1884	0.7744
JGG-1	13.21	10.94	0.8576	0.5614	12.0196	1.0415
RVG-205	11.66	9.47	0.7425	0.4291	10.5078	1.0213
RVG-201	14.18	9.05	0.7094	0.4984	11.3251	0.8027
VISHAL	15.71	10.83	0.8491	0.6609	13.0418	0.8672
JG-63	15.09	9.25	0.7254	0.5423	11.8139	0.7713
RVSSG-85	12.03	10.62	0.8328	0.4964	11.3029	1.1105
RVG-210	11.33	8.30	0.6512	0.3656	9.7000	0.9220
SAGL-161032	13.35	8.00	0.6274	0.4150	10.3338	0.7542
SAGL-163603	20.78	17.88	1.4025	1.4442	19.2780	1.0827
SAGL-161018	10.51	6.57	0.5149	0.2681	8.3058	0.7861
SAGL-163008	12.43	9.04	0.7091	0.4368	10.6027	0.9151
SAGL-161001	12.26	7.96	0.6241	0.3790	9.8760	0.8168
RVSSG-68	10.59	7.97	0.6248	0.3278	9.1852	0.9466
JG-315	23.28	21.27	1.6684	1.9243	22.2532	1.1498
JG-74	17.75	10.12	0.7933	0.6976	13.3987	0.7171
ICC-4958	19.94	19.71	1.5459	1.5272	19.8247	1.2438
Mean	16.04	12.75	1.00	0.84	14.27	0.99
Max	26.02	23.68	1.86	2.39	24.82	1.25
Min	10.51	6.57	0.51	0.27	8.31	0.72
CD $_{0.05}$	2.03	2.21	1.94	1.42	2.35	1.32

& Fig. 1. YI ranged between 0.51 (SAGL-161018) to 1.86 (SAGL-162371), STI from 0.84 (SAGL-161018) to 2.39 (SAGL-162371), GMP in between 8.31 (SAGL-161018) to 24.82 (SAGL-162371) and RDI from 0.72 (JG-74) to 1.25 (SAGL-22-112). Genotypes have more than one STI value considered as productive under normal and stressed conditions (28). In this study, several SAGL series genotypes, likewise SAGL-162371 (STI = 2.39), ICCV-201212 (1.50), reported higher values than others, which indicate higher yield with maintenance of yield stability across environments.in compression to the trait profile of genotype ICC4958, comparable to the reference drought-tolerant (26). Highest RDI SAGL-22-112, which displayed the highest RDI (1.25) despite moderate absolute yield, proposes a mechanism of relative stress tolerance and also maintains comparably higher yield than the population mean under drought conditions. Concluded results after study, all four drought indices, top performing genotypes are SAGL-162371, SAGL-162265, SAGL-152242 and ICC-4958, JG315. Considering this, the genotypes that were observed to have higher values for these indices might be proven to be the most tolerant. Research has demonstrated similar findings (40–43). The increased drought indices exhibited by genotypes, including SAGL-162371 (STI = 2.39, GMP = 24.82, YI = 1.86), are due to its superior yield regardless of whether it is irrigated or stressed, suggesting efficient moisture uptake and a negligible yield reduction in the terminal drought capability of the genotype. On the other hand, the reduced drought indices of SAGL-161018 (STI = 0.84, GMP = 8.31, YI = 0.51) are a result of poor yield, showing that it is predisposed to drought stress regardless of the types of environments.

Correlation coefficient between different drought indices and yield under stress and normal conditions

The relationship between different indices and grain yield was studied and the results showed that several indices, including STI, GMP, YI and RDI, were strongly linked to grain yield under both of the conditions, i.e., normal and drought conditions. This is important for future plant breeding programmes because it means that these indices can be used together to select plants that have high yields and can also handle stress well. The strong correlation of STI and GMP with yield under stress conditions is consistent with previous findings in chickpea and maize, suggesting that these indices could be proven useful in breeding programmes for different crops (40–42). In this context, all four drought indices are identified as the best for the selection of desired lines to the next stage of testing. Plants like SAGL-162371 and ICC-4958, which did well for all four indices, could be good candidates for further testing. On the other hand, plants that excel in only one area, such as RDI (e.g., SAGL-22-112), may have unique characteristics that are worth studying further, such as their root system or ability to adjust to drought. This study provides valuable insights for breeders looking to develop crops that can thrive in challenging conditions and highlights the importance of considering multiple factors when selecting plants for breeding programmes.

Molecular characterization

Gene-specific SSR molecular markers are highly polymorphic, associated with drought tolerance and help in targeted selection, better than genetic diversity analysis than neutral markers, as polymorphisms at these loci are more likely to reflect functional variation relevant to stress response (14). Out of 19 investigated SSR markers in the present investigation, 15 markers were found reproducible and polymorphic across 78 genotypes (Table 4 and Fig. 2). Whereas, 4 SSR markers were found to be monomorphic in nature, indicating their limited discriminatory power in this germplasm panel. A total of 43 alleles were considered as effective alleles for 15 markers varying from two to five alleles per locus, reflecting moderate allelic richness consistent with the narrow genetic base of cultivated chickpea genotypes (37). The mean of the major allele frequency of 0.5308 (53.08 %) indicated that no single allele dominated the population excessively, indicating the presence of molecular diversity. The genetic variation among SSR markers ranged between 0.2604 and 0.7245, with an average worth of 0.5457. The highest gene diversity was found in TS29 (0.7245) followed by TA 25 (0.6943), TA 110 (0.6654), TA 80 (0.6545), TA 200 (0.6427) and TR19 (0.6368) (Table 5; Fig. 2). These markers are very useful for identifying drought tolerant genotype with high allelic diversity, making more effective and informative candidates for future utilization in marker-assisted selection (MAS) programmes. Research has reported similar results (44, 45).

Polymorphism information content (PIC)

The PIC values were assessed for various SSR loci on the basis of the number of alleles and allele distribution. The PIC value provided a measure of diversity and frequency of the alleles among genotypes.

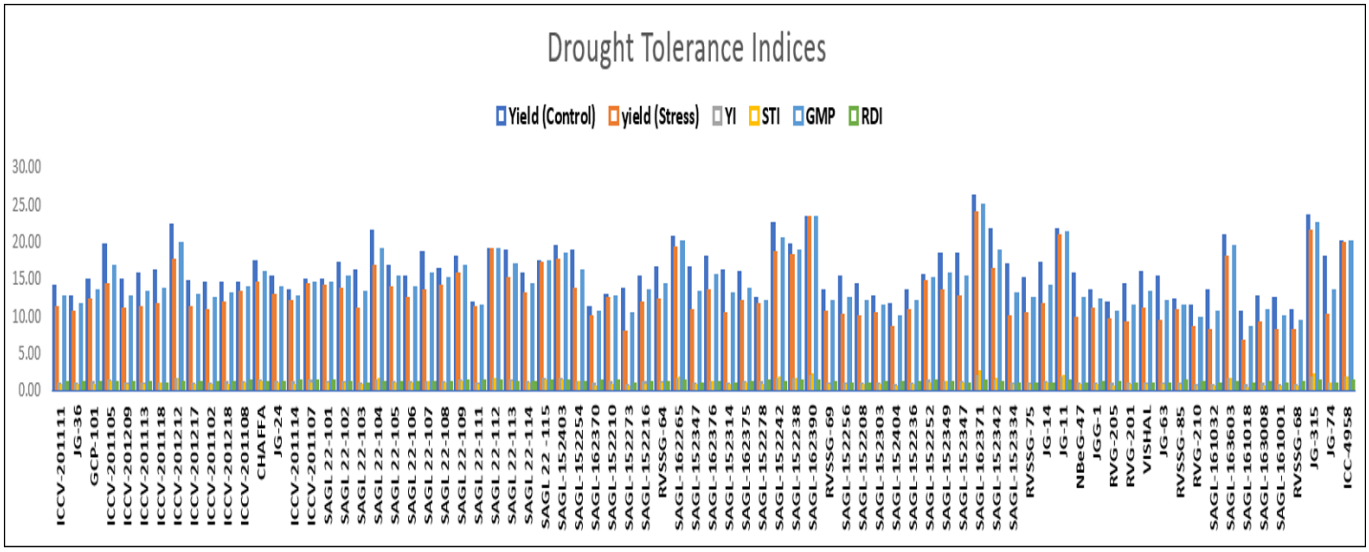


Fig. 1. Mean values of drought tolerance indices (STI, GMP, YI and RDI) and mean grain yield under irrigated (control) and drought stress conditions across 78 chickpea genotypes. STI: Stress tolerance index; GMP: Geometric mean productivity; YI: Yield index; RDI: Relative drought index

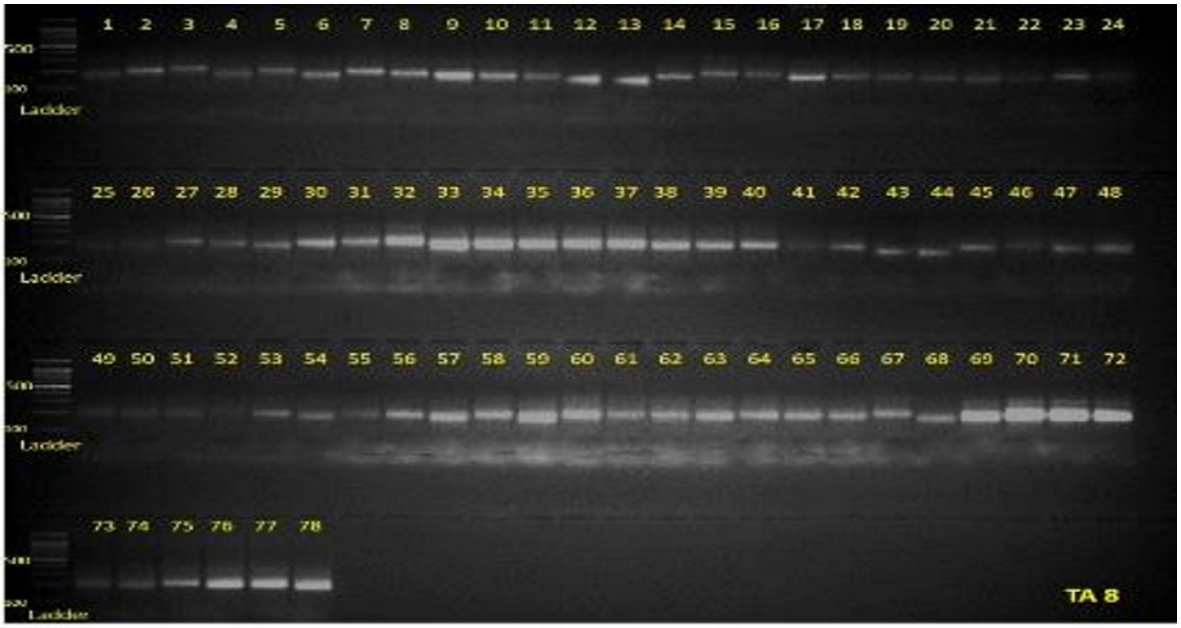
Table 4. Correlation coefficient between different drought indices and yield under stress and normal conditions

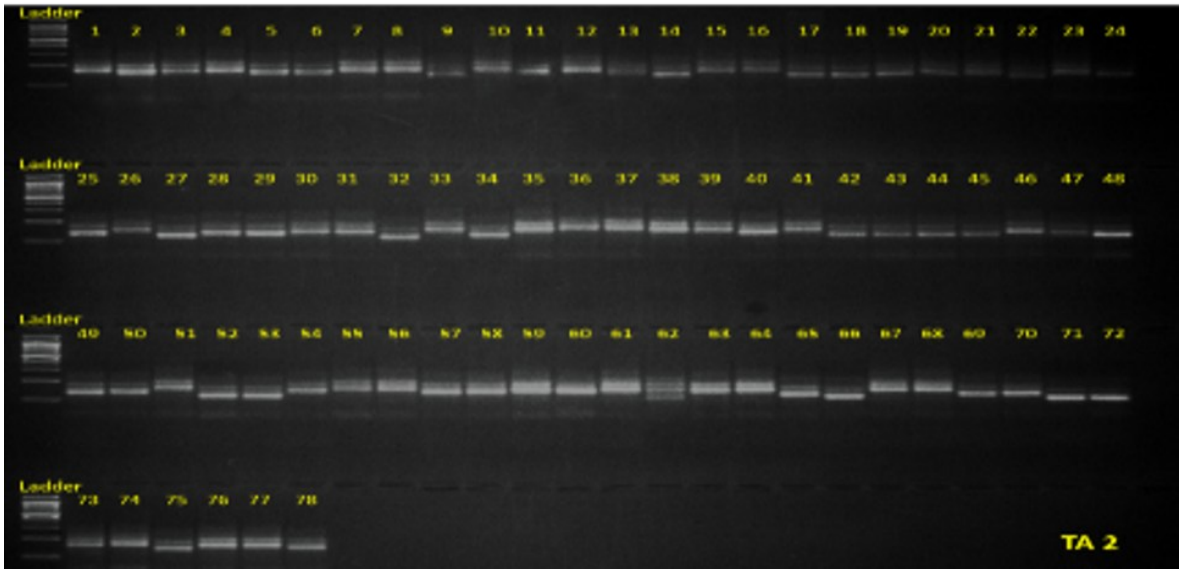
	Yield (control)	Yield (stress)	YI	STI	GMP	RDI
Yield (Control)	1.0000					
Yield (Stress)	0.8735**	1.0000				
YI	0.8735**	0.9989**	1.0000			
STI	0.941**	0.9738**	0.9738**	1.0000		
GMP	0.9559**	0.9778**	0.9778**	0.9908**	1.0000	
RDI	0.2302**	0.6662**	0.6662**	0.4981**	0.5011**	1

In addition to indicating the number of alleles at a locus, the PIC value, which ranges from 0 (monomorphic) to 1 (highly discriminative with multiple alleles at the same frequency), also indicates the molecular markers' ability to discriminate between associated frequencies of those alleles in the genotypes being investigated (46). The PIC value of the entire polymorphic markers during the present investigation ranged between 0.2265 (TAA170) and 0.6744 (TS29) with an average value of 0.4642. The highest PIC value was investigated for the marker TS29 (0.6744), followed by TA25 (0.6363), TA110 (0.5913), TA80 (0.5796), TA200 (0.5677), TR19 (0.5594), TA46 (0.5059), while the lowest was for the marker TA14 (0.3543), with slightly lower values observed in TR26 (0.2828) and TAA170 (0.2265). The observed results were similar to the findings (47, 48).

Cluster analysis of SSR markers

UPGMA (Unweighted pair group approach with arithmetic mean) approach based on pairwise genetic uniformity generated a dendrogram grouping all 78 chickpea genotypes into six different clusters (Table 6 & Fig. 3). Twenty genotypes made up Cluster I, eight genotypes in Cluster II, fifteen genotypes in Cluster III, two genotypes in Cluster IV, eleven genotypes in Cluster V and twenty genotypes in Cluster VI. Two more groups were created from Cluster I, with five and eighteen genotypes in each group, respectively. There are two groups in Cluster II, with two and six genotypes in each group, respectively. Cluster III was further split into two groups, with five and ten genotypes in each group, respectively. Three groups, comprising 3, 3 and 5 genotypes each, were created from Cluster V. The most significant finding from the cluster analysis was the composition of Cluster II: this group comprised ICC 4958 alongside JG315, JG14, RVSSG85, RVSSG75, SAGL-152334 and SAGL-162371. This indicated that these six genotypes share the greatest molecular similarity with





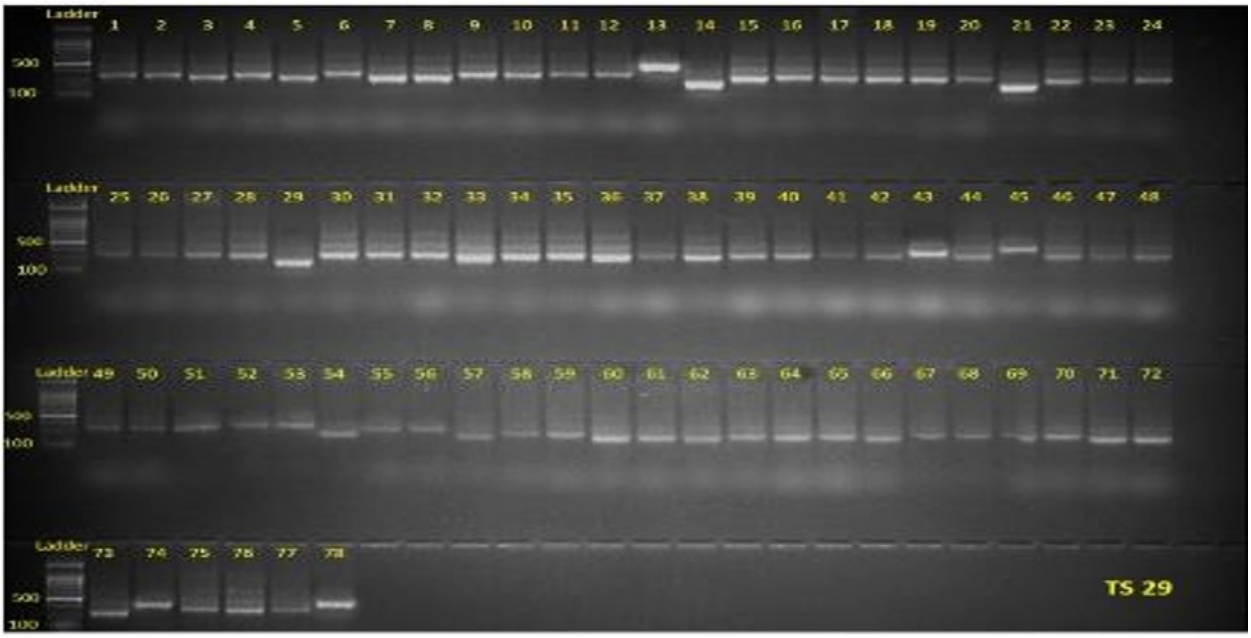


Fig. 2. Gel image representing PCR product of TA8, TA2 and TS29 SSR molecular markers with 78 chickpea genotypes. 1: ICCV-201111, 2: JG-36, 3: GCP-101, 4: ICCV-201105, 5: ICCV-201209, 6: ICCV-201113, 7: ICCV-201118, 8: ICCV-201212, 9: ICCV-201217, 10: ICCV-201102, 11: ICCV-201218, 12: ICCV-201108, 13: CHAFFA, 14: JG-24, 15: ICCV-201114, 16: ICCV-201107, 17: SAGL 22-101, 18: SAGL 22-102, 19: SAGL 22-103, 20: SAGL 22-104, 21: SAGL 22-105, 22: SAGL 22-106, 23- SAGL 22-107, 24: SAGL 22-108, 25: SAGL 22-109, 26: SAGL 22-111, 27: SAGL 22-112, 28: SAGL 22-113, 29: SAGL 22-114, 30: SAGL 22 -115, 31: SAGL-152403, 32: SAGL-152254, 33: SAGL-162370, 34: SAGL-152210, 35: SAGL-152273, 36: SAGL-152216, 37: RVSSG-64, 38: SAGL-162265, 39: SAGL-152347, 40: SAGL-162376, 41: SAGL-152314, 42: SAGL-162375, 43: SAGL-152278, 44: SAGL-152242, 45: SAGL-152238, 46: SAGL-162390, 47: RVSSG-69, 48: SAGL-152256, 49: SAGL-152208, 50: SAGL-152303, 51: SAGL-152404, 52: SAGL-152236, 53: SAGL-152252, 54: SAGL-152349, 55: SAGL-162389, 56: SAGL-162371, 57: SAGL-152342, 58: SAGL-152334, 59: RVSSG-75, 60: JG-14, 61: JG-11, 62: NBeG-47, 63: JGG-1, 64: RVG-205, 65: RVG-201, 66: VISHAL, 67: JG-63, 68: RVSSG-85, 69: RVG-210, 70: SAGL-161032, 71: SAGL-163603, 72: SAGL-161018, 73: SAGL-163008, 74: SAGL-161001, 75: RVSSG-68, 76: JG-315, 77: JG-74, 78: ICC-4958

Table 5. Different parameters analysed with drought-linked SSR markers

Marker	Major allele frequency	Numbers of genotype	Numbers of allele	Gene diversity	PIC value
TR19	0.4103	3	3	0.6368	0.5594
TA110	0.3590	3	3	0.6654	0.5913
TA8	0.5513	2	2	0.4947	0.3724
TA25	0.3974	5	5	0.6943	0.6363
TA80	0.3846	3	3	0.6545	0.5796
TA200	0.4359	3	3	0.6427	0.5677
TS29	0.3462	5	5	0.7245	0.6744
TR56	0.4872	3	3	0.5756	0.4839
TR26	0.7821	2	2	0.3409	0.2828
TA46	0.4872	3	3	0.5914	0.5059
TA2	0.6282	2	2	0.4671	0.3580
TAA170	0.8462	2	2	0.2604	0.2265
TA113	0.5641	2	2	0.4918	0.3709
TA130	0.6410	3	3	0.4849	0.3989
TA14	0.6410	2	2	0.4602	0.3543
Mean	0.5308	2.86	2.86	0.5457	0.4642

Table 6. Grouping of genotypes into clusters based on the similarity index of SSR markers

Cluster	Name of genotypes
Cluster I	SAGL 22-104, SAGL 22-105, SAGL 22-106, SAGL 22-107, SAGL 22-108, ICCV-201108, CHAFFA, JG-24, ICCV-201114, ICCV-201107, SAGL 22-101, SAGL 22-102, SAGL 22-103, ICCV-201217, ICCV-201102, SAGL-152314, SAGL-152349, SAGL-152342, RVG-210, SAGL-161032, SAGL-163603, SAGL-161018
Cluster II	ICC-4958, JG-315, JG-14, RVSSG-85, RVSSG-75, JG-63, SAGL-152334, SAGL-162371
Cluster III	SAGL-152208, SAGL-152303, SAGL-152404, SAGL-152236, SAGL-152347, JG-11, NBeG-47, JGG-1, RVG-205, RVG-201, VISHAL, RVSSG-68, JG-74, RVSSG-64, SAGL 22-109
Cluster IV	SAGL 22-113, SAGL-152252
Cluster V	ICCV-201111, JG-36, GCP-101, ICCV-201105, ICCV-201209, ICCV-201113, ICCV-201118, ICCV-201212, ICCV-201218, SAGL 22-111, SAGL 22-112
Cluster VI	SAGL 22-114, SAGL 22 -115, SAGL-152403, SAGL-152254, SAGL-162370, SAGL-152210, SAGL-152273, SAGL-152216, SAGL-162265, SAGL-152347, SAGL-162376, SAGL-162375, SAGL-152278, SAGL-152242, SAGL-152238, SAGL-162390, RVSSG-69, SAGL-152256, SAGL-162008, SAGL-161001

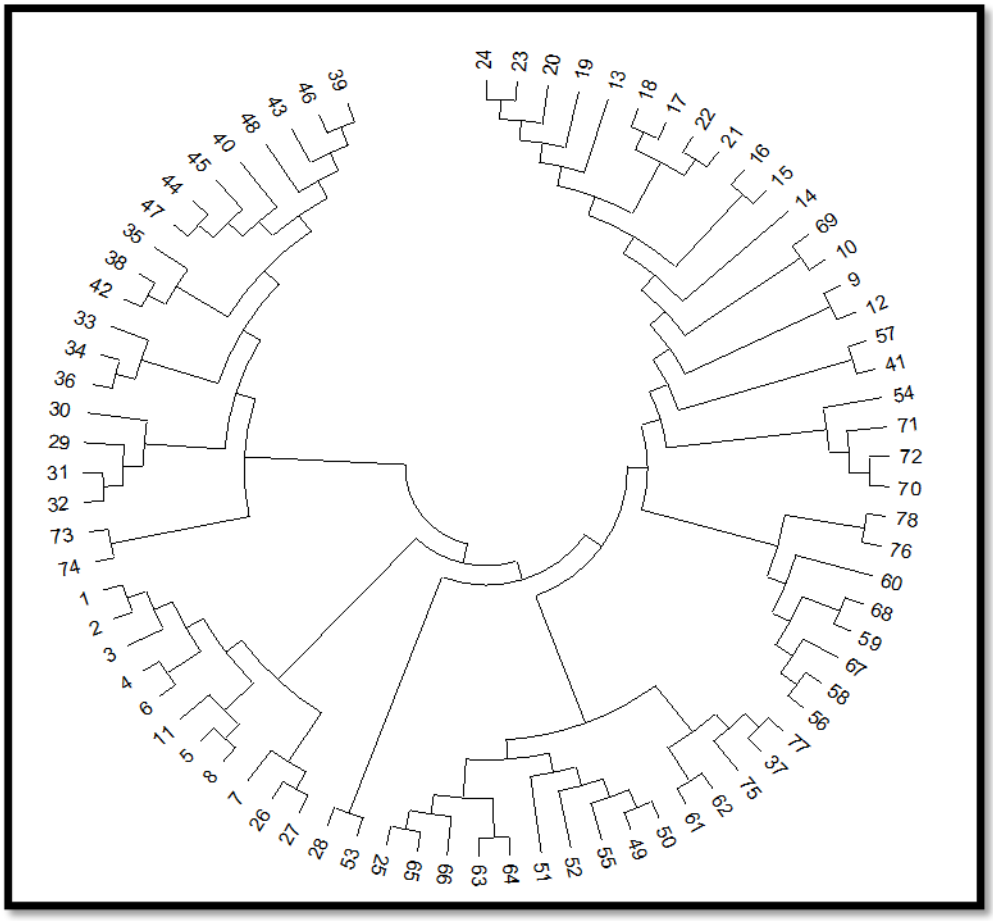


Fig. 3. Phylogenetic tree of 78 chickpea genotypes using the UPGMA. 1: ICCV-201111, 2: JG-36, 3: GCP-101, 4: ICCV-201105, 5: ICCV-201209, 6: ICCV-201113, 7: ICCV-201118, 8: ICCV-201212, 9: ICCV-201217, 10: ICCV-201102, 11: ICCV-201218, 12: ICCV-201108, 13: CHAFFA, 14: JG-24, 15: ICCV-201114, 16: ICCV-201107, 17: SAGL 22-101, 18: SAGL 22-102, 19: SAGL 22-103, 20: SAGL 22-104, 21: SAGL 22-105, 22: SAGL 22-106, 23: SAGL 22-107, 24: SAGL 22-108, 25: SAGL 22-109, 26: SAGL 22-111, 27: SAGL 22-112, 28: SAGL 22-113, 29: SAGL 22-114, 30: SAGL 22 -115, 31: SAGL-152403, 32: SAGL-152254, 33: SAGL-162370, 34: SAGL-152210, 35: SAGL-152273, 36: SAGL-152216, 37: RVSSG-64, 38: SAGL-162265, 39: SAGL-152347, 40: SAGL-162376, 41: SAGL-152314, 42: SAGL-162375, 43: SAGL-152278, 44: SAGL-152242, 45: SAGL-152238, 46: SAGL-162390, 47: RVSSG -69, 48: SAGL-152256, 49: SAGL-152208, 50: SAGL-152303, 51: SAGL-152404, 52: SAGL-152236, 53: SAGL-152252, 54: SAGL-152349, 55: SAGL-162389, 56: SAGL-162371, 57: SAGL-152342, 58: SAGL-152334, 59: RVSSG-75, 60: JG-14, 61: JG-11, 62: NBeG-47, 63: JGG-1, 64: RVG-205, 65: RVG-201, 66: VISHAL, 67: JG-63, 68: RVSSG-85, 69: RVG-210, 70: SAGL-161032, 71: SAGL-163603, 72: SAGL-161018, 73: SAGL-163008, 74: SAGL-161001, 75: RVSSG-68, 76: JG-315, 77: JG-74, 78- ICC-4958

the globally recognised drought-tolerance donor. Genotype ICC4958 has a widespread root system and is widely employed to improve drought tolerance by transferring QTL-hotspot regions utilising marker-assisted backcrossing in different genetic backgrounds. This convergence is precisely the kind of genotype breeders seek: a line that does not require repeated backcrossing to ICC 4958 but already carries a similar molecular profile while outperforming it agronomically in the present environment. These genotypes could therefore be regarded as drought-tolerant. The findings of this study may be helpful if breeders choose to employ these genotypes as recurrent parents in the MAS programme to develop drought tolerance. Previous studies on cluster analysis have also been reported (44, 45).

Principal coordinate analysis (PCoA)

Principal coordinate analysis (PCoA) based on the SSR data produced a scatter plot that divided 78 genotypes into two distinct groups, broadly corroborating the STRUCTURE-based population groupings (Fig. 4). The wide scatter of genotypes across the plot reflects the substantial within-population genetic variation identified by AMOVA. Genotypes belonging to the Cluster II group (ICC4958, SAGL-162371, JG315, JG14, SSG85, RVSSG75 and SAGL-152334) were located in a unique spatial area in the PCoA analysis, serving as additional multivariate evidence for the molecular distinctness of these genotypes from other genotypes (Fig. 4). Such differentiation can be attributed to allelic variations in the drought-related SSRs, since these are of great biological significance, considering that these marker genes regulate root architecture, osmotic adjustment and signalling processes under stress conditions in chickpeas (14, 26)

Population structure analysis

Population structure analysis categorized all investigated genotypes into two optimal sub- populations (K= 2), as indicated by the highest LnP (D) at K value (Fig. 5) All other accessions were classified as admixtures, whereas those with a membership fraction (Q) of 80 % or more were deemed pure (Fig. 5). Twelve pure and twenty-six admixture lines were evident at an 80 % threshold. Of the 52 pure lines, 26 belonged to group-1 (represented by the letter G1; red) and 26 belonged to group-2 (represented by the letter G2; green). Genotypes under these two populations were characterised as pure populations or admixture populations based on membership fractions: P1 showed 67.64 % pure and 32.35 % admixed individuals, while P2 showed 65.90 % pure and 34.09 % admixed individuals, indicating moderate levels of inter-population gene flow or shared ancestral backgrounds across the panel. These two sub-populations probably correspond to different breeding pedigrees among the

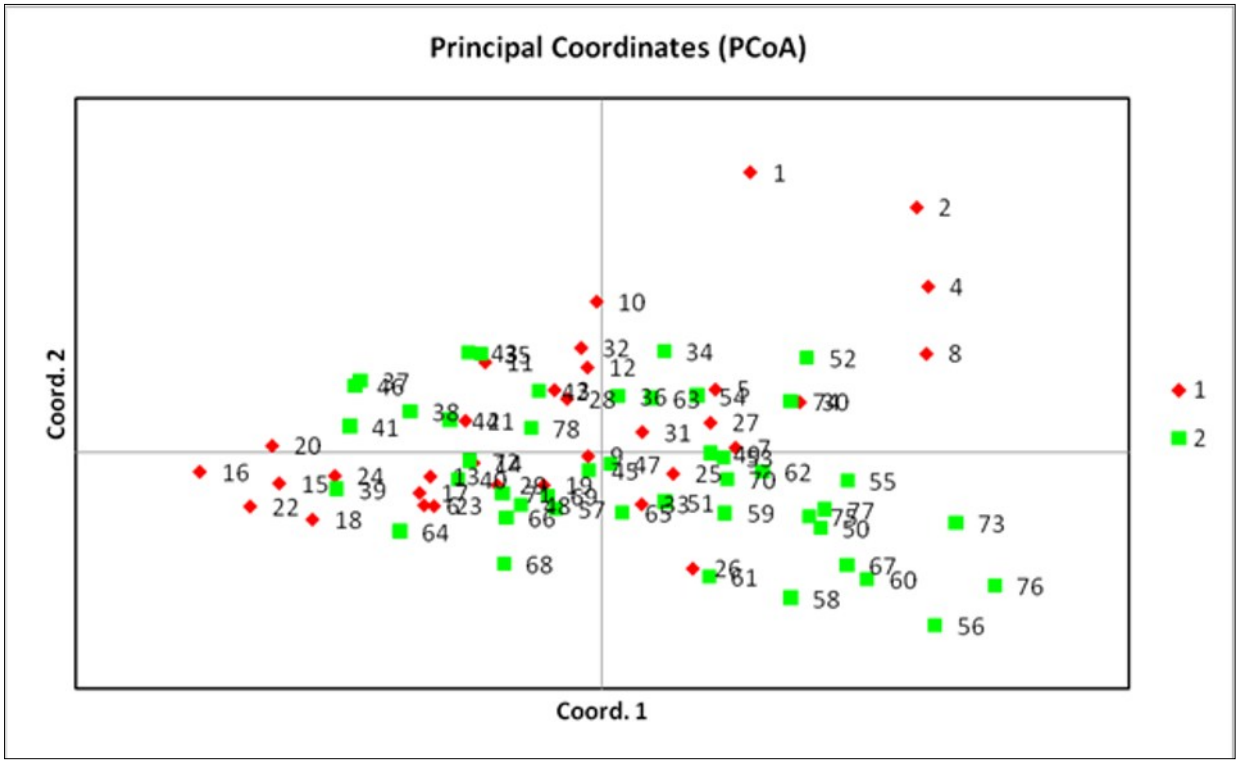


Fig. 4. Principal coordinates analysis (PCoA) grouped 78 chickpea genotypes.

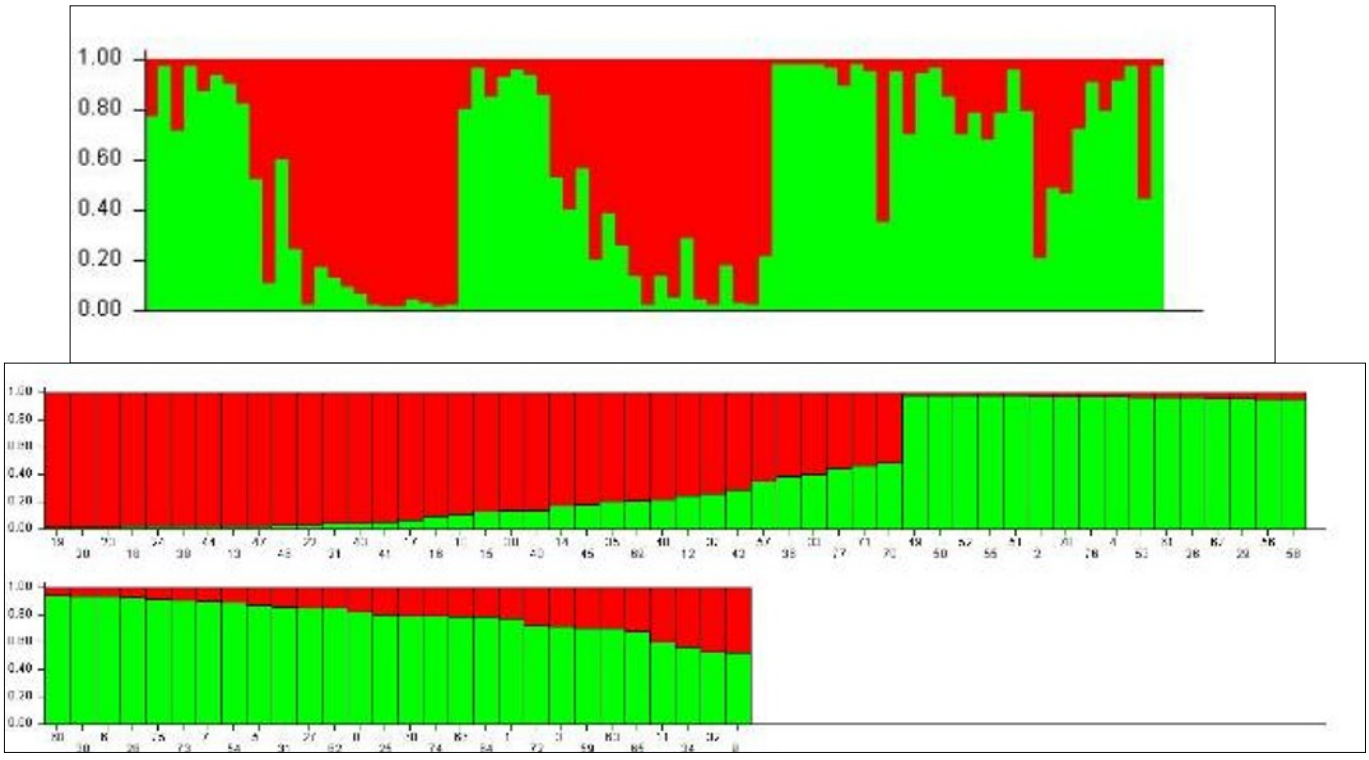


Fig. 5. Population structure (K = 2) inferred from STRUCTURE analysis for the 78 chickpea genotypes based on 15 SSR markers.

genotypes assessed, one made up of predominantly material originating from JNKVV Jabalpur and the other being lines obtained from RAK Sehore, which is in accordance with the breeding pedigrees shown in Table 1. It should be distinguished that there were admixture lines present in both of the sub-populations (about one-third in each), suggesting that past mixing between the two breeding lineages, with the advantage of having some introgressed favourable alleles from both of the populations, which is indeed of practical value to the breeder. The observed results were similar to the findings of Indian chickpea germplasm evaluations (38, 43).

Analysis of molecular variance (AMOVA)

Among the 78 genotypes of chickpeas, analysis of molecular variance (AMOVA) revealed that genetic differentiation within populations accounted for 91 % of the variance, whereas genetic differentiation between genotypes within populations explained 9 % of the variance (Fig. 6). This high degree of intrapopulation variation is typical for self-pollinated crops like chickpea and is attributed to the accumulated effect of many years of selection from a small genetic base (37). As a result, genetic diversity estimation has emerged as a crucial technique for identifying genetically distinct parents who possess desired traits (43). Long-term breeding and selection operations were aided by the molecular data-based

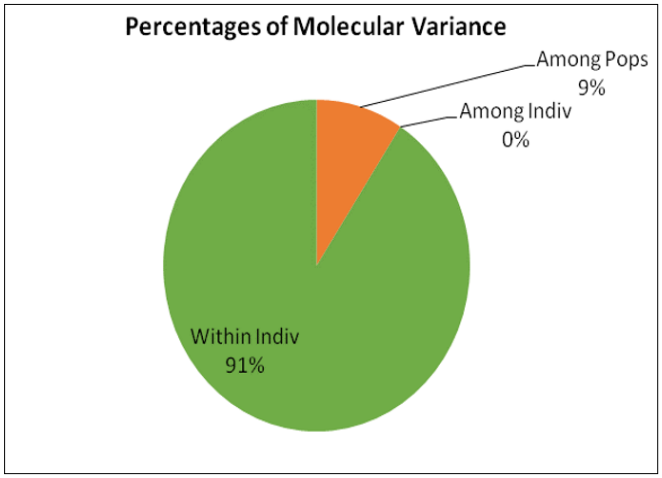


Fig. 6. The percentage of molecular genetic variations of 78 chickpea genotypes within populations, among individuals and populations revealed by SSR markers.

analysis of genetic diversity and genetic structure of the evaluated chickpea genotypes, which supported the morpho-biochemical property-based selection of potential parents. Several parents are preferred in chickpea crossbreeding to reduce the genetic instability of segregating populations (49, 50). Hybridisation between parents P_1 and P_2 populations may generate offspring which will incorporate the positive genes of both of the parents, which is a highly successful approach adopted for chickpea breeding in India (26). Considering both the phenotypic and molecular data, the current study proposes a hierarchical approach to germplasm utilisation to breed drought-tolerant cultivars. Genotypes viz., AGL-162371, JG315 and JG14 are the best in terms of having the highest value of STI and GMP index, ICC4958 molecular cluster membership and verified yield advantage under field drought conditions; thus, they can be used as parents to use in the crossing programme in the MAS-assisted introgression programme. Genotypes including SAGL-22-112, having the highest RDI score, SAGL-162265 and SAGL-152242, showed strong relative drought tolerance and are suitable for physiological evaluation and recurrent selection in germplasm collections. High-PIC markers, including TS29 and TA25, identified in the present study, represent easily accessible molecular tools for foreground selection of drought -tolerant alleles in BCs. Future research should focus on evaluating root morphology, canopy temperature and multilocation testing of these genotypes to apply results obtained at the molecular and index levels.

Conclusion

The investigation of integrated morphological drought indices and SSR molecular marker characterisation of 78 chickpea genotypes under drought conditions was conducted. STI, GMP, RDI and YI indices showed significant positive and higher correlation with grain yield under both normal and stressed conditions, validating their collective utility as reliable screening criteria. Genotype SAGL-162371 recorded the highest drought indices, reflecting its superiority in terms of high yield and drought tolerance under both environmental conditions. Marker TAA170 (84.62 %) displayed the main allele frequency. While markers TS29 and TA25 were determined to have the highest PIC value and gene diversity, making them preferable for foreground selection in a marker-assisted breeding programme. UPGMA cluster analysis revealed that genotypes, *viz*., SAGL-162371, JG315, JG14, RVSSG85, RVSSG75 and SAGL-152334, co-clustered with the established drought-tolerant donor, namely ICC4958, providing molecular corroboration for their phenotypic performance. A study of AMOVA observed 91 % genetic variation obtained within the population, indicating substantial exploitable diversity within the germplasm panel. Genotypes, i.e., SAGL-162371 and JG315 recommended as parents in a further hybridisation programme to develop a drought-resilience variety. while markers having high PIC values, viz., TS29 and TA25, are proposed as foreground selection tools. So, these combinations may be employed as useful sources or as a starting genetic material to develop drought-tolerant cultivars, along with having a higher yield potential, employing a molecular breeding approach.

Authors' contributions

RA contributed to the conceptualisation and experimentation. MKT contributed to the conceptualisation and methodology and assisted in the writing and editing of the manuscript. RKY helps in the experimentation. DKP and NT performed data analysis. NT performed data analysis. RM assisted in writing and editing the manuscript. AP assisted in the writing and editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this work, the authors used Grammarly to reduce grammatical errors. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

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