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RESEARCH ARTICLE

Elucidating genetic diversity and association mapping to identify SSR markers linked to 100 seed weight in chickpea (*Cicer arietinum* L.)

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Abstract

Chickpea, a cool-season grain legume enriched with high nutritive value is grown globally over 90 countries. Seed weight trait is one of the important quality parameters for fetching premium market price. Thus, improving seed traits, including high 100-seed weight (SW) is one of the major targets of chickpea breeding. A study of genetic variability, molecular diversity and marker-trait association (MTA) analysis for 100 SW was performed in a panel of 96 chickpea genotypes consisting of crop wild relatives, landraces, advanced breeding lines and released varieties. A wide range of genetic variability and high heritability for the studied trait indicated the great scope of improving this trait. Simple sequence repeat marker-based genetic diversity analysis grouped all the genotypes into two groups. This result was consistent with the result obtained from factorial and population structure analysis. To delineate the significant marker-trait association for 100 SW, association analysis was performed in the given panel of chickpea genotypes. The mixed linear model (MLM) was employed for detecting significant MTAs for 100 SW. Following MLM analysis, a total of seven significant MTAs was detected in the year 2016-17. While in the year 2017-18, MLM analysis showed three significant MTAs for 100 SW. Three markers TAA60, CakTpSSR02719, H1B04 markers exhibited significant MTA for both the years consistently. Thus, these genomic regions could be fine mapped in future for improving 100 SW in chickpea.

Keywords: Chickpea, landrace, marker-trait association, seed weight, SSR, wild relatives

Introduction

Chickpea is one of the important globally grown grain legume crops. Besides replenishing soil nitrogen content by symbiotically active rhizobacteria, it also supplies essential amino acids, vitamins and other micronutrients to the human populations, especially those residing across the underdeveloped countries globally (Bohra et al. 2014; Wallace et al. 2016). Seed size (measured through 100 SW) is one of the important yield-determining parameters in crop improvement, including chickpea. Thus improving 100 SW could be of immense importance for improving chickpea yield and quality traits. Several classical breeding approaches have been greatly devoted to capturing genetic

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diversity and working out the genetics of 100SW in chickpea (Upadhyaya et al. 2006; Bicer and Tuba 2008; Kivrak et al. 2020). Concomitantly advances in chickpea genomics allowed genetic dissection of 100 SW trait for improving chickpea yield (Jamalabadi et al. 2013; Kujur et al. 2014; Verma et al. 2015). However, the improvement of chickpea for this trait through genomics intervention remained limited. Thus, in the present investigation, we assessed the genetic variability for 100 SW traits in the years 2016-17and 2017-18 in a panel of 96 chickpea germplasm consisting of accessions, improved breeding lines, and crop wild relatives chickpea.

An association mapping analysis was performed through assaying 115 Simple Sequence Repeats (SSRs) existing across all the chickpea linkage groups in the given panel of chickpea genotypes to delineate the significant marker-trait association for 100 SW. The mixed linear model (MLM) was employed for detecting significant MTAs for 100 SW. Following MLM analysis, a total of seven significant MTAs were detected in the year 2016-17. While in the year 2017-18, MLM analysis showed three significant MTAs for 100 SW. Three markers, TAA60, CakTpSSR02719, and H1B04, consistently exhibited significant MTA for both years. Thus, these genomic regions could be fine mapped in future for improving 100 SW.

Materials and methods

Material

A panel of 96 chickpea genotypes containing 36 crop wild relatives, 35 landraces, seven advanced breeding lines and 16 released varieties of both *desi* and *kabuli* were evaluated in replicated trials for two consecutive years 2016-17 and 2017-18 (Supplementary Table S1). The experiment for 100 seed weight was designed and conducted at ICAR-Indian Institute of Pulses Research, Kanpur (Latitude: 26° 27′ 54.83° N, Longitude: 80° 20′ 59.10° E).

Statistical analysis

The analysis of variance (ANOVA) was performed using GenStat 17th Edition (VSN International, Hemel, Hempstead, UK) for individual environment. The trait data has been visualized through violin plot prepared by "ggplot2" package in R version 4.03 (R Project for Statistical Computing, http://www.r-project.org/). Broad sense heritability was estimated (Falconer and Mackay,1996) as $H^2=V_g/(V_g+V_e/n_r)$, where H^2 is broad sense heritability, V_g is genotypic variance, V_p is phenotypic variance, V_e is residual variance, and n_r is number of replications.

Genomic DNA isolation and SSR analysis

Two weeks-old seedling leaves from each genotype were taken for genotyping the entire panel of genotypes. Genomic DNA was isolated from all the genotypes using CTAB protocol (Murray and Thompson1980).

Polymerase chain reaction (PCR) protocol was followed as per Jha et al. (2018, 2019) and Bohra et al. (2020). A total of 180 SSR markers (genic and genomic) (Winter et al. 1999, 2000; Sethy et al. 2003, 2006; Gaur et al. 2011; Choudhary et al. 2012) and 70 newly synthesized Indel SSR markers with the known position on all the eight LGs were screened in the given set of genotypes, of which 115 SSRs showed polymorphism. All the polymorphic SSRs are enlisted in (Supplementary Table S2).

Molecular diversity and population structure analysis

The number of alleles per locus (Na), gene diversity (He) and polymorphism information content (PIC) were calculated with Power Marker v. 3.25 (Liu and Muse 2005). Neighbourhood joining tree and factorial analysis was performed with DARwin v. 6.0.13 with 1600 bootstrap value, (Perrier and Jacquemoud-Collet 2006). Likewise, STRUCTURE v. 2.3.4 (Pritchard et al. 2000) was used for identifying population structure (*Q*) and the subpopulation (*K*) in the given set of genotypes. To obtain optimum number of groups (K), STRUCTURE was run with *K* varying from 1 to 10 with five independent runs for each *K* value were conducted with 500000 Markov Chain Monte Carlo (MCMC) iterations with a 1000 burn-in periods. In parallel, the most probable *K* value was calculated according to the method suggested by Evanno et al. (2005) by using web tool STRUCTURE HARVESTER (Earl and von Holdt, 2012) (http://taylor0.biology.ucla.edu).

Association mapping and candidate gene analysis

The phenotypic data of 100 SW and the genotypic data were analyzed to discern significant MTAs. Mixed linear model based on Q and Q + K matrix was used. TASSEL v. 3.0 (Bradbury et al. 2007; Zhang et al. 2010) was run to underpin the MTAs, at $p = 0.05$ significance level. For investigating the candidate genes corresponding to the reported MTAs and the putative proteins encoded by these, we performed BLASTn search for the associated SSRs against the annotated chickpea reference genome (CDC frontier) (Varshney et al. 2013). Similarly, the possible proteins were predicted for the corresponding sequences by using InterPro (https://www.ebi.ac.uk/interpro/).

Results

Genetic variability for 100 SW

The genome wide association study (GWAS) panel consists of desi type, kabuli type and wild types, hence a significant genetic variation has been observed (Supplementary Fig. S1). In both years, the range for 100SW was very high (1.45-57.8 g). There was a considerable variation, even in each type. For example, in 45 desi type genotypes, the mean was

18.01 g with a range of 11.63-27.18 while in 13 kabuli types, the range was 25.58-56.90 g with a mean of 35.50 g. The 38 wild types included in this study also differed greatly with a minimum value of 1.45 g to a maximum of 19.55 g 100SW with a mean of 6.43 g. In this collection, out of 38 wild types of chickpea, 25 lines had 100SW below 3 g. Interestingly, except for *C. bijugam*, all the annual species of wild *Cicer* were included in the panel. There was a good amount of variation in the wild type *C. reticulatum* (9.80-1.955 g). High heritability (97.8% in 2017 and 98.7% in 2018) for 100SW was recorded in both years (Supplementary Table S3). In the violin plot, the entire collection of chickpea genotypes followed the normal distribution (Fig. 1).

SSR markers based molecular diversity analysis

Assaying 96 chickpea genotypes with 115 SSRs yielded a total of 577 alleles, with an average of 5.5 alleles per marker (Supplementary Table S3). The number of alleles ranged from 2 to 15, while the PIC values varied between 0.2 and 0.8. Similarly, gene diversity ranged from 0.24 to 0.86 with an average value of 0.62. As shown in Fig. 2, the entire 96 chickpea genotypes were clustered into two major groups based on unweighted neighbour-joining method. Cluster I contained 40 genotypes, while cluster II had 56 genotypes. Likewise, factorial analysis placed all the genotypes into two coordinates (Fig. 3).

Structure analysis

The population structure of the 96 chickpea genotypes was investigated using the Bayesian approach using the STRUCTURE program. The LnP (D) as well Evanno's ΔK values identified two genetically distinct populations (i.e., K = 2) (Supplementary Figs. S2 and S3).

Deciphering MTAs for 100SW

Mixed linear model (MLM) approach was used to detect significant MTAs for 100 SW. Following MLM analysis, seven markers (NCPGR46, TAA60, CakTpSSR02719, H1B04, and GA6) showed significant association with phenotypic variations (PVs) ranging from 12.2-21.2% in the year 2016-17 (Table 1). While in the year 2017-18, MLM analysis showed a significant association of three SSR markers TAA60, CakTpSSR02719, H1B04, TA1 and TA18 with PVs ranging from 6.7 to 22.2 % (Table 1). Fig. 4 depicted the Q-Q plot for 100SW evaluated in 2016-17 and 2017-18 based on MLM analysis. Three markers TAA60, CakTpSSR02719, H1B04 markers exhibited significant MTA for both years consistently and thus could be potentially used for marker-assisted selection for SW trait in chickpea.

Candidate gene identification

The candidate genomic regions showing significant association with 100 SW were BLAST searched for gene prediction against Kabuli chickpea's whole genome sequence, i.e. CDC frontier (Varshney et al. 2013). As a result, five candidate genes with putative function (Table 2) were predicted to reside within the genomic sequence displaying

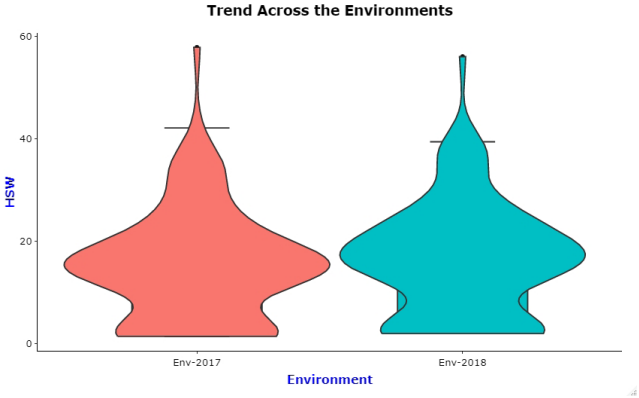

Fig. 1. The violin plot shows the phenotypic distribution of 100 seed weight in 96 chickpea genotypes

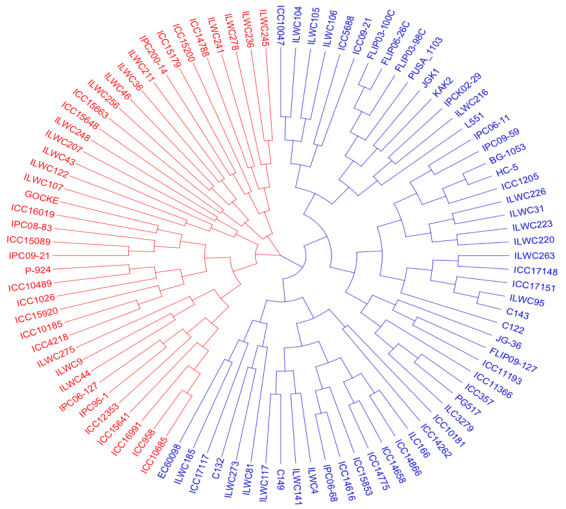

Fig. 2. Unweighted neighbour-joining tree displaying genetic relationship of 96 chickpea genotypes

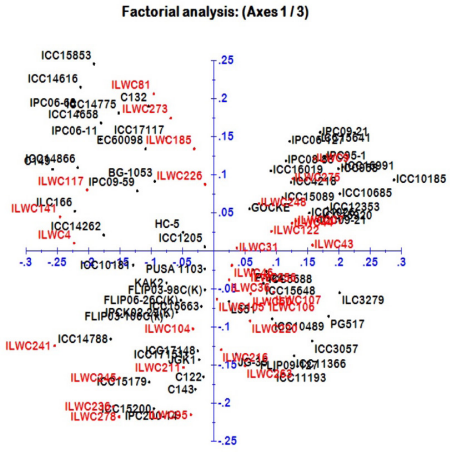

Fig. 3. Factorial analysis of 96 chickpea genotypes

significant MTAs for 100 SW.

Discussion

Genetic variability remains central to improving any traits, including 100 SW trait in chickpea. Wide range of genetic variability for 100 SW has been captured in the current study. This result remained consistent with the work reported previously (Bicer and Sakar 2008; Tsehaye et al. 2020). A high heritability of 100 SW trait could give us great opportunity for genetic improvement in chickpea yield (Tsehaye et al. 2020). Thus, in the current study, the high heritability of 100 SW trait could be potentially used to improve future genetic gain in chickpea.

Among the various molecular markers SSRs are one of the cheapest for molecular diversity analysis and marker-assisted breeding in various crops. In the present investigation, 577 alleles, with an average of 5.5 alleles per marker, were obtained. Similarly, gene diversity ranged from 0.24 to 0.86, with an average value of 0.62. These results were incongruent with the result suggested by other researchers (Upadhyaya et al. 2008; Jha et al. 2021). Population structure analysis categorized all *desi*, *kabuli* and crop wild relative genotypes into two groups. This result is in agreement with the result obtained by Upadhyaya et al. (2008); Bharadwaj et al. (2013) and Kujur et al. (2013) who evidenced the close evolutionary relationship of domesticated *desi* chickpea, *kabuli* chickpea and their close wild relative *C. reticulatum* based on molecular analysis. Increasing chickpea genomic repertoire allowed dissection of various traits of breeding importance including 100 SW (Basu et al. 2018; Garg et al. 2017; Rajkumar et al. 2018 for details Jha 2018). Previously, several QTLs for 100 SW were uncovered by biparental mapping approach (Kujuret al. 2014; Verma et al. 2015; Singh et al. 2016). However, genome-wide association mapping approach has been limitedly employed for unveiling the significant marker-trait association for this yield contributing trait in chickpea (Kujur et al. 2013; Dwivedi et al. 2017; Basu et al. 2018; Rajkumar et al. 2018). In the current study, TAA60 on LG2, H1B04 on LG3 and CakTpSSR02719 marker exhibited

Table 1. MTA analysis for SW traits for the year 2016-17 and 2017-18

Trait	Marker	LG	p value	PV%
SW_2017-Mean	NCPGR46	CaLG06	0.00989*	12.8
SW_2017-Mean	TAA60	CaLG02	0.01676*	19.4
SW_2017-Mean	CakTpSSR02719	-	0.02032*	13
SW_2017-Mean	H1B04	CaLG03	0.02737*	12.2
SW_2017-Mean	GA6	CaLG02	0.04583*	21.2
SW_2018-Mean	TAA60	CaLG02	0.0074*	22.2
SW_2018-Mean	CakTpSSR02719	-	0.00808*	15.6
SW_2018-Mean	NCPGR46	CaLG06	0.01469*	11.7
SW_2018-Mean	H1B04	CaLG03	0.0188*	13.2
SW_2018-Mean	TA1	CaLG01	0.03661*	13.2
SW_2018-Mean	TA18	CaLG07	0.04641*	6.7

* significant at 5% level

Table 2. Candidate genes underlying the markers intervals with their putative functions (based on InterPro)

Marker	Chromosome	Chrom-Blast	Position	Annotation	Gene	Function
NCPGR46	CaLG06	LG4	6753523	DRR	*Ca_03567*	IPR004147; ABC-1
TAA60	CaLG02	LG_1	28996900	Intergenic	-	
CakTpSSR02719	CaLG04	CaLG04	38680436	URR	*Ca_13099*	IPR002691; LIM binding protein
H1B04	CaLG03	C11167066	8505	DRR	*Ca_27914*	IPR007197; Radical SAM
GA6	CaLG02	LG8	1492393	DRR	*Ca_15022*	IPR001471; Pathogenesis-related transcriptional factor/ERF, DNA-binding
TA1	CaLG01	CaLG01	14798686	DRR	*Ca_07051*	IPR007087; Zinc finger, C2H2-type
TA18	CaLG07	scaffold3520	48185	scaffold	-	-

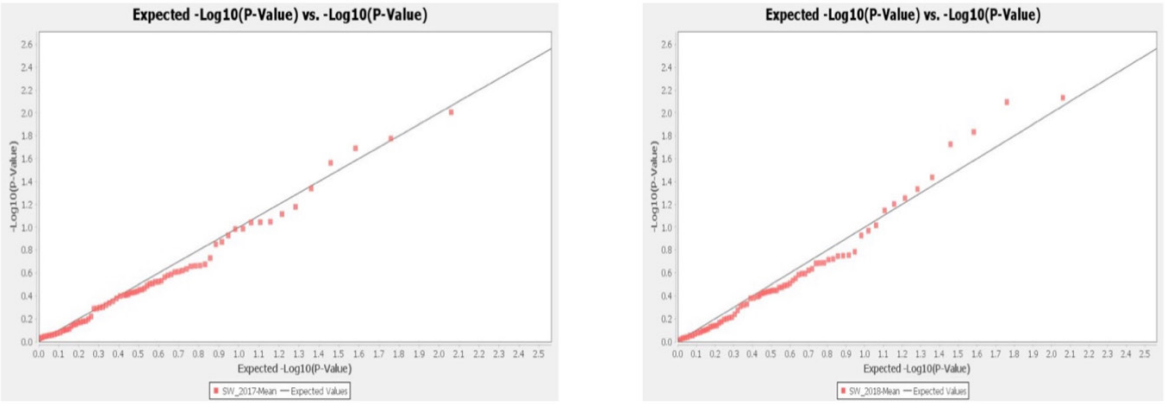

Fig. 4. MTA analysis of 100 seed weight based on MLM for the year 2016-17 and 2017-2018

significant MTA for both years 2016-2017 and 2017-18. Similarly, one QTL for 100 SWwas found on LG6 (Verma et al. 2015; Bajaj et al. 2015), one QTL on LG3 (Bajaj et al. 2015) and one QTL on LG2 (Gupta et al. 2015) was reported. In 2017-18, each significant MTAs were identified on LG1, LG2, LG3, LG6 and LG7, respectively. Similarly, two QTLs on LG1 (Kujur et al. 2014), one QTL on LG1 by employing QTL-seq approach in ICC4958 × ICC1882 mapping population (Singh et al. 2016), one QTL on LG1 (Gupta et al. 2015; Verma et al. 2015) and one major SW QTL *CaqSW1.1* (Das et al. 2015), one QTL on LG1 (Dwivedi et al. 2017), 5 QTLs on LG1 (Wang et al.2019) and three QTL on LG1 (Bajaj et al. 2015) were reported for this trait. While one QTL on LG2 and three QTLs on LG7 (Kujur et al. 2014; Verma et al. 2015) and five QTLs on LG3 (Wang et al. 2019) were mapped for this trait.

Decoding of chickpea genome sequence and advances in chickpea functional genomics have allowed delineating the underlying candidate gene(s) and their putative functions for the traits of agronomic relevance, including seed weight. Earlier, several underlying candidate genes viz., *Ca_04364*, *Ca_04607* (Singh *et al*.2016), *Ca00596*, *Ca19297* (Bajaj et al. 2015), *Ca00071* (Das et al. 2015), *Ca_12295, Ca_04364, Ca_04600, Ca_04601, Ca_04602* and *Ca_04607* (Garg et al. 2017), *Ca_23740* , *Ca_07830, Ca_04862, Ca_26707,* and *Ca_21133* (Rajkumar et al. 2018) encoding cell division kinase protein, component of DNA replication machinery, transmembrane protein, seed specific expression protein, F-box protein 5, expansin precursor, RAN GTPase-activating protein 1 *etc*., those regulating SW have been deciphered in chickpea. In the current study, a total of five candidate genes viz., *Ca_03567* (encoding ABC transporter protein) underlying NCPGR46 marker, *Ca_13099* (encoding LIM binding protein) underlying CakTpSSR02719, *Ca_15022* (ethylene responsive factor/pathogenesis related transcription factor) underlying GA6 marker, *Ca_27914* underlying H1B014 marker and *Ca_07051* (encoding C_2H_2 type zinc finger) underlying Ta1 marker were identified using the annotated chickpea genome sequence. Previously, Verma et al.(2015) reported c3hc4-type ring zinc finger protein-encoding candidate gene is related to seed weight in chickpea. Likewise, the participatory role of RING-H2 zinc-finger protein was reported for seed development in *Arabidopsis* (Xu and Quinn 2003). Considering the regulatory role of ERF/AP2 TF encoding candidate gene(s) contributing in seed size and seed weight has been reported earlier in *Arabidopsis* (Jokofuet al. 2005), in chickpea (Bajaj et al. 2015). Emphasizing on the role of ATP binding cassette (ABC) transporter protein-encoding gene-regulating seed weight trait has been demonstrated in chickpea (Basu et al. 2019) in soybean Karikariet al.(2020). *LIM* (Lin-11, Isl-1 and Mec-3 domains) genes are recognised to be involved in actin bundles formation and contribute in a major higher-order cytoskeletal assembly in plant (Park et al. 2014; Srivastava and Verma 2017). However, the role of LIM gene-regulating seed weight/size remains elusive. Thus, the *identified Ca_13099 (encoding LIM binding protein)* could be a novel gene contributing to seed weight in chickpea.

In summary, the association mapping analysis provided insight into the genetic basis of seed weight trait in chickpea. The identified significant MTAs and the underlying candidate genes need further in-depth analysis to explore their plausible role for seed weight in chickpea. Furthermore, the significant reported MTAs could be used for improving seed weight and related traits through the marker-assisted breeding approach.

Authors' contribution

Conceptualization of research (UCJ); Designing of the experiments (UCJ, RJ, SP, NPS); Contribution of experimental materials (BM, YK, AKS, SKC); Execution of field/lab experiments and data collection (RJ, VT, UCJ); Analysis of data and interpretation (PJP, HN); Preparation of the manuscript (UCJ, ST).

Supplementary materials

Supplementary Figs. S1 to S3 and Supplementary Tables S1 to S3 are provided.

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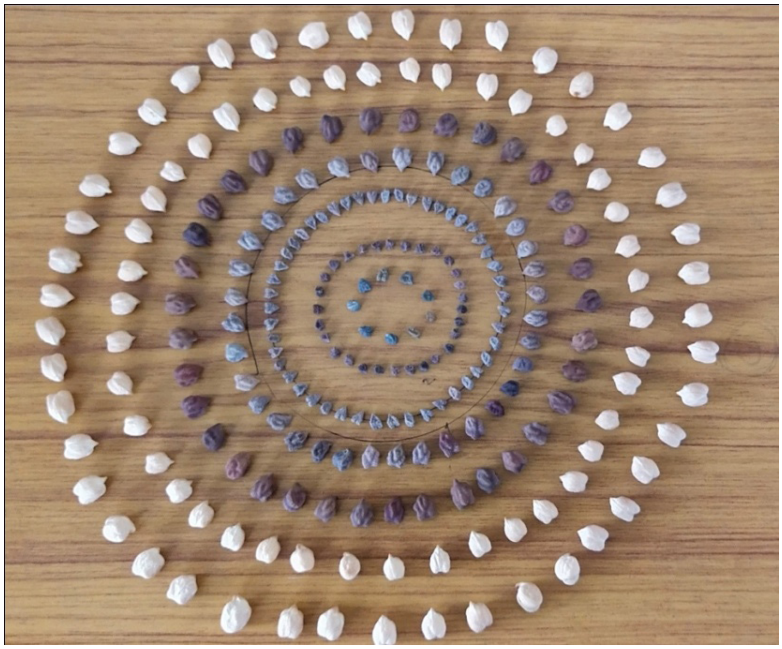
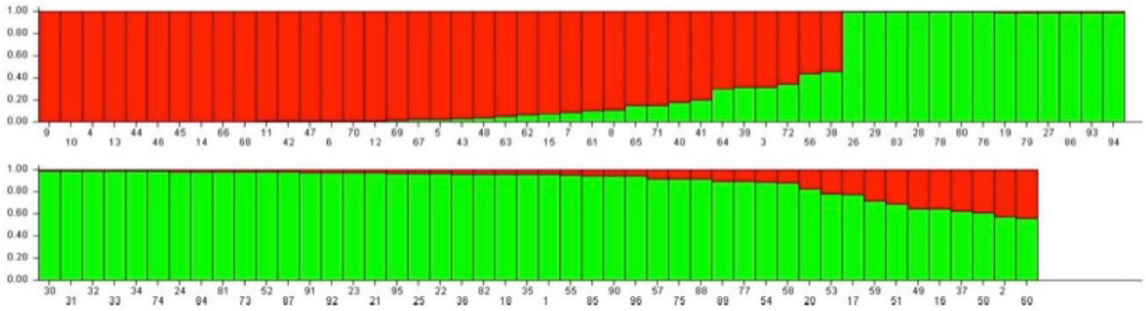
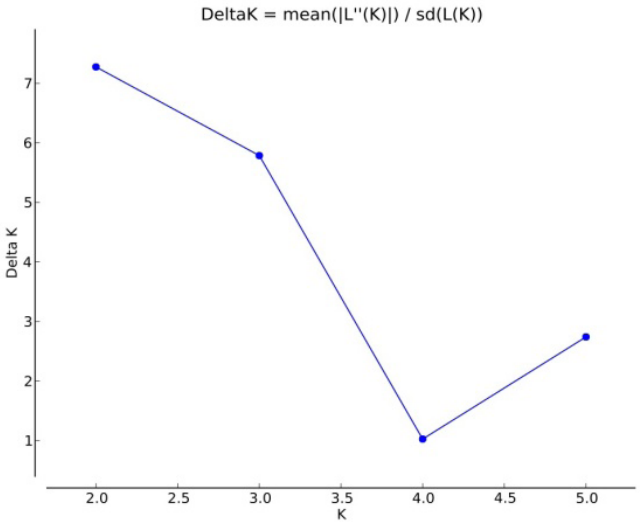


Supplementary Fig.S1. Genetic variability for 100 seed weight in the given set of chickpea genotypes



Supplementary Fig. S2. Population structure of 96 chickpea genotypes



Supplementary Fig. S3. Relationship between K and ΔK based on STRUCTURE analysis of 96 chickpea genotypes

Supplementary Table S1. List of chickpea genotypes

Sl.no.	Genotype	Status/Origin	*Type*
1	ICC10047	Accession, ICRISAT, India	Desi
2	ICC5688	Accession, ICRISAT, India	Desi
3	ICC09-21	Accession, ICRISAT, India	Desi
4	ICC10685	Accession, ICRISAT, India	Desi
5	ICC958	Accession, ICRISAT, India	Desi
6	ICC16991	Accession, ICRISAT, India	Desi
7	ICC15641	Accession, ICRISAT, India	Desi
8	ICC4218	Accession, ICRISAT, India	Desi
9	ICC10185	Accession, ICRISAT, India	Desi
10	ICC15920	Accession, ICRISAT, India	Desi
11	ICC1026	Accession, ICRISAT, India	Desi
12	ICC10489	Accession, ICRISAT, India	Desi
13	P-924	Accession, India	Desi
14	ICC15648	Accession, ICRISAT, India	Desi
15	ICC15663	Accession, ICRISAT, India	Desi
16	IPC200-14	Improved breeding line	Desi
17	ICC15179	Accession, ICRISAT, India	Desi
18	ICC15200	Accession, ICRISAT, India	Desi
19	ICC14788	Accession, ICRISAT, India	Desi
20	ICC10181	Accession, ICRISAT, India	Desi
21	ICC14262	Accession, ICRISAT, India	Desi
22	ILC166	ICARDA	Kabuli
23	ICC14866	Accession, ICRISAT, India	Desi
24	ICC14658	Accession, ICRISAT, India	Desi
25	ICC14775	Accession, ICRISAT, India	Desi
26	ICC15853	Accession, ICRISAT, India	Desi
27	ICC14616	Accession, ICRISAT, India	Desi
28	IPC06-68	Advanced breeding line, IIPR, India	Desi
29	IPC06-11	Advanced breeding line, IIPR, India	Desi
30	IPC09-59	Advanced breeding line, IIPR, India	Desi
31	BG-1053	Released variety, India	Kabuli
32	HC-5	Released variety, India	Desi
33	ICC1205	Accession, ICRISAT, India	Desi
34	JG-36	Released variety, India	Desi
35	FLIP09-127	ICARDA, Lebanon	Kabuli
36	ICC11193	Accession, ICRISAT, India	Desi
37	ICC11366	Accession, ICRISAT, India	Desi
38	ICC357	Accession, ICRISAT, India	Desi
39	PG517	Released variety, India	Kabuli
40	ILC3279	ICARDA, Lebanon	Kabuli
41	ICC12353	Accession, ICRISAT, India	Desi
42	IPC95-1	Accession, India	Desi
43	IPC06-127	Advanced breeding line, IIPR, India	Desi
44	IPC09-21	Advanced breeding line, IIPR, India	Desi
45	IPC08-83	Advanced breeding line, IIPR, India	Desi
46	ICC15089	Accession, ICRISAT, India	Desi
47	ICC16019	Accession, ICRISAT, India	Desi
48	GOCKE	ICARDA, Lebanon	Kabuli
49	FLIP03-100C	ICARDA, Lebanon	Kabuli
50	FLIP06-26C	ICARDA, Lebanon	Kabuli
51	FLIP03-98C	ICARDA, Lebanon	Kabuli
52	PUSA 1103	Released variety, India	Kabuli
53	JGK1	Released variety, India	Kabuli
54	KAK2	Released variety, India	Kabuli
55	IPCK02-29	Released variety, India	Kabuli
56	L551	Released variety, India	Kabuli
57	ILWC216	*C. reticulatum*	Wild
58	ILWC104	*C. reticulatum*	Wild
59	ILWC105	*C. reticulatum*	Wild
60	ILWC106	*C. reticulatum*	Wild
61	ILWC107	*C. reticulatum*	Wild
62	ILWC122	*C. reticulatum*	Wild
63	ILWC43	*C.judaicum*	Wild
64	ILWC207	*C.judaicum*	Wild
65	ILWC248	*C. pinnatifidum*	Wild
66	ILWC9	*C.pinnatifidum*	Wild
67	ILWC44	*C.judaicum*	Wild
68	ILWC275	*C.judaicum*	Wild
69	ILWC256	*C. judaicum*	Wild
70	ILWC46	*C. reticulatum*	Wild
71	ILWC36	*C. reticulatum*	Wild
72	ILWC211	*C.judaicum*	Wild
73	ILWC278	*C.judaicum*	Wild
74	ILWC236	*C.pinnatifidum*	Wild
75	ILWC245	*C.echinospermum*	Wild
76	ILWC241	*C. pinnatifidum*	Wild
77	ILWC4	*C. reticulatum*	Wild
78	ILWC141	*C. reticulatum*	Wild
79	C149	*C. arietinum*	Wild
80	ILWC117	*C.reticulatum*	Wild
81	ILWC81	*C.reticulatum*	Wild
82	ILWC273	*C.judaicum*	Wild
83	C132	*C. arietinum*	Desi
84	ICC17117	*C. yamashitae*	Wild
85	ILWC185	*C. judaicum*	Wild
86	EC60098	*C. cuneatum*	Wild
87	ILWC226	*C. pinnatifidum*	Wild
88	ILWC31	*C.judaicum*	Wild
89	ILWC223	*C.judaicum*	Wild
90	ILWC220	*C.judaicum*	Wild
91	ILWC263	*C.pinnatifidum*	Wild
92	ICC17148	*C. judaicum*	Wild
93	ICC17151	*C.judaicum*	Wild
94	ILWC95	*C.judaicum*	Wild
95	C143	*C. arietinum*	Desi
96	C122	*C. arietinum*	Desi

Supplementary Table S2. Summary statistics and genetic parameters of 96 chickpea genotypes evaluated during the year 2017 and 2018

Genetic Parameter	Mean	Median	Min	Max	Quartile 1	Quartile 3	MSS (g)	MSS (e)	GCV	H^2
Environment-2017	15.28+1.6	14.75	1.45	57.8	2.38	19.45	239.37	5.23	70.83	97.8
Environment-2018	16.20+1.2	22.25	2	56	2.88	22.25	246.69	3.09	67.68	98.7

MSS (g): Mean sum of square for genotype; MSS (e): Mean sum of square for error; H^2: Heritability

GCV: genetic coefficient of variation

Supplementary Table S3. Molecular diversity, number of alleles, gene polymorphism recorded in 96 chickpea genotypes

Marker	Linkage Group	Major Allele Frequency	Number of alleles	PIC	Gene diversity	Reference
CaINDEL31	LG4	0.74	5	0.42	0.44	Newly synthesized
CaINDEL14	LG4	0.50	7	0.56	0.63	Newly synthesized
NCPGR234	-	0.31	8	0.74	0.78	Gaur et al. (2011)
NCPGR231	LG4	0.32	6	0.73	0.77	Gaur et al. (2011)
NCPGR149	-	0.57	5	0.55	0.60	
CakTpSSR03637	LG4	0.46	9	0.68	0.72	-
CaINDEL24	LG8	0.70	3	0.34	0.43	Newly synthesized
CaINDEL12	LG1	0.44	4	0.55	0.62	Newly synthesized
CaINDEL44	LG8	0.82	2	0.25	0.29	Newly synthesized
CaINDEL37	LG5	0.48	3	0.46	0.56	Newly synthesized
CESSR172	LG2	0.28	9	0.79	0.81	Choudhary et al. (2012)
TS54	LG4	0.26	9	0.81	0.83	Winter et al. (2000)
TA2	LG4	0.23	15	0.84	0.85	Winter et al. (1999)
CaINDEL52	LG1	0.64	2	0.36	0.46	Newly synthesized
NCPGR76	LG4	0.53	3	0.50	0.58	Sethy et al. (2006)
CaINDEL48	LG8	0.70	2	0.33	0.42	Newly synthesized
CESSR114	LG4	0.23	9	0.79	0.82	Choudhary et al. (2012)
TA176	LG6	0.49	7	0.67	0.70	Winter et al. (1999)
NCPGR139	LG6	0.31	11	0.77	0.80	Gaur et al. (2011)
CaINDEL41	LG8	0.53	4	0.55	0.61	Newly synthesized
H2L102	LG5	0.36	6	0.68	0.73	Choudhary et al. (2012)
NCPGR199	LG4	0.53	5	0.54	0.61	Gaur et al. (2011)
TA80	LG4	0.31	5	0.70	0.74	Winter et al. (2000)
NCPGR200	LG6	0.30	11	0.77	0.79	Gaur et al. (2011)
H4F07	LG5	0.45	4	0.56	0.63	Gujaria et al. (2011)
GA102	LG7	0.65	6	0.51	0.54	Gaur et al. (2011)
CaINDEL18	LG4	0.85	2	0.22	0.25	Newly synthesized
CESSR432	LG5	0.70	3	0.41	0.46	Gaur et al. (2015)
H5A04	LG6	0.50	2	0.38	0.50	Lichtenzveig et al. (2005)
GA9	LG5	0.45	4	0.59	0.65	Winter et al. (2000)
NCPGR238	LG6	0.57	3	0.50	0.57	Gaur et al. (2011)
NCPGR202	LG6	0.34	10	0.77	0.79	Choudhary et al. (2012)
CaINDEL20	LG4	0.50	3	0.43	0.54	Newly synthesized
CESSR45	LG5	0.65	3	0.37	0.46	Choudhary et al. (2012)
CaINDEL17	LG4	0.50	3	0.55	0.62	Newly synthesized
CakTpSSR02719	LG4	0.35	5	0.64	0.70	-
TR31	LG3	0.64	3	0.37	0.47	Winter et al. (2000)

Cont...

Marker	Linkage Group	Major Allele Frequency	Number of alleles	PIC	Gene diversity	Reference
CaINDEL28	LG4	0.38	5	0.62	0.69	Newly synthesized
NCPGR165	LG1	0.54	5	0.54	0.60	Choudhary et al. (2012)
ICCM0297	LG1	0.49	4	0.56	0.63	Nayak et al. (2010)
TAA60	LG2	0.24	8	0.81	0.83	Winter et al. (1999)
TA113	LG1	0.48	3	0.49	0.58	Choudhary et al. (2012)
GA6	LG8	0.23	15	0.86	0.87	Choudhary et al. (2012)
H1B04	LG2	0.36	5	0.68	0.73	Choudhary et al. (2012)
NCPGR40	LG3	0.41	9	0.71	0.75	Choudhary et al. (2012)
NCPGR12	LG2	0.27	8	0.78	0.81	Choudhary et al. (2012)
TA64	LG3	0.44	5	0.62	0.68	Winter et al. (1999)
NCPGR274	LG6	0.69	3	0.37	0.45	Gaur et al. (2011)
CaINDEL8	LG4	0.51	3	0.53	0.61	Newly synthesized
NCPGR46	LG6	0.45	4	0.58	0.65	Sethy et al. (2006)
NCPGR232	LG5	0.34	10	0.75	0.78	Gaur et al. (2011)
NCPGR93	LG6	0.45	5	0.58	0.65	Choudhary et al. (2012)
NCPGR156	LG6	0.44	4	0.58	0.65	Choudhary et al. (2012)
CaINDEL6	LG4	0.49	3	0.50	0.59	Newly synthesized
NCPGR155	LG6	0.58	6	0.56	0.60	Choudhary et al. (2012)
H5G12	LG7	0.55	4	0.45	0.54	Choudhary et al. (2012)
STMS25	LG7	0.55	2	0.37	0.49	Winter et al. (2000)
CESSR43	LG4	0.68	3	0.42	0.48	Choudhary et al. (2012)
TA140	LG5	0.59	2	0.37	0.48	Winter et al. (1999)
NCPGR33	LG1	0.71	3	0.38	0.44	Sethy et al. (2006)
CaINDEL30	LG4	0.54	3	0.42	0.52	Newly synthesized
STMS7	LG5	0.56	5	0.55	0.61	Winter et al. (2000)
NCPGR225	LG3	0.58	3	0.40	0.50	Choudhary et al. (2012)
GA26	LG6	0.53	2	0.37	0.50	Choudhary et al. (2012)
CaINDEL15	LG4	0.58	3	0.40	0.50	Newly synthesized
CESSR139	LG3	0.32	4	0.69	0.74	Choudhary et al. (2012)
H2B061	LG2	0.32	5	0.70	0.74	Choudhary et al. (2012)
CESSR433	LG3	0.31	8	0.76	0.79	Choudhary et al. (2012)
NCPGR193	LG2	0.52	3	0.43	0.54	Choudhary et al. (2012)
NCPGR110	LG5	0.29	6	0.73	0.77	Choudhary et al. (2012)
NCPGR13	LG2	0.23	8	0.84	0.85	Choudhary et al. (2012)
CESSR105	LG3	0.51	2	0.37	0.50	Choudhary et al. (2012)
TR7	LG4	0.61	3	0.38	0.48	Winter et al. (1999)
CESSR131	LG3	0.67	3	0.36	0.45	Choudhary et al. (2012)
NCPGR255	LG7	0.59	3	0.38	0.49	Choudhary et al. (2012)
NCPGR206	LG6	0.52	3	0.39	0.51	Choudhary et al. (2012)
NCPGR267	LG6	0.83	3	0.25	0.28	Choudhary et al. (2012)
CaINDEL2	LG1	0.53	4	0.40	0.52	Newly synthesized
TA110	LG2	0.48	4	0.56	0.63	Winter et al. (2000)
TA18	LG7	0.50	3	0.49	0.58	Winter et al. (1999)
TA180	LG7	0.53	3	0.39	0.51	Winter et al. (1999)
CakTpSSR03923	LG7	0.60	4	0.39	0.49	-

Cont...

Marker	Linkage Group	Major Allele Frequency	Number of alleles	PIC	Gene diversity	Reference
NCPGR41	LG1	0.63	5	0.40	0.49	Choudhary et al.(2012)
CESSR47	LG4	0.71	3	0.34	0.42	Choudhary et al.(2012)
NCPGR43	LG3	0.27	6	0.76	0.79	Choudhary et al.(2012)
STMS4	LG3	0.45	4	0.58	0.65	Winter et al. (2000)
ICCM0159	LG3	0.47	5	0.58	0.65	Nayak et al.(2010)
NCPGR150	LG4	0.54	4	0.52	0.59	Choudhary et al.(2012)
NCPGR254	LG5	0.48	8	0.66	0.70	Choudhary et al.(2012)
NCPGR226	LG4	0.65	3	0.37	0.46	Choudhary et al.(2012)
TA46	LG4	0.46	4	0.57	0.64	Winter et al. (2000)
CESSR66	LG4	0.44	4	0.59	0.66	Choudhary et al.(2012)
ICCeM041	LG5	0.41	5	0.58	0.65	Varshney (2009)
NCPGR229	LG6	0.32	7	0.75	0.78	Choudhary et al.(2012)
STMS6	LG7	0.29	6	0.76	0.79	Winter et al. (2000)
CaINDEL10	LG8	0.53	4	0.54	0.60	Newly synthesized
ICCM0205	LG5	0.63	7	0.56	0.58	Nayak et al.(2010)
NCPGR249	LG7	0.40	5	0.68	0.72	Gaur et al. (2011)
H2B19	LG7	0.40	3	0.58	0.66	Lichtenzveig et al. (2005)
TA3	LG8	0.35	6	0.71	0.75	Winter et al. (1999)
ICCeM028	LG3	0.40	4	0.60	0.67	Gujaria et al.(2011)
CESSR50	LG4	0.40	3	0.59	0.66	Choudhary et al.(2012)
TAA58	LG7	0.38	3	0.58	0.66	Winter et al. (1999)
CESSR141	LG3	0.35	7	0.73	0.77	Choudhary et al.(2012)
STMS2	LG4	0.28	7	0.78	0.81	Winter et al.(1999)
GA34	LG6	0.39	5	0.61	0.67	Winter et al. (1999)
ICCeM015	LG2	0.39	4	0.60	0.67	Gujaria et al.(2011)
TS57	LG5	0.35	6	0.65	0.71	Choudhary et al.(2012)
NCPGR72	LG1	0.38	3	0.59	0.66	Choudhary et al.(2012)
TA203	LG1	0.41	4	0.58	0.65	Choudhary et al.(2012)
CaINDEL4	LG4	0.42	3	0.58	0.66	Newly synthesized
TR43	LG1	0.35	3	0.59	0.67	Winter et al. (1999)
CESSR20	LG3	0.44	6	0.63	0.69	Choudhary et al.(2012)
H4F09	LG8	0.47	3	0.56	0.63	Choudhary et al.(2012)
TA1	LG1	0.47	6	0.69	0.72	Winter et al. (2000)
CaINDEL32	LG4	0.38	3	0.59	0.66	Newly synthesized
CaINDEL16	LG4	0.36	3	0.59	0.66	Newly synthesized
TA59	LG2	0.38	3	0.59	0.66	Winter *et al*. (1999)
CESSR103	LG3	0.46	4	0.58	0.65	Choudhary *et al.*(2012)
GA11	LG1	0.47	3	0.57	0.64	Winter *et al*. (1999)
Mean		0.47	4.8	0.56	0.63	