



Yield plasticity and molecular diversity analysis in chickpea (*Cicer arietinum*)

SUPRIYA SACHDEVA¹, C. BHARADWAJ², SARVJEET SINGH³, MANISH ROORKIWAL⁴, VINAY SHARMA⁵, ANUPAM SINGH⁶ and RAJEEV VARSHNEY⁷

ICAR-Indian Agricultural Research Institute, Pusa, New Delhi 110 012, India

Received: 13 October 2018 ; Accepted: 3 December 2018

ABSTRACT

Genetic diversity among 40 chickpea (*Cicer arietinum* L.) genotypes was investigated using 125 microsatellite (SSR, simple sequence repeat) markers. Twenty five polymorphic markers with average genetic diversity and PIC (Polymorphic Information Content) value of 0.489 and 0.437, respectively, generated a total of 90 alleles. High PIC and gene diversity (H_E) values indicated good variability amongst the chickpea genotypes. Sequential Agglomerative Hierarchical Non-overlapping (SAHN) grouping revealed two main clusters with 29 genotypes in cluster I and 11 genotypes in cluster II. The Cluster analysis did not follow geographical diversity rather it was in agreement for genetic diversity with respect to seed type and parentage/pedigree. Grouping clearly delineated the diverse *kabuli* and *desi* genotypes. Molecular variance analysis also indicated 97% variation within the populations and 3% variation among the populations. Principal coordinate analysis (PCoA) divided all the 40 genotypes into three populations based on their seed type and pedigree. The 2D plot largely supported the dendrogram with similar pattern of clustering. It also indicated that the material used was diverse. Thus, the study proved that SSR markers are informative tools for assessing genetic diversity and can be recommended for characterization studies in chickpea.

Key words: Chickpea, Genetic diversity, Microsatellite markers, Molecular variance, Principal coordinate analysis, SAHN grouping.

Chickpea (*Cicer arietinum* L.) is a highly proteinaceous legume grown all over the world (Patil *et al.* 2017) and India produces 68% of total world production of around 8.88 mt in an area of 9.21 mha (Kumar *et al.* 2017). Low genetic diversity in the cultivated chickpea is one of the causes for narrow genetic base leading to lower yield gains in chickpea (Bharadwaj *et al.* 2011). Drought and heat both limit chickpea production resulting in 40–60% average yield losses globally (Sachdeva *et al.* 2017). Identification of divergent *kabuli* and *desi* pools and crossing between them has been suggested as a way for developing chickpea with broader genetic base by Santosh *et al.* 2017.

Molecular markers being stable and informative have been used for characterization of crop plants diversity (Bharadwaj *et al.* 2010, Satyavathi *et al.* 2005). These

are vital for marker assisted breeding programs targeting chickpea yield enhancement (Varshney *et al.* 2013a, Yadav *et al.* 2011, Bharadwaj *et al.* 2010) and to provide information on allelic variation in the breeding material (Jain *et al.* 2014). Microsatellites being abundant, highly polymorphic, co-dominant, multi-allelic and uniform in distribution across the genome are considered important in plant breeding (Cuevas and Prom 2013). Unique allelic profiles generated using the scored loci differentiates the genotypes based on the genetic data (Konsam *et al.* 2014). Thus, SSR markers were used in this study to understand the relationships among chickpea genotypes and assess the extent of genetic variability for use in future breeding programs.

MATERIALS AND METHODS

Forty chickpea genotypes including both *desi* and *kabuli* genotypes were selected from the training population set (Table 1). The material was obtained from Pulse Research Laboratory, Indian Agricultural Research Institute, New Delhi and sown under controlled conditions in plastic pots of 13 cm diameter at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi, temperature was maintained at 24°C during day and 18°C in the night. Yield traits, viz. seeds harvested from each plant, seed weight and yield of the plant were recorded for all the 40 genotypes.

¹Research fellow (supriyasachdeva19@gmail.com),

²Principal Scientist (chbharadwaj@yahoo.co.in), ⁶Research Fellow (mail: anupambiotech@gmail.com), Division of Genetics ICAR-IARI. ³Principal Scientist (sarvjeet1962@gmail.com) Division of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab. ⁴Research Scientist (M.Roorkiwal@cgiar.org), ⁷Programme Director (R.K.Varshney@cgiar.org) Genomics Lab, International Crops Research Institute for the Semi Arid Tropics, Patancheru, Hyderabad, Telangana. ⁵Ex-Head (vinaysharma30@yahoo.co.uk) Amity University, Rajasthan, Jaipur.

Table 1 Genotypes used in the study

Genotype	Biological status	Pedigree	Source	Seed type
ICCV 09313	Released variety	ICCV 92311 × ICC 14198	ICRISAT, India	Kabuli
ICCV10313	Released variety	ICCV 92337 × ICC 14194	ICRISAT, India	Kabuli
ICCV08310	Released variety	ICCV 95311 × ICC 17109	ICRISAT, India	Kabuli
ICCV097309	Released variety	(ICC 2588 × ICC 32) × [(ICCV 49 × ICC 15980) × ICCV 3]	ICRISAT, India	Kabuli
ICCV03311	Released variety	ICCV 92328 × [(ICCV 32 × ICC 12034) × ICC 19686]	ICRISAT, India	Kabuli
ICCV01309	Released variety	(ICC 4973 × ICC 14196) × ICCV 92329	ICRISAT, India	Kabuli
ICCV09312	Released variety	ICCV 92337 × ICC 7344	ICRISAT, India	Kabuli
ICCV9314	Released variety	ICCV 92311 × ICC 17109	ICRISAT, India	Kabuli
ICCV10304	Released variety	ICCV 92311 × ICC 14215	ICRISAT, India	Kabuli
ICCV10307	Released variety	ICCV 92311 × ICC 17109	ICRISAT, India	Kabuli
ICCV10306	Released variety	ICCV 92311 × ICC 17109	ICRISAT, India	Kabuli
ICCV10316	Released variety	ICCV 92337 × ICC 17109	ICRISAT, India	Kabuli
ICCV92337	Released variety	(ICCV 2 × ICC 12034) × ICC 7344	ICRISAT, India	Kabuli
ICCV00109	Released variety	ICC 18746 × ICCV 10	ICRISAT, India	Desi
ICCV03103	Released variety	[ICCV 92014 × JG 23] × BG 1032]	ICRISAT, India	Kabuli
ICCV09307	Released variety	ICCV 92337 × ICC 17109	ICRISAT, India	Kabuli
ICCV95423	Released variety	(ICC 7676 × ICC 32) × [(ICCV 49 × ICC 15980) × ICCV 3]	ICRISAT, India	Kabuli
ICCV97404	Released variety	(ICCV 32 × ICC 4967) × [(ICCV 49 × ICC 15980) × ICCV 3]	ICRISAT, India	Kabuli
ICCV10	Released variety	ICC 1376 × ICC 1443	ICRISAT, India	Desi
ICC1882	Genetic stock	Traditional landrace P1506-4 from ICRISAT	ICRISAT, India	Desi
BGD72	Released variety	P1231 × P1265	IARI, New Delhi	Kabuli
PUSA-1103	Released variety	(Pusa 256 × <i>Cicer reticulatum</i>) × Pusa 362	IARI, New Delhi	Desi
ICC4958	Genetic stock	GW 5/7, a drought tolerant breeding line from ICRISAT	ICRISAT, India	Desi
ICCV00301	Released variety	ICCV 92502 × ICCV 2	ICRISAT, India	Kabuli
ICCV00302	Released variety	FLIP 91-18C × ICCV 2	ICRISAT, India	Kabuli
ICCV01301	Released variety	GNG 1044 × (ICCV 32 × ICC 12034)	ICRISAT, India	Kabuli
L-550	Landrace	PBG7 × Rabat	PAU, Ludhiana	Kabuli
ICCV03403	Released variety	(ICC 4973 × ICC 14196) × ICCV 92329	ICRISAT, India	Kabuli
C-235	Released variety	IP 58 × C1234		Desi
ICCV03404	Released variety	(ICC 4973 × ICC 14196) × ICCV 92329	ICRISAT, India	Kabuli
ICCV03310	Released variety	BG 70 × ICCV 92329	ICRISAT, India	Kabuli
ICCV07301	Released variety	ICCV 95334 × (ICCV 2 × ICCV 98506)	ICRISAT, India	Kabuli
ICCV05312	Released variety	ICCV 2 × ICCV 92325	ICRISAT, India	Kabuli
ICCV5308	Released variety	ICCV 2 × ICCV 92311	ICRISAT, India	Kabuli
ICCV5313	Released variety	ICCV 2 × ICCV 92325	ICRISAT, India	Kabuli
ICCV4310	Released variety	(ICC 4973 × ICC 14196) × ICCV 92329	ICRISAT, India	Kabuli
PUSA-1003	Released variety	Mutant of L532	IARI, New Delhi	Kabuli
CSG8962	Released variety	Selection from GPF 7035	CSSRI, Karnal	Desi
ICCV4303	Released variety	(ICC 4973 × ICC 14196) × ICCV 92329	ICRISAT, India	Kabuli
ICCV2	Released variety	[(ICC 5003 × ICC 4953) × ICC 583] × (ICC 4973 × ICC 7347)	ICRISAT, India	Kabuli

Fresh young chickpea leaves (2 g) were used for genomic DNA isolation (Tapan *et al.* 2014). Quality and quantity of DNA was checked using 1% agarose (Sambrook *et al.* 2001) spectrophotometrically (Thermo Scientific, USA). A total of 125 SSR markers (Sigma-Aldrich) were used for genetic diversity analysis; only 25 markers were found to be polymorphic. Amplification was done in a 10 µl volume reaction master mix using a Veriti Thermal Cycler PCR (*Applied Biosystems*, Foster City, CA, USA). The PCR mix consisted of 1 µl of 20 ng genomic DNA, 1.6 µl of 10X TBE buffer, 1 µl of 10mM of dNTP mix, 1 µl each of forward and reverse primer and 0.3 µl of 3 U/µl Taq polymerase (Sigma-Aldrich) and the amplification was done as per PCR conditions described by Bhardwaj *et al.* (2011).

The amplicons were analyzed on 3% agarose gel containing EtBr (10 mg/ml) at a constant voltage of 120V for 3 h using horizontal gel electrophoresis system (Biorad, USA) in 1X TBE buffer. A 100bp DNA ladder (Thermo Scientific, USA) was used as standard to determine the approximate band size of the amplicons. Gel pictures were taken under UV light gel documentation system (UVITECH Imaging System, UK) and phenogram was generated based on Jaccard's coefficients (Jaccard 1908) by SAHN grouping method (Sokal and Sneath 1963) using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) program Version 2.1 (Rohlf 2000). Power Marker version 3.0 (Liu and Muse 2005) was used to assess diversity indices, viz. alleles generated, PIC values, gene diversity (H_E) and major allele frequency (Table 2).

Molecular variance was estimated in GenAlEx 6.5 software. The three broad populations based on seed type, viz. *kabuli* genotypes, *desi* genotypes, and *desi* genotypes of ICRISAT origin were subjected to principal coordinates analysis (PCoA). Distance matrix method based principal coordinates analysis (PCoA) was also done.

RESULTS AND DISCUSSION

Narrow genetic variability in chickpea has been a serious constraint in advancement for genetic improvement (Konsam *et al.* 2014). The repeated use of fewer numbers of elite lines with narrow genetic base for developing new breeding lines could have been one of the major reasons for this narrow genetic variability (Bharadwaj *et al.* 2011). Greater genetic diversity that can be obtained through the use of wider germplasm and use of wild relatives has been reported by many chickpea breeders (Bharadwaj *et al.* 2011). In this study, 125 SSR markers distributed all over the genome were used to characterize the chickpea genotypes and assess their genetic diversity. Among 125 SSR markers, only 25 were polymorphic and produced 3.6 alleles per locus on an average. PIC values and diversity indices were calculated for each SSR marker (Table 2). Maximum alleles (8) were detected for the locus TA136 while minimum (2) for GAA47. Thirteen highly informative polymorphic loci with high PIC values not less than 0.4 were generated and the highest PIC (0.7825)

was found for the primer TA136. Bharadwaj *et al.* (2010) also reported high PIC values in SSR analysis due to polymorphism of TAA motifs in chickpea with maximum DI (Diversity Index) and PIC value for TA136 (Udupa *et al.* 1999). This study could identify SSR markers that can be recommended for diversity analysis in chickpea. High DI and PIC values indicate greater amount of genetic variability at molecular level in chickpea lines analyzed and also the suitability of such SSR markers for characterization studies by many workers (Bharadwaj *et al.* 2010, Satyavathi *et al.* 2005). The most diverse genotypes were ICCV9314 and Pusa1103, with a similarity coefficient of 0.163. Such distant lines when crossed are expected to produce higher variability.

AMOVA indicated that 97% of the variations were within the populations while the remaining 3% variations were among the populations. The analysis of variance revealed significant differences in all the genotypes studied (Table 3), indicating existence of sufficient diversity among them which can be utilized to combine the desirable characters through *desi-kabuli* introgression breeding.

There was a wide variation among the genotypes for protein content and yield traits indicating the soundness of the material for diversity studies and using in crossing program (Table 4); these were further grouped on the basis of seed type, viz. *desi* and *kabuli*. These two gene pools represent diverse yet easily crossable lines having higher variation for yield traits, plant type, quality characters and tolerance to various stresses. The *desi* pool in this study showed higher leaf protein, higher yield in comparison to the *kabuli* pool with higher seed size (Table 5). The plant yield ranged from 4.2–46.10 g with an average value of 16.56 g. The mean 100-seed weight was found to be 28.74 g ranging from 11.19–41.76g.

The first and the second component of principal coordinate analysis (PCoA) accounted for 76.14% and 23.86% of the total variations, respectively, indicating the soundness of the biplot generated (Table 6). The PCoA biplot illustrates the differentiation among accessions (Fig 1). The chickpea genotypes from the different sources grouped into three populations may be due to different ancestors or different evolutionary processes or genetic exchanges representing three different gene pools. *Kabuli* and *desi* genotypes distinctly grouped indicating that they were diverse. Furthermore, the ICRISAT *desi* accessions within *desi* group sub-grouped themselves. This was in conformity with dendrogram generated by SAHN grouping wherein all the *desi* genotypes developed by ICRISAT were grouped at closer genetic distance to that of ICRISAT developed *kabuli* lines. The *kabuli* genotypes in cluster I and *desi* genotypes in cluster II represented the populations formed in PCoA. The PCoA done provides an insight into overall diversity unlike tree methods which tend to concentrate more on individual relations. The *kabuli* genotypes grouped in first lower quarter while the *desi* in the second quarter. Further, the *desi* genotypes developed by ICRISAT which were sourced from the training population grouped midway. Wider

Table 2 SSR markers used for diversity studies

Primer name		Primer sequence	No. of alleles produced	Major allele frequency	Gene diversity	PIC
TR43	F	AGGACGAAACTATTCAAGGTAAGTAGA	3	0.7	0.465	0.4199
	R	AATTGAGATGGTATTAATGGATAACG				
TA25	F	AGTTTAATTGGCTGGTTCTAAGATAAC	3	0.7692	0.3787	0.3434
	R	AGGATGATCTTTAATAAATCAGAATGA				
NC81	F	CCGAATGTCCATAAATCAAT	3	0.8875	0.2059	0.1958
	R	TGTTTACTGGGATAACTCC				
GAA47	F	CACTCCTCATGCCAACTCCT	2	0.5897	0.4839	0.3668
	R	AAAATGGAATAGTCGTATGGGG				
NCPGR69	F	GACCGAATGTCCATAAATCA	3	0.9125	0.1628	0.1553
	R	GGAGCTGGAAAACTACAGC				
NCPGR91	F	ATTGAATCCTTTCTGAACCG	2	0.7125	0.4097	0.3258
	R	CTGTTCTCTTTCTCCTCCG				
GA6	F	ATTTTCTCCGGTGTTCAC	3	0.6282	0.5322	0.4724
	R	AAACGACAGAGAGTGGCGAT				
NCPGR147	F	TGTATGAAAACACTTTGACTCATT	4	0.45	0.6747	0.617
	R	CGATGATATTCTCAGCGAAC				
TS29	F	AACATTCATGAACCTACCTCAACTTA	4	0.6375	0.5122	0.4451
	R	CCATATGAGTACACTACCTCTCGG				
TR31	F	CTTAATCGCACATTTACTCTAAAATCA	3	0.6081	0.4869	0.3808
	R	ATCCATTAACACGGTTACCTATAAT				
CaM1903	F	TGTGATGCAACCTAACAGTCA	4	0.4865	0.6015	0.5224
	R	CCATGTACACTTACACGGTAGAAGA				
CaM1502	F	TCAGAATGTCAAATTCAATTGTTG	3	0.6	0.515	0.4244
	R	TTGACTGCCACCAGTTACCA				
TA130	F	TCTTTCTTTGCTTCCAATGT	3	0.7436	0.3932	0.3335
	R	GTAATCCACGAGAAATCAA				
NCPGR74	F	TCCGTCACACATTTCTACT	5	0.4459	0.7199	0.6829
	R	CTTTTAGTTGGTCGAAGCC				
NCPGR103	F	ACAACCATATACTTTTGGCG	3	0.6579	0.5083	0.4557
	R	TTAGATGAAAAACGGGAGAA				
NCPGR77	F	TGGACTAACAAATACGACGA	3	0.7222	0.4398	0.3988
	R	AGGCCACCCTAAATTTTATT				
NCPGR107	F	AAACTCAATATTGCCCTTCA	3	0.725	0.4363	0.3955
	R	CCATAACTGGATTGAGCTTT				
NCPGR130	F	GATACTGGTGGAAAAATGGA	3	0.8125	0.3184	0.2901
	R	CAAGCTCTTTCAGAATTTGC				
NCPGR138	F	ATTCCAAATTGCTGTTGTTG	3	0.75	0.4013	0.3601
	R	TGTGGATTTAGTTGCAATG				
TA8	F	AAAATTTGCACCCACAAAATATG	3	0.7	0.4638	0.4175
	R	CTGAAAATTATGGCAGGGAAAC				
TR58	F	CTCTATATTGTTTGTTCGTTTTG	6	0.325	0.7616	0.7232
	R	TAAAATGTGTAGGGTGCAGAATAAATA				
TA136	F	AGATCATTGCAGAGAGTAATATTGGTT	8	0.3125	0.8075	0.7825
	R	TGCTGTGTGACCTATAACAATAAAAA				
H3A10	F	TTTAAGGCTTCAGGTATTGATTCT	7	0.5541	0.6377	0.6021
	R	TCACACATGCCAACTTAAAAATAAAA				
GAA50	F	TTCGTTCCCATCAACATTCA	3	0.7821	0.3646	0.3351
	R	CCCTCCCGTATTCATACCAA				
NCPGR99	F	ATCATGAAGCAAATCCTCAC	3	0.6154	0.547	0.488

Table 3 Analysis of molecular variance (AMOVA) for variation among and within forty genotypes of chickpea

Source of variation	Df	SS	MS	Estimated variance	%
Among pops	2	36.064	18.032**	0.458	3%
Within pops	37	565.161	15.275**	15.275	97%
Total	39	601.225		15.732	100%

**Significant at 1%, Df-degree of freedom, SS-sum of square, MS-mean of square.

genetic variability between *kabuli* and *desi* genotypes for base broadening and greater enhancement of productivity by crossing *desi* and *kabuli* lines was reported by Santosh *et al.* (2017).

SAHN clustering grouped the chickpea genotypes into two major clusters (Table 7, Fig 2). Out of two clusters, the larger cluster I comprised of 29 genotypes whereas cluster II comprised of 11 genotypes. The cluster I consisted of all genotypes which were *kabuli* type irrespective of their source of breeding. Contrastingly the cluster II was of

Table 4 Mean performance of chickpea genotypes for yield traits and soluble protein (leaf)

Genotypes	Protein content	Seeds/pod	100-seed weight (g)	Plant yield (g)
ICC1882	25.78±0.40	1.00±0	17.65 ± 0.27	22.2± 1.45
ICC4958	33.95±0.11	1.00±0	28.82 ± 0.35	18.44 ± 1.07
Pusa1103	30.64±0.61	1.18±0	21.97 ± 0.38	24.68 ± 0.99
BGD72	31.83±0.59	1.00±0	16.39 ± 0.19	46.10 ± 2.12
Pusa1003	24.71±0.36	1.02±0	16.58 ± 0.34	14.60 ± 1.40
CSG8962	33.60±0.17	1.03±0	11.19 ± 0.24	24.53 ± 2.29
C235	28.65±0.29	1.01±0	14.22 ± 0.19	14.20 ± 1.32
ICCV3310	31.46±0.12	1.00±0	33.17 ± 0.49	11.67 ± 1.26
ICCV3311	30.49±0.17	1.11±0	30.59 ± 0.37	11.50 ± 0.68
ICCV3403	30.53±0.08	1.03±0	30.93 ± 0.27	13.91 ± 1.36
ICCV3404	28.43±0.17	1.02±0	38.71 ± 0.34	16.87 ± 0.46
ICCV7301	29.47±0.11	1.04±0	37.29 ± 0.24	15.50 ± 1.40
ICCV4303	30.67±0.21	1.07±0	35.95 ± 0.26	12.71 ± 0.66
ICCV4310	29.76±0.21	1.06±0	33.61 ± 0.33	12.99 ± 1.36
ICCV5312	29.54±0.50	1.05±0	35.71 ± 0.27	4.20 ± 1.76
ICCV9312	29.86±0.11	1.05±0	37.29 ± 0.21	11.90 ± 2.13
ICCV9313	30.35±0.16	1.12±0	39.24 ± 0.19	7.06 ± 0.53
ICCV9314	28.95±0.21	1.14±0	36.45 ± 0.29	18.78 ± 2.41
ICCV10313	28.49±0.13	1.07±0	37.55 ± 0.24	36.50 ± 1.40
ICCV10	32.39±0.28	1.15±0	19.61 ± 0.15	15.47 ± 1.88
ICCV2	28.01±0.48	1.08±0	21.92 ± 0.36	17.68 ± 1.95
ICCV92337	30.31±0.09	1.09±0	30.93 ± 0.26	8.72 ± 1.39
ICCV8310	30.49±0.20	1.07±0	30.22 ± 0.34	8.73 ± 2.06
ICCV97309	32.10±0.33	1.23±0	24.66 ± 0.17	14.05± 1.17
ICCV1309	30.33±0.17	1.18±0	30.97 ± 0.23	14.28 ± 1.68
ICCV10304	29.86±0.09	1.21±0	22.68 ± 0.32	07.80± 0.26
ICCV10307	30.59±0.13	1.06±0	35.24 ± 0.25	8.19 ± 1.10
ICCV10306	31.20±0.24	1.24±0	35.53 ± 0.33	9.84 ± 2.09
ICCV10316	29.78±0.30	1.17±0	41.76 ± 0.33	13.65 ± 1.31
ICCV00109	29.71±0.10	1.18±0	20.87 ± 0.41	15.33 ± 1.35
ICCV3103	30.34±0.07	1.09±0	25.42 ± 0.40	10.57± 1.57
ICCV9307	31.27±0.35	1.07±0	38.94 ± 0.29	11.98 ± 2.08
ICCV95423	26.00±0.30	1.05±0	27.37 ± 0.35	41.36 ± 2.33
ICCV97404	29.62±0.21	1.11±0	25.46 ± 0.39	25.2 ± 3.67

Contd.

Table 4 (Concluded)

Genotypes	Protein content	Seeds/pod	100-seed weight (g)	Plant yield (g)
ICCV0301	30.68±0.27	1±0	17.95 ± 0.25	12.45± 2.45
ICCV0302	30.022±0.05	1±0	31.16 ± 0.48	12.52 ± 3.28
ICCV1301	29.39±0.20	1±0	26.54 ± 0.29	11.16 ± 2.46
L550	29.10±0.22	1.06±0	17.73 ± 0.28	16.70 ± 2.55
ICCV5308	30.04±0.60	1.04±0	37.66 ± 0.49	28.80 ± 2.43
ICCV5313	30.98±0.11	1.19±0	33.72 ± 0.29	19.45 ± 2.29
Mean	29.98	1.08	28.74	16.55
Max.	33.95	1.24	41.76	46.10
Min.	24.71	1.00	11.19	4.20
CV	0.007	1.20	1.06	1.01

Table 5 Differences in desi and kabuli genotypes of chickpea for yield related traits and soluble protein content (leaf)

Trait	Desi	Kabuli
Leaf protein	28.14	26.12
SPP	1.08	2.28
100 SW	19.49	30.7
Plant yield	23.82	15.02
SPPL	144.33	56.59

SPP- seeds/pod

Principal Coordinates (PCoA)

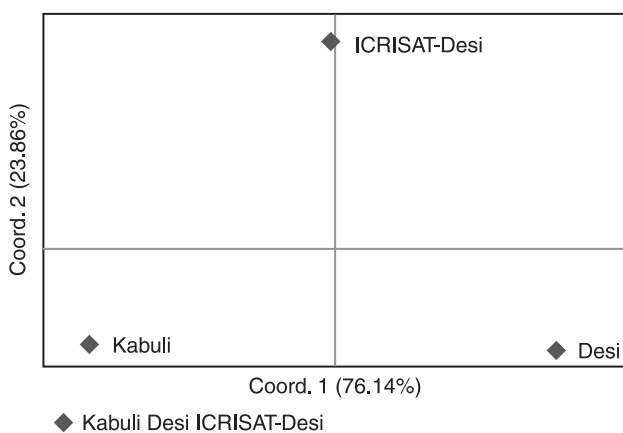


Fig 1 Principal coordinate analysis based on a squared Euclidean distance matrix between the individuals.

desi seed type having brown seed coat with smaller seed and owl head seed shape. Such distinctive separation was also reported by Konsam *et al.* (2014) who also correlated it to the probable differences in non-reducing raffinose family sugar differences. Bharadwaj *et al.* (2011) while studying phylogeny in a geographical collection of chickpea genotypes reported a similar distinction between desi and kabuli genotypes using molecular markers. Choumane *et al.* (2000) inferred that while using microsatellite markers for diversity analysis, close relatives and lines derived from the same cross tend to group together. The microsatellite flanking sequences in these lines are relatively conserved

Table 6 Percentage of variation explained by the first 3 axes

Axis	1	2	3
%	76.14	23.86	0.00
Cum %	76.14	100.00	100.00

Table 7 Clustering analysis of chickpea genotypes based on SAHN grouping

Major cluster	No. of genotypes	Minor cluster	Minor subgroup	Genotypes
I	28	I-A		ICCV10313, ICCV3311, ICCV5313, L550, ICCV1301, ICCV9307, ICCV3404, ICCV3103, ICCV97309, ICCV5312, ICCV10316, ICCV4303, ICCV3310, ICCV92337, ICCV9313, ICCV95423, ICCV1309, ICCV97404, ICCV4310, ICCV9312, ICCV10304, ICCV7301, ICCV8310, ICCV9314, ICCV10307, ICCV10306, ICCV3403, P1003
	1	I-B		C235
II	3	II-A	(i)	ICCV5308, ICCV2, ICCV0302
	2		(ii)	ICCV0301, ICCV00109
	2	II-B	(i)	ICC1882, ICCV10
	4		(i)	ICC4958, BGD72, CSG8962, P1103

and generally exist in same sequence, hence getting similar genotyping scores. Thus, these lines which may be phenotypically different are actually at molecular level very close and if selected in breeding program, tend to further narrow down the genetic base of chickpea. The grouping based on SSR data, SAHN grouping is in congruence with the PCoA 2D plot delineating kabuli and desi genotypes

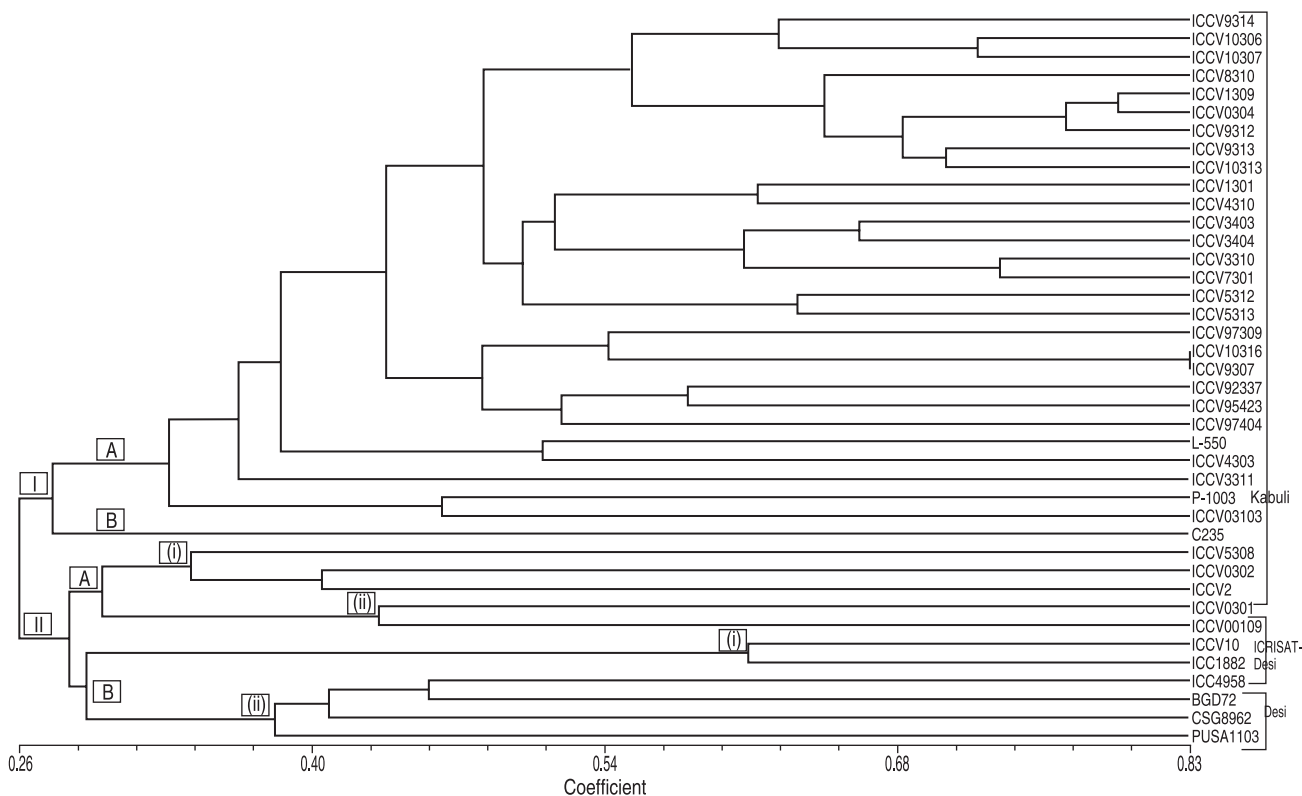


Fig 2 SAHN grouping based on molecular data showing genetic relatedness among the forty chickpea genotypes.

with minor deviations and indicated that the chickpea genotypes grouped in different clusters and there was significant diversity among them.

Breeders generally tend to concentrate more on phenotypic performance and variability which is generally due to genotype and environment interaction ($G \times E$ interaction). The various biometric tools help to evaluate only these $G \times E$ interactions, genotype effects and environmental effects. Though such genotypes appear phenotypically diverse, being similar at molecular level they do not help to broaden the genetic base and achieve greater yield gains (Glaszmann *et al.* 2010). The next step perhaps would be to identifying trait specific diverse germplasm and association of markers with trait of interest (Upadhyaya *et al.* 2011). Advances in the molecular tools available in this orphan semi-arid legume and subsequent sequencing of chickpea genome would greatly aid in the genomics assisted breeding of chickpea (Varshney *et al.* 2013b). This study would pave way for breeders to select superior parents for breeding program. It further indicates that SSR markers are good indicators of genetic divergence and the diverse chickpea genotypes identified could serve as important sources for enhancing the genetic potential of chickpea.

ACKNOWLEDGEMENTS

The authors acknowledge ICAR-IARI, Incentivizing Research on Agriculture (Component IV: Chickpea) Project funded by ICAR and AISRF, Department of Biotechnology for the financial, field and lab support.

REFERENCES

- Bharadwaj C, Chauhan S K, Rajguru G, Srivastava R, Satyavathi C T and Yadav S. 2010. Diversity analysis of chickpea (*Cicer arietinum* L.) using STMS markers. *Indian Journal Agricultural Science* **80**: 947–51.
- Bharadwaj C, Srivastava R, Chauhan S K, Satyavathi C T, Kumar J, Farqui A, Yadav S, Rizvi A H and Kumar T. 2011. Molecular diversity and phylogeny in geographical collection of chickpea (*Cicer* sp.) genotypes. *Journal of Genetics* **90**: e94–e100.
- Choumane W, Winter P, Weigand F and Kahl G. 2000. Conservation and variability of sequence tagged microsatellite sites (STMSs) from chickpea (*Cicer arietinum* L.) within the genus *Cicer*. *Theoretical and Applied Genetics* **101**: 269–78.
- Cuevas H E and Prom L K. 2013. Assessment of molecular diversity and population structure of the Ethiopian sorghum [*Sorghum bicolor* (L.) Moench] germplasm collection maintained by the USDA-ARS National Plant Germplasm System using SSR markers. *Genetic Resources and Crop Evolution* **60**: 1817–30.
- Glaszmann J C, Kilian B, Upadhyaya H D and Varshney R K. 2010. Accessing genetic diversity for crop improvement. *Current Opinion in Plant Biology* **13**: 1–7.
- Jaccard P. 1908. Nouvelles recherches sur la distribution florale. *Bulletin de la Societe Vaudoise des Sciences Naturelles* **44**: 223–70.
- Jaganathan D, Thudi M, Kale S, Azam S, Roorkiwal M, Gaur P M, Kishor P B K, Nguyen H, Sutton T and Varshney R K. 2015. Genotyping-by-sequencing based intra-specific genetic map refines a “QTL-hotspot” region for drought tolerance in chickpea. *Molecular Genetics and Genomics* **290**: 559–71.
- Jain M, Mohrana K C, Shankar R, Kumari R and Garg R. 2014. Genome-wide discovery of DNA polymorphisms in rice genotypes with contrasting drought and salinity stress response

- and their functional relevance. *Plant Biotechnology Journal* **12**: 253–64.
- Jiang G L. 2013. *Molecular markers and marker-assisted breeding in plants. Agricultural and Biological Sciences: Plant Breeding from Laboratories to Fields*, 1st edn. Andersen S B (Ed). Brookings, USA.
- Konsam S, Bharadwaj C, Ram G, Satyavathi C T and Jain P K. 2014. Molecular diversity of chickpea (*Cicerarietinum L.*) genotypes differing in their Raffinose family oligosaccharides, viz. raffinose and stachyose content as revealed through SSR markers. *Australian Journal of Crop Science* **8**: 1175–85.
- Kumar N, Bharadwaj C, Satyavathi C T, Pal M, Kumar T, Singhal T, Sachdeva S, Jain P K, Patil B S and Soren K R. 2017. Morpho-physiological characterization and grouping (SAHN) of chickpea genotypes for salinity tolerance. *Vegetos* **30**: 117–24.
- Kumar T, Bharadwaj C, Satyavathi C T and Jain P K. 2014. A high throughput, improved rapid and reliable genomic DNA extraction protocol from Chickpea (*Cicer arietinum L.*). *Vegetos* **26**: 185–90.
- Liu K and Muse S V. 2005. Power marker: an integral analysis environment for genetic marker analysis. *Bioinformatics* **21**: 2128–9.
- Patil B S, Desai P B, Vijayakumar G, Basavarajappa M P, Subramanya A E S, Bharadwaj C and Kulkarni V R. 2017. Dry root rot resistance in chickpea: Studies on genetic and molecular variations. *Vegetos (Special)* **30**: 23–8.
- Rohlf F J. 2000. NTSYS-PC. *Numerical Taxonomy and Multivariate Analysis System*. Version 2.10e. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook.
- Sachdeva S, Bharadwaj C, Sharma V, Kumar N, Bhat K V, Patil B S, Soren KR, Chaturvedi S K, Roorkiwal M, Chauhan S K and Varshney R. 2017. Morpho-physiological grouping of chickpea (*Cicer arietinum L.*) genotypes on the basis of their response to drought stress. *International Journal of Tropical Agriculture* **35**: 15–23.
- Sambrook J and Russell D W. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Santosh H B, Bharadwaj C, Santosh S, Raghavendra K P and Kumar J. 2017. Genetic diversity analysis for productivity enhancement through *desi-kabuli* introgression breeding in chickpea. *Electronic Journal of Plant Breeding* **8**: 125–33.
- Satyavathi C T, Bhat K V, Bharadwaj C, Tiwari S P and Chaudhary V. 2005. AFLP based DNA profiling and genetic diversity assessment of Indian soybean [*Glycine max (L.) Merrill*] varieties. *Genetic Resources and Crop Evolution* **53**: 1069–79.
- Sokal R R and Sneath P H A. 1963. *Principles of Numerical Taxonomy*. W H Freeman & Co., New York.
- Udupa S M, Robertson L D, Weigand F, Baum M and Kahl G. 1999. Allelic variation at (TAA)_n microsatellite loci in a world collection of chickpea (*Cicer arietinum L.*) germplasm. *Molecular and General Genetics* **261**: 354–63.
- Varshney R K, Song C, Saxena R K, Azam S, Yu S, Sharpe A G, Cannon S, Baek J, Rosen B D, Tar'an B, Millan T, Zhang X, Ramsay L D, Iwata A, Wang Y, Nelson W, Farmer A D, Gaur P M, Soderlund C, Penmetsa R V, Xu C, Bharti A K, He W, Winter P, Zhao S, Hane J K, Carrasquilla-Garcia N, Condie J A, Upadhyaya H D, Luo M C, Thudi M, Gowda C L, Singh N P, Lichtenzveig J, Gali K K, Rubio J, Nadarajan N, Dolezel J, Bansal K C, Xu X, Edwards D, Zhang G, Kahl G, Gil J, Singh K B, Datta S K, Jackson S A, Wang J and Cook D R. 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nature Biotechnology* **31**: 240–6.
- Varshney R K, Ribaut J M, Buckler E S, Tuberosa R, Rafalski J A and Langridge P. 2013. Can genomics boost productivity of orphan crops? *Nature Biotechnology* **30**: 1172–6.
- Yadav S, Bharadwaj C, Chauhan S K, Rizvi A H, Kumar J and Satyavathi C T. 2011. Analysis of genetic diversity in *Cicer* species using molecular markers. *Indian Journal of Genetics and Plant Breeding* **71**: 272–5.