International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-10, October 2016 Pages 79-83

Effectiveness of STMS Markers for Generating Unique DNA Profiles in Chickpea(Cicerarietinum L.) Germplasm

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***Abstract*— Chickpea is diploid (2n=2x=16) self-poillinated, third most important grain legume in world. India is largest producer and consumer of chickpea. Because of narrow genetic base, scope of improvement is much lesser in chickpea as compared to major cereal crops. Available germplasm must have to be systematically evaluated and uniquely identified with the help of modern technology like molecular markers. STMS markers has proved their worthiness in germplasm characterization in many major crops and they are proved to be useful in chickpea too. In the present study total 46 chickpea germplasm were uniquely identified with the help of 9 STMS loci. Out of them TA 72 was least polymorphic while TA 8 was highly polymorphic with 5 uniformly distributed diverse alleles. TA 8 has the maximum resolving power to distinguish between the different genotypes of chickpea germplasm.**

***Index Terms*— Chickpea, Molecular Markers, STMS, DNA Profiles**

I. INTRODUCTION

Chickpea is diploid (2n=2x=16) self-poillinated, third most important grain legume in world after dried beans and dried peas. Cultivation of chickpea is mainly confined to Asia contribute to the 90% of global area and production (FAOSTAT, 2004). Based on the size, colour and shape of the seed, germplasm of chickpea can be categorized into two groups, Kabuli type and Desi type. Kabuli type produce large, round, white or pale cream coloured seeds while Desi type have small, variably pigmented seeds that sharp edges with angular overall shape. Desi types are mainly cultivated in India who is the largest producer and consumer of chickpea. Kabuli types are mainly cultivated in Mediterranean region, South and Central America. Chickpea grain provides 18-22% protein that meet out the protein demand of poors or vegetarian classes. Chickpea also helps in maintaining soil productivity by fixing up to 141 Kg Nitrogen per hectare (Rupela, 1997). Chickpea germplasm severely limited bottleneck effect during its course of evolution in Fertile Crescent region thereby have narrow genetic base as very few survive the drastic climate change of that region. DNA marker technologies have revolutionized diversity analysis and establishment of identity ofdistinguished genotypes. The marker has stable phenotype, high polymorphism, independence from environment, quicker and convenient, sometimes cheaper in application rather than going for evaluation of certain quality trait which require costly biochemical testing. Out of the available range of molecular markers, it is Sequence Tagged Microsatellites Site (STMS) markers, increasingly preferred over existings becauseof their abundance, genomic coverage, reproducibility, co-dominance and high polymorphism. STMS markers are microsatellite sites based markers which utilized hypervariable hundreds base pair long regions of very short (≤ 4 base pair long) repetitive DNA sequences.

With the increasing number of germplasm, germplasm banks have redundancy of germplasm that leads to cost escalation for their maintenance. From breeding point of view, a researcher requires highly diverse genotypes to gain maximum by their crossing but most of the crosses yield mediocre recombinants because of poor choice of parents. STMS makers can be utilized for detecting polymorphism at molecular level that will help in generation of DNA fingerprint profiles and their categorization according to their molecular polymorphism.

II. MATERIALS AND METHODS

The present investigation undertaken at Genetics division of IARI, Pusa, New Delhi in 2005. 46 genotypes from chickpea and its wild relatives were collected that include 45 from Cicerarietinum and one from derivatives of interspecific hybrid of Cicerarietinum x Cicerreticulatumfrom the pulse block of the division. As chickpea is higly self-pollinated crop therefore samples can be bulked. DNA from the germplasm was extracted with the help of CTAB method of Doyle and Doyle, 1987 with minor modifications. The STMS primers were synthesized by the Gene Script Corporation under the DBT funded project on molecular mapping of chickpea genome. The most polymorphic primer sequence were selected from the what available in the public domain (Winter et al, 1999 and Huttelet al 1999). STMS marker analysis was completed in the following steps, 1). Genomic DNA isolation and quantification. 2). DNA amplification in PCR with help of STMS primers. 3). Gel electrophoresis of the amplified products. 4). SSR data entry and verification.STMS markers used in the study wasCaSTMS 25, TA 2, TA 8, TA 21, TA 43, TA 72, TA 80, TA 125, TR 58 and TS 45.

III. RESULT AND DISCUSSION

In the present study, a total of 11 STMS loci were analysed, covering various bin locations on different linkage group (Table 2). All the 11 STMS loci, in the genetic material under study were found to be highly polymorphic except TA-72.

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Table 1: STMS Allelic Profiles Of Selected Chickpea Germplasm

A. S.No.	*B. Genotypes*	**Primer name**								
		CaSTMS25	**TA 2**	**TA 8**	**TA 21**	**TA 43**	**TA 64**	**TA 72**	**TA 80**	**TA 125**
1	Pusa 212	a3	a1	-	a5	-	a3	a2	a1	a2, a3
2	Pusa 372	-	a2	a1	a6	a2, a3	a3	a2	a1	a2, a3
3	H 75-35	-	a2	-	a5	-	a2	a2	a1, a3	a1, a3
4	Pusa 361	a2	a4	a2	a3	-	a2	a2	-	a2, a3
5	IPC 92-132	-	a2	a3	-	a2	a2, a4	a2	a4	a2
6	Pant G 114	a3, a4	a2	a3	a3	a2, a3	a2	a2	a1	a2, a3
7	JC 62	a3	a2	a3	a5	a2	a4	a2	a1	a1
8	C 235	-	a2	a3	a3	a2	a2	a2	a3	a4
9	BG 365	a3	a2, a4	a4	a4	-	a3	a2	a4	a3
10	BG 391	a2	a2	a3	a5	a1, a3	a3	a2	a1	a2
11	IPC 92-1	a2	a2	-	a6	-	a3	a2	-	a3
12	BG 1101	-	a2	a4	a5	a2	a2	a2	a4	a4
13	Pusa 1003	a3, a4	a1, a4	a4, a5	a2	a2	a2	a2	a4	a4
14	FG 711	a3	a3, a4	a4	a5	-	a5	a2	a2	a3
15	ICCV 96030	a2	a3, a4	a3, a5	a5	-	a5	a2	a4	a3
16	FG 712	a2	a1, a3, a4	a3	a2	a3	a4	a2	a1, a3	a2
17	BG 1044	a2	a1	a3	a6	a3	a4	a2	a4	a2
18	Pusa 1103	a2, a4	a3, a4	-	a5	a3	-	a2	-	a2
19	GCP 9504	a2	a3, a4	a3	a5	-	a4	a2	a5	a2
20	Pusa 256	a2	a4	-	a5	a3	a3	a2	a5	a5
21	ICRISAT 3077	-	a2, a4	-	a3	a2	a4	a2	a4	a4
22	FLIP 90-166	a2	a2	-	a3	a3	a1	a2	a5	a2
23	IPCK 96-3	a2	a4	-	a5	a3	a4	a2	a2	a3
24	ICC 12450	a2	a2, a4	a3	a5	a3	a4	a1	a3	a3
25	ICCV 88506	a2	a2, a4	a4	a3	-	a4	-	a3	-
26	BG 1107	a3, a4	a2	a3	a3	a2, a3	a4	a2	a5	a2
27	ICRISAT 3073	a3	a1	-	a2, a3	a3	a2	a2	a1	a2
28	IPC 927	a2	a1, a5	a4	a3	a3	a2	a2	a5	a3
29	Pusa 362	a3	a5	a4	a2	-	a3	a2	a5	a3
30	F 2-27	a1	a5	-	a1	-	a3	a2	a1, a5	a3
31	SAKI 95-116	a4	a5	-	a3	-	a4	a2	a4	-
32	IPC 97-7	a2	a5	-	a1	a3	a1	a2	a5	-
33	*C. reticulatium*	a3	a3, a5	a4	a1	a3	a4	a2	a2	-
34	BG 1072	-	a1, a3, a5	a3	a2	a1, a3	a3	a2	a2, a3	a2
35	CSG 8962	a2	a5	a1	a2	a3	a2	a2	a3	a3
36	HOO-108	a2	a1, a5	a2	a3	a3	a1	a2	a3	a4
37	ICRISAT 3070	a4	a2, a4	-	a2	a3	a1	a2	a4	-
38	ICC 4958	a3	a2, a4	a2	a1	a3	a1	a2	-	a3
39	ILC 3279	a2	a2	a3	a2	a3	a2	-	a5	-
40	BG 376	a2	a2, a4	a3	a1, a2	a3	a2	a2	a4	-
41	Mexico local	a2	a2, a4	-	a2	a3	a2	-	a5	a3
42	MCD 3	a3	a2, a4	a3	-	a3	-	a2	a4	a3
43	BGD 1005	-	a2, a4	a3	a1	-	-	a2	a2, a4	-
44	ILC 202	a3	a2, a4	-	a1	-	-	-	a5	a5
45	Wilt resistant selection	a2	a2, a4	a2, a3, a4	a1	-	a2	a2	a2	a3
46	BG 313	a2	-	-	a1	-	a2	a2	a5	-

Data from 9 STMS loci were only utilized for further statistical analysis due to missing data (more than 30%) in 2 STMS loci viz, TR 48 and TS 45. A total of 40 alleles were found for the 9 STMS loci with an average of 9.4 per locus. The highest number of alleles were observed in TA21 (six alleles) followed by *TA-2* (5 alleles),*TA-8* (5 alleles), TA-64 (5 alleles), *TA80* (5 alleles), *TA125* (5 alleles), CaSTMS (4 alleles)and *TA72* (2 alleles). Out of these 2 alleles of *TA72* a single allele was present in most of the entries *i.e.* a_2 allele in 45 entries out of the selected 46 entries without any null allele (Table 1).

Table 1 provides the summarized data regarding the allele number, allele frequencies and allele distribution in various entries. Considering the homozygosity of the populations occurrence of more than 2 bands in some of the germplasm lines varieties for different STMS loci (Fig. 1) was surprising; for instance double bands were observed in Pant G 114 for TA 125, in BG 1072 forTA80 etc.Based on the allele frequencies, the PIC (polymorphism information content) values were estimated for different STMS loci analyzed (Table 2). The PIC values ranged from 0.043 to 0.820. TA 72 showed the lowest PIC value because of very uneven distribution of 2 alleles, while TA 8 showed maximum PIC (0.820) because of the well distributed 5 alleles among the genotypes of *Cicer*. The average PIC value across all the loci analyzed was 0.5. Three markers out of nine markers showed higher PIC value than the average PIC value.

Estimation of size range of allele was approximately estimated, as the resolution power Resophore gel is relatively less, compared to that of polyacrylamide gel. Some STMS markers were found to have high discriminative power for differentiation of *Cicer* germplasm lines as the present study demonstrates that in the present study, 9 out of 40 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 deviation from the expected pattern. Chickpea germplasm/ varieties are assumed to be highly homozygous and thereby should reveal only a single band (allele) per locus for a large majority of them, if not all. However, double bands could clearly seen in many of the lines as Pusa 212, Pusa 372, H75-35, Pusa 361, IPC 92-132, Pant G 114, BG 365, BG 391, Pusa 1003, ICCV 960 30, FG 712, Pusa 1103, GCP 9504, ICRISAT 3077, ICC 1245, ICCV 88506, BG 1107, ICRISAT 3073, IPC 927, F_2-27, *Cicerreticulatum*, BG 1072, H00-108, ICRISAT 3070, Mexico local, MCD 3, BGD 1005 and wilt resistant selection.

Molecular markers have been utilized for variety of purposes including construction of linkage maps examining genetic relationships between individuals and identification of crop cultivars. These are considered as valuable tools for plant breeding programmers as well as for studies related to evolution and biodiversity conservation. Among the various DNA based markers, the microsatellite or STMS markers have become highly popular and the 'The markers system of choice in diverse crop plants owing to their abundance in the genome, robustness, reproducibility, hypervariability and codominance (Powell et al., 1996). Application of STMS markers in genetic analysis of chickpea, started with an initial study of Huttelet al. (1999).

Since then, the power and potential of SSR markers for a wide range of applications in genetic and breeding of chickpea has been well demonstrated by several researchers (e.g. Huttel 1999, Winter et al, 1999 and Flandez-galvez, 2003, W Choumane 2000 etc.), but still substantial number of chickpea microsatellites are not available in public domain. Microsatellite genotypic data from a no. of loci have potential to provide unique allelic profiles or DNA fingerprints for establishing genotypes identity.

While carrying out STMS profiling, due consideration was given to stratified sampling of polymorphic STMS loci covering bin location on various chromosomes. The STMS polymorphism were assayed using a DNA pooling strategy, although it is not supposed to do as all the genotypes under study are supposed to be pure lines.

This comprehensive characterization of potentially useful and elite germplasm lives of chickpea in increasingly emphasized and pleaded to counter thedemand of plant varietal registration and protection. For finer discrimination of elite germplasm lines of chickpea as well as diversity analysis utility of STMS marker has been demonstrated at larger scale on a large set of chickpea germplasm maintained at IARI, New Delhi.

Results from the present study support the observations several workers about the potential utility of STMS in characterizing chickpea germplasm (Huttel 1999, Winter et al, 1999 and Flandez-galvez, 2003, W Choumane 2000). There was reasonably high rate of polymorphism for at least four markers four markers namely TA8, TA21, TA80 and TA125 out of nine STMS markers/ loci in the present study point towards the scope for further utilization of these markers for chickpea germplasm characterization. The occurrence of unique alleles or rare STMS alleles provides an immense opportunity for generation of comprehensive fingerprint database. In the present study "null" alleles were also observed, which could be done to mutations in the primer binding site leading to non-amplification. The PIC value is influence by the occurrence of variants per locus as well as relative distribution of the alleles. The range of alleles per locus found was two to six (Table 2). For example, TA72 with two alleles has PIC value of 0.043 and TA8 with five alleles has more PIC value as 0.820,which indicates the significance of distribution of alleles across the genome.

In the present study most of the STMS primers consistently amplified bands in many of the germplasm lines at the same locus. The reasons for occurrence of two bands deviating from the expected single band for a homozygous genotype could be residual heterozygosity, contamination of DNA or amplification of similar sequence in two separate genomic regions. The first probability in likely for cases where few genotypes as Pusa 212, Pusa 316 etc. showed double bands (Table 1). It could be due to residual heterozygosity, contamination of seed stock or presence of multiple copies of the gene in the genome. Thus, molecular characterization can gain very useful information to chickpea breeder.

The present study has proved that STMS makers highly impressive in establishing the unique identity chickpea germplasm.

Table 2: Polymorphism Information Content (PIC) of STMS loci across various germplasm/ variety analysed

Primer	Repeat class of	Linkage Group	Repeat at the STMS	Alleles	Number of genotypes sharing	Frequency of STMS alleles	PIC	Allele size(bp)
CaSTMS 25	Di-repeat	11	(CT)19	a1	2	0.047	0.676	230-290
TA 2	Tri-repeat	2	(TAA) 16TGA	a1	7	0.100	0.713	150-300
TA 8	Tri-repeat	6	(TAA) 44	a1	2	0.057	0.820	320-390
TA 21	Tri-repeat	5	(TAA) 51	a1	9	0.191	0.712	100-250
TA 43	Tri-repeat	3	(TAA) 19	a1	2	0.740	0.712	180-240
TA 64	Tri-repeat	1	(TAA) 39	a1	6	0.130	0.791	200-300
TA 72	Tri-repeat	2	(ATT) 36	a1 a2	1 46	0.425 0.957	0.043	210-230
TA 80	Tri-repeat	4	(TTA) 23	a1 a2 a3 a4 a5	10 7 8 12 13	0.200 0.140 0.160 0.240 0.260	0.814	180-280
TA 125	Tri-repeat	1	(TAA) 33	a1 a2 a3 a4 a5	1 16 20 5 3	0.022 0.355 0.444 0.111 0.666	0.674	200-280

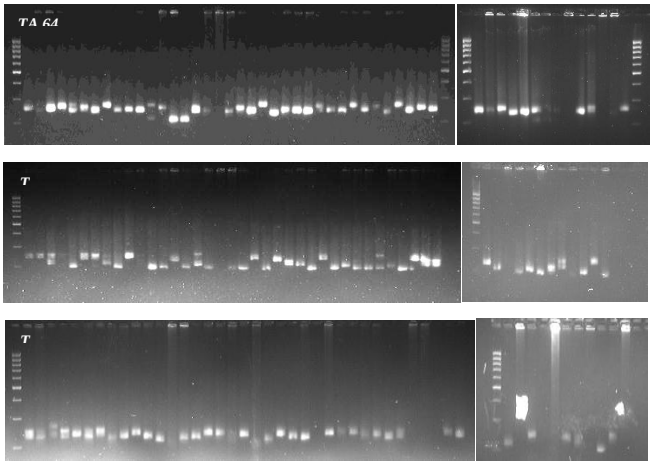

Fig.1: Gel Electrophoregrams of TA 29, TA 80 and TA 125 of 46 genotypes of chickpea in the order as they appeared in the Table 1

ACKNOWLEDGEMENT

The author fondly appreciate the financial support provided by the DBT under project Molecular Mapping of Chickpea Genome.

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