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Molecular and phenotypic diversity among chickpea (*Cicer arietinum*) genotypes as a function of drought tolerance

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Abstract. Diversity as a function of drought tolerance may be identified by morphological characters, and molecular tools used to find the most divergent genotypes for breeding programs for drought tolerance in future. The narrow genetic base of chickpea can be circumvented by using diverse lines in breeding programs. Forty chickpea genotypes were studied for their morphological and molecular diversity with an objective of identifying the most diverse drought-tolerant lines. In total, 90 alleles were detected with 3.6 alleles per locus. Polymorphism information content (PIC) values ranged from 0.155 to 0.782 with an average value of 0.4374 per locus. The size of amplified products ranged from 160 bp to 390 bp. Primer TA136 with eight alleles showed the highest PIC value of 0.7825, indicating its ability to differentiate the genotypes at molecular level. DARwin neighbour-joining tree analysis based on dissimilarity estimates was done for the molecular data and sequential agglomerative hierarchical non-overlapping (SAHN) grouping for the morphological data. It could clearly discriminate the tolerance and the sensitivity of genotypes. Two-dimensional principal coordinates analysis (PCoA) plot indicated good diversity for drought tolerance. The genetic similarity coefficients ranged from 0.115 (genotypes BGD72 to ICCV 5308) to 0.828 (genotypes ICCV 10316 to ICCV 92337).

Additional keywords: genetic diversity, membrane stability index, molecular markers, relative water content.

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Introduction

Chickpea (*Cicer arietinum* L.; 2n = 2× = 16), a member of the Fabaceae, is the most essential legume crop after dry beans (*Phaseolus vulgaris*) with a genome size of ~738 Mb and 28 269 genes (Varshney *et al*. 2013). Chickpea is grown mostly on residual soil moisture mainly from the previous wet season in the semi-arid regions of the world (Gaur *et al*. 2012). Globally, an area of 8.25 Mha is used for chickpea cropping, producing 7.33 Mt (Project Coordinator's Report 2015–16), and ~70% of global production is in India (FAOSTAT 2012).

The average productivity of chickpea is very low and has remained stagnant for some time. The low productivity is due to various biotic and abiotic stresses and low diversity among cultivated varieties. Drought is one of the major limiting factors in chickpea production globally, and is estimated to reduce chickpea yield by up to 50% (Kumar *et al*. 2015). Growth and photosynthesis are primarily affected by drought stress, and to minimise these yield losses it is vital to evaluate parameters of growth such as chlorophyll index, plant height, relative water content (RWC), membrane stability index (MSI), biomass, 100-seed weight and plant yield, and to understand the morphological and physiological basis of yield variation.

Breeding for drought tolerance in chickpea is limited by absence of good selection indices, particularly morphological and physiological responses that can be effectively used. Molecular markers are highly reproducible and they have been frequently used to discern traits, to assess genetic diversity and in characterisation studies (Satyavathi *et al*. 2006). They have become the integral component of chickpea breeding programs (Bharadwaj *et al*. 2010; Yadav *et al*. 2011; Varshney *et al*. 2013). Simple sequence repeats (SSRs) have been extensively used in plant genetics and breeding because of their co-dominant nature, high reproducibility and relative abundance, multi-allelic nature, high degree of polymorphism and extensive genome coverage (Varshney *et al*. 2005; Bharadwaj *et al*. 2010; Choudhary *et al*. 2012). Furthermore, SSR genotypic data from several loci also

provide distinctive allelic profiles for establishing genotype identity (Bharadwaj *et al*. 2010).

The objective of the present study was to assess the genetic diversity of chickpea genotypes by using morphological and microsatellite markers, to identify high-yielding and drought-stress-tolerant genotypes for use in future crop-improvement programs. Breeding for drought tolerance is constrained by the absence of selection indices that can be used for introducing stress tolerance. Hence, there is an urgent need to discern the morphological and physiological responses of chickpea lines to drought stress and select tolerant genotypes for crossing programs.

Materials and methods

The plant material consisted of 40 chickpea genotypes available at Pulse Research Laboratory, Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi (Table 1). These include released varieties, breeding lines and selected lines from the International Training population obtained from ICRISAT. The study was conducted during 2015–16 at the National Phytotron Facility, IARI, New Delhi (28°08′N, 77°12′E), under glasshouse conditions, with the diurnal temperature maintained at 24°C and nocturnal temperature 18°C.

Soil selection and stress treatment

The experimental soil, with electric conductivity 0.4 dS m^{-1} and pH 8.1, was taken from the IARI field. A completely randomised design was used for the experiment with each genotype sown in three replicates in plastic pots 6 cm by 6 cm under two different conditions: irrigated and stressed. The drought stress was imposed at 35 days after sowing. Plants were maintained well and watered regularly before being subjected to stress at the pre-flowering stage, imposed as per Mafakheri *et al*. (2010). Data on morphological and physiological parameters including chlorophyll index, plant height, RWC,

Table 1. List of genotypes for study

No.	Variety	Source	Pedigree
1	ICCV09313	ICRISAT, Hyderabad	ICCV92311 × ICC14198
2	ICCV10313	ICRISAT, Hyderabad	ICCV92337 × ICC14194
3	ICCV08310	ICRISAT, Hyderabad	ICCV95311 × ICC17109
4	ICCV097309	ICRISAT, Hyderabad	(ICC2588 × ICCC32) × [(ICCC49 × ICC15980) × ICCV3]
5	ICCV03311	ICRISAT, Hyderabad	ICCV92328 × [(ICCC32 × ICC12034) × ICC19686]
6	ICCV01309	ICRISAT, Hyderabad	(ICC4973 × ICC14196) × ICCV92329
7	ICCV09312	ICRISAT, Hyderabad	ICCV92337 × ICC7344
8	ICCV9314	ICRISAT, Hyderabad	ICCV92311 × ICC17109
9	ICCV10304	ICRISAT, Hyderabad	ICCV92311 × ICC14215
10	ICCV10307	ICRISAT, Hyderabad	ICCV92311 × ICC17109
11	ICCV10306	ICRISAT, Hyderabad	ICCV92311 × ICC17109
12	ICCV10316	ICRISAT, Hyderabad	ICCV92337 × ICC17109
13	ICCV92337	ICRISAT, Hyderabad	(ICCV2 × ICC12034) × ICC7344
14	ICCV00109	ICRISAT, Hyderabad	ICC18746 × ICCV10
15	ICCV03103	ICRISAT, Hyderabad	[ICCV92014 × JG23) × BG1032]
16	ICCV09307	ICRISAT, Hyderabad	ICCV92337 × ICC17109
17	ICCV95423	ICRISAT, Hyderabad	(ICC7676 × ICCC32) × ((ICCC49 × ICC15980) × ICCV3)
18	ICCV97404	ICRISAT, Hyderabad	(ICCC32 × ICC4967) × [(ICCC49 × ICC15980) × ICCV3]
19	ICCV10	ICRISAT, Hyderabad	ICC1376 × ICC1443
20	ICC1882	ICRISAT, Hyderabad	Traditional landrace P1506-4 from ICRISAT
21	BGD72	IARI, New Delhi	P1231 × P1265
22	Pusa1103	IARI, New Delhi	(Pusa256 × *Cicer reticulatum*) × Pusa362
23	ICC4958	ICRISAT, Hyderabad	GW 5/7, a drought tolerant breeding line from ICRISAT
24	ICCV00301	ICRISAT, Hyderabad	ICCV92502 × ICCV2
25	ICCV0302	ICRISAT, Hyderabad	FLIP 91-18C × ICCV2
26	ICCV01301	ICRISAT, Hyderabad	GNG1044 × (ICCC32 × ICC12034)
27	L550	Ludhiana	PBG7 × Rabat
28	ICCV03403	ICRISAT, Hyderabad	(ICC4973 × ICC14196) × ICCV92329
29	C235	Ludhiana	IP58 × C1234
30	ICCV03404	ICRISAT, Hyderabad	(ICC4973 × ICC14196) × ICCV92329
31	ICCV03310	ICRISAT, Hyderabad	BG70 × ICCV92329
32	ICCV07301	ICRISAT, Hyderabad	ICCC95334 × (ICCV2 × ICCV98506)
33	ICCV05312	ICRISAT, Hyderabad	ICCV2 × ICCV92325
34	ICCV5308	ICRISAT, Hyderabad	ICCV2 × ICCV92311
35	ICCV5313	ICRISAT, Hyderabad	ICCV2 × ICCV92325
36	ICCV4310	ICRISAT, Hyderabad	(ICC4973 × ICC14196) × ICCV92329
37	Pusa1003	IARI, New Delhi	Mutant of L532
38	CSG8962	Karnal	Selection from GPF7035
39	ICCV4303	ICRISAT, Hyderabad	(ICC4973 × ICC14196) × ICCV92329
40	ICCV2	ICRISAT, Hyderabad	[(ICC5003 × ICC 4953) × ICC 583] × (ICC4973 × ICC7347)

MSI, biomass, 100-seed weight and plant yield were recorded. After the stress was terminated, plants were watered regularly until harvest.

Physiological parameters

Plant height was recorded manually, with a ruler, from three randomly selected, healthy-looking plants. The height was calculated from the surface of the soil to the highest tip of the plant when held up.

The top three completely open, healthy leaves were collected from three plants for calculation of RWC. A 400-mg sample of leaves was placed in a Petri dish containing distilled water, for 4 h at room temperature, and turgid weight was recorded. Leaves were oven-dried at 60°C for 72 h, and then the weight was quickly recorded as plant dry weight to avoid retention of atmospheric moisture. RWC was calculated for all 40 genotypes via the formula of Barrs and Weatherley (1962):

$$\mathrm{RWC} = (\mathrm{FW} - \mathrm{DW}/\mathrm{TW} - \mathrm{DW}) * 100$$

where FW is fresh weight, DW is dry weight, and TW is turgid weight.

A 400-mg sample of fresh leaves was placed in a test tube and immersed in distilled water. It was then incubated at 45°C for 30 min in a water bath and electrical conductivity (EC1) was recorded with an electrical conductivity meter. The test tube was kept in the water bath at 100°C for 10 min and a final conductivity reading (EC2) was taken. The MSI was calculated using given formula (Blum and Ebercon 1981):

$$\mathrm{MSI} = 1 - (\mathrm{EC1}/\mathrm{EC2}) * 100$$

Chlorophyll index was calculated via a chlorophyll meter. Readings were taken randomly from three plants at around 12 : 00 (midday), using the SPAD-502Plus (Konica Minolta, Osaka, Japan).

Yield and yield-related data, i.e. biomass, 100-seed weight and plant yield, were also recorded. Drought tolerance was measured by calculating the drought susceptibility index (DSI) given by Fischer and Maurer (1978):

$$\mathrm{DSI} = (1 - \mathrm{Yd}/\mathrm{Yp})/\mathrm{D}$$

where Yd is grain yield of the genotype under moisture-stress condition, Yp is grain yield of the genotype under irrigated condition, and D is mean yield of all strains under moisture stress condition/mean yield of all strains under irrigated condition.

Statistical analyses

All parameters were then statistically analysed and the mean value of the samples from the three replications was taken into account for data analysis. Analysis of variance (ANOVA) for a completely randomised design was done as per standard methods (Panse and Sukhatme 1964) and the statistical significance was calculated for each of the parameter by comparing the tabulated and calculated *F*-values at $P = 0.05$ and 0.01.

Genomic studies

Genomic DNA was extracted by using the modified CTAB method (Kumar *et al*. 2013). For each cultivar. A sample of fresh young leaves (2 g) was taken and crushed in a Geno/Grinder (SPEX SamplePrep, Metuchen, NJ, USA) to obtain a clear lysate, which served as the starting material for subsequent steps for DNA extraction. After DNA purification, the DNA concentration was checked on 0.8% agarose gel (Sambrook and Russell 2001) and quality was detected with NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Dilutions of 20 ng μL^{-1} were prepared from the stock DNA samples.

SSR amplification

In total, 125 SSR markers synthesised by Bioneer, Daejeon, South Korea, were used to study diversity among the genotypes. These primers were used to amplify DNA of the 40 chickpea genotypes by using a G-STORM thermal cycler (Labtech, Palaiseau, France). The PCR program comprised an initial denaturation at 94°C for 6 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C−58°C (based on primer annealing temperature) for 1 min and 72°C for 2 min, with a final extension at 72°C for 10 min before cooling to 4°C. Reaction master mix (10 µL) was prepared containing 1 µL template DNA, 1 µL primer, 1 µL 4-deoxyribonucleotide triphosphate, 0.12 µL $MgCl_2$, 1.25 µL *Taq* DNA buffer and 0.3 µL *Taq* DNA polymerase (Banglore Genei, Banglore, India). The PCR products were analysed by agarose gel electrophoresis.

Diversity analysis

Polymorphic bands of each SSR marker were scored in the binary format at each level of a particular locus, and the resulting data were analysed using DARwin version 5.0.128 (http://darwin.cirad.fr/) (Bharadwaj *et al*. 2011). Binary data obtained by SSR markers was used to calculate Jaccard's coefficients (Jaccard 1908) between a pair of genotypes. On the basis of the similarity matrix generated on binary data and on morphological data, they were grouped by using the sequential agglomerative hierarchical non-overlapping (SAHN) clustering method and neighbour joining (NJ) tree analysis. Bootstrap analysis was done for the node construction by using 1000 bootstrap values (Perrier *et al*. 2003). Principal coordinates analysis (PCoA) was also performed based on the presence and absence of each allele in the data matrix, and the two principal coordinates were used to observe the dispersion of genotypes. The binary matrix can also be converted to appropriate formats required for specific programs. PowerMarker version 3.0 (Liu and Muse 2005) was used for calculating basic statistics and diversity studies including the total number of alleles (N_A), major allele, gene diversity (H_E), allele frequency, availability, heterozygosity (H_O) and polymorphism information content (PIC).

Results

Physiological parameters

The ANOVA for the parameters studied is presented in Table 2. All seven traits showed significant variation under the drought-stress environment, indicating considerable diversity in the material used for the study. The mean performance of 40 genotypes for the seven traits along with their individual

Table 2. ANOVA for the seven morphological and physiological traits studied under drought-stress conditions in chickpea genotypes
$^{**}P < 0.01$

Source of variation	Replicate	Treatment	Error
d.f.	2	39	78
Plant height	1133.075	50.097**	54.102
Chlorophyll index	3488.633	247.985**	213.445
Relative water content	5908.945	186.078**	244.551
Membrane stability index	5470.295	239.482**	260.005
Biomass	106 294.10	83 059.352**	44 255.170
100-seed weight	993.566	141.449**	96.201
Plant yield	19 841.960	16 886.938**	8952.237

standard deviations and overall mean are compiled for normal conditions (Table 3) and drought-stress conditions (Table 4). Mean plant height under normal conditions was 64.98 cm, with a minimum of 45.28 cm (ICCV3403) and a maximum of 78.7 cm (ICC4958), whereas under drought stress, plant height ranged from 39.82 cm (ICC1882) to 74.1 cm (ICCV97309) with an average of 59.36 cm. Under normal conditions, mean chlorophyll index was 53.62 SPAD units, ranging from 44.53 units (ICCV9314) to 67.1 units (ICCV10307), whereas under drought stress, mean chlorophyll index was 46.77 SPAD units, ranging from 24.23 units (ICCV3404) to 61.36 units (ICCV00109). Highly significant variation was observed

Table 3. Mean performance of 40 chickpea genotypes evaluated for morphological and physiological traits under normal conditions
Chl index, Chlorophyll index; RWC, relative water content; MSI, membrane stability index

Genotype	Plant height (cm)	Chl index (SPAD units)	RWC	MSI	Biomass (g)	100-seed wt (g)	Plant yield (g)
ICC1882	27.33 ± 0.34	50.70 ± 0.48	61.69 ± 0.14	47.60 ± 0.10	689.48 ± 5.79	17.65 ± 0.27	216.77 ± 1.45
ICC4958	33.0 ± 0.45	61.33 ± 0.22	81.38 ± 0.25	78.70 ± 0.14	730.66 ± 6.53	28.72 ± 0.35	185.23 ± 1.07
Pusa1103	31.0 ± 0.22	55.55 ± 0.41	72.55 ± 0.15	68.45 ± 0.19	518.03 ± 8.43	21.97 ± 0.38	248.27 ± 0.99
BGD72	30.0 ± 0.98	53.03 ± 0.46	72.66 ± 0.13	70.68 ± 0.11	588.55 ± 10.12	16.39 ± 0.19	461.43 ± 2.12
Pusa1003	29.33 ± 0.56	61.41 ± 0.26	50.88 ± 0.07	51.30 ± 0.07	420.63 ± 7.72	16.58 ± 0.34	144.73 ± 1.40
CSG8962	33.0 ± 1.19	55.05 ± 0.57	80.48 ± 0.14	71.08 ± 0.25	694.78 ± 8.17	11.19 ± 0.24	241.40 ± 2.29
C235	29.66 ± 1.28	47.67 ± 0.43	62.91 ± 0.08	57.37 ± 0.68	320.87 ± 8.03	14.22 ± 0.19	143.70 ± 1.32
ICCV3310	36.0 ± 0.98	61.20 ± 0.35	63.40 ± 0.12	67.31 ± 0.28	463.10 ± 12.40	33.17 ± 0.49	120.59 ± 1.26
ICCV3311	33.33 ± 0.79	53.55 ± 1.93	72.77 ± 0.09	76.38 ± 0.21	520.14 ± 12.34	30.59 ± 0.37	114.52 ± 0.68
ICCV3403	32.33 ± 0.56	47.72 ± 1.20	70.07 ± 0.24	45.29 ± 0.15	472.29 ± 7.59	30.93 ± 0.27	145.8 ± 1.36
ICCV3404	30.33 ± 1.36	53.85 ± 2.35	45.38 ± 0.17	65.93 ± 0.19	463.07 ± 8.15	38.71 ± 0.34	168.25 ± 0.46
ICCV7301	32.0 ± 0.45	58.28 ± 0.29	62.33 ± 0.15	66.76 ± 0.27	363.88 ± 8.88	37.29 ± 0.24	155.70 ± 1.40
ICCV4303	22.66 ± 1.13	62.20 ± 0.29	69.66 ± 0.14	56.42 ± 0.23	539.35 ± 13.51	35.95 ± 0.26	130.05 ± 0.66
ICCV4310	31.0 ± 0.22	56.73 ± 0.29	68.66 ± 0.16	59.53 ± 0.16	284.78 ± 5.02	33.61 ± 0.33	128.42 ± 1.36
ICCV5312	31.66 ± 0.47	59.50 ± 0.26	69.53 ± 0.16	73.53 ± 0.14	467.56 ± 13.7	35.71 ± 0.27	50.43 ± 1.76
ICCV9312	30.0 ± 0.23	45.40 ± 0.89	70.50 ± 0.18	56.86 ± 0.23	380.0 ± 4.66	37.29 ± 0.21	125.41 ± 2.13
ICCV9313	33.33 ± 0.72	54.82 ± 0.18	65.38 ± 0.12	65.89 ± 0.15	461.07 ± 12.57	39.24 ± 0.19	71.61 ± 0.53
ICCV9314	29.33 ± 0.47	44.53 ± 0.53	72.43 ± 0.26	70.40 ± 0.30	354.60 ± 10.80	36.45 ± 0.29	183.34 ± 2.41
ICCV10313	28.33 ± 0.85	47.07 ± 0.33	83.64 ± 0.22	71.12 ± 0.21	699.37 ± 4.76	37.55 ± 0.24	365.70 ± 1.40
ICCV10	32.33 ± 0.34	53.60 ± 0.73	82.21 ± 0.10	73.33 ± 0.17	361.40 ± 9.14	19.61 ± 0.15	161.90 ± 1.88
ICCV2	30.0 ± 0.81	56.87 ± 0.21	68.43 ± 0.3	47.39 ± 0.49	703.19 ± 5.42	21.92 ± 0.36	167.59 ± 1.95
ICCV92337	28.33 ± 0.69	47.20 ± 0.18	66.32 ± 0.25	67.65 ± 0.08	422.85 ± 7.66	30.93 ± 0.26	85.28 ± 1.39
ICCV8310	30.0 ± 0.22	49.83 ± 0.56	71.68 ± 0.17	57.20 ± 0.21	356.75 ± 12.55	30.22 ± 0.34	88.54 ± 2.06
ICCV97309	28.0 ± 0.59	52.53 ± 0.46	69.02 ± 0.15	78.33 ± 0.13	687.49 ± 9.85	24.66 ± 0.17	146.44 ± 1.17
ICCV1309	34.33 ± 1.02	54.53 ± 0.32	51.35 ± 0.16	65.47 ± 0.32	854.0 ± 14.61	30.97 ± 0.23	150.36 ± 1.68
ICCV10304	32.33± 0.56	50.07 ± 1.17	63.28 ± 0.16	70.60 ± 0.14	382.70 ± 15.75	22.68 ± 0.32	78.86 ± 0.26
ICCV10307	32.66 ± 0.34	67.10 ± 1.39	65.01 ± 0.25	66.21 ± 0.28	456.63 ± 9.82	35.24 ± 0.25	81.10 ± 1.10
ICCV10306	34.0 ± 0.59	55.07 ± 0.86	69.89 ± 0.05	68.42 ± 0.30	398.42 ± 4.34	35.53 ± 0.33	104.67 ± 2.09
ICCV10316	33.33 ± 0.34	50.87 ± 0.65	65.13 ± 0.24	61.63 ± 0.35	447.18 ± 12.43	41.76 ± 0.33	138.47 ± 1.31
ICCV00109	30.33 ± 0.34	49.93 ± 1.31	64.45 ± 0.29	62.59 ± 0.16	474.56 ± 8.46	20.87 ± 0.41	153.94 ± 1.35
ICCV3103	29.0 ± 0.22	63.20 ± 0.31	70.57 ± 0.13	67.61 ± 0.17	311.67 ± 4.58	25.42 ± 0.40	106.88 ± 1.57
ICCV9307	30.66 ± 0.34	54.4 ± 1.48	69.43 ± 0.18	74.21 ± 0.26	408.30 ± 4.68	38.94 ± 0.29	117.61 ± 2.08
ICCV95423	32.33 ± 0.47	48.17 ± 0.28	64.82 ± 0.21	62.64 ± 0.39	391.70 ± 2.97	27.37 ± 0.35	417.28 ± 2.33
ICCV97404	28.33 ± 0.34	46.20 ± 0.16	56.50 ± 0.13	67.24 ± 0.33	655.73 ± 15.11	25.46 ± 0.39	237.0 ± 3.67
ICCV0301	31.33 ± 0.72	50.55 ± 0.38	60.32 ± 0.15	60.13 ± 0.40	568.0 ± 13.0	17.95 ± 0.25	120.93 ± 2.45
ICCV0302	23.0 ± 0.81	55.43 ± 1.17	63.73 ± 0.09	56.05 ± 0.24	413.11 ± 7.93	31.16 ± 0.48	121.03 ± 3.28
ICCV1301	29.0 ± 0.22	56.13 ± 0.31	56.69 ± 0.1	69.07 ± 0.24	349.85 ± 9.80	26.54 ± 0.29	123.51 ± 2.46
L550	26.66 ± 0.56	47.13 ± 1.45	65.22 ± 0.17	60.65 ± 0.22	695.48 ± 5.28	17.73 ± 0.28	162.17 ± 2.55
ICCV5308	28.33 ± 0.34	53.10 ± 0.42	68.37 ± 0.16	65.01 ± 0.39	135.88 ± 4.44	37.66 ± 0.49	275.79 ± 2.43
ICCV5313	30.33± 0.34	53.30 ± 0.99	71.23 ± 0.21	77.38 ± 0.14	416.07 ± 5.74	33.72 ± 0.29	191.71 ± 2.29
Mean	30.48	53.62	66.10	64.98	483.06	28.74	165.81
Max.	36.0	67.10	83.64	78.70	854.0	41.76	461.43
Min.	22.66	44.53	45.38	45.29	135.88	11.19	50.43
CV	1.94	1.24	0.25	0.37	1.90	1.07	1.02

Table 4. Mean performance of 40 chickpea genotypes evaluated for morphological and physiological traits under drought-stress conditions
Chl index, Chlorophyll index; RWC, relative water content; MSI, membrane stability index; DSI, drought susceptibility index

Genotype	Plant height (cm)	Chl index (SPAD units)	RWC	MSI	Biomass (g)	100-seed wt (g)	Plant yield (g)	DSI
ICC1882	23.66 ± 1.02	45.13 ± 1.06	49.09 ± 0.24	41.70 ± 0.44	612.99 ± 8.75	14.91 ± 0.34	164.73 ± 1.27	0.274
ICC4958	31.33 ± 0.47	53.03 ± 0.24	78.88 ± 0.37	73.75 ± 0.62	672.13 ± 11.74	25.18 ± 0.73	177.40 ± 2.51	0.048
Pusa1103	29.0 ± 0.59	46.20 ± 0.43	68.06 ± 0.24	61.63 ± 0.18	426.82 ± 12.78	17.87 ± 0.38	226.97 ± 4.92	0.098
BGD72	28.66 ± 0.72	42.27 ± 0.59	69.29 ± 0.54	67.37 ± 0.38	591.53 ± 6.62	14.13 ± 0.19	401.43 ± 2.95	0.149
Pusa1003	30.33 ± 0.34	41.33 ± 0.35	60.08 ± 0.19	45.94 ± 0.24	120.83 ± 3.74	13.63 ± 0.37	62.50 ± 0.53	0.649
CSG8962	29.33 ± 0.69	56.13 ± 0.18	71.28 ± 0.16	71.36 ± 0.26	374.27 ± 61.51	10.53 ± 0.23	214.30 ± 1.36	0.128
C235	26.67 ± 1.16	53.07 ± 0.43	60.39 ± 0.13	45.57 ± 0.32	108.67 ± 2.86	10.80 ± 0.28	47.60 ± 0.53	0.764
ICCV3310	31.33 ± 0.34	40.37 ± 1.06	62.10 ± 0.45	65.38 ± 0.18	56.43 ± 1.21	28.0 ± 0.59	45.84 ± 1.15	0.708
ICCV3311	30.66 ± 0.34	41.20 ± 0.57	64.61 ± 0.24	73.37 ± 0.17	155.40 ± 3.49	22.90 ± 0.56	95.87 ± 1.12	0.186
ICCV3403	30.0 ± 0.22	36.83 ± 1.05	65.55 ± 0.48	42.29 ± 0.38	53.67 ± 2.64	25.04 ± 0.44	116.74 ± 2.51	0.228
ICCV3404	21.66 ± 0.47	24.23 ± 1.57	42.64 ± 0.48	62.74 ± 0.36	121.30 ± 2.48	30.46 ± 0.83	120.72 ± 3.74	0.323
ICCV7301	30.0 ± 0.22	56.93 ± 0.34	59.21 ± 0.23	64.84 ± 0.40	121.30 ± 4.32	24.55 ± 1.00	74.65 ± 1.24	0.595
ICCV4303	21.66 ± 0.47	29.47 ± 2.31	66.29 ± 0.22	53.46 ± 0.59	40.53 ± 2.15	30.96 ± 0.61	78.26 ± 2.08	0.455
ICCV4310	30.33 ± 0.34	53.20 ± 0.39	65.40 ± 0.35	55.06 ± 0.28	101.3 ± 2.29	25.85 ± 0.41	58.03 ± 0.64	0.626
ICCV5312	30.66 ± 0.27	45.60 ± 0.80	64.49 ± 0.61	68.36 ± 0.80	73.73 ± 1.58	30.16 ± 0.55	41.73 ± 0.66	0.197
ICCV9312	30.0 ± 0.22	54.97 ± 0.39	66.93 ± 0.41	53.95 ± 0.43	227.37 ± 6.27	30.66 ± 0.91	63.81 ± 0.61	0.561
ICCV9313	32.33 ± 0.34	44.20 ± 0.84	58.77 ± 0.39	59.65 ± 0.17	304.43 ± 4.35	31.91 ± 0.77	61.90 ± 0.94	0.155
ICCV9314	27.0 ± 1.17	27.70 ± 2.03	71.47 ± 0.19	53.21 ± 0.28	122.63 ± 5.41	30.45 ± 0.41	168.85 ± 2.13	0.090
ICCV10313	26.33 ± 0.69	54.47 ± 0.29	74.90 ± 0.37	63.86 ± 0.21	368.67 ± 10.03	31.96 ± 0.77	253.41 ± 3.32	0.351
ICCV10	24.66 ± 1.24	52.13 ± 0.21	75.97 ± 0.38	69.23 ± 0.33	212.07 ± 4.68	16.15 ± 0.22	134.77 ± 2.51	0.191
ICCV2	30.0 ± 0.22	47.62 ± 0.72	56.98 ± 0.40	43.75 ± 0.66	311.50 ± 4.61	17.53 ± 0.18	132.14 ± 2.81	0.242
ICCV92337	27.66 ± 0.56	44.27 ± 0.78	60.98 ± 0.62	63.01 ± 0.59	164.93 ± 1.41	25.87 ± 0.34	75.99 ± 1.19	0.125
ICCV8310	32.33 ± 0.56	46.40 ± 0.59	64.61 ± 0.89	50.89 ± 0.24	268.10 ± 13.34	22.99 ± 0.68	51.87 ± 2.68	0.473
ICCV97309	22.33 ± 0.56	41.63 ± 1.08	62.69 ± 0.69	74.11 ± 0.60	310.37 ± 8.20	20.25 ± 0.36	136.99 ± 2.48	0.074
ICCV1309	29.0 ± 0.22	55.20 ± 0.90	49.0 ± 0.27	62.32 ± 0.60	79.37 ± 2.03	28.17 ± 0.54	73.76 ± 1.79	0.582
ICCV10304	24.33 ± 0.69	31.87 ± 1.84	61.34 ± 0.26	65.89 ± 0.62	173.07 ± 3.09	17.51 ± 0.32	66.93 ± 1.02	0.173
ICCV10307	25.33 ± 0.56	40.50 ± 1.10	59.32 ± 0.30	60.41 ± 0.20	304.83 ± 4.16	30.43 ± 0.41	62.54 ± 0.94	0.261
ICCV10306	23.0 ± 0.81	56.47 ± 1.38	63.41 ± 0.93	59.21 ± 0.28	153.27 ± 2.17	28.87 ± 0.61	75.00 ± 1.79	0.324
ICCV10316	25.0 ± 0.67	56.47 ± 0.30	61.12 ± 0.46	54.88 ± 0.21	410.40 ± 8.45	34.46 ± 0.89	74.83 ± 2.21	0.525
ICCV00109	22.66 ± 0.47	61.37 ± 1.25	59.22 ± 0.26	54.69 ± 0.56	282.10 ± 6.13	17.55 ± 0.45	54.35 ± 0.56	0.739
ICCV3103	23.33 ± 0.34	56.80 ± 0.46	41.86 ± 0.26	61.44 ± 0.31	198.43 ± 2.50	19.16 ± 0.35	59.96 ± 0.63	0.502
ICCV9307	28.0 ± 0.45	53.80 ± 0.45	61.65 ± 0.32	70.31 ± 0.28	165.93 ± 2.84	33.84 ± 0.93	54.51 ± 2.00	0.613
ICCV95423	28.0 ± 0.39	53.07 ± 0.67	61.21 ± 0.23	59.24 ± 0.27	212.27 ± 4.51	23.14 ± 0.60	156.54 ± 2.02	0.714
ICCV97404	15.66 ± 1.16	46.13 ± 0.50	52.74 ± 0.53	61.91 ± 0.44	320.30 ± 7.79	18.73 ± 0.76	159.55 ± 2.26	0.373
ICCV0301	26.66 ± 0.34	49.67 ± 0.28	54.25 ± 0.87	52.06 ± 0.49	130.57 ± 6.48	13.22 ± 0.27	49.01 ± 0.79	0.680
ICCV0302	25.33 ± 0.56	54.60 ± 0.18	58.03 ± 0.45	51.45 ± 0.21	141.63 ± 7.71	24.67 ± 1.12	58.29 ± 1.91	0.592
ICCV1301	25.0 ± 0.59	50.0 ± 0.05	53.33 ± 0.61	60.59 ± 0.21	205.27 ± 4.06	21.13 ± 0.64	119.07 ± 3.98	0.041
L550	18.66 ± 0.52	46.70 ± 0.52	61.50 ± 0.31	53.56 ± 0.45	619.32 ± 13.02	15.91 ± 0.40	73.99 ± 1.39	0.621
ICCV5308	17.0 ± 0.81	29.40 ± 2.36	62.97 ± 0.42	52.12 ± 0.48	73.60 ± 2.94	29.98 ± 0.72	246.78 ± 2.45	0.120
ICCV5313	26.66 ± 0.47	50.75 ± 0.19	63.09 ± 0.48	71.94 ± 0.67	90.66 ± 0.92	29.76 ± 0.42	115.41 ± 2.63	0.455
Mean	26.79	46.78	61.62	59.42	237.05	23.48	112.68	
Max.	32.33	61.37	78.88	74.11	672.13	34.46	401.43	
Min.	15.67	24.23	41.86	41.70	40.53	10.53	41.73	
CV	2.09	1.64	0.66	0.65	2.80	2.30	1.67	

for plant yield, ranging from 50.43 g (ICCV5312) to 461.43 g (BGD72) with an average value of 165.81 under normal conditions, whereas under drought stress, plant yield varied from 41.72 g (ICCV5312) to 401.43 g (BGD72) with an average of 112.67 g. The 100-seed weight ranged from 11.19 g (CSG8962) to 41.76 g (ICCV10316) with a mean value of 28.74 g under normal condition, and from 10.53 g (CSG8962) to 34.46 g (ICCV10316) with a mean value of 23.48 g under drought-stress conditions. Therefore, drought stress considerably affected yield parameters. DSI under drought stress ranged from 0.048 (ICC4958) to 0.764 (C235) differentiating the tolerant and sensitive genotypes. Genotypes with lower DSI namely P1103 (0.098), CSG8962 (0.128) and BGD72 (0.149) may be used in future for developing drought resilient genotypes.

Under drought stress, there was significant decrease in the mean of most of the characters under study. Maximum reduction was seen in biomass (50.92%), followed by plant yield (32.04%) and 100-seed weight, which showed a reduction of 18.30% under drought-stress conditions (Table 5).

The SAHN grouping based on the quantitative traits under drought-stress environment grouped the 40 genotypes into three major clusters (Fig. 1). Cluster II was the largest, comprising 33 genotypes, and cluster III was smallest with two genotypes (ICCV3404 and ICCV3103). Cluster I contained five genotypes. Cluster II could be divided into two subclusters (IIa, IIb).

In subcluster IIb, two intra-subclusters IIb(i) and IIb(ii) could be identified (Table 6). Cluster IIa had the most tolerant genotypes, i.e. ICC4958, ICCV10, ICCV10313 and ICCV97309, grouping together, whereas the most susceptible genotypes were grouped in clusters I and III. Cluster II, in general, comprised the tolerant and moderately tolerant genotypes. These genotypes had a stable MSI and relative decrease of MSI under stress was very low.

SSR data analyses

In total, 125 SSR markers were used to describe and evaluate the level of genetic diversity among the 40 chickpea genotypes. Among these 125 primer pairs, 25 located all over the genome were identified as polymorphic. In total, 90 alleles were detected, with an average number of 3.6 alleles per locus (Table 7).

Table 5. Per cent reduction of different traits under stress

Trait	Overall mean under normal conditions	Overall mean under stress conditions	%Decrease
Plant height (cm)	64.98	59.37	8.64
Chlorophyll index (SPAD units)	53.62	46.78	12.76
Relative water content	67.0	61.62	8.03
Membrane stability index	64.98	59.42	8.57
Biomass (g)	483.06	237.05	50.93
100-seed weight (g)	28.74	23.48	18.30
Plant yield (g)	165.81	112.68	32.05

The analysis of SSR profiles in Table 7 also includes the major allele frequency, gene diversity, availability, heterozygosity and PIC values. The highest number of alleles (8.0) was detected for the marker TA136, followed by H3A10 (seven alleles) and TR58 (six alleles). Amplified fragments produced by markers TA136 and NC147 (four alleles) are depicted in Fig. 2. A high level of gene diversity was shown among the 25 loci across the 40 chickpea genotypes, with H_E values ranging from 0.1628 to 0.8075 with an average of 0.4891 (Table 7). Sixteen of the markers studied produced three alleles and they were robust enough to distinguish precisely the chickpea genotypes, meaning they have potential to be used for molecular characterisation.

PIC value

The PIC values provide a measure of allelic diversity and frequency among genotypes. The level of polymorphism among the 40 chickpea genotypes was estimated by calculating PIC values for each of the SSR markers. The PIC value of each marker evaluated on the basis of its alleles varied widely among the primers, ranging from 0.1553 to 0.7825 with an average value of 0.4374 (Table 7), and the size of amplicons ranged from 160 bp to 390 bp. Primer TA136 showed the highest PIC (0.7825) followed by TR58 (0.7232), NC74 (0.6829) and NC147 (0.6170). PIC values of an SSR marker thus give an estimate of discriminatory power of that marker

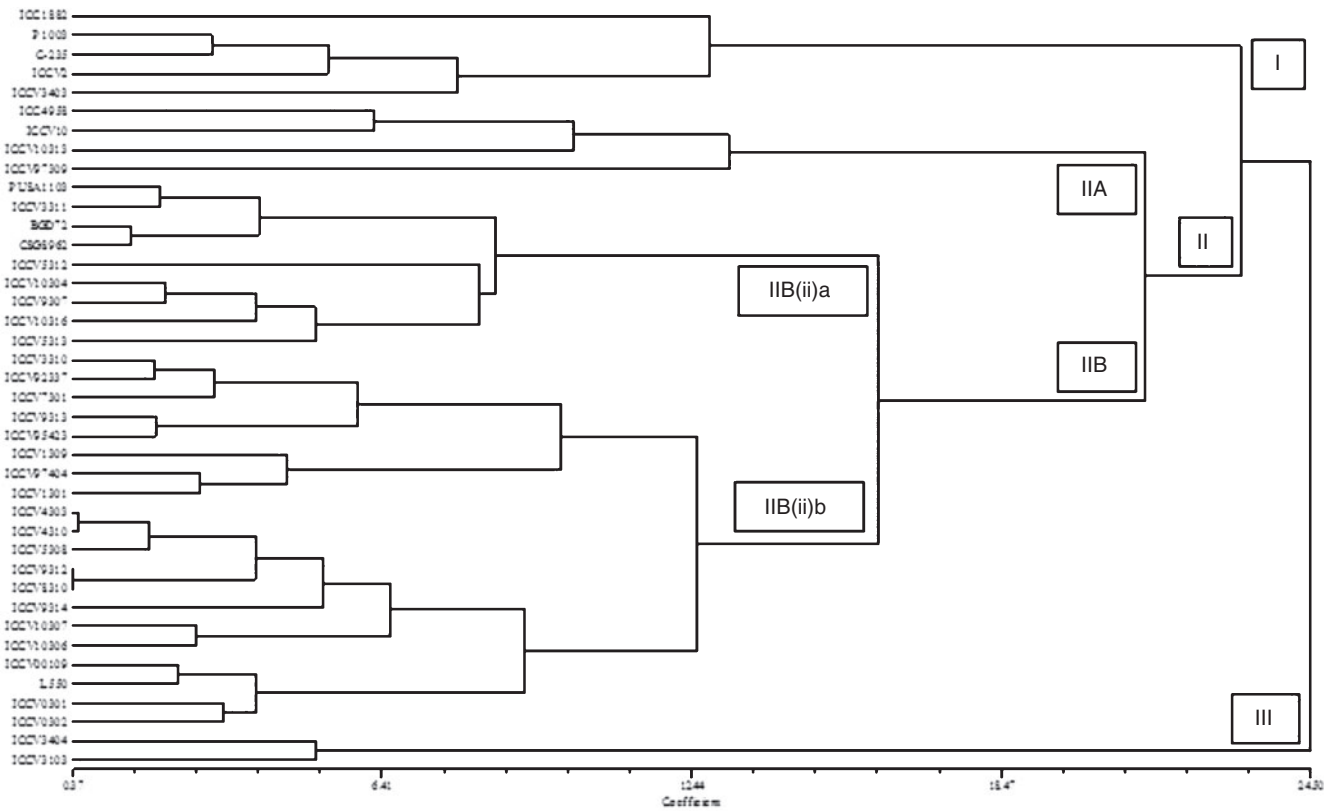

Fig. 1. SAHN grouping based on morphological and physiological parameters showing genetic relatedness among the 40 chickpea genotypes under a drought-stress environment.

Table 6. Clustering based on morphological data of the 40 chickpea genotypes under drought stress environment
Number of genotypes in each cluster is in parentheses. RWC, Relative water content; MSI, membrane stability index

Major cluster	Subcluster	Minor cluster	Genotypes	Average values	
				RWC	MSI
I (5)			ICC1882, Pusa1003, C235, ICCV2, ICCV3403	58.52	43.78
II (33)	IIA (4)		ICC4958, ICCV10, ICCV10313, ICCV97309	75.14	71.86
	IIB (29)	IIB(i) (9)	Pusa1103, ICCV3311,BGD72,CSG8962, ICCV5312, ICCV10304, ICCV9307, ICCV10316, ICCV5313	66.21	68.45
		IIB(ii)a (8)	ICCV3310, ICCV92337, ICCV7301, ICCV9313, ICCV95423, ICCV1309, ICCV97404, ICCV1301	58.40	63.49
		IIB(ii)b (12)	ICCV4303, ICCV4310, ICCV5308, ICCV9312,ICCV8310, ICCV9314, ICCV10307, ICCV10306, ICCV00109, L550, ICCV0301, ICCV0302	65.58	55.29
III (2)			ICCV3404, ICCV3103	42.84	62.96

Table 7. Summary of genetic variation statistics for 25 SSR markers among 40 chickpea genotypes
PIC, Polymorphism information content

Marker name	Major allele frequency	Allele number	Availability	Gene diversity (H_E)	Heterozygosity (H_O)	PIC
TR43	0.70	3.0	1.0	0.4650	0.050	0.4199
TA25	0.7692	3.0	0.975	0.3787	0.1538	0.3434
NC81	0.8875	3.0	1.0	0.2059	0.0250	0.1958
GAA47	0.5897	2.0	0.975	0.4839	0.0	0.3668
NC69	0.9125	3.0	1.0	0.1628	0.0250	0.1553
NC91	0.7125	2.0	1.0	0.4097	0.0250	0.3258
GA6	0.6282	3.0	0.975	0.5322	0.0256	0.4724
NC147	0.450	4.0	1.0	0.6747	0.150	0.6170
TS29	0.6375	4.0	1.0	0.5122	0.0250	0.4451
TR31	0.6081	3.0	0.925	0.4869	0.0270	0.3808
CaM1903	0.4865	4.0	0.925	0.6015	0.2162	0.5224
CaM1502	0.60	3.0	1.0	0.5150	0.0	0.4244
TA130	0.7436	3.0	0.975	0.3932	0.0	0.3335
NC74	0.4459	5.0	0.925	0.7199	0.1892	0.6829
NC103	0.6579	3.0	0.950	0.5083	0.0	0.4557
NC77	0.7222	3.0	0.90	0.4398	0.0	0.3988
NC107	0.7250	3.0	1.0	0.4363	0.0	0.3955
NC130	0.8125	3.0	1.0	0.3184	0.2750	0.2901
NC138	0.7500	3.0	1.0	0.4013	0.0500	0.3601
TA8	0.70	3.0	1.0	0.4638	0.0	0.4175
TR58	0.3250	6.0	1.0	0.7616	1.0	0.7232
TA136	0.3125	8.0	1.0	0.8075	0.9750	0.7825
H3A10	0.5541	7.0	0.925	0.6377	0.2162	0.6021
GAA50	0.7821	3.0	0.975	0.3646	0.2051	0.3351
NC99	0.6154	3.0	0.975	0.5470	0.0	0.4880
Mean	0.6451	3.60	0.976	0.4891	0.1453	0.4374

and their utility in genetic studies (Bharadwaj *et al*. 2011). The genetic diversity values (He) also highlight the effectiveness of SSR loci information.

Cluster analysis based on DARwin grouping

A tree was constructed by using the unrooted NJ tree analysis, and DARwin analysis was used to construct the similarity matrix. The radial branching grouped the 40 genotypes into three major clusters (Fig. 3 and Table 8). Of the three clusters, the largest was cluster III, which comprised 23 genotypes, whereas cluster I emerged as smallest with five genotypes (ICCV2, Pusa1003, C235, L550 and ICC1882). Cluster II with 12 genotypes contained the most tolerant genotypes, i.e. ICC4958, CSG8962, ICCV7301, ICCV4303, ICCV0301, Pusa1103, BGD72, ICCV10304, ICCV0302, ICCV10313, ICCV10 and ICCV3311, grouping together. The most susceptible genotypes were grouped in cluster I. Cluster III, in general, comprised the moderately tolerant genotypes. In addition, these genotypes had very low deviation in their growth parameters under drought-stress conditions relative to normal conditions.

Genetic relationship and distance

Genetic relationships between the genotypes were explained by the Jaccard's coefficient calculated using the molecular data.

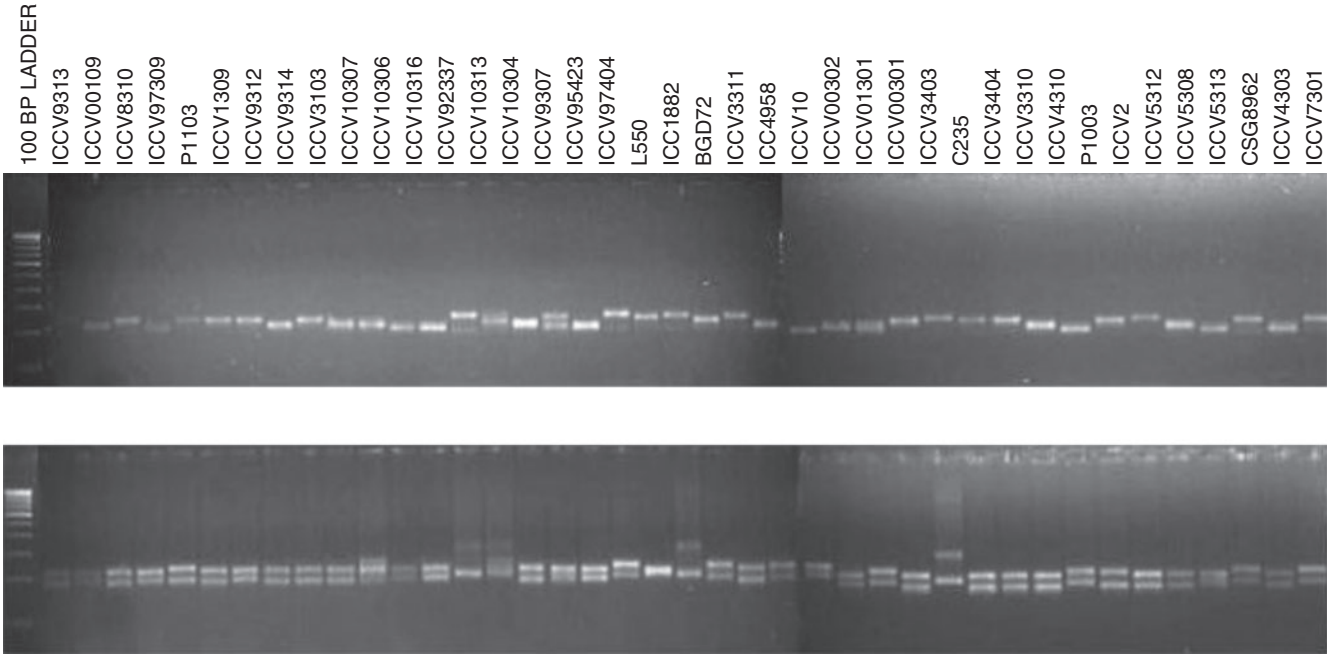

Fig. 2. Upper gel depicts the amplified fragments produced by marker TA136 and lower gel depicts the amplified fragments produced by marker NC147.

The SSR markers showed varying degrees of genetic relatedness among the chickpea genotypes. Similarity coefficients ranging from 0.115 to 0.828 were obtained, with an average value of 0.385. ICCV10316 and ICCV92337 had the maximum similarity coefficient of 0.828, followed by ICCV3103 and ICCV1309 (0.793), and ICCV3103 and ICCV9312 (0.793). All of these genotypes were grouped together in same cluster. The largest distance was observed between ICCV5308 and BGD72, which showed the least genetic similarity of 0.115, followed by ICCV3311 and ICC4958 (0.128), and then 0.136 between L550 and ICCV8310 and 0.137 between ICCV10313 and ICCV9314. The lowest genetic distance values between genotypes indicated that these genotypes were less distantly related to each other.

Cluster analysis using principal coordinates analysis

The PCoA scatter plot, which provides the spatial representation of genetic distances among genotypes, grouped the 40 genotypes into five clusters (Fig. 4 and Table 9). The first two coordinates explained 17.56% of the total variance. The first, second and third clusters based on PCoA comprised all of the genotypes of clusters I and II that were generated by using SAHN grouping. However, the fourth and fifth PCoA clusters comprised all of the genotypes of cluster III of the SAHN grouping. PCoA done on the basis of the Eigen vectors clearly delineated the grouping of tolerant genotypes and susceptible genotypes in different clusters, i.e. clusters I–III and clusters IV and V, respectively (Fig. 4 and Table 9). The two-dimensional plot obtained from PCoA using SSR data largely supported the morphological-based dendrogram, with a few exceptions, and the clustering pattern clearly suggested that there was considerable similarity in the grouping and that material used was diverse.

Discussion

Formulation of a successful breeding program requires knowledge of the nature and magnitude of genotypic and phenotypic diversity present in the test material. Diverse lines may provide greater genetic gains in crossing programs (Bharadwaj *et al*. 2001). Chickpea is the third-most important pulse crop in Asia is a rich and economical source of proteins, vitamin and minerals (Varshney *et al*. 2013). Low genetic diversity in chickpea has constantly slowed chickpea-enhancement programs (Bharadwaj *et al*. 2011); however, the economic importance of chickpea necessitates the need to study the genetic diversity among cultivated chickpea lines. This diversity can be assessed from morphological and physiological traits and through use of molecular markers (Da Silva *et al*. 2015).

All of the characters studied showed highly significant variation in their mean sum of squares, demonstrating the presence of sufficient diversity as indicated by *F*-test. Such a base population will help in identification of genotypes based on their performance that can be used for recombination and advancement of generations (Bharadwaj *et al*. 2001). Kumar *et al*. (2015) also observed remarkable variation in morphological, physiological and phenological characters and yield and its components in chickpea. Genetic study of drought-tolerance parameters is prerequisite for breeders in selection of desired genotypes. Variation in drought tolerance is dependent on genotype and its ability to withstand stress through various mechanisms such as higher RWC and MSI (Bharadwaj *et al*. 2001).

In the present study, apart from yield parameters, data on plant height, chlorophyll index, RWC, MSI, biomass, 100-seed weight were recorded to ascertain the drought-tolerance ability of a genotype (Kumar *et al*. 2015; Kumar *et al*. 2016). Genotypic

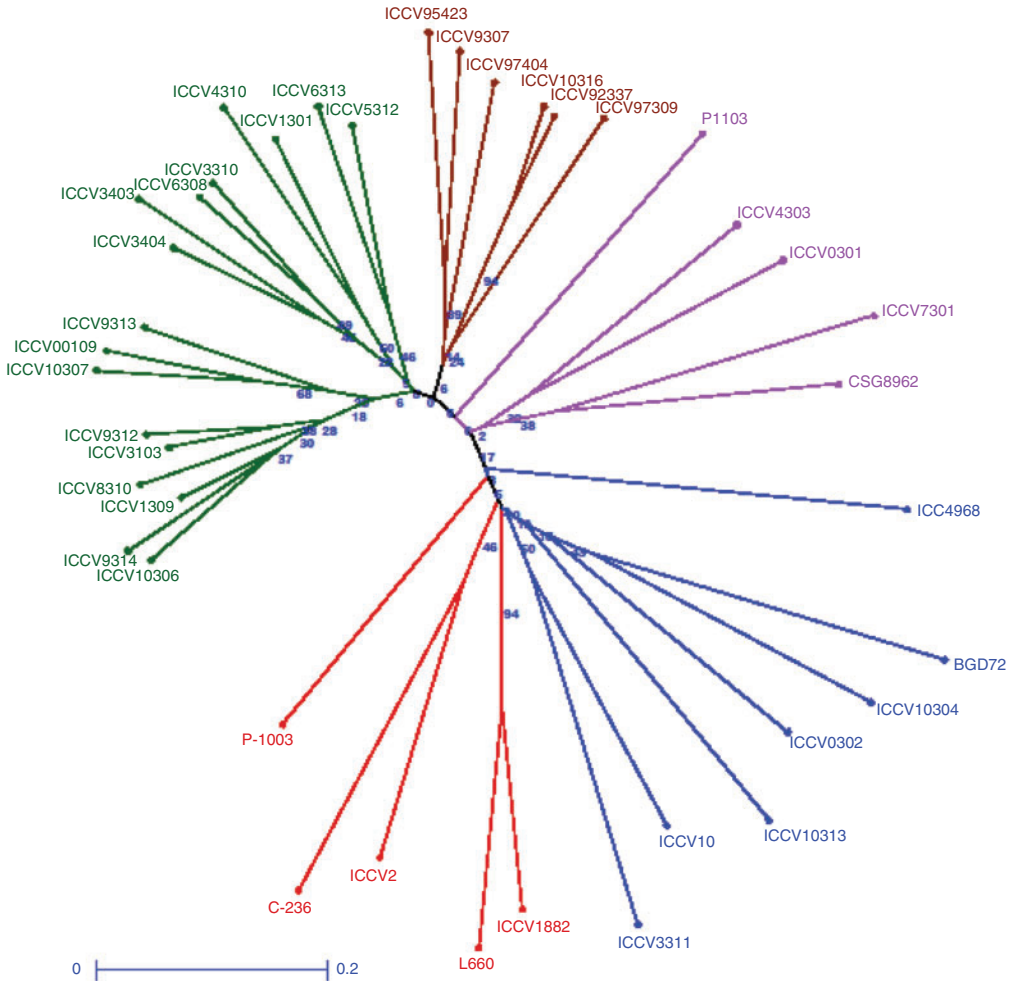

Fig. 3. Clustering of the 40 chickpea genotypes based on DARwin grouping.

Table 8. Clustering analysis based on DARwin grouping of the 40 chickpea genotypes under drought stress environment
RWC, Relative water content; MSI, membrane stability index

Major cluster	No. of genotypes	Genotypes	RWC	MSI
I	5	ICC1882, Pusa1003, C235, ICCV2, L550	57.73	46.14
II	12	ICC4958, ICCV10, ICCV10313, Pusa1103, ICCV3311, BGD72, CSG8962, ICCV10304, ICCV4303, ICCV7301, ICCV0301, ICCV0302	68.21	65.19
III	23	ICCV3404, ICCV3103, ICCV97309, ICCV5312, ICCV9307, ICCV10316, ICCV5313, ICCV3310, ICCV92337, ICCV9313, ICCV95423, ICCV1309, ICCV97404, ICCV1301, ICCV4310, ICCV5308, ICCV9312, ICCV8310, ICCV9314, ICCV10307, ICCV10306, ICCV00109, ICCV3403	61.82	61.15

performance varied substantially between drought-stress and non-stress conditions, with a decrease in most parameters under stress conditions. ICC4958, CSG8962, Pusa1103 and BGD72 had better RWC and MSI under stress and their yield reductions were lowest in drought-stress conditions, making them the most tolerant genotypes. Genotypes showing minimum reduction in yield under stress conditions may be used as ideal donors for drought tolerance. Different physiological mechanisms help different genotypes to withstand drought-stress conditions, providing enormous opportunities to breeders by combining traits when developing drought-tolerant genotypes.

In the 1980s, RWC was widely used as a criterion to measure the water status of plants and assess their drought-tolerance. Wheat genotypes with high RWC can better withstand drought stress (Schonfeld *et al*. 1988). In most plant species, osmoregulation is a key mechanism for conserving turgor pressure against water loss, which helps plant to continue water absorption and retain the metabolic activities

(Gunasekera and Berkowitz 1992). Zlatko Stoyanov (2005) found that the osmotic potential and turgor pressure in first leaf of bean decreased significantly when drought stress was applied for 14 days with soil potentials reaching up to –0.9 MPa. Ramos *et al*. (2003) also indicated that RWC of bean leaves under drought stress was much lower than in control plants. The RWC of the stem of bean plants exposed to drought stress and at 10, 14 and 18 days after withholding irrigation was found to be significantly lower than in control plants (Lazcano-Ferrat and Lovat 1999). Gaballah *et al*. (2007) observed that antitranspirants when applied to two sesame genotypes prevented water transpiration from leaves, leading to an increase in RWC.

Researchers also reported characters such as MSI, biomass and yield to be highly affected by drought conditions (Leport *et al*. 1999). Decreases in biomass and seed yield are the major concerns in terms of compromised biological processes under drought-stressed conditions at different levels (organ, cellular and molecular level). Drought tolerance is a complex trait influenced by several factors including days to flowering and maturity, yield, shoot biomass production, early shoot growth vigor, root length density, total transpiration, root : shoot ratio and transpiration efficiency (Varshney *et al*. 2011). It involves interactions between various stress factors and molecular, biochemical and physiological phenomena (Razmjoo *et al*. 2008). Several biotic and abiotic stresses limit chickpea growth and development; although chickpea grows well under receding soil-moisture conditions, drought and salinity are the most important stresses leading to yield losses.

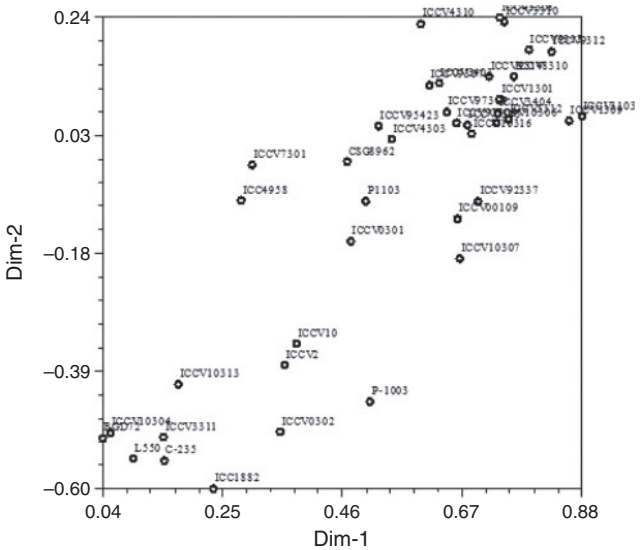

Fig. 4. Principal coordinates analysis 2D plot of the 40 chickpea genotypes based on 25 SSR markers data.

With the anticipated scarcity of water in the future, terminal drought will continue to limit the chickpea production, with an estimated ~40–60% decrease in average productivity (Kumar *et al*. 2017). Under such a scenario, it is pertinent to identify genotypes that have a lower DSI but at the same time a higher yield and high biomass. Such lines can directly be used for yield improvement in niche areas. ICC4958 and ICCV97309 have very high drought tolerance owing to their MSI values and have average or above-yields, and could be used as ideal donors (Tables 3 and 4). Root-trait quantitative trait loci have already been recognised in ICC4958 (Varshney *et al*. 2014*b*) and it has been used in marker-assisted backcrossing in chickpea for improving drought tolerance. In addition, BGD72, Pusa1103 and CSG8962 not only have high MSI values but also higher yield under stress, low DSI and high biomass, indicating their flexibility to produce higher yield even under vegetative and terminal drought-stress conditions (Table 4).

Low genetic diversity among genotypes is restraining genetic improvement of chickpea, and an insight into the genetic base of genotypes would help breeders to plan future crossing programs aimed toward broadening the genetic base of chickpea varieties. Understanding the degree of genetic variation among the chickpea lines at the molecular level may give an idea of genetic relatedness and identify the germplasm sources that have valuable genes for agronomically desirable traits (Choudhary *et al*. 2012). Such studies are crucial for developing various breeding strategies for chickpeas. Very low polymorphism has been detected by DNA-based markers, which is a serious constraint in the development of genetic maps or tagging of important traits in chickpea. In the present evaluation of 125 SSR markers in 40 chickpea varieties and selected lines from International Training population, ICRISAT, 90 alleles were obtained in total with an average of 3.6 alleles per locus. SSR data can therefore provide distinctive allelic profiles for establishing genotype identity (Bharadwaj *et al*. 2010). Allelic frequency, number of alleles, availability,

Table 9. Clustering based on principal coordinates analysis of 40 chickpea genotypes
RWC, Relative water content; MSI, membrane stability index

Major cluster	No. of genotypes	Genotypes	Average values	
			RWC	MSI
I	24	ICCV97309, ICCV4303 ICCV5312, ICCV9307, ICCV10316, ICCV5313, ICCV10306, ICCV00109, ICCV3310, ICCV92337, ICCV9313, ICCV95423, ICCV1309, ICCV10307, ICCV97404, ICCV1301, ICCV4310, ICCV5308, ICCV9312, ICCV9314, ICCV8310, ICCV3103, ICCV3403, ICCV3404	62.02	60.96
II	6	ICC4958, ICCV7301, CSG8962, ICCV10, ICCV0301, Pusa1103	69.30	66.52
III	4	BGD72, ICCV10304, ICCV3311, ICCV10313	69.01	68.69
IV	3	Pusa1003, ICCV2, ICCV302	58.86	47.67
V	3	ICC1882, C235, L550	57.35	46.55

gene diversity (H_E), heterozygosity and PIC values were estimated for different SSR markers. The H_E values ranged from 0.1628 to 0.8075, with TA136 showing maximum H_E and PIC values. High H_E values indicate the amount of genetic variation in the chickpea lines, and high PIC values show the suitability of microsatellite markers for diversity studies.

The genetic relationships among the genotypes, as obtained by the SSR data, were evaluated by means of dissimilarity matrix. On the basis of dissimilarity estimates, genotypes were grouped by SAHN clustering method, DARwin grouping and NJ tree analysis and were presented in a 2D PCoA scatter plot. The matrices of genetic dissimilarity estimated between the chickpea genotypes provided similarity coefficients ranging from 0.115 to 0.828 with an average of 0.385, indicating considerable molecular genetic diversity among the genotypes. The highest genetic similarity was observed between ICCV103016 and ICCV92337, with a similarity coefficient of 0.828. The most diverse accessions, on the two extremes of the SAHN dendrogram and NJ tree analysis, were ICCV5308 and BGD72, with a similarity coefficient of 0.115. DARwin tree constructed on the basis of morphological parameters (RWC and MSI) was in congruence with the PCoA 2D plot with minor deviations. The fact that the genotypes do not form a group based on SAHN clustering, NJ tree analysis and PCoA plot demonstrates that there is significant diversity among the genotypes and can assist in increasing the narrow base of chickpea lines.

The diverse lines identified in the present study can perhaps appeal to breeders when designing breeding programs for yield resilience under drought-stress conditions and for obtaining greater genetic gains. Thus, both phenotypic and molecular data proved to be efficient tools for discerning diversity among the genotypes and identifying high-yielding drought-tolerant chickpea lines.

Conflicts of interest

The authors declare no conflicts of interest.

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