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Assessment of genetic diversity among 125 cultivars of chickpea (*Cicer arietinum* L.) of Indian origin using ISSR markers

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Abstract: Utilization of diverse germplasm is required to enhance the genetic variability of cultivars. Opportunity to generate favorable gene combinations can be provided by genetically diverse lines; thus, the probability of creating unique cultivars increases. Genetic diversity analysis of chickpea can give important indications for understanding species relationships and may help in developing and planning breeding programs. Thus, the present investigation was performed to assess the genetic diversity in 125 cultivars of chickpea of Indian origin, among which 42 are resistant and 13 are susceptible to Fusarium wilt and Ascochyta blight. Out of 40 ISSR primers, 26 primers generated 213 polymorphic loci. On average, 9 loci per marker were found; among these, 8 were polymorphic. The average polymorphic information content was 0.72, ranging from 0.26 to 0.91. The resolving power of the ISSR primers varied from 0.34 to 10.24. Cluster analysis using the UPGMA algorithm showed 3 major clusters, supported by STRUCTURE analysis. Genetic diversity analysis in terms of Shannon's index and Nei's gene diversity for resistant, susceptible, and miscellaneous cultivars revealed higher values for miscellaneous cultivars, indicating more variability among these cultivars in comparison to resistant and susceptible cultivars. AMOVA results among groups and among cultivars were 6% and 94%, respectively, while the estimated gene flow was 8.964. These values indicated high genetic variability among the studied cultivars and can be used to select good parental material in breeding programs and determine its correlation with the relationships based on pedigree data and morphological traits.

Key words: *Cicer arietinum*, genetic polymorphism, molecular markers, analysis of molecular variance, gene flow

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important cool season food legume worldwide after dry bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). Chickpea seed production has been increasing since the 1990s and rose from 7 million tons in 1990 to 11 million tons in 2012. This increase is mainly due to better yields, which reached about 0.9 t/ha worldwide in 2011 (FAO, 2013). It is a diploid plant (2n = 2x = 16) with an estimated haploid genome size of 738 Mb (Varshney et al., 2013). It is cultivated in about 33 countries and regions including Central-West Asia, South Europe, Ethiopia, North Africa, and Australia (FAO, 2013; ICRISAT, 2013). It has high nutritive value and serves as an important cheap source of protein in developing countries in addition to improving land fertility (Saeed et al., 2011). The chickpea seed is a good source of carbohydrates and proteins, which collectively constitute 80% of the total dry seed weight (Talebi et al., 2008; Aggarwal et al., 2013). Moreover, chickpea pod covers and seed coats are used as fodder (Tahir and Karim, 2011). Chickpea is also an important food for people to use to improve major food-related health problems (Charles et al., 2002; Jukanti et al., 2012). However, more research is necessary to increase the benefits of this valuable food legume through breeding (Milan et al., 2006).

Modern plant breeding and agricultural systems have narrowed the base for the genetic diversity of cultivated chickpea (Robertson et al., 1997). Therefore, it is time to explore new sources of variation that might be used in plant breeding programs. Knowledge of genetic diversity is important for gene bank management and breeding programs such as gene tagging and marker-assisted selection (MAS). Genetic diversity among the parents is a prerequisite for ensuring the chance of improved segregate selection for various characters (Dwevedi et al., 2009). Criteria for the assessment of genetic variability can include

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morphological traits (Upadhaya et al., 2007) and molecular markers (Sharma et al., 1995). Molecular markers have proved to be valuable tools for the characterization and assessment of genetic variability within and between species and populations (Talebi et al., 2008).

Various molecular markers are available for the identification of cultivars and analysis of genetic diversity. The random amplified polymorphic DNA (RAPD) marker system has been used for evaluating genetic diversity in chickpea (Ahmad, 1999; Sant et al., 1999; Sudupak et al., 2002). However, little genetic diversity was detected using RAPD markers (Simon and Muehlbauer, 1997; Singh et al., 2002). Inter simple sequence repeats (ISSR) markers are more consistent than the RAPD markers, as they generate a greater number of polymorphic loci per primer (Ratnaparkhe et al., 1998a; Aggarwal et al., 2011). Unlike RAPDs, ISSR markers are detected using longer semiarbitrary SSR primers at highly stringent conditions in PCR; therefore, they are reproducible and highly polymorphic DNA markers (Bornet and Branchard, 2001; Alam et al., 2009). ISSR is based on amplification of genomic segments flanked by inversely oriented and closely spaced SSR loci using microsatellite core-unit–bearing oligonucleotide primers that could be either nonanchored or anchored to 5' or 3' end of the repeats with 1–3 random nucleotides (Zietkiewicz et al., 1994; Sudupak, 2004). This technique is fast and cost-efficient, and does not require prior knowledge of sequence.

ISSR markers have been employed to analyze genetic diversity and relationships in a number of crops (Ajibade et al., 2000; Raina et al., 2001; Bart et al., 2002). In chickpea, Ratnaparkhe et al. (1998b) reported that the ISSR technique is helpful in finding markers closely linked to a disease-resistant gene. In addition, these markers have been successfully used to study diversity and phylogenetic relationships in chickpea for the last decade (Iruela et al., 2002; Rajesh et al., 2003; Rao et al., 2007; Bhagyawant and Srivastava, 2008; Aggarwal et al., 2011). However, there have been very few reports investigating the level of genetic variation in chickpea cultivars according to their resistance/susceptibility to Fusarium wilt and Ascochyta blight. Therefore, the present study was carried out to determine genetic diversity among 125 chickpea cultivars, 42 resistant and 13 susceptible to Fusarium wilt and Ascochyta blight, using ISSR markers.

2. Materials and methods

2.1. Plant materials and DNA isolation

One hundred and twenty-five chickpea cultivars of Indian origin (Table 1) were grown in randomized

Table 1. List of chickpea cultivars used in the present study.

Sr. no.	Genotype	Sr. no.	Genotype	Sr. no.	Genotype	Sr. no.	Genotype	Sr. no.	Genotype
1	ICCV4958	26	Avrodhi	51	RSGK-6(k)	76	GPF-2	101	Pusa 391
2	Katila	27	CSG 8962	52	JG-64	77	JGG-1	102	SAKI9516
3	PDG 84-16	28	Pusa 372	53	ICCV-10	78	PG 12	103	GCP 105
4	BG 276	29	HK 98-155	54	BushyMutant	79	RSG-2	104	RAU 52
5	Tyson	30	RSG 973	55	Hima	80	Chaffa	105	Pusa 240
6	H-208	31	RSG 888	56	BG 396	81	PDG-3	106	Sadabahar
7	HC-3	32	HC-1	57	BG 1006	82	GNG1292	107	RSG-11
8	E 100 Ym	33	Pusa 256	58	IPC 92-39	83	JG 11	108	Pusa 329
9	GNG 663	34	Pusa 362	59	IPC 98-12	84	KWR 108	109	Dohadyellow
10	C-235	35	Vishal	60	ICCV14880	85	JG 218	110	Pusa 1003
11	DCP 92-3	36	H04-45	61	IPC 99-18	86	Phule G-5	111	JG 130
12	Radhey	37	HC-5	62	IPC2000-33	87	Pant G114	112	B 108
13	RSG 963	38	H03-56	63	IPC 2001-2	88	Pusa 312	113	BGD 75
14	Pusa 261	39	Gaurav	64	IPC 95-1	89	K 850	114	C 214
15	Annegiri	40	ICC 4958	65	PG 96006	90	GCP 101	115	C 15
16	RSG 931	41	Amethyst	66	IPC 97-67	91	BGM 413	116	C 20
17	GNG 146	42	WR-315	67	IPC 94-94	92	Virat(k)	117	C 16
18	BGM 408	43	ICCV92944	68	IPC2000-41	93	PBG-5	118	M 1
19	Pusa 267	44	ICCV96030	69	IPC2000-45	94	PDG 4	119	M 2
20	Vijay	45	L 551	70	RSG 807	95	RSG 44	120	H04-57
21	HK 94-134	46	Pusa 1053	71	Pusa 209	96	Pusa 212	121	H04-44
22	GNG 469	47	L 550(k)	72	CSJD-844	97	GL 769	122	H04-87
23	JG 315	48	ICCV-2	73	GG-2	98	Vaibhava	123	H04-11
24	BGD 72	49	JG 74	74	RS-10	99	KPG 59	124	Digvijay
25	PBG-1	50	JKG-1(k)	75	Pusa 244	100	ICCV 37	125	PantG186

blocks designed in 3 replicates at the research farm of the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. All cultivars were categorized into 3 groups/populations (Table 2) depending upon their resistance/susceptibility to Fusarium wilt and Ascochyta blight. Out of the 125 cultivars, 42 are resistant and 13 are susceptible to Fusarium wilt and Ascochyta blight. Genomic DNA was isolated from leaves of 3–4-week-old seedlings using the modified CTAB method of Thompson and Murray (1980). The quality and concentration of DNA were measured by NanoDrop spectrophotometer (ND-100) and electrophoresis using 0.8% agarose gel.

Table 2. Grouping of chickpea cultivars in response to Fusarium wilt/Ascochyta blight.

Sr. no.	Groups	Response to Fusarium wilt/Ascochyta blight
1–42	Group 1	Resistant
43–55	Group 2	Susceptible
56–125	Group 3	Miscellaneous

2.2. ISSR-PCR

A set of 40 ISSR primers were obtained from Alpha DNA (Montreal, Quebec, Canada; H4C3N9) (Table 3), and PCR amplification was performed in in a thermal cycler (MJ Research). Amplification reactions were performed in volumes of 15 µL containing 1X PCR buffer, 200 µM of each dNTP, 2.5 mM $MgCl_2$, 0.4 µM primer, 1 unit of Taq polymerase (Sigma-Aldrich), and 20 ng of template

Table 3. Sequence of primers used for ISSR amplification and their GC content, annealing temperature (T_A), total number of loci (TL), number of polymorphic loci (NPL), percentage of polymorphic loci (PPL), total fragments amplified (TF), resolving power (Rp), and polymorphic information content (PIC).

No.	Primer sequence (5'~3')	GC (%)	T_A (°C)	TL	PPL	TF	Rp	PIC
807	AGAGAGAGAGAGAGAGT	47.0	52.3	13	92.3	363	5.81	0.886
808	AGAGAGAGAGAGAGAGC	52.9	55.0	17	100	513	8.21	0.905
809	AGAGAGAGAGAGAGAGG	52.4	55.0	18	100	238	3.81	0.915
810	GAGAGAGAGAGAGAGAT	47.0	53.5	11	100	240	3.84	0.875
811	GAGAGAGAGAGAGAGAC	52.9	56.0	14	100	205	3.28	0.863
812	GAGAGAGAGAGAGAGAA	47.0	53.8	9	100	128	2.05	0.814
813	CTCTCTCTCTCTCTCTT	47.0	48.0	3	66.6	148	2.37	0.575
814	CTCTCTCTCTCTCTCTA	47.0	38.1	7	85.7	250	4.00	0.730
815	CTCTCTCTCTCTCTCTG	52.9	51.6	9	100	84	1.34	0.815
816	CACACACACACACACAT	47.0	51.4	8	100	96	1.54	0.728
817	CACACACACACACACAA	47.0	51.4	1	100	21	0.34	0
818	CACACACACACACACAG	52.9	50.4	5	100	147	2.35	0.718
819	GTGTGTGTGTGTGTGTA	47.0	49.6	4	100	66	1.06	0.662
820	GTGTGTGTGTGTGTGTC	52.9	52.4	4	75	131	2.10	0.504
821	GTGTGTGTGTGTGTGTT	47.0	53.5	4	100	40	0.64	0.229
823	TCTCTCTCTCTCTCTCC	52.9	50.0	11	90.9	268	4.29	0.794
824	TCTCTCTCTCTCTCTCG	52.9	50.1	2	100	54	0.86	0.499
825	ACACACACACACACACT	47.0	48.9	3	100	79	1.26	0.450
826	ACACACACACACACACC	52.9	55.0	9	100	200	3.20	0.851
827	ACACACACACACACACG	52.9	54.3	16	100	157	2.51	0.913
829	TGTGTGTGTGTGTGTGC	52.9	54.0	4	75	122	1.95	0.264
830	TGTGTGTGTGTGTGTGG	52.9	55.9	12	83.3	433	6.93	0.849
834	AGAGAGAGAGAGAGAGAT	44.4	48.5	11	63.6	542	8.67	0.866
835	AGAGAGAGAGAGAGAGAC	50.0	51.0	15	80	460	7.36	0.843
836	AGAGAGAGAGAGAGAGTA	44.4	55.9	10	60	640	10.24	0.870
840	GAGAGAGAGAGAGAGAAT	44.4	55.8	12	100	283	4.53	0.797
	Total			232		5908		

DNA. The first cycle consisted of denaturation for 5 min at 94 °C, primer annealing at 48–56 °C (depending on the primer) for 45 s, and extension at 72 °C for 1 min. In the next 35 cycles the denaturation period was 45 s at 94 °C, annealing and extension parameters were the same as in the first cycle, and final extension was at 72 °C for 10 min. Amplification products were electrophoresed on 1.4% (w/v) agarose gel in 1X Tris-Borate EDTA (TBE) buffer at 90 V for 1 h, stained with ethidium bromide, and documented on a gel documentation system (SynGene, Germany); 1 kb and 100 bp ladders were used as molecular size markers. All PCR reactions were run in duplicate, and only reproducible and clear bands were scored.

2.3. Data analysis

The banding patterns obtained from ISSR were scored as present (1) or absent (0), and each was treated as an independent character. The data were subjected to cluster analysis by the neighbor-joining (NJ) method, and a dendrogram was generated using DARWIN (version 5.0.158). In addition, statistically unbiased clustering of data was performed using STRUCTURE (version 2.3.1) (Evann et al., 2005). POPGENE was used to calculate within-species diversity (Hs), total genetic diversity (Ht), and Nei's unbiased genetic distance among cultivars (Nei, 1978). Data for Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL), and percentage polymorphic loci (PPL) across 125 cultivars were also analyzed (Zhao et al., 2006). Furthermore, the ISSR data was subjected to a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) among the cultivars. Diversity index (*DI*), effective multiplex ratio (*EMR*), and marker index (*MI*) of ISSR marker was calculated according to Powell et al. (1996). The resolving power of the ISSR primers was calculated according to Prevost and Wilkinson (1999). The resolving power (*Rp*) of a primer is: $Rp = \Sigma\ IB$, where *IB* (band informativeness) takes the value of: $1 - [2 \times (0.5 - P)]$, *P* being the proportion of the 125 cultivars containing the band. Similarly, the polymorphism information content (PIC) of a primer is calculated as:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 \ ,$$

where p_i is the frequency of an individual genotype generated by a given ISSR primer, and summation extends over *n* alleles.

3. Results and discussion

The 26 ISSR primers selected in the study generated a total of 232 ISSR bands (an average of 9 bands per primer), out of which 213 were polymorphic (91.8%). The number of bands varied from 2 to 15 with sizes ranging from 120 to 2000 bp. Amplification results of 26 primers seem to indicate that microsatellites more frequent in *Cicer* contain the repeated di- (AG)n, (GA)n, (CA)n, (CT)n, (TC)n, (TG)n, and (AC)n. The resolving power (Rp) of the ISSR primers of the 26 nucleotides ranged from 0.34 to 10.24 (Table 3).

The complete data set of 5908 bands was used for cluster analysis based on bootstrapping and NJ method. The NJ-generated dendrogram (Figure 1) consisted of 1 mega cluster and 2 mini clusters; cluster II was extensively divided into mini clusters. Cluster I consists of 9 cultivars (90, 73, 87, 89, 88, 81, 77, 78, and 84), while cluster III comprised 33 cultivars (60, 14, 20, 21, 19, 22, 8, 11, 15, 10, 12, 17, 18, 6, 7, 9, 16, 4, 5, 71, 68, 70, 44, 45, 61, 72, 69, 67, 63, 62, 66, 64, and 65). The other 83 cultivars in cluster II were further subdivided into 10 subgroups. The estimated likelihood of the clustering of cultivars using STRUCTURE analysis was found to be optimal when K = 3; ΔK reached its maximum value when K = 3 (Figure 2), suggesting that all the cultivars fell into 1 of the 3 clusters. The cultivars were more likely distributed (at high probability) with respect to response towards diseases despite small interference (Figure 3).

Relatively high genetic variation was detected among the cultivars. Genetic diversity analysis in terms of Na, Ne, H, I, Ht, Hs, and PPL for resistant, susceptible, and miscellaneous cultivars revealed higher values for miscellaneous, indicating more variability among these cultivars in comparison to resistant and susceptible ones (Table 4). AMOVA among groups (6%) and among cultivars (94%) indicated that there are more variations within the population (Table 5). Higher genetic variability occurred across cultivars, as the estimated gene flow was 8.964 (Table 6). The overall value of Shannon's index was 0.369, and the value of Nei's genetic diversity was 0.231. The values of the mean diversity index (DI) and mean effective multiplex ratio (EMR) are 0.721 and 7.659, respectively. Further, the marker index of ISSR was obtained within a good range (0.639), indicating ISSR is a powerful molecular marker for genetic characterization of *Cicer arietinum* cultivars; this is further supported by the grouping pattern in the NJ-generated dendrogram.

Ratnaparkhe et al. (1998a) studied inheritance of ISSR polymorphisms in a cross of cultivated chickpea (*Cicer arietinum* L.) and a closely related wild species (*Cicer reticulatum* L.) using primer that anneals to a simple repeat of various lengths, sequences, and nonrepetitive motifs. The majority of primers are dinucleotides, which upon testing provide useful banding patterns. Twenty-two primes were used for analysis and yielded a total of 31 segregating loci. Moreover, all ISSR loci showed nearly complete agreement with expected Mendelian ratios. Ratnaparkhe et al. (1998b) also observed an abundance of dinucleotides and trinucleotide repeats at the Fusarium

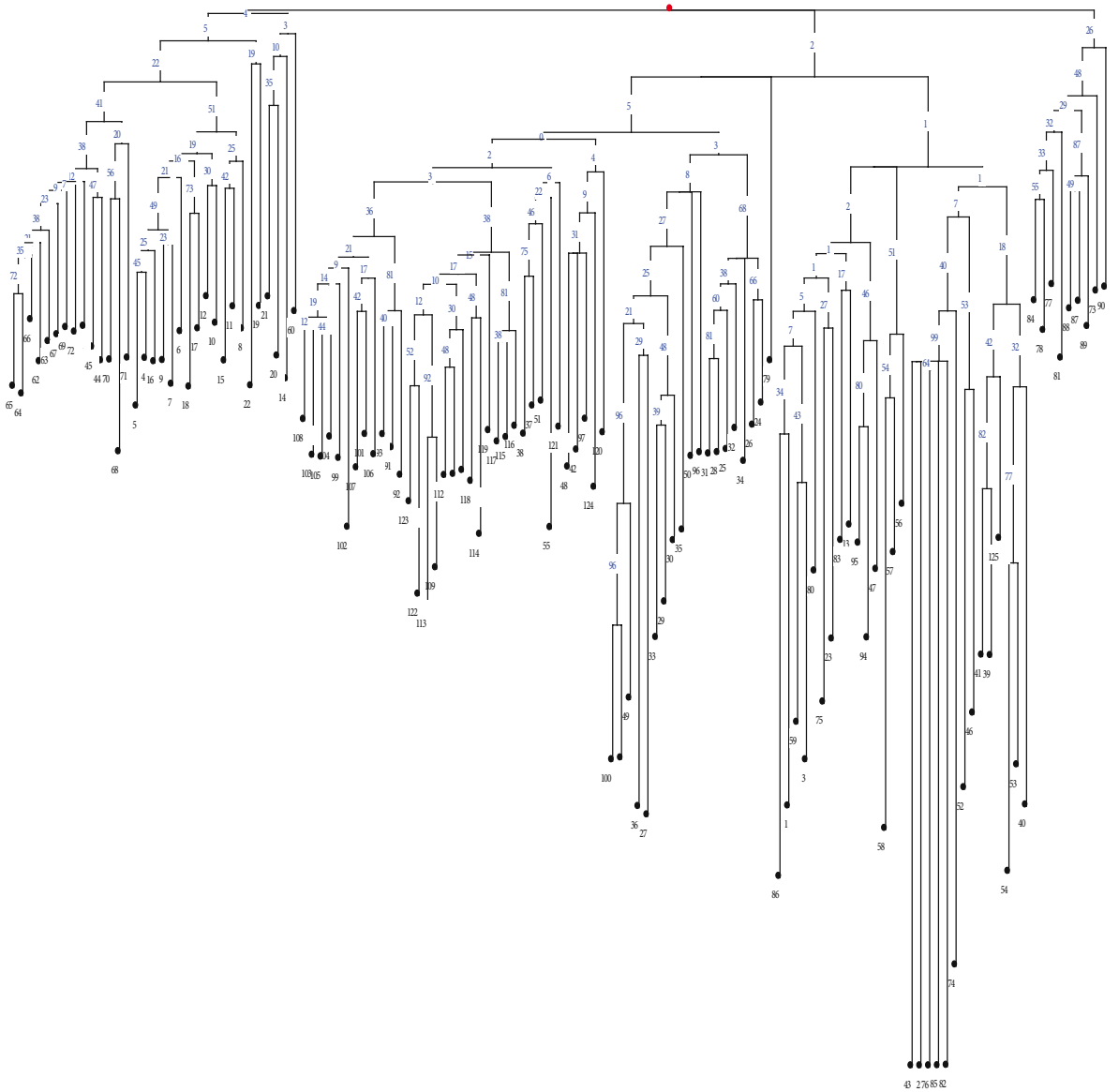

Figure 1. NJ tree representing clustering of cultivars along with supported bootstrap values based on ISSR profiling.

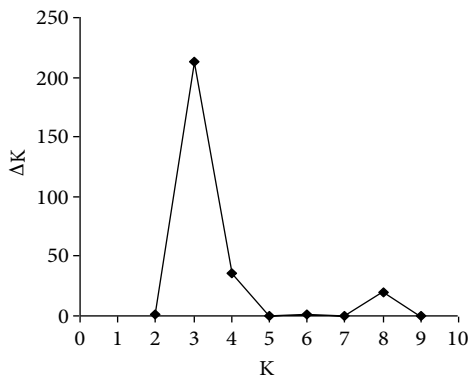

Figure 2. Relationship between K and ΔK based on STRUCTURE analysis of chickpea cultivars using ISSR data.

wilt disease resistance gene cluster. The repeat $(AC)_8T$ amplified a marker, UBC-825_{1200}, which was closer to the gene than other markers for resistance to Fusarium wilt race 4. These results specified that ISSR markers can provide important information for the design of other primers.

Iruela et al. (2002) used a total of 52 markers (RAPD + ISSR) to study the variation within *Cicer arietinum* and found that the Jaccard's similarity index obtained from these data varied between 0.19 and 0.97. Only 31 fragments were found to be polymorphic out of 234; thus, little polymorphism was detected (13.2%). The other species with more than 1 accession showed average values of similarity between 0.45 and 0.78, with *C. bijugum* and

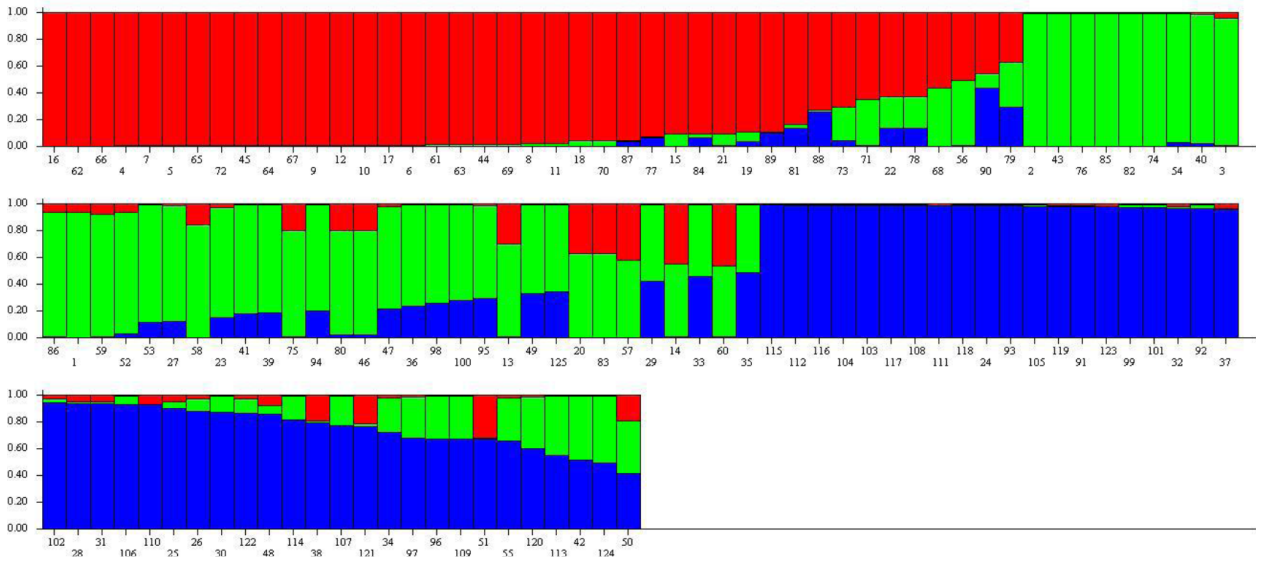

Figure 3. STRUCTURE analysis of chickpea cultivars based on ISSR data showing grouping of cultivars when K = 3.

Table 4. Summary of genetic variation statistics for all loci of ISSR among the chickpea cultivars with respect to their resistance/susceptibility to Fusarium wilt and Ascochyta blight.

Response to diseases	Sample size	Na	Ne	H	I	Ht	PPL
ISSR							
Resistant	42	1.747 (0.436)	1.342 (0.343)	0.210 (0.183)	0.324 (0.256)	0.209 (0.033)	74.68
Susceptible	13	1.556 (0.498)	1.309 (0.349)	0.186 (0.191)	0.282 (0.276)	0.186 (0.037)	55.79
Miscellaneous	70	1.888 (0.316)	1.367 (0.316)	0.231 (0.166)	0.363 (0.228)	0.231 (0.028)	88.84

Na = observed number of alleles; Ne = effective number of alleles; H = Nei's gene diversity; I = Shannon's information index; Ht = total genetic diversity; PPL = percentage of polymorphic loci.

Table 5. Summary of analysis of molecular variance (AMOVA) based on ISSR analysis. Levels of significance are based on 1000 iteration steps.

Source of variation	D.F.	S.S.D.	M.S.	Variance component	Percentage	P-value
Among groups	2.00	177.87	88.93	1.79	6	-
Among cultivars	122.00	3188.87	26.14	26.14	94	<0.001

D.F.: degree of freedom; S.S.D.: sum of square deviation; M.S.: mean square deviation; P-value: probability of null distribution.

Table 6. Overall genetic variability across all 125 cultivars of chickpea based on ISSR markers.

Na	Ne	H	I	Ht	Hs	Gst	Nm	DI	EMR	MI
2.000	1.364	0.231	0.369	0.231	0.219	0.053	8.964	0.721	7.659	0.639
–	(0.310)	(0.159)	(0.211)	(0.025)	(0.023)					

Na = observed number of alleles; Ne = effective number of alleles; H = Nei's gene diversity; I = Shannon's information index; Ht = heterogeneity; Hs = homogeneity; Gst = gene differentiation; Nm = gene flow (Nm = 0.5(1 – Gst)/Gst); DI = diversity index; EMR = effective multiplex ratio; MI = marker index.

C. cuneatum having the lowest genetic diversity (0.78 and 0.74, respectively). These findings confirm the low level of genetic diversity within chickpea compared to the wild species. Rajesh et al. (2002) selected 13 wild species of *Cicer* (6 annual and 7 perennial) and amplified using 25 ISSR primers. The UPGMA and AMOVA tests were performed to quantify the genetic variability. They also used 4 cultivars of *C. arietinum*, i.e. Vijay, JG62, ICC4958, and V65R. Fifteen primers out of 25 generated a total of 115 reproducible bands, giving an average of 6.6 polymorphic bands per primer. In an AMOVA analysis among populations, a total variance of 51.10% was found within populations, while 48.90% of total variance was found among populations.

PCR products of 6 SSR primers in *Cicer* accessions were treated as dominant DNA markers by Sudupak (2004) and were exploited to compute the distances among accessions and species. On average, 25 bands per primer with product sizes ranging from 0.2 to 2.5 kb were observed. Among *C. arietinum* accessions, only 3 loci were polymorphic, revealing the grouping of accessions. ISSR variation among species was common with expected gene diversity of 0.24 and 100% polymorphic loci, but variation within species was low. Among the species, *C. pinnatifidum* was the most polymorphic followed by *C. reticulum*, while *C. arietinum* had the lowest level of ISSR variation.

Rao et al. (2007) tested genetic relationships between 19 chickpea cultivars and 5 accessions of its wild progenitor *C. reticulum* by using RAPD and ISSR markers. Six primers out of 10 were found to be polymorphic and generated a total of 64 bands; among these, 80% of bands were polymorphic. Among cultivated chickpea varieties, 56.3% polymorphic bands were found, while 65.6% polymorphic bands were found among wild accessions. From the UPGMA dendrogram, it is discernible that the material taken for the analysis can be divided into 2 major clusters. Wild accessions are grouped into 1 cluster and all chickpea cultivars in another cluster that is subdivided into groups and subgroups. The similarity coefficient varied from 0.84 to 0.96 in cultivars, while it varied from 0.71 to 0.81 in wild accessions. The ISSR study clearly signified that, even with 6 polymorphic primers, reliable estimation of genetic diversity could be attained.

Bhagyawant and Srivastava (2008) performed the genetic fingerprinting of chickpea cultivars using ISSR primers. Twelve *Cicer* cultivars were screened using 10 ISSR primers for PCR studies. A total of 492 bands were amplified across 12 cultivars with 7 primers, revealing an average of 70.28 bands per primer and 5.85 bands per primer per genotype. The total number of loci amplified by 3' anchored repeats varied from 36 to 96. The primers based on poly (ATG) and (GAA) motifs produced at least 36 bands, whereas primers (AC)T and (AC)TT produced a maximum of 96 bands.

A molecular evaluation of 5 chickpea varieties was conducted by Tahir and Karim (2011) to access the genetic diversity and relationship of chickpea cultivars using RAPD and ISSR markers. All 5 primers used were polymorphic and generated 6.6 bands per primer in a total of 33 bands. The varieties shared 36.4% common bands and 63.6% polymorphic bands with ISSR primers. The Jaccard similarity matrix varied from 0.16 to 1.00 in chickpea varieties.

Genetic fingerprinting of 115 chickpea cultivars was performed by Aggarwal et al. (2011) using 6 ISSR primers. Out of 6 primers, 3 were found to be polymorphic and generated reproducible fragments of sizes ranging from 0.15 to 3 kb. A total of 1527 scorable gel positions were observed, showing 72.3% polymorphism. The dendrogram based on UPGMA divided all cultivars into 5 clusters, while the Jaccard's similarity coefficient ranged from 0.01 to 0.90.

The extent of polymorphism detected in the present study is higher than in previously reported ISSR markers (Iruela et al., 2002; Rajesh et al., 2002; Sudupak, 2004; Rao et al., 2007; Tahir and Karim, 2011). This may be due to the high resolving power of ISSR primers used for detecting polymorphism. Since chickpea has little genetic diversity due to obligatory self-pollination and an extensively monotonous genome, it was necessary to screen many ISSR markers to detect sufficient DNA polymorphism. In the present scenario ISSR markers are the efficient marker system because of their ability to expose various informative loci from a single amplification. Similar studies were conducted by Bornet and Branchard (2001), by Fernandez et al. (2002) in barley, and by Qian et al. (2001) in rice. The idea behind studying such a large collection was to provide better knowledge about genetic variability so that this information proves to be informative in the management of genetic resources in chickpea. Genetic information obtained from ISSR data can be used in categorizing chickpea cultivars and can also harmonize the genetic studies generated from morphological traits. Further, the genetic divergence that exists between chickpea cultivars can be used efficiently for gene tagging and plant breeding.

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References

Aggarwal H, Rao A, Rana JS, Singh J, Kumar A, Chhokar V, Beniwal V (2011). Inter simple sequence repeats reveal significant genetic diversity among chickpea (*Cicer arietinum* L.) cultivars. J Plant Sci 6: 202–212.

Aggarwal H, Singh J, Khaket TP, Chhokar V (2013). Genetic diversity in chickpea using various molecular markers: first step towards molecular breeding. Int J Adv Res 1: 393–398.

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

Ajibade SR, Weeden NF, Chite SM (2000). Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111: 47–55.

Alam A, Naik PK, Mishra GP (2009). Congruence of RAPD and ISSR markers for evaluation of genomic relationship among 28 populations of *Podophyllum hexandrum* Royle from Himachal Pradesh, India. Turk J Bot 33: 1–12.

Bart S, Melchinger AE, Lubberstedt T (2002). Genetic diversity in *Arabidopsis thaliana* L. Heynh investigated by cleaved amplified polymorphic sequence (CAPS) and inter-simple sequence repeat (ISSR) markers. Mol Ecol 11: 495–505.

Bhagyawant SS, Srivastava N (2008). Genetic fingerprinting of chickpea (*Cicer arietinum* L.) germplasm using ISSR markers and their relationships. Afr J Biotechnol 7: 4428–4431.

Bornet B, Branchard M (2001). Nonanchored inter-simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol Biol Rep 19: 209–215.

Charles MT, Dominique R, Kumar J, Dangi OP (2002). A preliminary study of the functional properties of chickpea leaves. In: Annual Meeting of the Canadian Society of Food and Nutrition. Edmonton, Alberta, Canada, pp. 89–96.

Dwevedi KK, Gaibriyal M (2009). Assessment of genetic diversity of cultivated chickpea (*Cicer arietinum* L.). Asian Journal of Agricultural Sciences Sci 1: 7–8.

Evann 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.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

FAO (2013). FAOSTAT. Food and Agriculture Organization of the United Nations. Website http://faostat.fao.org/default.aspx.

Fernandez ME, Figueiras AM, Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor Appl Genet 104: 845–851.

ICRISAT (2013). Chickpea (*Cicer arietinum* L.). Website http://www.icrisat.org/crop-chickpea.htm.

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.

Jukanti AK, Gaur PM, Gowda CL, Chibbar RN (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. Br J Nutr 108: S11–S26.

Millan T, Clarke HJ, Siddique KHM, Buhariwalla HK, Gaur PM, Kumar J, Gil J, Kahl G, Winter P (2006). Chickpea molecular breeding: new tools and concepts. Euphytica 147: 81–103.

Murray MG, Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321–4325.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individual. Genetics 89: 583–590.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breeding 2: 225–238.

Prevost A, Wilkinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98: 107–112.

Qian W, Ge S, Hang DY (2001). Genetic variation within and among populations of a wild rice *Oryza granulata* from china detected by RAPD and ISSR markers. Theor Appl Genet 102: 440–449.

Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM (2001). RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome 44: 763–772.

Rajesh PN, Sant VJ, Gupta VS, Muehlbauer FJ, Rajesh PK (2002). Genetic relationships among annual and perennial wild species of *Cicer* using inter simple sequence repeat (ISSR) polymorphism. Euphytica 129: 15–23.

Rao AS, Usha Rani P, Deshmukh PS, Kumar PA, Panguluri SK (2007). RAPD and ISSR fingerprinting in cultivated chickpea (*Cicer arietinum* L.) and its wild progenitor *Cicer reticulatum* L. Genet Resour Crop Ev 54: 1235–1244.

Ratnaparkhe MB, Santra DK, Tullu A, Muehlbauer FJ (1998a). Inheritance of inter simple sequence repeat polymorphism and linkage with Fusarium wilt resistance gene in chickpea. Theor Appl Genet 96: 348–353.

Ratnaparkhe MB, Tekeoglu M, Muehlbauer FJ (1998b). Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor Appl Genet 97: 515–519.

Robertson LD, Ocampo B, Singh KB (1997). Morphological variation in wild annual *Cicer* species in comparison to the cultigen. Euphytica 95: 309–319.

Saeed A, Hovsepyan H, Darvishzadeh R, Imtiaz M, Panguluri SK, Nazaryan R (2011). Genetic diversity of Iranian accessions, improved lines of chickpea (*Cicer arietinum* L.) and their wild relatives by using simple sequence repeats. Plant Mol Biol Rep 29: 848–858.

Sant VJ, Patankar AG, Sarode ND, Mhase LB, Sainani MN, Deshmukh RB, Ranjekar PK, Gupta VS (1999). Potential of DNA markers in detecting divergence and in analyzing heterosis in Indian elite chickpea cultivars. Theor Appl Genet 98: 1217–1225.

Sharma PC, Winter P, Bunger T, Huttel B, Weigand F, Weising K, Kahl G (1995). Abundance and polymorphism of di-, tri- and tetra-nucleotide tandem repeats in chickpea (*Cicer arientinum* L.). Theor Appl Genet 90: 90–96.

Simon CJ, Muehlbauer FJ (1997). Construction of a chickpea linkage map and its comparison with map of pea and lentil. J Hered 88: 115–119.

Singh PC, Prasad D, Singhal V, Randhawa GJ (2002). Analysis of genetic diversity in *Cicer arietinum* L. using RAPD markers. Journal of Plant Biochemistry and Bioechnologyt 11: 109–112.

Sudupak MA (2004). Inter and intra-species inter simple sequence repeat (ISSR) variations in the genus *Cicer*. Euphytica 135: 229–238.

Sudupak MA, Akkaya MS, Kence A (2002). Analysis of genetic relationships among perennial and annual *Cicer* species growing in Turkey using RAPD markers. Theor Appl Genet 105: 1220–1228.

Tahir NAR, Karim HFH (2011). Determination of genetic relationships among some varieties of chickpea (*Cicer arietinum* L.) in Sulaimani by RAPD and ISSR markers. Jord J Biol Sci 4: 77–86.

Talebi R, Fayaz F, Mardi M, Pirsyedi SM, Naji AM (2008). Genetic relationships among chickpea (*Cicer arietinum*) elite lines based on RAPD and agronomic markers. Int J Agric Bio 10: 301–305.

Upadhaya HD, Dwivedi SL, Gowda CLL, Singh S (2007). Identification of diverse germplasm lines for agronomic traits in chickpea (*Cicer arietinum* L.) core collection for use in crop improvement. Field Crop Res 100: 320–326.

Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Tar'an B et al. (2013). Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nature Biotechnol 31: 240–248.

Zhao WG, Zhang JQ, Wang YH, Chen TT, Yin YL, Huang YP, Pan YL, Yang YH (2006). Analysis of genetic diversity in wild populations of mulberry from western part of Northeast China determined by ISSR markers. J Genet Mol Biol 17: 196–203.

Zietkiewicz E, Rafalski JA, Labuda D (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176–183.