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Diversity Analysis among Chickpea Genetic Stock as Revealed Through STMS Marker Analysis

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Genetic diversity analysis of chickpea germplasm can provide useful information for the selection of parental material and thus help in planning breeding strategies.In the present study, a total of 57 STMS loci were analyzed to discern the variability among 87 chickpea lines consisting of released varieties and elite germplasm. A total of 87 alleles were found for the 19 STMS loci with an average of 4.57 alleles per locus. PIC value ranged from 0.94 to 0.10 and the heterozygosity ranged from 0.11 to 0.94, indicating good variability among the material as well as polymorphism generated. All the genotypes could cluster into six distinct groups with one genotype remaining unclustered. Greater gains can be obtained by crossing lines MPJG-2000-108 with SBD 377 for *Desi* and PG 0515 with ILC 212 for *Kabuli* improvement. Base broadening through *Kabuli* × *Desi* introgression with greater gains can be obtained by using ICC 4516 and ILC 212 as parents.

Key Words: Chickpea, Cluster Analysis, Molecular markers, PCR, STMS markers

Introduction

Chickpea (*Cicer arietinum* L.; Family: Fabaceae) is a self-pollinated, diploid (2n=16), cool season pulse crop with a genome size of ~738-Mb and an estimated 28,269 genes (Varshney *et al.,* 2013). It is widely grown in more than 50 countries representing all the continents (Upadhyay *et al.,* 2011). Worldwide chickpea ranks third among legumes (Food and Agricultural Organization, 2010) *i.e.* almost 15% of the total pulse production of world. In the duration of 2010, the worldwide chickpea area was about 12.0 million ha, with 10.9 million metric tons of production with the yield of 911 kg/ha (FAOSTAT, 2012). India is the world's major producer, the annual production is around 7.58 Mt, grown in the area of approximately 8.32 mha, which is the world's 68% production of total chickpea and the average yield is approximately 912 kg/ha (FAOSTAT, 2012). More than 95% of the area of production and consumption of chickpea is shared by the developing countries. Chickpea is grown mainly in South East Asian countries. *Kabuli* (white seeds) and *Desi* (brown seeds) are the two main types of cultivated chickpea, presenting two diverse gene pools (Nawroz and Hero, 2011).

Chickpea has a very narrow genetic base which is limiting the genetic improvement of chickpea through breeding efforts. The level of natural variation among cultivated chickpea and wild accessions at molecular level is greatly aids in increasing the efficiency of breeding programme (Bharadwaj *et al*., 2011). This is because the phenotypic variability is largely an account of 'G × E' interaction where as the variability at molecular level is devoid of the interference by environment. Diversity analysis is essential to understand *per se* the variability present in germplasm collection that can be practically put to use in plant breeding programmes for recombination breeding. Simple Sequence Repeats (SSRs) are the preferred markers in most areas of molecular genetics as they are highly polymorphic even between closely related lines require very low amount of DNA and are very transferable across populations. SSRs are generally co-dominant markers and are most useful for studies on population genetics and mapping (Jarne and Lagoda, 1996; Goldstein and Pollock, 1997). SSR genotypic data from a number of loci have potential to provide distinctive allelic profiles for establishing genotypes identity (Bharadwaj *et al*., 2010, 2011; Chaudhary *et al*., 2012). Keeping the above points in mind an

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investigation was planned to discern the variability of diverse chickpea lines consisting of elite germplasm and cultivated lines, so that most diverse parents for crossing programme can be identified and diversity of the material can be analyzed.

Materials and methods

A total of 87 elite chickpea genetic stock obtained from Chickpea Project, Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012 were used in this study. The genotypes were designated as GS-1 to GS-87 (Table 1).

DNA Isolation and Genotyping

Isolation of DNA was carried out by as per Kumar *et al*., 2013. A total of 50 sequence tagged microsatellite site (STMS) loci were screened in the accessions of which only 19 were polymorphic (Table 2). The STMS markers were synthesized as per the sequences of (Winter *et al.,* 2000; Bharadwaj *et al*., 2010) from Bioneer, Daejeon, South Korea. BioRad MyCycler thermal cycler, Richmond, USA was used to carry out amplifications in 10 μL volume reaction mixture. This mixture contained 1 μL of 20 ng plant genomic DNA, 1.6 μL of 10×Tris buffer (15mM MgCl2 and gelatine), 1 μL of 10 mM dNTP mix, 1.0 μL each of forward and reverse primer and 0.3 μL of 3 UμL^{-1}*Taq* polymerase. PCR was performed with following conditions 50s at 90^{o}C followedby 18 cycles of denaturation at 94^{o}C for 20s, annealing for 50s at 50^{o}C (Touch down of 0.5^{o}C for every repeat cycle) and1 min elongation at 72^{o}C for 50s. Further 20 cycles of denaturationat 94^{o}C for 20s, annealing for 50s at 55^{o}C and 50s elongation at 72^{o}C were given and final extension at 72^{o}C for7 min were performed. The resolution of PCR products was done on three per cent metaphor gels (Lonza) (Fig. 1).

The polymorphic bands were scored in a spread sheet format with '0' representing absence of band and '1' representing the presence of band 'Null allele' for any specific marker in any genotype was again considered as absence of band (designated as '0'). The data was analyzed in NTSYS-PC software (version 2.21b). Bootstraps were done using Free Tree and Tree view software. For Clustering, UPGMA was used based on the similarity matrix generated on combined data. Polymorphic information content for each STMS primer pair was calculated.

Results

In the present study, a total of 57 STMS loci were analyzed, covering various bin locations on different linkage groups of which 19 were polymorphic (Table 2). A lower level of polymorphism is expected in chickpea which is having a narrow level of diversity compared to other crops and here it was 33.3%. All the 19 STMS loci, in the genetic material under study were found to be highly polymorphic. Excellent polymorphism was revealed by most of these STMS markers. Data from all the 19 STMS loci were utilized for statistical analysis. A total of 87 alleles were found with an average of 4.57 alleles per locus. The highest numbers of alleles were observed in TA194 (five alleles), TA 14, TA80, TA113, TA117 (four alleles each), TA14, TA71, TA110, CaSTMS2, CaSTMS15 and NCPGR4 (three alleles each).

Polymorphism information content (PIC) of each marker system was calculated for each marker and *locus* using the polymorphism information content (Lynch and Walsh, 1998) which gives an estimate of the discriminating power of a *locus* by taking into account not only the number of alleles that are expressed but also their relative frequencies. PIC ranged from 0.11 to 0.94. Highest PIC was observed for NCPGR7 and lowest for TA71.

Some STMS markers were found to have high discriminative power for differentiation of chickpea genetic stocks as the present study demonstrates that 19 out of 57 STMS alleles were found to be unique or rare; unique or rare allele is one with a frequency less than or equal to 0.10. The present findings also indicated instances where the STMS profiles for some of the genotypes displayed maximum variation pattern. Chickpea is highly self-pollinated and should, therefore, reveal lower polymorphism for majority of the genotypes, thus the occurrence of dialleles is also very less with a few primers only and is in concurrence with the reports (Singh *et al.,* 2008; Ahmad *et al.,* 2010; Singh *et al.,* 2014) of a narrow genetic base of chickpea. It has been The STMS data was utilized for estimating pair wise genetic similarities among various entries using Jaccard's coefficient (1908) method. The genetic similarity matrix was further analyzed using UPGMA clustering algorithm by software programme NTSYS pc version 2.21b. The dendogram derived from this analysis is depicted in Fig. 2. The dendogram clearly showed 5 large clusters, 1 small clusters and 1 genotype remained ungrouped (GS39).The

Table 1. Description of 87 elite germplasm accessions used in the present investigation.

Genetic Stock No.	Name	Source of collection	Type	Characteristics	100 SW(g)
1	MPJG-2000-108	JNKVV, Jabalpur	Desi	Improved high yielding breeding line	23.0
2	ICC 4516	ICRISAT, Hyderabad	Desi	Improved high yielding breeding line	11.6
3	KTP-1	IIPR, Kanpur	Desi	Improved high yielding breeding line	26.5
4	IPC-2000-20	IIPR, Kanpur	Desi	Improved high yielding breeding line	23.2
5	IPC-2000-37	IIPR, Kanpur	Desi	Improved high yielding breeding line	14.7
6	IPC-2004-52	IIPR, Kanpur	Desi	Improved high yielding breeding line	13.2
7	IPC-92-39	IIPR, Kanpur	Desi	Improved high yielding breeding line	20.75
8	IPCK-2002-111-2	IIPR, Kanpur	Desi	Improved high yielding breeding line	24.6
9	ICCV 6105	ICRISAT, Hyderabad	Desi	Extra bold, 100 seed weight 43.4g	40.3
10	DGS 730	IIPR, Kanpur	Desi	Improved high yielding breeding line	20.9
11	Basal Pod Mutant	JNKVV, Jabalpur	Desi	Bushy, anthocyanin, lateral spreading	16.8
12	PG0515	MPKVV, Rahuri	Kabuli	Extra bold	60.52
13	Cut ward curve mutant	JNKVV, Jabalpur	Desi	Mutant Stock	14.83
14	Double Podded	IARI, New Delhi	Desi	Very high frequency of double flower green seeded, late, axial pigmentation	17.25
15	IPC 718	IIPR, Kanpur	Desi	Desi, medium bold, multi flower, good poddy, medium tall.	13.7
16	BIO-107	NRCPB, New Delhi	Desi	Soma clonal variants	108.7
17	BIO-108	NRCPB, New Delhi	Desi	Soma clonal variants	15.64
18	BIO-110	NRCPB, New Delhi	Desi	Thick primary branches, Anthocyanin pigment rich stems	28.2
19	ILC 5498	ICARDA, Syria	Kabuli	Erect plant type	22.04
20	ILC 5598	ICARDA, Syria	Desi	Erect plant type	23.0
21	IPC-2004-46	IIPR, Kanpur	Desi	Improved high yielding breeding line	17.65
22	IPC-2006-3	IIPR, Kanpur	Desi	Erect, basal pigmentation	40.08
23	IPC-2006-11	IIPR, Kanpur	Desi	Improved high yielding breeding line	47.76
24	IPC-2006-13	IIPR, Kanpur	Desi	Improved high yielding breeding line	21.23
25	IPC-2006-129	IIPR, Kanpur	Desi	Erect type	24.73
26	IPC-2007-22	IIPR, Kanpur	Desi	Erect type	32.2
27	JGM-7	JNKVV, Jabalpur	Desi	Anthocyanin pigmentation in stem	14.85
28	DG 702	IARI New Delhi	Desi	Erect	19.92
29	DG 724	IARI New Delhi	Desi	Improved high yielding breeding line	18.8
30	DG 733	IARI New Delhi	Desi	Improved high yielding breeding line	18.32
31	DG 752	IARI New Delhi	Desi	Improved high yielding breeding line	16.54
32	HK01-36	CCSHAU, Haryana	Kabuli	Improved high yielding breeding line	12.12
33	HK01-203	CCSHAU, Haryana	Kabuli	Improved high yielding breeding line	34.08
34	HK-00-290	CCSHAU, Haryana	Kabuli	Improved high yielding breeding line	28.0
35	Double Pod High Density	IARI, New Delhi	Desi	Erect type with higher number of double flowers and double pods	10.5
36	EC565214	ICRISAT, Hyderabad	Desi	Erect type	
37	EC565211	ICRISAT, Hyderabad	Desi	Erect type	17.1
38	Kabuli Dark Brown	IARI New Delhi	Kabuli	Dark brown Kabuli seed type	18.1
39	Market Collection-1(K)	IARI New Delhi	Kabuli	Bold seeded	51.4
40	Gokcee (FLIP 87-8C)	ICARDA, Syria	Kabuli	Drought tolerant line from ICARDA	28.54
41	JG-2003-14-2	JNKVV, Jabalpur	Desi	Erect, compact	32.14
42	JG2-14-11	JNKVV, Jabalpur	Desi	High yielding, small seeded breeding line	22.44
43	BGM-408	IARI, New Delhi	Desi	Variety developed through mutation breeding	24.54
44	BGM-417	IARI, New Delhi	Desi	Variety developed through mutation breeding	12.23
45	BGM-547	IARI, New Delhi	Desi	Variety developed through mutation breeding	22.4
46	ICC-5434	ICRISAT, Hyderabad	Desi	Spreading Type	23.84

Contd.

Table 1 Contd.

Genetic Stock No.	Name	Source of collection	Type	Characteristics	100 SW(g)
47	ICC-16644	ICRISAT, Hyderabad	Kabuli	Improved breeding line	29.4
48	IPC-2006-125	IIPR, Kanpur	Desi	Erect	25.32
49	HC-5	IIPR, Kanpur	Desi	Erect	28.38
50	CSJK-25	CSKVV, Kanpur	Kabuli	Extra bold	28.27
51	IGK-I	IGKVV, Raipur	Kabuli	Extra bold	29.22
52	MNK-1	UAS, Raichur	Kabuli	Extra bold	29.21
53	PCS-10	IARI, New Delhi	Desi	Improved breeding line	34.08
54	PA-0590	IARI, New Delhi	Desi	Improved breeding line	13.98
55	Roasted Type	IARI, New Delhi	Desi	Seed suitable for parching use	30.66
56	Out warded curve	IARI, New Delhi	Desi	Mutant stock for leaflet shape	15.14
57	Pinnate leaf	IARI, New Delhi	Kabuli	Mutant stock for leaflet shape	27.91
58	Crack seed	IARI, New Delhi	Desi	Simple leaf, Mutant stock for seed type	29.9
59	Vareigata (D)	IARI, New Delhi	Desi	Mutant stock for leaflet shape	15.93
60	Fasiata Mutant	IARI, New Delhi	Desi	Mutant stock for leaflet shape	13.57
61	DG F_6-1209	IARI, New Delhi	Kabuli	Simple leaf mutant stock for leaflet shape	25.5
62	DG F_5-205	IARI, New Delhi	Kabuli	Simple leaf mutant stock for leaflet shape and white flowered	13.01
63	AKG-70	Akola, Maharashtra	Desi	Improved breeding line	15.84
64	ICC-12825	ICRISAT, Hyderabad	Kabuli	Small seed	11.76
65	ICC-17256	ICRISAT, Hyderabad	Desi	Improved breeding line	21.92
66	ICC-16341	ICRISAT, Hyderabad	Desi	Open flower	10.6
67	ICC-16129	ICRISAT, Hyderabad	Desi	Open flower	10.7
68	ICC-13925	ICRISAT, Hyderabad	Desi	Improved breeding line	11.6
69	ICC-17109	ICRISAT, Hyderabad	Kabuli	Extra large seed (100 Seed weight 65g)	44.1
70	Harigantars	IIPR, Kanpur	Desi	Black seed, erect, early, long internodes, Local land race	11.23
71	SBD-377	IARI, New Delhi	Desi	Extra bold simple leaf desi type	48.2
72	ILC-212	ICARDA, Syria	Kabuli	Small seeded	41.4
73	C-235	ICARDA, Syria	Desi	Small seeded	11.28
74	TDB	IARI, New Delhi	Desi	Tuberculated, desi, bold seeded	27.12
75	C-214	IARI, New Delhi	Desi	Improved breeding line	18.0
76	GL-769	IARI, New Delhi	Desi	Improved breeding line	14.7
77	BG-2073	IARI, New Delhi	Desi	Improved breeding line	13.96
78	K-850	Kanpur	Desi	Improved breeding line	26.72
79	CSG-8962	CSSRI, Karnal	Desi	Salinity tolerant	12.24
80	Biogreen	NRCPB, New Delhi	Pea type	Pea type kabuli with green seed coat colour	12.25
81	JG-315	JNKVV, Jabalpur	Desi	Wilt resistant donor for all the races except *foc*1	14.22
82	DG-5033	IARI, New Delhi	Desi	Extra bold desi	33.21
83	BGD-132	IARI RS Dharwad	Kabuli	Lateral spreading	23.4
84	BGD-9812	IARI RS Dharwad	Desi	Lateral spreading	13.08
85	RSG-143-1	RAU, Jodhpur	Desi	Breeding line tolerant to high temperature	26.2
86	JG-11	JNKVV, Jabalpur	Desi	Double poded high yielding line	22.1
87	DG-5066	IARI, New Delhi	Desi	Extra bold seed	21.26

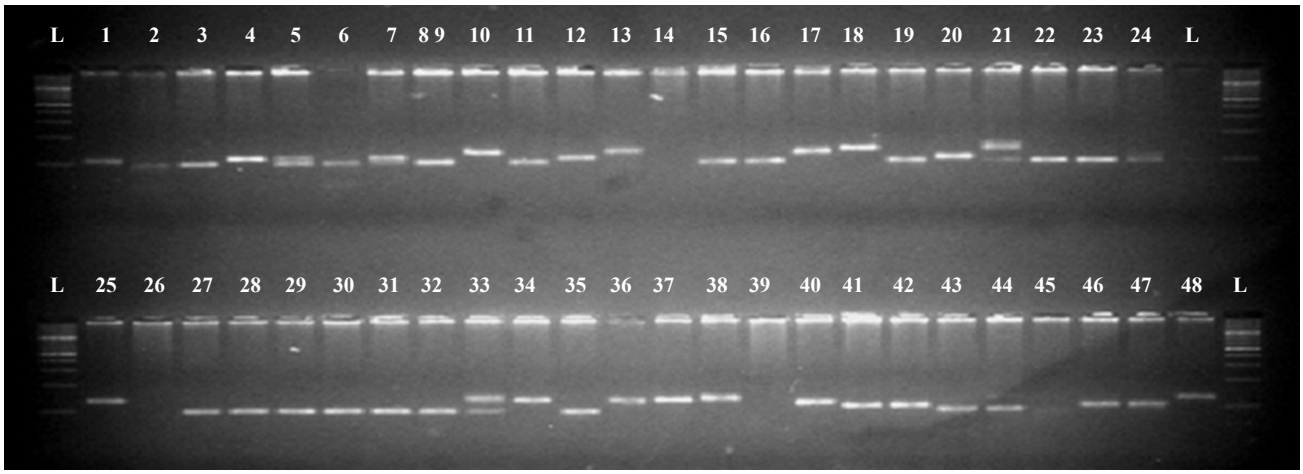

Fig. 1(a) SSR-PCR amplification products of chickpea with accessions using SSR primer TA194

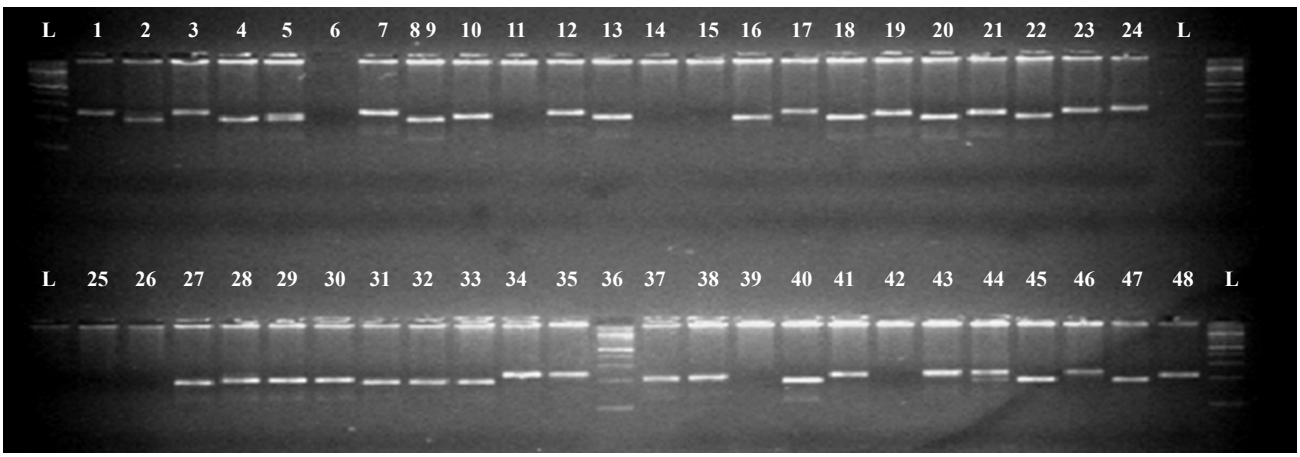

Fig. 1(b) SSR-PCR amplification products of chickpea with accessions using SSR primer TA80

Table 2. SSR primers used for chickpea germplasm analysis

S. No.	Primer name	Linkage group	PIC value	Heterozygosity
1	TA103	LG2	0.33	0.42
2	TA3	LG9	0.18	0.20
3	TA14	LG4	0.47	0.53
4	TA71	LG5	0.11	0.11
5	TA186	LG4	0.21	0.23
6	TA194	LG2	0.68	0.72
7	TA203	LG1	0.17	0.17
8	TA200	LG2	0.59	0.59
9	TA80	LG6	0.59	0.66
10	TA96	LG7	0.25	0.29
11	TA113	LG 1	0.45	0.53
12	TA117	LG2	0.47	0.56
13	TA110	LG2	0.40	0.52
14	CaSTMS2	a	0.10	0.11
15	CaSTMS15	a	0.51	0.60
16	NCPGR4	LG6	0.44	0.52
17	NCPGR6	LG6	0.84	0.84
18	NCPGR7	LG4	0.94	0.94
19	NCPGR12	LG7	0.27	0.32

Source: Bharadwaj *et al.,* 2010, 2011; a – from Huttel *et al*.; Varshney *et al.,* 2013.

cluster I, II, III, IV, V and VI comprised of 36, 5, 13, 11, 19 and 2 genotypes respectively. Maximum Jaccard's correlation was seen for the genotypes IPC-2000-20 (GS4) and IPC-2000-37 (GS5) while the genotypes BGD-132 (GS83) and BGD-9812(GS84) were having highest similarity index, while the genotypes SBD377 (GS71) and ILC-212(GS72) have shown the highest dissimilarity with all the other genotypes and distinctly formed a separate cluster (cluster VI) (Fig. 2).

Discussions

Among the various DNA based markers, microsatellite or STMS markers are highly accepted and have been used in the diverse crop plants owing to their abundance in the genome (Powell *et al.,* 1996). The application of STMS markers in genetic analysis of chickpea, started with an initial study of (Huttel *et al.,* 1999) and after that, the power and potential of SSR markers for a broad

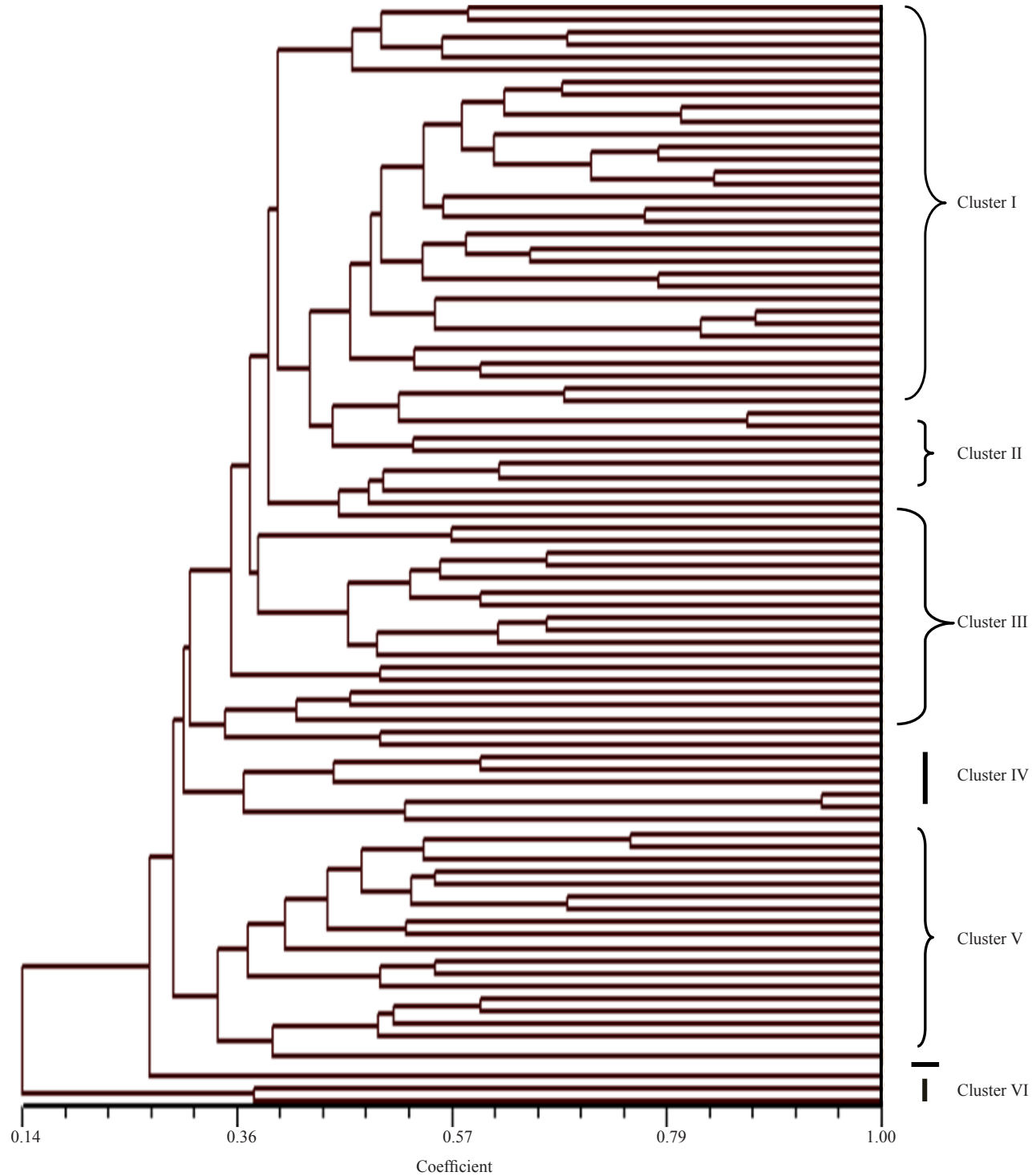

Fig. 2. Jaccard's similarity grouping of 87 chickpea genotypes

range of applications in genetic and breeding of chickpea has been well demonstrated by a number of researchers (Huttel *et al.,* 1999; Winter *et al*., 2000; Flandez-galvez *et al*., 2003; Choumane *et al*., 2000). Microsatellite genotypic data from a number of loci have potential to give unique allelic profiles or DNA fingerprints for establishing genotypes identity (Bharadwaj *et al*., 2010). A narrow genetic base in chickpea warrants immediate base broadening efforts. Though morphological diversity is generally used by the breeders as a criteria in making

Table 3. Clustering of genotypes based on UPGMA analysis of 87 genetic stocks of chickpea using SSR markers

S. No.	Cluster No.	No. of genotypes	Names of genotypes
1.	I	36	GS1, GS2, GS3, GS4, GS5, GS6, GS7, GS8, GS10, GS11, GS13, GS14, GS15, GS16, GS18, GS19, GS20, GS23, GS24, GS25, GS26, GS27, GS28, GS29, GS30, GS31, GS41, GS42, GS43, GS44, GS45, GS49, GS59, GS75, GS85, GS86.
2.	II	5	GS9, GS12, GS21, GS37, GS40.
3.	III	13	GS17, GS22, GS32, GS36, GS35, GS38, GS46, GS48, GS55, GS56, GS61, GS68, GS70.
4.	IV	11	GS57, GS69, GS65, GS58, GS60, GS47, GS52, GS87, GS50, GS51, GS53.
5.	V	19	GS33, GS34, GS67, GS64, GS66, GS79, GS80, GS73, GS78, GS74, GS63, GS77, GS76, GS54, GS81, GS82, GS62, GS83, GS84.
6.	VI	2	GS71, GS72.

crosses, it is clearly known that the manifestations of 'G × E' interactions make closely related individuals to appear diverse and thus there are greater chances of these being used in crossing programmes. Knowledge of molecular diversity in the material helps discern this diversity and in identification of parents for crossing programme (Bharadwaj *et al.,* 2010).

For acceleration and optimizing the long process of creating new chickpea cultivars molecular markers are included as analyzing tools. Molecular markers are considered as good candidates for classifying genotypes in different groups and thus assessing genetic distances as well as genetic expected gains. RAPD were earlier used. However owing to greater reliability and repeatability, SSR markers are now being increasingly used for discerning genetic diversity.

Nineteen STMS primer pairs could amplify 1-4 loci per primer pair generating 4.57allels per locus on an average. Contrary to the fact that chickpea is a self pollinated crop and should generate lower polymorphism. World chickpea germplasm has a narrow genetic base (Nguyen *et al.*, 2004) and lacks the desirable traits needed for ready utilization in varietal improvement programs. A narrow genetic base and sexual incompatibility with other *Cicer* wild types, which carry the sources for various desirable traits, contribute to the limited progress in the improvement of chickpea yield (Chaudhary *et al*., 2012). The presence of multiple alleles may have occurred to the fact that there is a very high residual heterozygotic balance conserved due to *Desi* × *Kabuli* introgression that played a major role in chickpea evolution. This may be one of the causes for obtaining multiple bands using SSR markers (Singh *et al.,* 2008). A high degree of molecular polymorphism was exhibited by all the markers studied indicates the markers that have been used for diversity analysis were sound. The PIC ranged from 0.10 to 0.94 and heterozygosity ranged from 0.11 to 0.94. The Jacards similarity matrix dendogram constructed using the UPGMA method showed that all the clusters were dissimilar and grouped into seven major clusters. A critical examination of these clusters with indicates that the grouping was primarily based on seed size. Cluster VI has SBD 377 and ILC 212. SBD 377, a simple leaf mutant developed at IARI had an ICARDA line in its pedigree PRR1, a derivative from FLIP 90-166, an ICARDA line and thus would have got clustered in proximity with GS72 *i.e*. ILC 212. The market collection GS39, a bold seeded *kabuli* type remained un-grouped as it was very bold in its size and does not represent a released cultivar but market collection obtained under the ISOPOM trial. Cluster two comprised mostly either bold seeded or erect types. Thus plant architecture also played an important role. However, contrary to earlier workers reports, it is clearly noticed that the place where the cultivars were developed did not play a major role in grouping. This may be due to the fact that, the elite breeding lines included in this study obtained from different centers were developed from germplasm either obtained from ICRISAT or ICARDA. In the previous study of Bharadwaj *et al*. (2011) specific lines of ICARDA, ICRISAT and IARI were used where all the lines from ICARDA and wild species were grouped as a distinct cluster. The breeder's generally use diverse sources selected based on morphological traits and their observation in making crosses. Similar results were obtained by Choudhary *et al*. (2012).

Results from the present study support the observations of several workers about the potential utility of STMS in characterizing asparagus lines (Huttel *et al.,* 1999; Winter *et al*., 2000; Flandez-galvez *et al*., 2003; Choumane *et al*., 2000). There was reasonably high rate of polymorphism for at least ten markers namely TA194, TA80, TA113, TA117, TA14, TA110, NC6, NC7, CaSTMS15 and NCPGR4 out of 19 STMS markers loci in the present study. This pointed towards the scope for further utilization of these markers for characterization of different cultivars of chickpea. The STMS polymorphism were assayed using a DNA pooling

strategy, although it is not supposed to do as all the genotypes under study are pure lines (Flandez-Galvez *et al*., 2003) demonstrated the power and potential of SSR markers for a wide range of applications in genetic and breeding of chickpea. Molecular markers being easily reproducible they have become favourite tools with breeders and biotechnologists to discern the traits as well as to study diversity among cultivars (Satyavathi *et al*., 2005). However, no correlation could be derived from PIC and allele numbers in this study. Further, Greater gains can be obtained by crossing lines MPJG-2000-108 with SBD 377 for *desi* improvement and PG 0515 with ILC 212 for *kabuli* improvement. These genotypes have been identified as most diverse in the present study. Pre-breeding and Base broadening through *kabuli* × *desi* introgression for greater gains is an important activity of the breeders. This increases the diversity obtained in the succeeding generations to carry out further selections as there is enormous amount of variation that is seen in these generations for seed size, seed type and other traits. In the present study greater base broadening can be achieved by using ICC 4516 and ILC 212 as parents in the breeding programme.

This study helped to determine the genetic relationship between elite genetic stock of chickpea based on STMS marker data, and these results greatly contribute to germplasm bank management, conservation programs, and breeding purposes. The occurrence of unique alleles or rare STMS alleles provides an immense opportunity for generation of comprehensive fingerprint database. The present investigation also gives an idea of the interrelationship among the genotypes and highlights the need for helpful supplementation of pedigree data and other morphological data with the database generated by STMS marker to efficiently discover the genetic inter-relationship among the genotypes, fingerprint the varieties for their protection and most importantly select parents for a sound breeding programme.

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