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Population structure and association analysis of heat stress relevant traits in chickpea (*Cicer arietinum* L.)

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Abstract

Understanding genetic diversity and population structure is prerequisite to broaden the cultivated base of any crop. In the current investigation, we report discovery of a total of 319 alleles by assaying 81 SSRs on 71 chickpea genotypes. The cluster analysis based on Jaccard coefficient and unweighted neighbor joining algorithm categorized all genotypes into two major clusters. Cultivars grown within the same agro-climatic zones were clustered together, whereas the remaining genotypes particularly advanced breeding lines and accessions assigned to another cluster. Population structure analysis separated the entire collection into two subpopulations ($K = 2$) and the clustering pattern remained in close agreement with those of distance-based methods. Importantly, we also discovered marker trait association for membrane stability index (MSI) and leaf chlorophyll content measured as SPAD chlorophyll meter reading (SCMR), the two important physiological parameters indicative of heat stress (HS) tolerance in chickpea. Association analysis using both general linear and mixed linear models of the mean phenotypic data of traits recorded in 2016 and 2017 uncovered significant association of NCPGR206 and H2L102 with the MSI trait. Likewise, SSR markers GA9, TR31 and TA113 exhibited significant association with SCMR trait. The genomic regions putatively linked with two traits may be investigated in greater detail to further improve knowledge about the genetic architecture of HS tolerance in chickpea.

Introduction

Globally, chickpea (*Cicer arietinum* L.) is the third most important grain legume crop after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.). A total of

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14.2 Mt chickpea is harvested annually from 14.8 m ha area worldwide with an average productivity of 0.96 t ha^{-1} (FAO 2014). With nearly 80% contribution to global chickpea production, India together with Southern and South-Eastern Asia remains the largest producer (Gaur et al. 2012). Like other grain legumes, chickpea plays a key role in addressing malnutrition related issues by offering plant-based dietary protein coupled with essential micronutrients and vitamins (Graham and Vance 2003). Also, chickpea helps enriching soil fertility through symbiotically fixing the atmospheric nitrogen to soil (Graham and Vance 2003).

Rigorous breeding efforts have led to the development and release of several high-yielding chickpea cultivars adapted to various agro-climatic zones in India (AICRP on Chickpea 2014). Potential yield of chickpea, however, remains unrealized and this may be due to several reasons including the vulnerability of this crop to a range of biotic and abiotic stresses (Jha et al. 2014a). Genetic bottleneck associated with the domestication process followed by human-led selection has caused significant loss in genetic

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diversity of chickpea (Abbo et al. 2003). At the same time, intensive breeding efforts focusing mainly on the development of high-yielding genotypes have resulted in narrow genetic base of cultivated gene pool, thus rendering these cultivars prone to a variety of biotic and abiotic stresses (Upadhyaya et al. 2008). In this respect, information about the genetic diversity available in crop's gene pools facilitates broadening the genetic base of chickpea and assists accelerating genetic gains of the crop. To analyze genetic diversity for guiding selection of diverse parental combinations for hybridization, the role of molecular marker has been evident across different crops.

Several attempts have been made to investigate the genetic diversity in chickpea using diverse DNA marker systems such as random amplified polymorphic DNA (RAPD) (Ahmad 1999; Iruela et al. 2002), amplified fragment length polymorphism (AFLP) (Nguyen et al. 2004; Shan et al. 2005), inter-simple sequence repeat (ISSR) (Choudhary et al. 2013a; Aggarwal et al. 2015), simple sequence repeat (SSR) (Upadhyaya et al. 2008; Bharadwaj et al. 2010; Sefera et al. 2011; Keneni et al. 2012; Choudhary et al. 2012a; Ghaffari et al. 2014; Saxena et al. 2014; Hajibarat et al. 2014, 2015; De Giovanni et al. 2017) and single nucleotide polymorphism (SNP) (Bajaj et al. 2015a). Among the various DNA marker systems, SSRs are still preferred by the research community due to their co-dominant inheritance, high reproducibility and greater abundance across the genome (Gupta and Varshney 2000). Assessment of genetic diversity in chickpea so far has primarily focused on accessions of primary gene pool including *Cicer reticulatum* and *C. echinospermum* (Upadhyaya et al. 2008; Choudhary et al. 2012a, 2013a). However, studies on population structure and genetic diversity of high-yielding chickpea varieties adapted to different agro-climatic zone of India, along with advanced breeding lines remain limited. In the current study, we here conducted an SSR-based diversity analysis of 44 chickpea varieties cultivated across diverse agro-climatic zones in India along with 27 genotypes that cover germplasm accessions, advanced breeding lines and landraces. Given the fact that up to 53 kg/ha of chickpea yield is lost due to heat stress (HS) (Kalra et al. 2008; Jha et al. 2014a, b), we recorded data on membrane stability index (MSI) and SPAD chlorophyll meter reading (SCMR) in the panel. The MSI and SCMR represent the two key physiological traits related to HS tolerance in chickpea. Finally, we performed association mapping to discover putative MTAs explaining variation for the studied traits.

Materials and methods

A set of 71 *desi* chickpea genotypes comprising 44 varieties and 27 genotypes including accessions from ICRISAT Hyderabad, advanced breeding lines (from IIPR, Kanpur, and JNKVV, Jabalpur, India) and landraces was used in the present study (see Supplementary File Table 1). Importantly, the panel also involved heat tolerant and sensitive genotypes as reported by previous researchers (Krishnamurthy et al. 2011; Jha and Shil 2015; Gaur et al. 2016).

DNA extraction and SSR analysis

Genomic DNA was extracted from plant leaves following the CTAB method. A total of 120 SSR markers were screened in the collection, of which 81 SSRs yielded polymorphic fragments. The SSRs used here are reported previously by different research groups (Winter et al. 1999, 2000; Sethy et al. 2003, 2006; Gaur et al. 2011; Choudhary et al. 2012b) and correspond to all eight linkage groups (LGs) of chickpea (Table 1).

PCR analysis

PCR was carried out in a 10 µl reaction mixture that contained 5.9 µl of sterilized distilled water, 1.00 µl template DNA (25 ng), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 1.00 µl 10 × PCR buffer (10 mM Tris–Hcl, 50 mM KCl, pH 8.3), 1.00 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5 U/µl) (Thermo Fisher Scientific Mumbai, India, Pvt. Ltd.) using G-40402 thermo cycler (G-STORM, Somerset, UK). A touchdown PCR profile was used for amplification with initial denaturation at 94 °C for 5 min followed by 10 cycles of touch down 61–51 °C, 30 s at 94 °C, annealing for 30 s at 61 °C (the annealing temperature for each cycle being reduced by 1 °C per cycle) and extension for 30 s at 72 °C. This was accompanied by 40 cycle of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, elongation at 72 °C for 45 s, and 10 min of final extension at 72 °C. Amplified fragments were resolved in 3% agarose gel using 0.5 × TBE running buffer and images were analyzed with Quantity one software (Bio-Rad, CA 94547, USA).

Genetic diversity and population structure analysis

The genetic diversity parameters viz., number of alleles per locus (Na), gene diversity (He) and polymorphism information content (PIC) were analyzed by Power Marker v. 3.25 (Liu and Muse 2005). With 1000 bootstrap value, neighborhood joining tree analysis was performed with DARwin v. 6.0.13 (Perrier and Jacquemoud-Collet 2006). DARwin v.

Table 1 Allele number (Na), gene diversity (He) and polymorphism information content recorded in 71 genotypes with 81 SSR markers

No.	Marker	PIC	No. of alleles (Na)	Gene diversity (He)	LG group	References
1	CakTpSSR03637	0.526	3	0.59	–	–
2	Cak TpSSR2543	0.508	3	0.57	–	–
3	NCPGR149	0.73	6	0.77	–	Gaur et al. (2011)
4	NCPGR231	0.693	5	0.74	LG4	Gaur et al. (2011)
5	NCPCR234	0.709	5	0.75	–	Gaur et al. (2011)
6	NCPGR136	0.663	5	0.71	LG1	Gaur et al. (2011)
7	CESSR172	0.421	3	0.53	LG2	Choudhary et al. (2012b)
8	STMS10	0.581	3	0.66	LG3	Winter et al. (1999)
9	GA105	0.307	2	0.38	LG3	Winter et al. (2000)
10	ICCeM018	0.415	3	0.53	LG3	Gujaria et al. (2011)
11	TR19	0.711	7	0.75	LG2	Winter et al. (2000)
12	TA2	0.696	5	0.74	LG4	Winter et al. (1999)
13	TS54	0.812	8	0.83	LG4	Winter et al. (2000)
14	NCPGR76	0.371	2	0.49	LG6	Gaur et al. (2011)
15	CESSR164	0.479	4	0.57	LG4	Choudhary et al. (2012b)
16	CESSR114	0.374	2	0.50	LG4	Choudhary et al. (2012b)
17	TS53	0.762	7	0.79	LG5	Winter et al. (2000)
18	NCPGR139	0.633	6	0.68	LG6	Gaur et al. (2011)
19	TA176	0.685	7	0.72	LG6	Winter et al. (1999)
20	H2L102	0.436	3	0.51	LG5	Choudhary et al. (2012b)
21	TAAS	0.7	5	0.74	LG5	Winter et al. (2000)
22	NCPGR199	0.528	4	0.60	LG4	Gaur et al. (2011)
23	NCPGR200	0.802	7	0.83	LG6	Gaur et al. (2011)
24	TA80	0.749	6	0.78	LG 6	Winter et al. (2000)
25	GA102	0.528	3	0.60	LG7	–
26	H4F07	0.493	5	0.53	LG6	Gujaria et al. (2011)
27	CESSR432	0.37	3	0.47	LG5	Choudhary et al. (2012b)
28	NCPGR56	0.411	3	0.46	LG5	Sethy et al. (2006)
29	NCPGR 238	0.352	2	0.46	LG6	Gaur et al. (2011)
30	GA9	0.682	5	0.73	LG6	Winter et al. (2000)
31	H5A04	0.515	4	0.57	LG6	Choudhary et al. (2012b)
32	NCPGR202	0.632	5	0.69	LG6	Gaur et al. (2011)
33	CESSR45	0.413	3	0.49	LG1	Choudhary et al. (2012b)
34	CESSR159	0.352	3	0.42	LG1	Choudhary et al. (2012b)
35	TR31	0.506	3	0.59	LG3	Winter et al. (1999)
36	CakTpSSR02719	0.545	4	0.61	–	–
37	CakTpSSR04076	0.395	3	0.51	–	–
38	CakTpSSR03090	0.609	4	0.67	–	–
39	NCPGR165	0.649	4	0.70	LG1	Gaur et al. (2011)
40	ICCM0297	0.55	3	0.63	LG1	Nayak et al. (2010)
41	TA113	0.59	6	0.64	LG1	Winter et al. (1999)
42	TAA60	0.54	3	0.61	LG2	Winter et al. (2000)
43	GA6	0.785	7	0.81	LG2	–
44	NCPGR40	0.656	5	0.71	LG2	Sethy et al. (2006)
45	TA64	0.801	6	0.83	LG3	Winter et al. (1999)
46	NCPGR12	0.431	3	0.54	LG3	Sethy et al. (2003)
47	NCPGR274	0.534	4	0.61	LG3	Gaur et al. (2011)
48	NCPGR220	0.533	4	0.60	LG3	Gaur et al. (2011)
49	NCPGR232	0.664	5	0.71	LG5	Gaur et al. (2011)
50	NCPGR46	0.58	3	0.65	LG6	Sethy et al. (2006)

Table 1 (continued)

No.	Marker	PIC	No. of alleles (Na)	Gene diversity (He)	LG group	References
51	NCPGR156	0.381	3	0.50	LG6	Gaur et al. (2011)
52	NCPGR155	0.375	2	0.50	LG6	Gaur et al. (2011)
53	STMS25	0.391	3	0.51	LG7	Winter et al. (1999)
54	H5G12	0.375	2	0.50	LG7	Choudhary et al. (2012b)
55	TA140	0.454	3	0.54	LG7	Winter et al. (2000)
56	CESSR43	0.375	2	0.50	LG1	Choudhary et al. (2009)
57	NCPGR33	0.596	4	0.67	LG1	Sethy et al. (2006)
58	NCPGR225	0.362	2	0.47	LG3	Gaur et al. (2011)
59	H1B04	0.547	5	0.61	LG3	Choudhary et al. (2012b)
60	TA106	0.797	8	0.82	LG6	Winter et al. (2000)
61	NCPGR93	0.699	5	0.74	LG6	Sethy et al. (2006)
62	GA26	0.614	4	0.68	LG6	Winter et al. (2000)
63	STMS7	0.385	3	0.49	LG5	Winter et al. (1999)
64	NCPGR6	0.541	4	0.60	LG1	Sethy et al. (2003)
65	TA8	0.636	5	0.69	LG1	Winter et al. (1999)
66	CESSR433	0.369	2	0.49	LG1	Choudhary et al. (2012b)
67	CESSR139	0.364	3	0.46	LG1	Choudhary et al. (2012b)
68	H2B061	0.371	2	0.49	LG2	Gujaria et al. (2011)
69	NCPGR193	0.433	3	0.54	LG2	Gaur et al. (2011)
70	NCPGR13	0.298	2	0.36	LG2	Sethy et al. (2003)
71	NCPGR110	0.491	3	0.56	LG2	Gaur et al. (2011)
72	TR7	0.447	3	0.55	LG6	Winter et al. (2000)
73	CESSR105	0.39	3	0.50	LG3	Choudhary et al. (2012b)
74	NCPGR267	0.347	2	0.45	LG6	Gaur et al. (2011)
75	NCPGR206	0.352	3	0.44	LG6	Gaur et al. (2011)
76	NCPGR255	0.415	3	0.53	LG7	Gaur et al. (2011)
77	NCPGR52	0.701	4	0.75	LG7	Sethy et al. (2006)
78	TA110	0.74	6	0.78	LG2	Winter et al. (2000)
79	NCPGR41	0.556	3	0.63	LG7	Sethy et al. (2006)
80	TA18	0.367	3	0.44	LG7	Winter et al. (2000)
81	TA180	0.69	5	0.74	LG7	Winter et al. (2000)

6.0.13 was also used for factorial analysis, while principal coordinate analysis (PCoA) was performed using GenAlEx v. 6.502 (Peakall and Smouse 2012). To determine population structure (Q) and the subpopulation (K) in the given set, model-based analysis was conducted with STRUCTURE v 2.3.4 (Pritchard et al. 2000). By applying admixture model, five independent runs were conducted with 200000 Markov Chain Monte Carlo (MCMC) iterations for each K value ranging from 1 to 10 with a burn-in length of 200. In parallel, the best K value was obtained according to the ΔK method of Evanno et al. (2005) by processing the STRUCTURE results by STRUCTURE HARVESTER (Earl and von Holdt 2012) (http://taylor0.biology.ucla.edu).
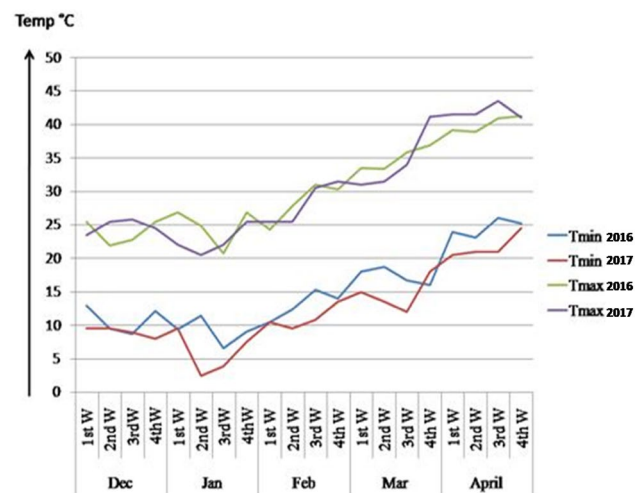

Fig. 1 Mean weekly minimum and maximum day temperature recorded during crop growing period under late sown condition

Analysis of MSI and SCMR in the panel

The data on these two traits were recorded at reproductive stage from 71 genotypes for consecutive 2 years (2016 and 2017) under late sown condition. The mean weekly temperature during crop growth is shown in Fig. 1. The genotypes were grown in augmented design along with three checks in five blocks. The third leaf from the top of each genotype was selected for recording observations on MSI and SCMR. The MSI was estimated based on electrolyte leakage under stress (Sullivan 1972) and was calculated using the following formula given by Blum and Ebercon (1979). MSI = 100 − membrane injury index (MII),where MII is calculated as a ratio of C1 and C2, with C1 and C2 representing the electrolytes measured at 40 and 80 °C, respectively. Similarly, SCMR is used as an indirect measure of leaf chlorophyll content. Three SCMR measurements were recorded in third fully expanded young leaf from the top at reproductive stage using a non-destructive, portable SPAD-502 chlorophyll, meter and averaged subsequently.

Statistical analysis

Analysis of variance (ANOVA) was performed for year-wise data for the two traits using SAS v.9.2. A combined analysis of variation over years was carried out for partitioning the phenotypic variance into year, genotype, and error variances. To study the genotypic variation in the given panel of genotypes, various descriptive statistics including mean, median, standard deviation (SD), standard error, coefficient of variation and broad sense heritability were computed based on the phenotypic data recorded for the two traits in the genotypes.

Association mapping to detect significant MTAs

The phenotypic data on MSI and SCMR traits and the genotypic data were analyzed to discover significant MTAs. Both models viz., general linear (GLM) and mixed linear (MLM) based on Q and Q + K matrix, respectively, were employed. TASSEL v. 3.0 (Bradbury et al. 2007; Zhang et al. 2010) was used to detect MTAs, and $P = 0.05$ was considered as a significance threshold.

Putative candidate gene analysis

To find the possible candidate genes that correspond to the reported MTAs and the putative proteins encoded by these, we performed BLASTn search for the associated SSRs against the reference genome sequence of chickpea (CDC frontier) (Varshney et al. 2013). In parallel, the proteins were predicted for the corresponding sequences using InterPro (IPR).

Results

SSR analysis

In the current study, genotyping of 71 chickpea genotypes with 81 SSRs provided a total of 319 alleles with an average of 3.9 alleles per marker (Table 1). The number of alleles ranged from 1 to 8, and the maximum number of alleles was obtained for the markers TS53, TA106 and TS54 (Table 1). The PIC values ranged from 0.29 to 0.81 with an average of 0.53. Similarly, gene diversity varied between 0.3 and 0.8 with a mean value of 0.6. Interestingly, a comparative (decade wise) analysis of different cultivars released over the last 50 years suggested an overall improvement of genetic diversity based on estimated gene diversity with the values ranging between 0.36 and 0.83 (Table 2).

Genetic diversity analysis and relationships among the chickpea genotypes

The cluster analysis based on unweighted neighbor joining method clearly separated 71 genotypes into two major clusters (Fig. 2). Cluster I contained 41 released cultivars that are widely adapted to various agro-climatic zones in India, along with six improved lines and one landrace (Katila). The second cluster harbored 19 advanced breeding lines, one landrace Barwan and three released cultivars viz., Pusa 512, Pusa 391 and Pusa 408. Further, examination at sub-cluster level suggested that most of the genotypes harbored within the same sub-cluster are recommended for cultivation in the same agro-climatic zone of India. For instance, cultivars Pusa 256 and Pusa 372 belonging to sub-cluster (a) were released for both North West Plain Zone (NWPZ) and Central Zone (CZ). Likewise, RSG 11 and Pusa 261 existing in sub-cluster (d) were released for NWPZ. The majority of varieties belonging to sub-clusters (e)–(i) were released for NWPZ (see Fig. 2). Importantly, KWR 108 and JG 16 from sub-cluster (c) were released for North East Plain Zone (NEPZ) of India. While sub-cluster (b) containing cultivars

Table 2 Comparison of undertaken released chickpea cultivars as per the period of release

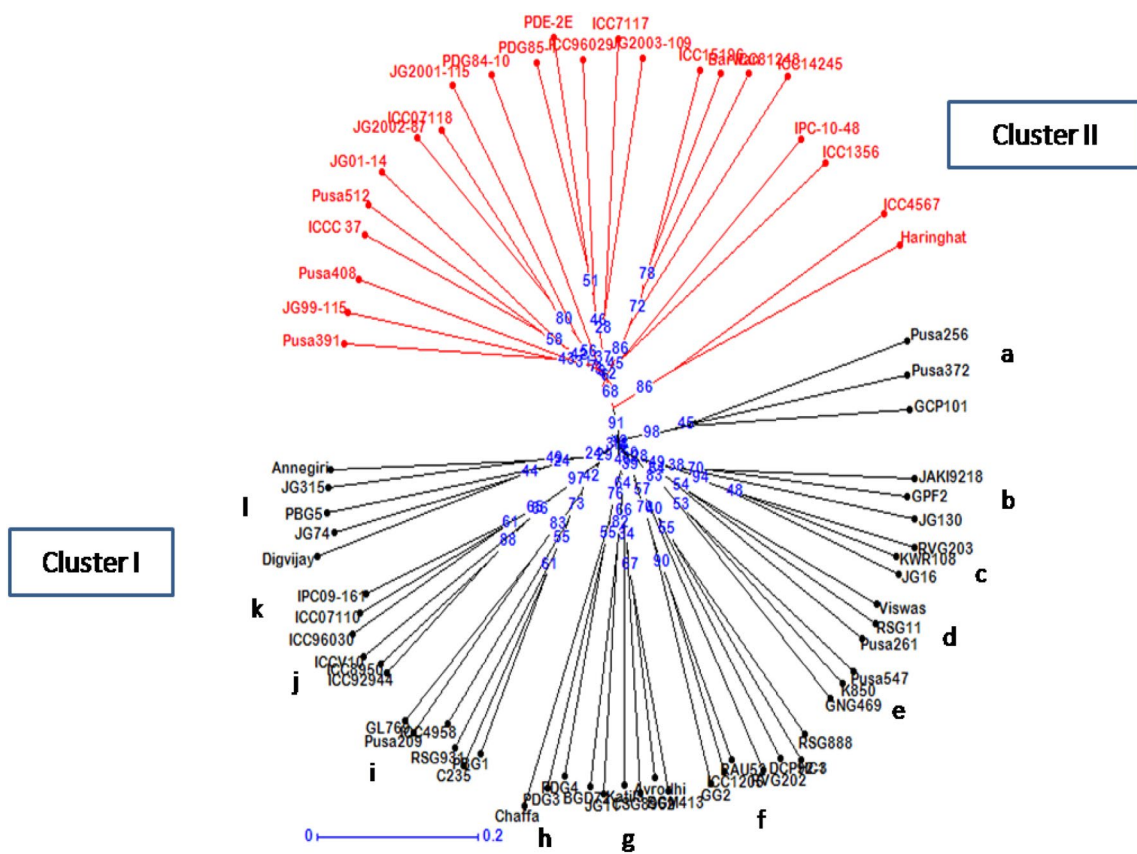| Period of release	No. of genotypes/varieties	Total no. of alleles	Avg. no. of alleles/marker	PIC
All genotypes	71	319	3.9	0.53
All cultivars	44	299	3.7	0.51
2001–2012	9	242	2.9	0.47
1990–2000	15	267	3.3	0.48
1980–1990	15	261	3.2	0.47
Before 1970	5	213	2.6	0.43

Fig. 2 Neighbour joining phylogenetic tree using SSR marker data in 71 chickpea genotypes. Bootstrap values are indicated at the node of each cluster
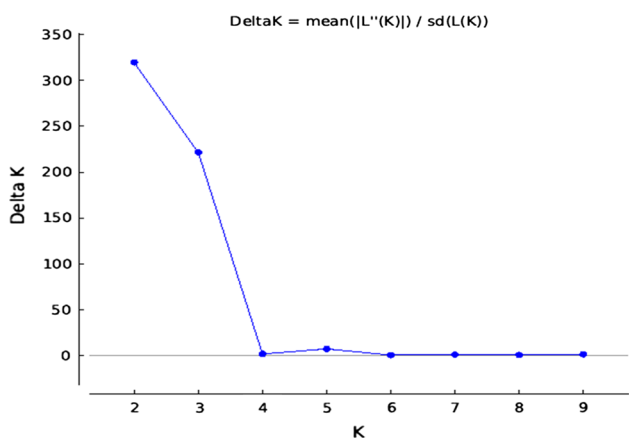

Fig. 3 Relationship between K and ΔK based on STRUCTURE analysis of chickpea genotypes based on SSR marker data

JAKI 9218 and JG130 and sub-cluster (j) containing ICCV 10 and ICCV 92944 were released for CZ and South Zone (SZ). Similarly, most of the cultivars existing in sub-cluster (l) were released for CZ.

Structure analysis

We dissected the population structure of the germplasm panel using Bayesian-based clustering approach with K values ranging from 1 to 10, which led the genotypes clustering into two distinct subpopulations (see Fig. 1 in Supplementary File). This pattern corroborated with the highest peak found at $K = 2$ (Fig. 3). The first subpopulation comprised the released cultivars, whereas the rest of the genotypes remained in the second subpopulation representing predominantly the advanced breeding lines and germplasm accessions. The clustering pattern inferred from factorial analysis agreed with the results obtained from STRUCTURE analysis. The genotypes existing in subpopulation of STRUCTURE are contained in quadrants I and IV of factorial analysis (Fig. 4), whereas the genotypes in subpopulation 2 of STRUCTURE were represented by quadrants II and III. Similarly, as shown in Fig. 5, two clusters were also recorded in PCoA with the PC1 and PC2 explaining 9.45 and 6.42%, respectively of the total variance.

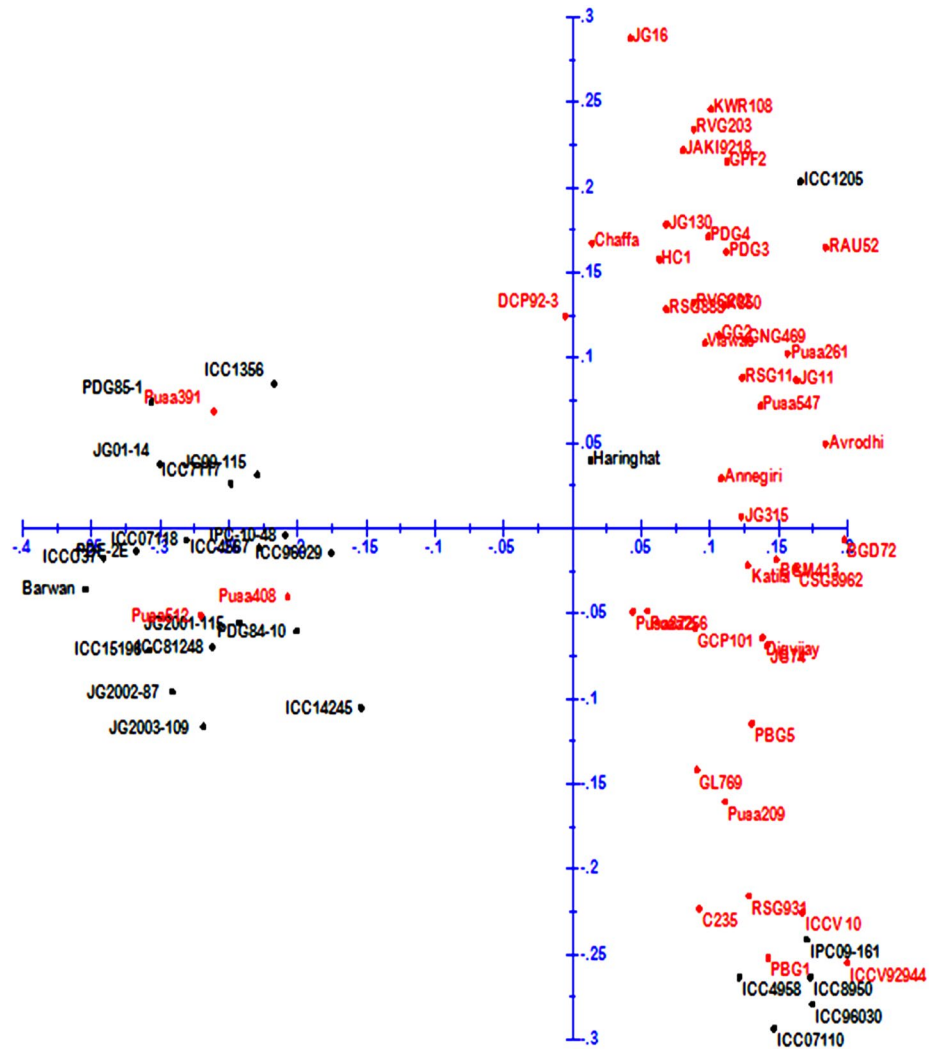**Fig. 4** Factorial analysis of 71 genotypes based on 81 SSR markers

Fig. 5 PCoA analysis of 71 genotypes, PC1 explaining 9.45% and PC2 explaining 15.87% of variance, respectively
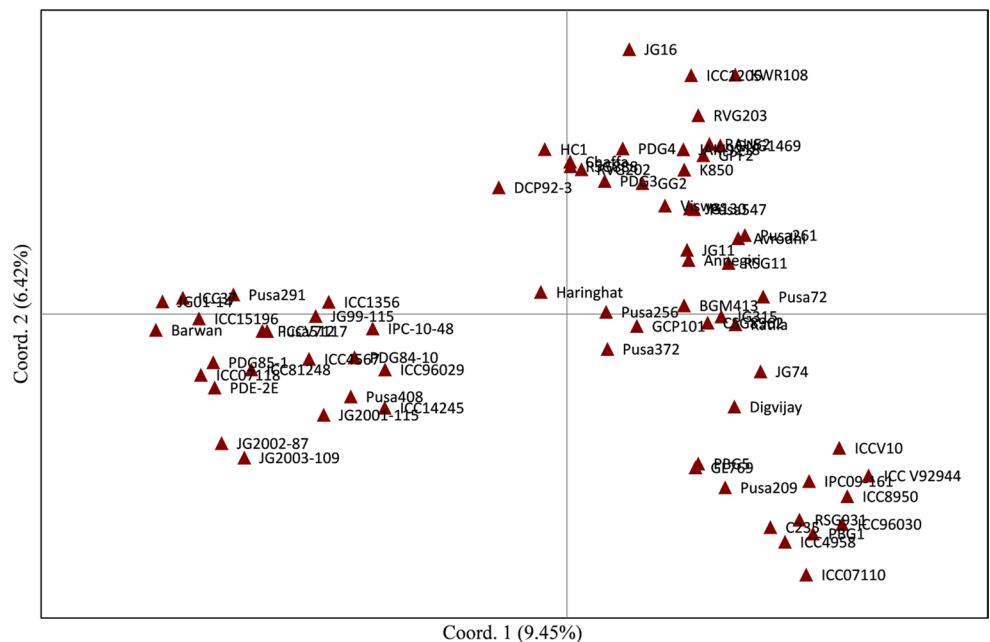
Table 3 Combined ANOVA for MSI and SCMR traits under late sown conditions (2016 and 2017)

Source of variation	df	MSI 2016–2017 Mean of squares MSI	SCMR 2016–2017 Mean of squares SCMR
Year	1	59.67	47.082935
Genotype	70	155.8**	158.325869**
Error	70	53.26	80.862299
h^2 (Broad sense)		0.7417	0.4893

**P < 0.01

Analysis of MSI and SCMR

Combined ANOVA of MSI and SCMR recorded over 2 years suggested significant genetic difference among the genotypes (Table 3). For the two traits, values for the range, median, standard error, and coefficient of variation are given in Table 4. The frequency distributions for the two traits are shown in (Figs. 2 and 3 see in Supplementary File).

Association analysis

We attempted to discover MTAs through analyzing the genotyping data of 81 SSRs and two traits with GLM and MLM approaches. Concerning analysis of MSI (year 2016) with GLM, the markers NCPGR267, NCPGR206, H2L102, TA64, NCPGR156, and NCPGR238 showed significant associations, with phenotypic variation (PV) ranging from 6.1 to 20.2% (Table 5). On the other hand, analysis with MLM approach for MSI (year 2016) uncovered significant association of five SSR markers (NCPGR267, NCPGR206, H2L102, TS53 and NCPGR156) with the trait accounting for PVs in the range of 8.5 and 22.2% (Table 6). Importantly, association of three SSR markers (NCPGR267, H2L102 and NCPGR206) with MSI trait was evident by both GLM and MLM in 2 years (2016 and 2017).

Likewise for SCMR data (2016), GLM revealed that the SSRs GA9, CESSR172, TA113, NCPGR199, CESSR114, ICCM0297 and NCPGR267 had significant association with the trait. In case of MLM, association of two SSR markers ICCM0297 and CESSR172 with the SCMR could be established. In 2017, GLM analysis suggested that TR31 and CESSR433 markers had significant association with SCMR (P values 0.04308 and 0.04755). The significant association

Table 5 Significant MTA and their effects on MSI and SCMR traits by Q GLM approach of association mapping

Trait	Year	Marker	LG group	P value	PV%
MSI	2016	NCPGR267	LG5	0.0004	17
MSI		NCPGR206	LG2	0.00252	12.6
MSI		H2L102	LG6	0.04448	11.2
MSI		TA64	LG6	0.02062	20.2
MSI		NCPGR156	LG6	0.01094	15.2
MSI		NCPGR238	LG6	0.03756	6.1
MSI	2017	NCPGR 267	LG5	0.00139	13.8
MSI		H2L102	LG6	0.01282	14.7
MSI		NCPGR206	LG2	0.0423	5.9
MSI	Mean phenotypic	NCPGR206	LG2	0.00866	9.7
MSI	Data of 2016–2017	H2L102	LG6	0.02105	13.4
SCMR	2016	GA9	LG5	0.01191	19.7
SCMR		CESSR172	LG1	0.00422	17.7
SCMR		TA 113	LG1	0.03007	17.7
SCMR		NCPGR199	LG1	0.03453	15.6
SCMR		CESSR114	LG3	0.03455	9.4
SCMR		ICCM0297	LG5	0.0205	10.8
SCMR		NCPGR267	LG5	0.03812	6
SCMR	2017	TR31	LG2	0.04308	6.5
SCMR		CESSR433	LG4	0.04755	5.5
SCMR	Mean phenotypic	TA113	LG1	0.00697	22.2
SCMR	Data of 2016–2017	GA9	LG5	0.00856	20.7

P = 0.05

of the SSR marker TR31 with SCMR trait was obtained in both MLM and GLM in the year 2017. Two important markers NCPGR206 and H2L102 showed consistently significant MTA for MSI trait recorded exclusively in the year 2016 and 2017 and mean data of 2016 and 2017 given the both GLM and MLM analysis. The quantile–quantile (Q–Q) plot of these calculated P values is depicted in Fig. 6 considering both GLM and MLM and the markers showed association with the studied traits by deviating from the null expectation (Fig. 6). The significant MTA between the marker GA9 and SCMR trait was evident by both methods across 2 years. The SSR markers viz., NCPGR206, H2L102 and GA9 showing stable MTAs with MSI and SCMR traits could be potentially

Table 4 Descriptive statistics for two traits recorded in 71 genotypes

Trait	Minimum	Maximum	Mean	Median	Standard deviation	Mean standard error	CV%
MSI (%)	19.56	75.3	41.4	37.4	15.1	1.8	35.09
SCMR	11.1	57.1	27.3	24.9	9	1.07	22.67

Table 6 Significant MTA and their effects on MSI and SCMR traits by Q + K MLM approach of association mapping

Trait	Year	Marker	LG group	P value	PV%
MSI	2016	NCPGR267	LG5	0.00112	16.5
MSI		NCPGR206	LG2	0.01758	8.5
MSI		H2L102	LG6	0.02399	14.3
MSI		TS 53	LG7	0.03182	22.2
MSI		NCPGR 156	LG6	0.0365	12.8
MSI	2017	NCPGR267	LG5	0.00268	13.8
MSI		H2L 102	LG6	0.02129	14.7
MSI		NCPGR206	LG2	0.04754	5.9
MSI	Mean phenotypic	NCPGR206	LG2	0.02589	7.5
MSI	Data of 2016–2017	NCPGR267	LG5	0.00132	16
MSI		H2L102	LG6	0.01711	15.5
SCMR	2016	ICCM 0297	LG5	0.04255	9.5
SCMR		CESSR 172	LG1	0.04478	12.2
SCMR	2017	TR31	LG2	0.02917	7.6
SCMR	Mean phenotypic	TR31	LG2	0.03894	6.8
SCMR	Data of 2016–2017	GA9	LG5	0.04221	17.5

$P = 0.05$

harnessed in future chickpea breeding for improving HS tolerance.

Candidate gene identification

The SSR markers showing significant MTAs were BLASTed against the Kabuli genome (CDC frontier) sequence of chickpea (Varshney et al. 2013) for gene prediction. For MSI, two proteins including ATPase/AAA-type/ATP-binding protein, and zinc finger/C2H2-type were predicted from the sequence showing homology with the SSR markers showing association with the trait. Similarly for SCMR, five proteins viz., Domain of unknown function DUF1084, DNA glycosylase/AP lyase, H2TH DNA-binding, zinc finger, Nitrilase/cyanide hydratase and apolipoprotein N-acyl-transferase and Aquaporin NIP were predicted (Table 7).

Discussion

Knowledge about the genetic diversity within crop's gene pool is a prerequisite for improving gains in breeding programs of crops (Upadhyaya et al. 2008; Ghaffari et al. 2014). Introduction of novel alleles to the cultivated pool holds the potential to break the yield ceiling (Choudhary et al. 2012a).
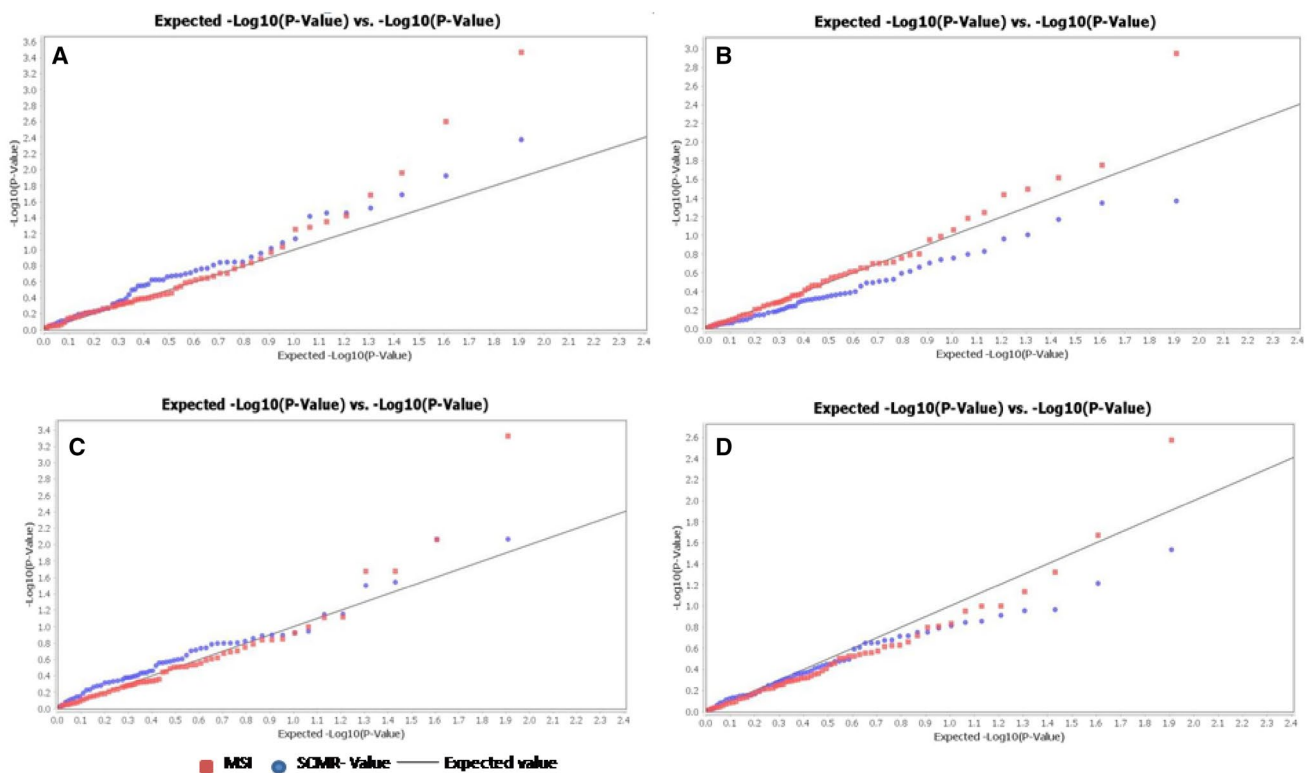

Fig. 6 a, **b** Quantile–Quantile (Q–Q) plots for MSI and SCMR (tested by GLM). **c**, **d** Quantile–Quantile (Q–Q) plots for MSI and SCMR (tested by MLM) witnessing significant association of markers with the given traits by deviating from the expected null distribution of P values, assuming no associations, represented as solid line; distribution of P values observed

Table 7 Putative candidate gene underlying the significant MTAs and their putative functions

Trait	Marker	Candidate gene	LG group	P value	PV%	Marker effect	Putative proteins (based on InterPro)
MSI	NCPGR267	*Ca_05237*	CaLG6	0.00112	16.5	13.54	ATPase, AAA-type, ATP binding
MSI	H2L102	*Scaffold*	–	0.02399	14.3	–	–
MSI	TS53	*Scaffold*	–	0.03182	22.2	–	–
MSI	NCPGR156	*Ca_18128*	CaLG2	0.0365	12.8	–	–
MSI	NCPGR238	*Ca_14617*	CaLG6	0.03756	6.1	6.97364	Zinc finger, RING-type, zinc ion binding
SCMR	GA9	*Ca_05159*	CaLG5	0.01191	19.7	3.83725	Domain of unknown function DUF 1084
SCMR	CESSR172	*Ca_02896*	CaLG1	0.00422	17.7	9.12119	DNA glycosylase/AP lyase, H2TH DNA-binding
SCMR	TA113	*Ca_02459*	CaLG1	0.03007	17.7	7.19934	Zinc finger, C2H2-type, Zinc ion binding
SCMR	NCPGR199	*Scaffold*	–	0.03453	15.6	–	–
SCMR	CESSR 114	*Ca_07065*	CaLG1	0.03455	9.4	–	Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase
SCMR	ICCM 0297	–	–	0.0205	10.8	–	–
SCMR	NCPGR267	*Ca_05237*	CaLG6	0.03812	6	–	ATPase, AAA-type, ATP binding
SCMR	TR31	*Ca_21333*	CaLG3	0.04308	6.5	4.9576	Aquaporin NIP, Major intrinsic protein
SCMR	CESSR433	*Ca_14730*	CaLG1	0.04755	5.5	–	Putative uncharacterized protein

In this respect, molecular markers such as SSR are promising in discerning genetic diversity at DNA level. Also, the marker systems have been efficiently recruited for identifying important MTAs to accelerate trait improvement in chickpea (Hüttel et al. 1999; Udupa et al. 1999; Upadhyaya et al. 2008; Bharadwaj et al. 2011; Sefera et al. 2011; Choudhary et al. 2012a). In the present study, a total of 319 alleles with an average of 3.9 alleles per locus were recovered from 71 chickpea genotypes assayed with 81 SSRs. The results suggested the presence of moderate level of genetic diversity in the studied panel. This finding showed agreement with the previous reports (Ghaffari et al. 2014; Hajibarat et al. 2015), which also suggested lesser genetic diversity among cultivated chickpea genotypes. A moderate value of average PIC and lower count for average alleles per locus noted here might be due to inclusion of cultivated genotypes. Inclusion of various chickpea wild species and landraces could produce higher PIC value and higher number of allele count per locus as suggested by Upadhyaya et al. (2008) and Ghaffari et al. (2014).

The level of heterozygosity ranged from 0.3 (GA105) to 0.83 (TS54) with a mean of 0.6, which was in accordance with the values obtained by Upadhyaya et al. (2008) and Hajibarat et al. (2015). A positive association between number of alleles and PIC value observed in this study was congruent with the results reported previously by other researchers in chickpea (Upadhyaya et al. (2008) and Sefera et al. (2011). Increasing trends of genetic diversity based on PIC values calculated in the cultivars released in the early decades of seventies and post decades of eighties reflected in the our study were similar with the results obtained by Sefera et al. (2011). Additionally, Thudi et al. (2016) have also evidenced increase in genetic diversity based on the presence of

higher copy number variations (CNVs) in chickpea varieties released after the year 2002. A decade wise analysis of 59 Indian pigeonpea varieties with 60 SSR markers showed a near constant genetic diversity over the last 50 years (Bohra et al. 2017). A trend of depicting progressive increase in genetic diversity was reported in rice using SSR analysis of varieties released from 1970 to 2000 in India (Choudhary et al. 2013b). Moreover, results of UPGMA and sub-cluster analysis indicated limited genetic diversity among the cultivars released for the same agro-climatic zone.

Population structure analysis unravels the existing genetic diversity across the collection. In recent years, previous studies have shed light into the genetic structure of chickpea using SSR and SNP markers (Kujur et al. 2013; Diapari et al. 2014; Thudi et al. 2014; Bajaj et al. 2015a; De Giovanni et al. 2017). In our analysis, the patterns arising from STRUCTURE were consistent with those obtained from distance-based clustering methods (NJ and PCoA analyses). Of the two major clusters, released varieties grouped within one cluster, while the other group retained advanced breeding lines and accessions. A previous report in chickpea divided the entire genotypes into two distinct groups: *desi* and *kabuli* types (Upadhyaya et al. 2008). More recently, population structure analysis of 103 chickpea accessions yielded three distinct subpopulations (De Giovanni et al. 2017).

Elucidating the genetic architecture of complex traits in crops is crucial to accelerated trait improvement and association mapping has emerged as a robust technique in this respect (Huang and Han 2014). In chickpea, genome-wide genetic variants showing association with a range of agriculturally important traits including drought, HS, zinc and iron content, 100 seed weight, seed coat color, seed protein content, flowering time were discovered by employing

association genetics (Thudi et al. 2014; Diapari et al. 2014; Bajaj et al. 2015b; Upadhyaya et al. 2015, 2016a, b). Stress conditioned by heat is reported to cause severe deterioration in chickpea yield (see Jha et al. 2017). In the context, identification of DNA markers that could enable rapid selection of the traits underlying HS tolerance is of great significance. The MSI and SCMR traits have been extensively studied as indicators to improve HS tolerance in several crops such as sorghum (Sullivan 1972), wheat (Talukder et al. 2014), etc. Our observation of significant genetic variation for these two traits is in line with the earlier findings documented in chickpea with respect to MSI and SCMR under HS (Jha et al. 2015; Thudi et al. 2014). High heritability was recorded for MSI (74.1%) in comparison to SCMR (48.9%).

To the best of our knowledge, this study is the first to report MTAs for MSI and SCMR in chickpea, two important physiological parameters associated with HS tolerance. Importantly, we found three SSR markers NCPGR206, NCPGR267, and H2L102 that consistently showed significant association with MSI. Similarly, the SSRs TR31 and GA9 on LG2 and LG5, respectively, had significant MTA. Considering MSI and chlorophyll content as important physiological parameters for drought tolerance in wheat, Elshafei et al. (2013) identified five SRAP markers linked to chlorophyll content QTL with PV up to 53%. The authors also reported another five SRAP markers linked with the QTL underlying MSI trait, which explained up to 44% PV. Recently, five QTLs associated with plasma membrane damage trait were mapped in wheat on chromosomes 7A, 7B and 7D based on the analysis of a bi-parental mapping population under HS (Talukder et al. 2014). Also, seven QTLs explaining PV up to 30.8% were identified on chromosomes 6A, 7A, 1B and 1D in wheat for SCMR chlorophyll content under HS. An earlier study aimed to dissect traits relevant to drought and HS in chickpea revealed more than 300 MTAs, the majority of which were related to 100-seed weight trait. Importantly, the MTAs for two important traits viz., 100-seed weight and $\delta^{13}C$ coincided with the "QTL-hotspot" of Varshney et al. (2014) on LG4.

Limited studies have been performed that describe the candidate genes and their corresponding function for membrane stability and chlorophyll-related traits in relation to HS tolerance in plants (Jespersen et al. 2017). In our current study, the candidate gene *Ca_05237* underlying MSI and SCMR-associated NCPGR267 marker region was predicted to encode ATPase/AAA protein. Overexpression of *ZmSKD1* gene encoding putative ATPase/AAA protein exhibiting drought tolerance has been recorded in tobacco (Xia et al. 2013). Given the reported involvement of zinc finger proteins (ZNP) in regulation of abiotic stress tolerance in plants, the markers NCPGR238 and TA113 associated with MSI and SCMR were found to reside within the genomic region that encodes zinc finger or RING-type protein. Role

of zinc finger or RING-type protein in conferring HS tolerance has also been reported (Liu et al. 2015; Zhang et al. 2012). Contribution of aquaporins towards plant's tolerance to abiotic stresses including HS has been documented in various crops (Afzal et al. 2016; Obaid et al. 2016), and we observed SCMR-associated marker TA113 coincided with genomic region encoding aquaporin NIP/major intrinsic protein. Likewise, SCMR-associated CESSR172 marker region was predicted to encode DNA glycosylase/AP lyase protein, which is involved in imparting tolerance to abiotic stresses as elucidated from its higher expression in Arabidopsis (Chen et al. 2012). However, we advocate further confirmation for the MTAs established in the present study in other genetic backgrounds prior to deploying these DNA markers in breeding chickpea for HS tolerance.

Conclusion

We conclude that the genetic diversity among cultivars and advanced breeding lines along with accessions could be exploited for broadening the genetic base of chickpea and breeding superior chickpea cultivars. After validation of the preliminary results of association mapping for MSI and SCMR might be helpful for screening of HS tolerance genotype. The putatively linked genomic regions may be investigated in greater detail for better understanding of HS tolerance in chickpea.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abbo S, Berger J, Turner NC (2003) Evolution of cultivated chickpea: four bottlenecks limit diversity and constrain adaptation. Funct Plant Biol 30:1081–1087

Afzal Z, Howton TC, Sun Y, Mukhtar MS (2016) The roles of aquaporins in plant stress responses. J Dev Biol 4:1–22

Aggarwal H, Rao A, Kumar A, Rana JS, Naik PK, Chhokar V (2015) Assessment of genetic diversity among 125 cultivars of chickpea (*Cicer arietinum* L.) of Indian origin using ISSR markers. Turk J Bot 39:218–226

Ahmad F (1999) Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual Cicer species. Theor Appl Genet 98:657–663

AICRP on Chickpea (2014): Project Coordinator's Report, 2014–2015. Indian Institute of Pulses Research, Kanpur, India

Bajaj D, Das S, Badoni S, Kumar V, Singh M, Bansal KC, Tyagi AK, Parida S (2015a) Genome-wide high-throughput SNP discovery and genotyping for understanding natural (functional) allelic diversity and domestication patterns in wild chickpea. Sci Rep 5:12468

Bajaj D, Das S, Upadhyaya HD, Ranjan R, Badoni S, Kumar V, Tripathi S, Gowda CL, Sharma S, Singh S, Tyagi AK, Parida SK (2015b) A genome wide combinatorial strategy dissects complex genetic structure of seed coat color in chickpea. Front Plant Sci 6:979

Bharadwaj C, Chauhan SK, Rajguru G, Srivastava R, Satyavathi TC, Yadav S, Rizvi AH, Solanki RK (2010) Diversity analysis of chickpea (*Cicer arietinum* L.) using STMS markers. Indian J Agric Sci 80:947–951

Bharadwaj C, Srivastava R, Chauhan SK, Satyavathi CT, Kumar J, Faruqui A, Yadav S, Rizvi AH, Kumar T (2011) Molecular diversity and phylogeny in geographical collection of chickpea (*Cicer* sp.) accessions. J Genet 90:e94–e100

Blum A, Ebercon A (1979) Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci 21:43–47

Bohra A, Jha R, Pandey G, Patil PG, Saxena RK, Singh IP, Singh D, Mishra RK, Mishra A, Singh F, Varshney RK, Singh NP (2017) New hypervariable SSR markers for diversity analysis, hybrid purity testing and trait mapping in Pigeonpea [*Cajanus cajan* (L.) Millspaugh]. Front Plant Sci 8:1–15

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635

Chen H, Chu P, Zhou Y, Li Y, Liu J, Ding Y, Tsang EWT, Jiang L, Wu K, Huang S (2012) Overexpression of AtOGG1, a DNA glycosylase/AP lyase, enhances seed longevity and abiotic stress tolerance in Arabidopsis. J Expt Bot 63:4107–4121

Choudhary S, Sethy NK, Shokeen B, Bhatia S (2009) Development of chickpea EST-SSR markers and analysis of allelic variation across related species. Theor Appl Genet 118:591–608

Choudhary P, Khanna SM, Jain PK, Bharadwaj C, Kumar J, Lakhera PC, Srinivasan R (2012a) Genetic structure and diversity analysis of the primary gene pool of chickpea using SSR markers. Genet Mol Res 11:891–905

Choudhary S, Gaur R, Gupta S, Bhatia S (2012b) EST-derived genic molecular markers: development and utilization for generating an advanced transcript map of chickpea. Theor Appl Genet 124:1449–1462

Choudhary P, Khanna SM, Jain PK, Bharadwaj C, Kumar J, Lakhera PC, Srinivasan R (2013a) Molecular characterization of primary gene pool of chickpea based on ISSR markers. Biochem Genet 51:306–322

Choudhary G, Ranjitkumar N, Surapaneni M, Deborah DA, Vipparla A, Anuradha G, Siddiq EA, Vemireddy LR (2013b) Molecular genetic diversity of major Indian cultivars over decadal periods. PLoS One 8:e66197

De Giovanni C, Pavan S, Taranto F, De Rienzo V, Miazzi MM, Marcotrigiano AR, Mangini G, Montemurro C, Ricciardi L, Lotti C (2017) Genetic variation of a global germplasm collection of chickpea (*Cicer arietinum* L.) including Italian accessions at risk of genetic erosion. Physiol Mol Biol Plants 23:197

Diapari M, Sindhu A, Bett K, Deokar A, Wartkentin TD, Taran B (2014) Genetic diversity and association mapping of iron and zinc concentrations in chickpea (*Cicer arietinum* L.). Genome 57:459–468

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Elshafei AA, Saleh M, Al-Doss AA, Moustafa KA, Al-Qurainy FH, Barakat MN (2013) Identification of new SRAP markers linked to leaf chlorophyll content, flag leaf senescence and cell membrane

stability traits in wheat under water-stressed condition. AJCS 7:887–893

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

FAOSTAT (2014) FAO statistics division. http://faostat3.fao.org/compare/E. Accessed 27 2016

Gaur R, Sethy NK, Choudhary S, Shokeen B, Gupta V, Bhatia S (2011) Advancing the STMS genomic resources for defining new locations on the intraspecific genetic linkage map of chickpea (*Cicer arietinum* L.). BMC Genom 12:117

Gaur PM, Jukanti AK, Varshney RK (2012) Impact of genomic technologies on chickpea breeding strategies. Agronomy 2:199–221

Gaur PM, Samineni S, Sajja SB, Varshney RK, Chaturvedi SK, Jayalakshmi V, Babbar A, Mannur DM, Kumar AGV (2016) Enhancing resilience of chickpea to climate change. In: Symposium on Physiological approaches to enhance productivity in pulses under changing climate, April 2016, JNKVV, Jabalpur India, pp 18–23

Ghaffari P, Talebi R, Keshavarz F (2014) Genetic diversity and geographical differentiation of Iranian landrace, cultivars and exotic chickpea lines as revealed by morphological and microsatellite markers. Physiol Mol Biol Plant 20:225–233

Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877

Gujaria N, Kumar A, Dauthal P, Dubey A, Hiremath P, BhanuPrakash A, Farmer A, Bhide M, Shah T, Gaur PM, Upadhyaya HD, Bhatia S, Cook DR, May GD, Varshney RK (2011) Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (*Cicer arietinum* L.). Theor Appl Genet 122:1577–1589

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetics and plant breeding with emphasis on bread wheat. Euphytica 1(13):163–185

Hajibarat Z, Saidi A, Hajibarat Z, Talebi R (2014) Genetic diversity and population structure analysis of landrace and improved chickpea (*Cicer arietinum*) genotypes using morphological and microsatellite markers. Environ Expt Biol 12:161–166

Hajibarat Z, Saidi A, Hajibarat Z, Talebi R (2015) Characterization of genetic diversity in chickpea using SSR markers, start codon targeted polymorphism (SCoT) and conserved DNA-derived polymorphism (CDDP). Physiol Mol Biol Plants 21:365–373

Huang X, Han B (2014) Natural variations and genome-wide association studies in crop plants. Annu Rev Plant Biol 65:531–551

Hüttel B, Winter P, Weising K (1999) Sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Genome 42:210–217

Iruela M, Rubio J, Cubero JI, Gil J, Milan T (2002) Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. Theor Appl Genet 104:643–651

Jespersen D, Belanger FC, Huang B (2017) Candidate genes and molecular markers associated with heat tolerance in colonial Bentgrass. PLoS One 12(2):e0171183

Jha UC, Shil S (2015) Association analysis of yield contributing traits of chickpea genotypes under high temperature condition. Trends Biosci 8:2335–2341

Jha UC, Chaturvedi SK, Bohra A, Basu PS, Khan MS, Debmalya B (2014a) Abiotic stresses, constraints and improvement strategies in chickpea. Plant Breed 133:163–178

Jha UC, Bohra A, Singh NP (2014b) Heat stress in crop plants: its nature, impacts and integrated breeding strategies to improve heat tolerance. Plant Breed 133:679–701

Jha UC, Basu PS, Singh DK (2015) Genetic variation and diversity analysis of chickpea genotypes based on quantitative traits under high temperature stress. Intl J Bioresour Stress Manag 6:700–706

Jha UC, Bohra A, Jha R, Parida S (2017) Integrated 'omics' approaches to sustain major global grain legume productivity under heat stress. Plant Breed. https://doi.org/10.1111/pbr.12489

Kalra N, Chakraborty D, Sharma A, Rai HK, Jolly M, Chander S, Kumar PR, Bhadraray S, Barman D, Mittal RB, Lal M, Sehgal M (2008) Effect of temperature on yield on some winter crops in northwest India. Curr Sci 94:82–88

Keneni G, Bekele E, Imtiaz M, Dagne K, Getu E, Assefa F (2012) Genetic diversity and population structure of Ethiopian chickpea (*Cicer arietinum* L.) germplasm accessions from different geographical origins as revealed by microsatellite markers. Plant Mol Biol Rep 30:654–665

Krishnamurthy L, Gaur PM, Basu PS, Chaturvedi SK, Tripathi S, Vadez V, Varshney RK, Gowda CLL (2011) Large genetic variation for heat tolerance in the reference collection of chickpea (*Cicer arietinum* L.) germplasm. Plant Genet Res 9:59–69

Kujur A, Bajaj D, Saxena MS, Tripathi S, Upadhyaya HD, Gowda CLL, Singh S, Jain M, Tyagi AK, Parida SK (2013) Functionally relevant microsatellite markers from chickpea transcription factor genes for efficient genotyping applications and trait association mapping. DNA Res 20:355–374

Liu K, Muse SV (2005) Power marker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129

Liu Q, Wang Z, Xu X, Zhang H, Li C (2015) Genome-wide analysis of C2H2 zinc-finger family transcription factors and their responses to abiotic stresses in poplar (*Populus trichocarpa*). PLoS One 10:e0134753

Nayak SN, Zhu H, Varghese N, Datta S, Choi HK, Horres R, Jüngling R, Singh J, Kishor PB, Sivaramakrishnan S, Hoisington DA, Kahl G, Winter P, Cook DR, Varshney RK (2010) Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. Theor Appl Genet 120:1415–1441

Nguyen TT, Taylor PW, Redden RJ, Ford R (2004) Genetic diversity estimates in Cicer using AFLP analysis. Plant Breed 123:173–179

Obaid AY, Sabir JS, Atef A, Liu X, Edris S, El-Domyati FM, Mutwakil MZ, Gadalla NO, Hajrah NH, Al-Kordy MA, Hall N, Bahieldin A, Jansen RK (2016) Analysis of transcriptional response to heat stress in *Rhazyastricta*. BMC Plant Biol 16:252

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539

Perrier X, Jacquemoud-Collet JP (2006) DARwin Software. Paris: Centre de Cooperation Internationale en Recherche Agronomique Pour le De'veloppement (CIRAD)

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Saxena MS, Bajaj D, Kujur A, Das S, Badoni S, Kumar V, Singh M, Bansal KC, Tyagi AK, Parida SK (2014) Natural allelic diversity, genetic structure and linkage disequilibrium pattern in wild chickpea. PLoS One 9:e107484

Sefera T, Abebie B, Gaur PM, Assefa K, Varshney RK (2011) Characterization and genetic diversity analysis of selected chickpea cultivars of nine countries using simple sequence repeat (SSR) markers. Crop Pasture Sci 62:177–187

Sethy NK, Shokeen B, Bhatia S (2003) Isolation and characterization of sequence-tagged microsatellite sites markers in chickpea (*Cicer arietinum* L.). Mol Ecol Notes 3:428–430

Sethy NK, Shokeen B, Edwards KJ, Bhatia S (2006) Development of microsatellite markers and analysis of intra specific genetic variability in chickpea (*Cicer arietinum* L.). Theor Appl Genet 1(12):1416–1428

Shan F, Clarke HC, Plummer JA, Yan G, Siddique KHM (2005) Geographical patterns of genetic variation in the world collection of wild annual Cicer characterized by amplified fragment length polymorphisms. Theor Appl Genet 110:381–391

Sullivan CY (1972) Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. In: Rao NGP, House LR (eds) Sorghum in the seventies. Oxford and IBH publishing Co., New Delhi

Talukder SK, Babar MA, Vijayalakshmi K, Poland J, Prasad PV, Bowden R, Fritz A (2014) Mapping QTL for the traits associated with heat tolerance in wheat (*Triticum aestivum* L.). BMC Genet 15:97

Thudi M, Upadhyaya HD, Rathore A, Gaur PM, Krishnamurthy L, Roorkiwal M, Nayak SN, Chaturvedi SK, Basu PS, Gangarao NV, Fikre A, Kimurto P, Sharma PC, Sheshashayee MS, Tobita S, Kashiwagi J, Ito O, Killian A, Varshney RK (2014) Genetic dissection of drought and heat tolerance in chickpea through genome-wide and candidate gene-based association mapping approaches. PLoS One 9:e96758

Thudi M, Chitikineni A, Liu X, He W, Roorkiwal M, Yang W, Jian J, Doddamani D, Gaur PM, Rathore A, Samineni S, Saxena RK, Xu D, Singh NP, Chaturvedi SK, Zhang G, Wang J, Datta SK, Xu X, Varshney RK (2016) Recent breeding programs enhanced genetic diversity in both desi and kabuli varieties of chickpea (*Cicer arietinum* L.). Sci Rep 6:38636

Udupa SM, Robertson LD, Weigand F, Baum M, Kahl G (1999) Allelic variation at $(TAA)_n$ microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. Mol Genet Genomics 261:354–363

Upadhyaya HD, Dwivedi SL, Baum M, Varshney RK, Udupa SM, Gowda CLL, Hoisington D, Singh S (2008) Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). BMC Plant Biol 8:106

Upadhyaya HD, Bajaj D, Das S, Saxena MS, Badoni S, Kumar V, Tripathi S, Gowda CL, Sharma S, Tyagi AK, Parida SK (2015) A genome-scale integrated approach aids in genetic dissection of complex flowering time trait in chickpea. Plant Mol Biol 89:403–420

Upadhyaya HD, Bajaj D, Narnoliya L, Das S, Kumar V, Gowda CL, Sharma S, Tyagi AK, Parida SK (2016a) Genome-wide scans for delineation of candidate genes regulating seed-protein content in chickpea. Front Plant Sci 7:302

Upadhyaya HD, Bajaj D, Das S, Kumar V, Gowda CL, Sharma S, Tyagi AK, Parida SK (2016b) Genetic dissection of seed-iron and zinc concentrations in chickpea. Sci Rep 6:24050

Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S et al (2013) Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat Biotechnol 31:240–246

Varshney RK, Thudi M, Nayak SN, Gaur PM, Kashiwagi J, Krishnamurthy L, Jaganathan D, Koppolu J, Bohra A, Tripathi S, Rathore A, Jukanti AK, Jayalakshmi V, Vemula A, Singh SJ, Yasin M, Sheshshayee MS, Viswanatha KP (2014) Genetic dissection of drought tolerance in chickpea (*Cicer arietinum* L.). Theor Appl Genet 127:445–462

Winter P, Pfaff T, Udupa SM, Huttel B, Sharma PC, Sahi S, Arreguin-Espinoza R, Weigand F, Muehlbauer FJ, Kahl G (1999) Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol Gen Genet 262:90–101

Winter P, Benko-Iseppon AM, Hüttel B et al (2000) A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163

Xia Z, Wei Y, Sun K, Wu J, Wang Y et al (2013) The maize AAA-type protein SKD1 confers enhanced salt and drought stress tolerance in transgenic tobacco by interacting with lyst-interacting protein 5. PLoS One 8:e69787

Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM (2010) Mixed linear model approach adapted for genome-wide association studies. Nat Genet 42:355–360

Zhang X, Li J, Liu A, Zou J, Zhou X et al (2012) Expression profile in rice panicle: insights into heat response mechanism at reproductive stage. PLoS One 7:e49652