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Short Communications

Molecular analysis for genetic structure of biotic and abiotic stress resistant genotypes in chickpea (*Cicer arietinum* L.)

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Molecular characterization of biotic (*Ascochyta* blight, *Fusarium* wilt & dry root rot) and abiotic (drought & salinity) resistant genotypes of chickpea was carried out using a specific set of 20 polymorphic STMS (sequence tagged microsatellite site) markers. The number of alleles ranged from 1 to 3 alleles per locus. The PIC (polymorphism information content) value ranged from 0.0 to 0.656 with an average of 0.268, indicating the considerable efficiency of markers for studying the polymorphism level. The primer GA-33 showed maximum PIC value (0.656), while the ten primers had the 0.0 value. The dendogram derived from the analysis had six clusters. Many pairs showed the maximum similarity (0.983), whereas accessions ICC-1392 and ICC-2065 showed the minimum similarity (0.900). The conservation of genotypes ICC-2580, ICC-1392 and ICC-2065 was recommended for their utilization in Middle-east Asia region chickpea improvement programme.

Keywords: Chickpea, microsatellite marker, molecular diversity, PIC, STMS

Chickpea (*Cicer arietinum* L.; Family: *Fabaceae*) is a self-pollinated diploid with 2X=16 chromosomes and genome size of 732 Mb. It is the 3rd important food legume in terms of the cultivated area (11.7 million ha) and total annual production (9.3 million tonnes)[1]. The average global productivity of chickpea is about 0.8 tonnes ha^{-1}, which is far below the actual yield potential because the crop is subjected to a number of fungal diseases throughout the growing season. *Ascochyta* blight, *Botrytis* grey mould, *Fusarium* wilt, black root rot, dry root rot, pod borer and leaf miner are the important biotic stresses; while drought, salinity and fluctuations in temperature (both extremes) are the major abiotic stresses, imposing the major constraints to chickpea productivity. Chickpea is very sensitive to salinity and it affects about 100 million ha of arable land worldwide and about 13 million ha in India[2]. It has been previously stated that there is too little variability for salinity tolerance in chickpea to undertake a successful breeding programme for salinity tolerance. Drought stress is also one of the most serious constraints for the productivity of chickpea. Several physiological, morphological and phonological traits have been listed to play a significant role in crop adaptation to drought stress during soil drying. The root traits, such as, biomass, length density and depth, have been proposed as the main drought avoidance traits to contribute to seed yield under terminal drought environments. There is an urgent need for a major breakthrough in the new technology front to increase yield levels through morpho-physiological changes in plant type and successful hybrid technology, development of multiple disease resistant varieties and tolerance to abiotic/biotic stresses.

The development of core and mini core collections has been suggested as a gateway for the utilization of genetic diversity in crop improvement[3]. More recently a composite collection of 3000 chickpea accessions has been developed[4]. The composite collection consisted of core collection and representative sample of unique accessions from ICARDA, beside sources of resistance to biotic and abiotic stresses, for early maturity, multi-seeded pods, double podded, large-seed size, high seed protein, nodulation and responsiveness to high input conditions. The major objective of the present study was to analyze the level of diversity at microsatellite loci level within the monitored set of chickpea genetic resources and to identify genotypes for biotic/abiotic stresses for their use in chickpea breeding programmes.

Twenty chickpea accessions, a subset of mini core germplasm/landraces of India, Algeria and Iran obtained from ICRISAT, Hyderabad were used as plant material. These germplasm lines/landraces were

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resistant to biotic and abiotic stresses (Table 1). Genomic DNA was isolated from young leaves using CTAB method[5] with some modifications. Twenty STMS markers were used for amplifications (Table 2). PCR analyses were performed in the Eppendorf Mastercycler gradient in a total volume of 10 µL, containing 25 ng of template DNA, 1× buffer (Bangalore Genei, India), 1 mM $MgCl_2$ (Bangalore Genei, India), 0.125 mM dNTPs (Bangalore Genei, India), 0.5 µM of each primers (Integrated DNA Technology, Imperial Life Sciences, USA), 1U/µL *Taq* DNA polymerase ((Bangalore Genei, India). The thermal profile cycling conditions of denaturation at 95°C for 20 sec; annealing at 52-70°C for 50 min and elongation at 72°C for 50 min for 35 cycles was preceded by an initial denaturation at 95°C for 2 min. In the end, a final extension at 72°C for 7 min was given. The amplicons were resolved on 2% agarose gel containing ethidium bromide (0.5 µg/mL) in 1× TBE buffer at 50 volts. Frequencies of incidence of all polymorphic alleles were calculated and used for determination of polymorphic information content[6]. For each STMS marker, polymorphism information content (PIC) was determined[7]. The similarity matrix was further analyzed by using NTSYS-pc version 2.2 to produce an agglomerative hierarchical classification[8] by employing UPGMA (Unweighted Paired Group method using Arithmetic Mean) with average linkage[9].

In the genetic material under study, CaSTMS12, GA22, GA26 and GA33 STMS markers were found to be highly polymorphic (Fig. 1). Three alleles were observed in primers CaSTMS12, GA22, GA26 and GA33, followed by two alleles in CaSTMS20, GA2, GA8, GA11, GA40 and GA43, and finally one allele in the primers CaSTMS21, CaSTMS23, CaSTMS24, GA13, GA14, GA20, GA21, GA31 and GA102 (Table 2). Primer GA33 had maximum number (54) of genotypes

Table 1—List of 20 chickpea accessions resistant to biotic and abiotic stress used in the study

No.	Genotypes	Source	Stresses
1	ICC-1356	India	*Fusarium* wilt
2	ICC-1392	India	*Fusarium* wilt
3	ICC-1397	India	*Fusarium* wilt, salinity tolerant
4	ICC-1398	India	*Fusarium* wilt
5	ICC-1422	India	Drought tolerant
6	ICC-1431	India	Salinity tolerant, *Fusarium* wilt
7	ICC-1510	India	Salinity sensitive, *Fusarium* wilt
8	ICC-1710	India	Dry root rot, salinity tolerant
9	ICC-1715	India	Salinity sensitive, *Fusarium* wilt
10	ICC-1882	India	Drought tolerant
11	ICC-1915	India	*Ascochyta* blight, salinity tolerant
12	ICC-1923	India	Salinity sensitive, *Fusarium* wilt
13	ICC-2065	India	Fusarium wilt, salinity tolerant
14	ICC-2072	India	*Fusarium* wilt, salinity tolerant
15	ICC-2210	Algeria	*Fusarium* wilt
16	ICC-2242	India	Dry root rot
17	ICC-2263	Iran	Salinity sensitive
18	ICC-2277	Iran	Dry root rot, salinity tolerant
19	ICC-2507	Iran	Salinity sensitive
20	ICC-2580	Iran	Salinity tolerant

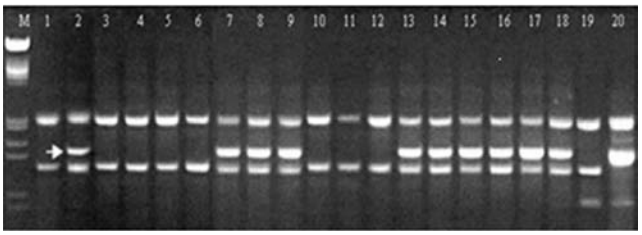

Fig. 1—STMS profiling of 20 accessions of chickpea. [M, Marker; Lanes 1-20, Accessions as in Table 1]

Table 2—PIC of STMS loci across various genotypes

Primers	Alleles	No. of genotypes sharing alleles	Frequency of STMS alleles	PIC
CaSTMS12	a_1	2	0.033	0.532
	a_2	20	0.483	
	a_3	20	0.483	
CaSTMS13	a_3	20	1	0
CaSTMS20	a_2	20	0.5	0.5
	a_3	20	0.5	
CaSTMS 21	a_3	20	1	0
CaSTMS 23	a_3	20	1	0
CaSTMS 24	a_3	20	1	0
GA2	a_2	20	0.5	0.5
	a_3	20	0.5	
GA8	a_2	20	0.5	0.5
	a_3	20	0.5	
GA11	a_2	20	0.5	0.5
	a_3	20	0.5	
GA13	a_3	20	1	0
GA14	a_3	20	1	0
GA20	a_3	20	1	0
GA21	a_3	20	1	0
GA22	a_1	11	0.248	0.57
	a_2	17	0.415	
	a_3	18	0.443	
GA26	a_1	7	0.115	0.599
	a_2	20	0.44	
	a_3	20	0.44	
GA31	a_3	20	1	0
GA33	a_1	14	0.231	0.656
	a_2	20	0.381	
	a_3	20	0.381	
GA102	a_3	20	1	0
GAA40	a_2	20	0.5	0.5
	a_3	20	0.5	
GAA43	a_2	20	0.5	0.5
	a_3	20	0.5	

sharing alleles, followed by 42 genotypes sharing alleles in case of primer CaSTMS12, and minimum 40 genotypes in case of primers like CaSTMS13, GA13, GA14, GA20, GA21 and GA31. On the basis of genotypes sharing alleles, the frequency of alleles for the primer GA33 was calculated to be 0.231, 0.381 and 0.381. Based on the allele frequencies, the PIC values were estimated for different STMS loci. The PIC values ranged from 0.0 to 0.656. The PIC value ranging from '0' (monomorphic) to '1' (highly discriminative with many alleles in equal frequency) is an indication of discriminative power of marker not only for number of alleles at a locus but also for relative frequencies of those alleles in the genotypes under study. On the basis of number of genotypes sharing alleles and their frequencies, the highest value of PIC (0.656) was found for the primer GA 33 because of well distributed presence of three alleles across the genotypes of *C. arietinum.* The lowest PIC (0.00) value is indicative of no allelic variation among genotypes and it was observed for the primers CaSTMS13, CaSTMS21, CaSTMS23, CaSTMS24, GA13, GA14, GA20, GA21, GA31 and GA102. The low PIC value was observed probably due to poor distribution of alleles in the genome. On the basis of allele amplification, the number of alleles present in a genotype shows the polymorphism level; as primer CaSTMS12 amplified 3 alleles, while primer CaSTMS21 did only single alleles. Finally the PIC was calculated to measure the informativeness and polymorphism of 20 STMS loci. On the basis of PIC values, we can identify the genotypes sharing alleles and the distribution of alleles in the genome.

STMS data were used to make pair wise comparison of the accessions based on shared and unique products with NTSYS-PC (version 2.2s). Genetic relationship among accessions were evaluated by similarity matrix based on Jaccard's, which ranged from 0.900-1.00 indicating a close relationship amongst themselves. The maximum similarity among 20 genotypes was exhibited with a similarity coefficient value of 0.983 between many pairs of chickpea accessions, *viz.*, ICC-1392 *vs* ICC-1356, ICC-1397 *vs* 1392, ICC-1422 *vs* ICC-1356, etc. Whereas the minimum similarity with a coefficient value of 0.900 was exhibited by the accessions ICC-1392 and ICC-2065. The similarity coefficient was used for cluster analysis following the UPGMA algorithm irrespective of low genetic variation amongst them for their very specific utility.

The results of cluster analysis was displayed in the form of a 2-dimensional diagram known as a dendrogram (Fig. 2). The dendrogram illustrates the merges or divisions, which have been made at successive levels. The resultant phenogram grouped 20 accessions into three distinct clusters with different sub clusters, *viz.*, IA, IB, II, IIIA, IIIB and IIIC. Cluster analysis is an important approach to determine genetic variability by using established computer algorithms developed in the fields of multivariate statistics. The basic aim of cluster analysis is to group individuals or objects based on the characteristics they

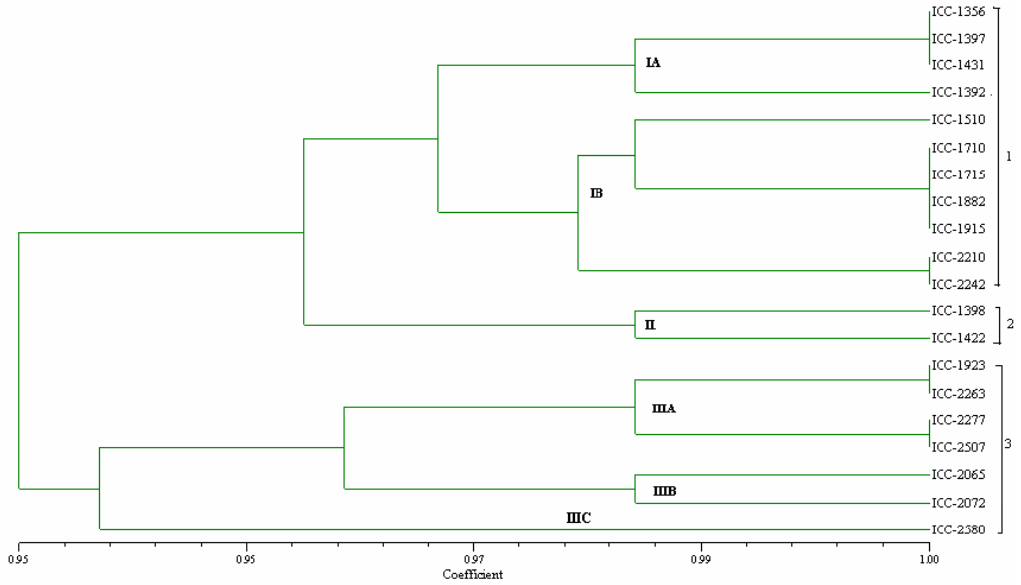

Fig. 2—UPGMA based cluster analysis of chickpea using STMS primers.

possess, so that the individuals with similar descriptions are mathematically gathered into same cluster. The cluster-I comprised of 11 genotypes, which were further grouped into two subclusters, *viz*., IA and IB. Cluster IA comprised of 4 genotypes, *viz*., ICC-1356, ICC-1397, ICC-1431 and ICC-1392; while subcluster IB was represented by seven genotypes, *viz*., ICC-1510, ICC-1710, ICC-1715, ICC-1882, ICC-1915, ICC-2210 and ICC-2242. Of these 11 genotypes, the 9 genotypes ICC-1356, ICC-1397, ICC-1431, ICC-1710, ICC-1715, ICC-1882, ICC-1915, ICC-2210 and ICC-2242 formed three different small clusters, which showed the similarity coefficient value of 1.00 indicating that genotypes of these small clusters are similar with each other probably due to common ancestors. Cluster II was represented by only 2 genotypes ICC-1398 and ICC-1422 with a similarity coefficient value of 0.983, indicating their origin from different but closely related parents. Cluster III contained 6 genotypes, which were further divided into 3 subclusters, IIIA IIIB and IIIC. Subcluster IIIA was represented by two different groups having four genotypes, namely, ICC-1923, ICC-2263, ICC-2277 and ICC-2507, which showed similarity coefficient value of 1.00 indicating common parent. Subcluster IIIB was represented by two genotypes ICC-2065 and ICC-2072. These two genotypes showed similarity coefficient of 0.983 indicating their origin from different but closely related parents. The genotype ICC-2580 was placed in a separate subcluster IIIC and remained isolated having the similarity index of 0.917 with the genotype ICC-1392, indicating their origin from diverse parents. The most common similarity coefficient value showed among all 20 genotypes was 0.983. The Genotype ICC-2580 placed individually at the end of the cluster III showed the similarity coefficient value of 0.967 with ICC-2210, ICC-2242, ICC-2277 and ICC-2507, while 0.917 with ICC-1392. The genotype ICC-1392 exhibited the lowest similarity coefficient value of 0.90 with ICC-2065. Thus, the results show that the genotypes ICC-2580, ICC-2065 and ICC-1392 could be utilized for hybridization programmes for combining some desirable traits due to considerable degree of variability available between them.

In conclusion, the primers CaSTMS 12, GA 22, GA 26 and GA 33 being polymorphic with 3 alleles per locus can be used for molecular characterization and diversity analysis of chickpea. The diversity analysis further indicates that the ICC-2580, a soil salinity tolerant landrace from Iran, and ICC-1392/ICC-2065, *Fusarium* wilt resistant germplasms from India should be conserved and be utilized for mapping/cloning of genes along with their utilization in hybridization programmes in order to generate sources of abiotic (salinity tolerant) and biotic (*Fusarium* wilt) stresses in chickpea improvement in the Middle-east Asia region.

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