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Molecular marker-based linkage map of chickpea (*Cicer arietinum*) developed from *desi* × *kabuli* cross

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ABSTRACT

A molecular marker-based linkage map of chickpea (*Cicer arietinum* L.) was developed from a *desi* × *kabuli* cross of 'BGD 112' and 'FLIP 90-166' using Sequence-tagged Microsatellite (STMS) Markers. Both the parents representing the cultivated chickpea, the map loci marked indicates usable polymorphism for genetic as well as loci studies. A total of 250 STMS markers were used to study the parental polymorphism and 49 of which had showed polymorphism were used for genotyping F2 lines. Linkage analysis revealed 8 linkage groups mapped by 33 loci by these markers covering a distance of 471.1 cM of map distance with an average marker density of 14.2 cM at a LOD of 3.0. The molecular map using *desi* × *kabuli* cross throws insights into variability and diversity that can be utilized directly by the breeders unlike that generated using wide crosses as the loci that this map has marked, has a direct utility in marker-assisted breeding.

Key words: Chickpea, Linkage map, Molecular markers, STMS markers

In the warm environments of central and southern India, chickpea (*Cicer arietinum* L.) is challenged by fusarium wilt, a major yield reducing disease, while in north-western India, owing to cooler environments it is exposed to severe foliar disease ascochyta blight.

Conventional breeding approaches have not greatly improved yield. Chickpea breeders throughout the world are focusing on increasing yield by pyramiding genes for resistance/ tolerance into elite germplasm. Molecular markers have been shown to play a crucial role in crop improvement programmes. Such markers serve as efficient and powerful tools for marker-assisted selection (MAS) of agronomically important traits. Molecular marker technologies help in improving the efficiency of breeding several fold since selection is not directly on the trait of interest but on the molecular marker tightly linked to the trait, thereby accelerating the generation of new varieties, especially when the characters are difficult to score. In addition to these applications, DNA markers also provide new insights into genome analysis, help in germplasm characterization, phylogenetic analysis and genetic diagnostics (Bharadwaj *et al.* 2010, Tara Satyavathi *et al.* 2005). Availability of molecular markers and molecular genetic linkage maps are the pre-requisites for undertaking molecular breeding activities. Simple sequence repeats (SSR) are tandem repeats of 1–5 nucleotides which are widely distributed in eukaryotic genomes. Simple sequence repeats markers are becoming preferred due to their properties of co-dominance, reproducibility and high variability and are being widely employed for molecular mapping. Keeping the above points in mind, our study was mainly involved in use of the already reported SSR markers in the public domain for the construction of a genetic linkage map of chickpea.

MATERIALS AND METHODS

The plant material consisted of 103 F2 lines developed from an intra-specific cross of 'BGD 112' an Indian *desi* germplasm line with 'FLIP 90-166', a *kabuli* germplasm line from ICARDA, Aleppo, Syria and both the parents. Leaf DNA of about 100 mg of fresh young leaf tissue was collected from the winter crop of 2007–08, immediately frozen in liquid nitrogen and stored at –80ºC. Isolation of DNA was carried out using the CTAB method with minor modification. Sequence tagged microsatellite (STMS) primers from Bioneer, South Korea were used. Biorad MyCycler thermal cycler, USA was used to carry out amplifications in 10 µl volumes which had 20–25 ng plant genomic DNA, 10×Tris

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buffer (15 mM MgCl2 and Gelatine) of Banglore Genei, India, 10 mM dNTP mix, 1.0 µl primer and 0.3 µl of 3U µ/litre *Taq* (Banglore Genei, India). Polymerase chain reaction (PCR) analysis was taken up by having preparation of 150 seconds at 90ºC, followed by 18 cycles of denaturation at 94ºC for 20 sec., annealing for 50 sec. at 50ºC (touch down of 0.5ºC for every repeat cycle) and 1 min. elongation at 72ºC for 50 sec. Further 20 cycles of denaturation at 94ºC for 20 sec., annealing for 50 sec. at 55ºC and 50 sec. elongation at 72ºC were given and finally extension at 72ºC for 7 min. were performed.

Parental polymorphism was initially studied between the two parental lines 'BGD 112' and 'FLIP 90-166' using 250 STMS markers in 2008–09, selected from those reported by various workers (Winter *et al.*1999, 2000; Sethy *et al.*2006 a, b and Choudhary *et al.* 2006) and separated on 3% metaphor agarose (Cambrex, Rockland, USA).

The polymorphic markers were genotyped in 2008–09 in all the 103 F2 lines. The polymorphic bands were scored in a spread sheet format with 'A' representing the first parental band, 'B' representing the second parental band and 'H' representing the heterozygote individuals. The data were loaded in a mapmaker ver.3.06, a LOD score of 3.0 and a maximum recombination fraction of 0.25 were employed as the criteria of linkage to form the linkage groups.

RESULTS AND DISCUSSION

The survey of parental polymorphism with 250 STMS primers revealed polymorphism by 49 markers (19.6%). These markers were used for genotyping in all the 103 F2 lines. Linkage analysis revealed 8 linkage groups that mapped 33 loci by these markers (Fig. 1). These 33 loci covered a distance of 471.1 cM with an average marker density of 14.2 cM at a LOD score of 3.0.

The current genetic map constructed using of 33 loci covering 471.1cM of map distance with a marker density of 14.2cM. Of these 24 markers are present within 50 cM of distance from each other. Though maps of Winter *et al.* (1999, 2000) covered 7 cM but being in wide cross, a tendency of these markers was observed to skew towards the wild parent (*C.reticulatum* authority). In the present study both the parents being from the cultivated chickpea (*C. arietinum*); absence of skewedness indicates better applicability. This can be the reason that though in the present study, we had used a large number of markers reported by the earlier workers, lower polymorphism was seen as most of the markers reported by earlier workers had coded for the genomic regions of the wild parent which is absent in the cross used. In the studies of Winter *et al.* (2000), 68% of the alleles were from *C. reticulatum.* Use of many of these STMS markers may not have shown polymorphism in the present study as they may not be coding for the genomic sequences of the *C.arietinum* genome. Thus only forty nine STMS markers of the 250 markers used in the study (19.6%) have produced polymorphism. Thirtythree of these fortynine STMS markers (67%) could be mapped into the *C.arietinum* genome, both the parents being of *C.arietinum,* indicates that when they are mapped onto important traits of yield and disease resistance or other characters, these markers will have wider applicability in marker-assisted breeding programmes. Among the 49 markers that showed polymorphism in the parents only 28% have shown segregation distortion displaying χ^2 values above 10. However 16 markers (32%) were unlinked. It has been reported by earlier workers too that segregation distortion was less related to class of markers. Such distortion is generally due to the genomic regions where they nest (Winter *et al.* 2000). The physical size of the genome is about 750 Mbp, which would infer that 360 bp would be covered under 1cM. The map is involving *C.arietinum* × *C.arietinum* parents, both being cultivated and hence a low level of polymorphism is expected and has been reported *kabuli* × *desi* cross (Cobos *et al.* 2005) and cultivated chickpea (Bharadwaj *et al.* 2010). The map has eight linkage groups probably indicating the eight chromosomes (Fig 1). However large regions still need to be covered by markers indicating future line of work. In some of the STMS under study, there were multiple bands from the same locus. Such multiple bands were also reported by Winter *et al.* (1999) where it was inferred that these bands arise due to the presence of cryptic sites both on upstream and downstream and sometimes between the priming sites. One interesting feature of this map is that clustering was seen only on linkage group 4. Most of the earlier maps reported by various workers (Radhika *et al.* 2007, Milan *et al.* 2010) have indicated markers having skewed segregation in 3–4 clusters as these workers have used wide crosses where such skewedness is expected. Further such skewedness may become pronounced in RILs than in F$_2$ founder population.

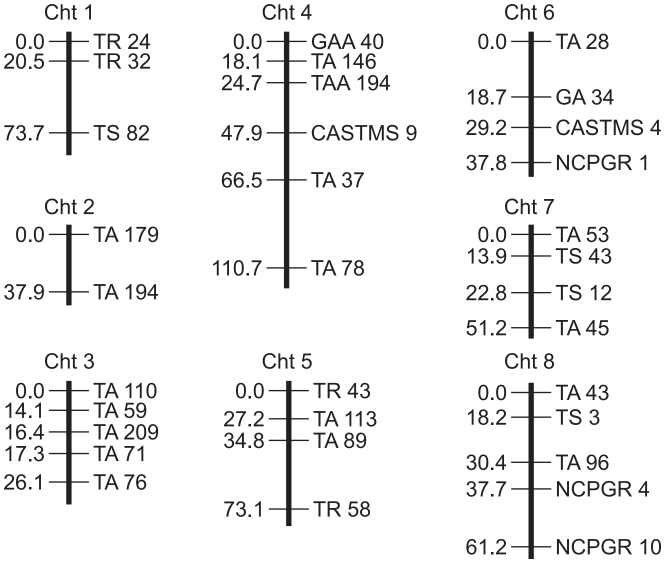

Fig 1 Linkage map of chickpea develped from *desi*×*kabuli* cross

In the present study the STMS markers were very useful in mapping and also with regards to their transferability to chickpea cultivars as they represent usable variability. The molecular map using *desi* × *kabuli* cross throws insights into variability and diversity that can be utilized directly by the breeders unlike that generated using wide crosses as the loci this map has marked has a direct utility in marker-assisted breeding. Though the map generated is not a high density map but it is hoped that it will kick start the chickpea genome analysis.

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