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An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged microsatellite site and resistance gene analog markers

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Abstract An intraspecific linkage map of the chickpea genome based on STMS as anchor markers, was established using an F_2 population of chickpea cultivars with contrasting disease reactions to *Ascochyta rabiei* (Pass.) Lab. At a LOD-score of 2.0 and a maximum recombination distance of 20 cM, 51 out of 54 chickpea-STMS markers (94.4%), three ISSR markers (100%) and 12 RGA markers (57.1%) were mapped into eight linkage groups. The chickpea-derived STMS markers were distributed throughout the genome, while the RGA markers clustered with the ISSR markers on linkage groups LG I, II and III. The intraspecific linkage map spanned 534.5 cM with an average interval of 8.1 cM between markers. Sixteen markers (19.5%) were unlinked, while 11 chickpea-STMS markers (20.4%) deviated significantly ($P< 0.05$) from the expected Mendelian segregation ratio and segregated in favor of the maternal alleles. However, ten of the distorted chickpea-STMS markers were mapped and clustered mostly on LG VII, suggesting the association of these loci in the preferential transmission of the maternal germ line. Preliminary comparative mapping revealed that chickpea may have evolved from *Cicer reticulatum*, possibly via inversion of DNA sequences and minor chromosomal translocation. At least three linkage groups that spanned a total of approximately 79.2 cM were conserved in the speciation process.

Keywords Chickpea · STMS markers · Genetic map · Comparative mapping · Resistance gene analogs

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Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world (FAO 1994). Major chickpea growing countries include India, Turkey, Canada, Pakistan, Australia and Mexico. In 2000, Australia was the largest chickpea exporter (FAOSTAT Database 2000). Aside from being the highest valued pulse crop (Panagiotopoulos 2000), chickpea is an important rotation crop in Australia, particularly in the Victorian wheatbelt. The crop fixes and provides atmospheric nitrogen and reduces the potential inoculum for soil borne root diseases such as take-all and cereal cyst nematode. A major biotic factor, which limits chickpea production worldwide, is the fungal disease ascochyta blight caused by the ascomycete *Ascochyta rabiei* (Pass.) Lab. (Saxena and Singh 1987; Singh et al. 1992). The disease can cause crop loss from 10% to complete crop failure. Consequently, breeding efforts have been directed towards the development of high yielding chickpea cultivars with durable resistance to ascochyta blight via broad-based gene pyramiding (Singh 1997; Van Rheenen and Haware 1997). While marker-assisted breeding is the effective approach to pyramid resistance sources of broad genetic background (Van Rheenen and Haware 1997), genetic mapping provides the information needed to implement DNA marker-assisted selection (Paterson 1996).

The advances in molecular marker technology have accelerated the progress of genome mapping in chickpea. Linkage maps have been developed based on interspecific crosses between selected chickpea cultivars and *Cicer reticulatum* accessions (Kazan et al. 1993; Simon and Muehlbauer 1997; Winter et al. 1999, 2000; Banerjee et al. 2001). *C. reticulatum* was identified as the wild *Cicer*

progenitor of chickpea (Ladizinsky and Adler 1976). Molecular markers associated with quantitative trait loci (QTL) for resistance to ascochyta blight, fusarium wilt and some morphological traits have also been located on the interspecific linkage maps (Kazan et al. 1993; Simon and Muehlbauer 1997; Ratnaparkhe et al. 1998; Santra et al. 2000; Tekeoglu et al. 2000; Winter et al. 2000; Banerjee et al. 2001).

The use of interspecific rather than intraspecific populations for genome mapping in chickpea was primarily due to the extremely low level of genetic polymorphism detected within the cultivated gene pool (Ahmad et al. 1992; Udupa et al. 1993; Labdi et al. 1996). However, a genetic map constructed from an interspecific cross may not represent the true recombination-distance (cM) map order of the cultivated genome. Due to the uneven recombination of homoeologous chromosomes during meiosis, DNA markers for linkage analysis would have a high degree of segregation distortion in the mapping population resulting in biased estimation of the linkage marker distance. In *Solanum*, 40% and 27% marker distortion were observed in inter- and intraspecific backcross populations, respectively (Gebhardt et al. 1991). In an extreme case, Xu et al. (1997) reported that an interspecific recombinant inbred line (RIL) population of tomato had the highest frequency of marker distortion (73%), whereas an intraspecific F_2 population of *Cupea lanceolata* had the lowest marker distortion (5.4%). The progressive selfing in the development of RIL populations has compounded the cumulative effect of both the genetic (G), environment (E) and G × E factors, which govern the preferential transmission of either parental allele in the progenies (Xu et al. 1997). Furthermore, the construction of genetic maps based on wide crosses has the disadvantage of identifying loci that may be polymorphic only between more divergent genotypes but not between more closely related genotypes. Such maps thus have little direct application in breeding programs that exploit intraspecific variation within the cultivated forms. A genetic linkage map constructed from a cross within the cultivated gene pool, especially in the framework of targeting traits of breeding interest, would therefore be most desirable.

Chickpea is a self-pollinated diploid ($2n = 2x = 16$) annual grain legume with a genome size of approximately 750 Mbp (Arumuganathan and Earle 1991); slightly less than the well-characterized tomato genome (950 Mbp). Assuming the same recombination rate as tomato, a 1-cM genetic distance in the chickpea map equates to 500 Kbp in physical distance. Therefore, 70 to 100 evenly distributed markers should be sufficient to detect any particular locus in the chickpea genome.

Markers generated by sequence tagged microsatellite site (STMS) primer pairs are particularly suitable for genome analysis. Aside from being highly polymorphic, STMS markers are PCR-based, may provide single-locus detection, may be co-dominantly inherited, may utilize non-radioisotope detection and offer the potential for automated application in plant breeding (Mansfield et al. 1994). One-hundred and seventy four STMS loci have been characterized from the chickpea genome and have proven to be polymorphic in chickpea at an intraspecific level (Hüttel et al. 1999; Winter et al. 1999). Winter et al. (2000) also demonstrated the suitability of these chickpea-STMS markers for genome mapping in a *Cicer* interspecific population, which resulted in the construction of the most extensive linkage map currently available for a *C. arietinum* × *C. reticulatum* hybrid genome. Although recombination distances cannot be assumed to be equal between intraspecific chickpea and interspecific *Cicer* hybrid maps, the use of common (anchor) markers would allow for a map-based assessment of linkage conservation and colinearity between these genomes.

The mapping of resistance gene analogs (RGA) on linkage maps has also been used as a candidate-gene approach to identify genes for resistance to various pathogens (Kanazin et al. 1996; Leister et al. 1996;Yu et al. 1996; Feuillet et al. 1997). Although not all amplified products may correspond to a functional disease resistance gene, RGA primers have been shown to amplify the conserved sequences of leucine-rich repeats (LRR), kinase and/or nucleotide-binding sites (NBS), thereby targeting genes for disease resistance or other important signal-transduction processes in plants (Bent 1996). RGA screening has also been successfully applied in various QTL analyses. Byrne et al. (1996) was able to link the candidate genes involved in the flavone synthesis pathway of maize with the host defense response phenotype associated with a QTL for resistance to corn earworm.

The main objective of this study was the development of an intraspecific linkage map of the chickpea genome based primarily on chickpea-STMS as anchor markers. Molecular markers associated with quantitative trait loci for ascochyta blight resistance in chickpea (Santra et al. 2000) and resistance gene analogs (Chen et al. 1998) were also integrated into the map. The possible evolutionary genetic inference of the speciation of chickpea from *C. reticulatum* at the chromosomal level was also discussed.

Materials and methods

Plant material and DNA extraction

Eighty five F_2 progenies of chickpea derived from an intraspecific cross between desi cultivars ICC12004 (ascochyta blight resistant) and Lasseter (ascochyta blight highly susceptible; Meredith personal communication) were used as a mapping population. The parents and F_1 plants were confirmed as homozygous/homogeneous lines and genuine hybrids respectively, by STMS analysis (Galvez et al. 2000).

Plant materials were propagated in the glasshouse at the Victorian Institute for Dryland Agriculture (VIDA), Victoria, Australia, and genomic DNA was isolated from young leaves according to a modified micropreparation procedure of the cetyltrimethyl-ammoniumbromide (CTAB) DNA isolation protocol (Taylor et al. 1995).

DNA amplification and polymorphism screen

Molecular markers screened in this study included 110 chickpea-STMS as anchor markers, two ISSR, three RAPD, 28 field pea-STMS and 21 RGA markers. The chickpea-STMS markers had been used in the construction of the *C. arietinum* × *C. reticulatum* genomic map (Winter et al. 2000). These markers were chosen evenly among the eight large linkage groups of this map to represent the basic chromosome number of chickpea. The RAPD and ISSR markers have been reported by Santra et al. (2000) to be associated with QTL for ascochyta blight resistance in chickpea. Whereas, the field pea-STMS markers were among the newly characterized microsatellite markers for field pea developed through the Agrogene® field pea consortium (Ford et al. 2002; Agrogene, France) and have been shown to have a high transferability to the chickpea genome (Pandian et al. 2000). The RGA marker primer sequences have been previously described (Chen et al. 1998).

The polymerase chain reaction (PCR) with chickpea-STMS primer pairs was performed as described by Hüttel et al. (1999) and Winter et al. (1999), with some modifications. The PCR was carried out with a PTC-100 or PTC-200 thermocycler (MJ Research, Inc., USA) in 15-μL reaction volumes. In order to increase the screening efficiency of markers, microsatellites with compatible annealing temperatures of primer pairs and no overlapping size of amplification products were multiplexed in the PCR. The total reaction volume, primer concentration and amount of DNA sample were optimized for each microsatellite combination as recommended by Ribaut et al. (1997). PCR products were electrophoresed on either 3% Metaphor agarose (FMC BioProducts, Rockland, USA) or 5% denaturing polyacrylamide gel (PAGE) in Tris-borate EDTA (TBE) running buffer (Sambrook et al. 1989). The resolved PCR products were then detected by ethidium bromide staining (agarose; Sambrook et al. 1989) and silver staining (PAGE; Promega Corp., USA). Bands that could not be certainly scored as codominant markers were otherwise scored as STMS-dominant markers.

The PCR conditions optimized by Santra et al. (2000) were used for the analysis of ISSR, RAPD and field pea-STMS markers. Whereas, RGA analysis was basically that of Chen et al. (1998). The PCR volume for these marker systems was also adjusted to 15-µL and performed on a PTC-100 or PTC-200 (MJ Research Inc., USA) thermocycler. Except for the RGA analysis, PCR products were resolved on 2% agarose and detected by ethidium bromide staining. The multi-loci amplification of RGA primers necessitated resolution of PCR products on PAGE.

Parents and the F_1 plant were screened for DNA polymorphism using the described marker systems. Polymorphic markers were tested for segregation using representative (16) F_2 individuals. Only markers that segregated in the sample F_2 s were mapped in the whole mapping population.

Marker nomenclature

Except for the heterologous STMS-locus resolved from PCR-multiplexing, all markers were designated after the name of their PCR primers as previously described (Chen et al. 1998; Winter et al. 1999, 2000; Santra et al. 2000). The heterologous STMS-locus was given the mapping experiment number and 'sp' (to denote subproduct) as its marker name, e.g. M51sp. Furthermore, markers generated from the same primer (pairs) were identified by the addition of lower case letters. For dominant markers, the approximate sizes of the fragments in base pairs (bp) were added as subscripts; with the fragment size's from ICC12004 italicized.

Inheritance and linkage analysis

Chi-square analysis ($P < 0.05$) was applied to test the segregation of the mapped markers against the expected Mendelian segregation ratio for co-dominant and dominant inheritance in an F_2 population. Mapmaker V3.0 (Lander et al. 1987) was used for linkage analysis. Primary linkage groups were constructed by two-point analysis (LOD > 2.0) using the "group" command, relative to the placement of anchor markers (Winter et al. 2000). The best order of markers in each linkage group was established using the "map", "order", "try" and "sequence" commands; and validated by the strict linkage threshold criteria of the "ripple" multipoint analysis function. Map distances were calculated in cM by applying the "Kosambi" function.

Results

Polymorphism and markers for mapping

Of the 164 primers/primer pairs screened between the parents, only 65 (39.6%) revealed DNA polymorphism, generating a total of 82 reproducible and segregating markers for linkage analysis (Table 1). The transferability of field pea-STMS primers was high (data not shown); however, only one out of 28 primer pairs produced a reliable polymorphic amplicon. Chickpea-STMS and RGA primers detected 44.5% and 52.4% intraspecies polymorphisms respectively, and generated the highest number of markers for mapping. Fourteen chickpea-STMS markers were generated from the multiplexed PCR of one triplex and five duplex combinations of primer

Table 1 Polymorphism screen and number of markers generated for mapping using different marker types

Marker type and PCR amplification[a]	Primers (pairs) screened	Primers yielding polymorphism	Markers for mapping (ave./primer)	Alleles/loci detected	
				Number (ave./marker)	Size range in bp (mean)
Chickpea-STMS	110	49 (44.5%)	54 (1.1)	107 (about 2)	125–385 (235)
single PCR		36	40		
multiplex PCR:					
triplex		1 combination	3		
duplex		5 combination	11		
ISSR	2	2 (100%)	3 (1.5)	3 (1)	404–1159 (889)
RAPD	3	2 (66.7%)	3 (1.5)	3 (1)	857–3024 (1901)
Field pea-STMS	28	1 (3.6%)	1 (1.0)	1 (1)	52
RGA	21	11 (52.4%)	21 (1.9)	21 (1)	114–1338 (443)
Total	164	65 (39.6%)	82 (1.3)	N/A	N/A

[a] Chickpea-STMS markers were amplified either by single or multiplexed PCR reaction. Other marker types were all amplified by single PCR

Table 2 Segregation distortion and number of unlinked markers

Marker type	No. of markers	Distorted segregation (χ^2 at $P < 0.05$) (%)	Unlinked markers (%)		
			Mendelian segregation	Distorted segregation	Total
Chickpea-STMS	54	11 (20.4%)	2	1	3 (5.6%)
ISSR	3	1 (33.3%)	0	0	0 (0.0%)
RAPD	3	3 (100%)	N/A	3	3 (100%)
Field pea-STMS	1	1 (100%)	N/A	1	1 (100%)
RGA	21	6 (28.6%)	5	4	9 (42.9%)
Total	82	22 (26.8%)	7	9	16 (19.5%)

Table 3 Segregation ratios of chickpea-STMS markers that deviated from the expected 1:2:1 Mendelian ratio and frequency of the maternal alleles in the mapping population

Marker	Segregation ratios		Chi-square ($P < 0.05$)[a]	Frequency of maternal alleles (%)
	Expected	Observed		
TR29	21:42:21	34:34:16	10.8	61
TA114	21:42:21	38:34:11	20.3	66
TS72	18:35:18	28:28:14	8.4	60
TA14	21:42:21	33:33:18	9.2	59
TA130	20:41:20	28:43:10	8.3	61
TAA55	21:41:21	28:43:11	7.2	60
TA179	17:34:17	26:29:12	7.1	60
TS43	20:40:20	30:36:14	7.2	60
TS57	20:40:20	34:29:16	13.8	61
TR1	18:37:18	29:34:11	9.2	62
TS53	21:42:21	31:37:15	7.1	60
Mean (range)			9.9 (7.1–20.3)	61 (59–66)

[a] $\chi^2_{2,.05} = 5.99$

Table 4 General features of the intraspecific map

Linkage group (LG)	Size (cM)	Number of mapped markers			Ave. marker density (cM)	Over-all distribution of markers
		Mendelian segregation	Disorted segregation	Total		
I	197.7	17	2	19	10.4	Dense sub-cluster, joined with widely spaced markers
II	97.2	8	1	9	10.8	Dense markers at distal end
III	81.2	7	3	10	8.1	Dense and even coverage
IV	57.7	5	0	5	11.54	Sparse but even coverage
V	44.2	4	2	6	7.4	Acceptable marker density and even coverage
VI	31.1	8	1	9	3.4	Dense sub-cluster at the central region, but acceptable over all distribution
VII	19	2	4	6	3.2	Good marker density and distribution
VIII	6.4	2	0	2	6.4	Only two markers
Total/ave.	534.5	53	13	66	8.1	

pairs. Of these, only one marker (M51sp) resolved from the TAA60 and TR56 primer combination, did not correspond to the expected size in single PCR runs. M51sp was then classified as a heterologous STMS marker. The ISSR and RAPD markers detected 100% and 66.7% polymorphisms respectively, although there were only two and three primers tested.

Inheritance and segregation distortion

The chi-square test ($P < 0.05$) identified 22 (26.8%) markers that did not segregate in accordance with the expected Mendelian inheritance (Table 2). All marker types exhibited segregation distortion. However, in the extreme case, the field pea-STMS and RAPD markers showed 100% aberrant segregations. Chickpea-STMS markers had the lowest percentage (20.4%) of anomalous markers, which all segregated in favor of the alleles from the maternal parent Lasseter (Table 3).

Linkage and correlation with segregation distortion

After linkage analysis, 16 out of 82 markers (19.5%) remained unlinked (Table 2). These were mostly RGA markers, and all of the field pea-STMS and RAPD markers. Only 5.6% of the chickpea-STMS markers were not mapped into any linkage group.

The aberrant segregation of markers did not correlate with the number of unlinked markers. As shown in Table 2, only one out of 11 anomalous chickpea-STMS markers was not mapped. The other two unmapped chickpea-STMS markers had Mendelian inheritance in the mapping population. Likewise, four unmapped RGA markers had aberrant segregations while five segregated in accordance with the expected segregation ratio.

General features of the map

The general features of the intraspecific map are summarized in Table 4. At a LOD-score of 2.0 and a maximum recombination distance (r) of 20 cM, 66 markers comprised of 51 chickpea-STMS, three ISSR and 12 RGA, were mapped into eight linkage groups that spanned 534.5 cM of the chickpea genome at an average marker density of 8.1 cM. The linkage groups were numbered in Roman numerals (LG I to VIII) to differentiate them from the Arabic numbering of the previous *C. arietinum* × *C. reticulatum* linkage groups (Winter et al. 2000). LG I represented the largest linkage group in terms of size and the number of markers mapped. On the other hand, LG VIII was made up of only two STMS markers spaced at 6.4 cM and was the shortest among the eight linkage groups. LG size was not correlated to the number of linked markers in the group. For instance, LG II and VI had the same number of markers, but LG II covered 97.2 cM while LG VI only 31.1 cM. However, the size of a LG and number of markers together, provided an estimation of marker density. LG II had an average marker density of 10.8 cM, while LG VI, the second most-dense linkage group, had a much higher marker density (3.4 cM). LG VII was the densest linkage group with an average marker density of 3.2 cM, but was very small (19 cM). On the other hand, LG IV was made up of five widely spaced markers (11.5 cM), and was the sparsest linkage group. Finally, marker density was not affected by the marker type. All the marker types were densely and sparsely located in different regions of the mapped genome (Fig. 1). LG III, V, VI and VII were evenly and densely covered with markers of different types.

Distribution of markers

The chickpea-STMS markers were distributed throughout the genome (Fig. 1). The markers covered the eight linkage groups at a satisfactory marker density. On the other hand, the three ISSR markers mapped adjacent to the RGAs in the central region of LG I and the distal end of LG II. The RGA markers mainly clustered on LG III, although a few were also located on LG I, LG II, IV and VI.

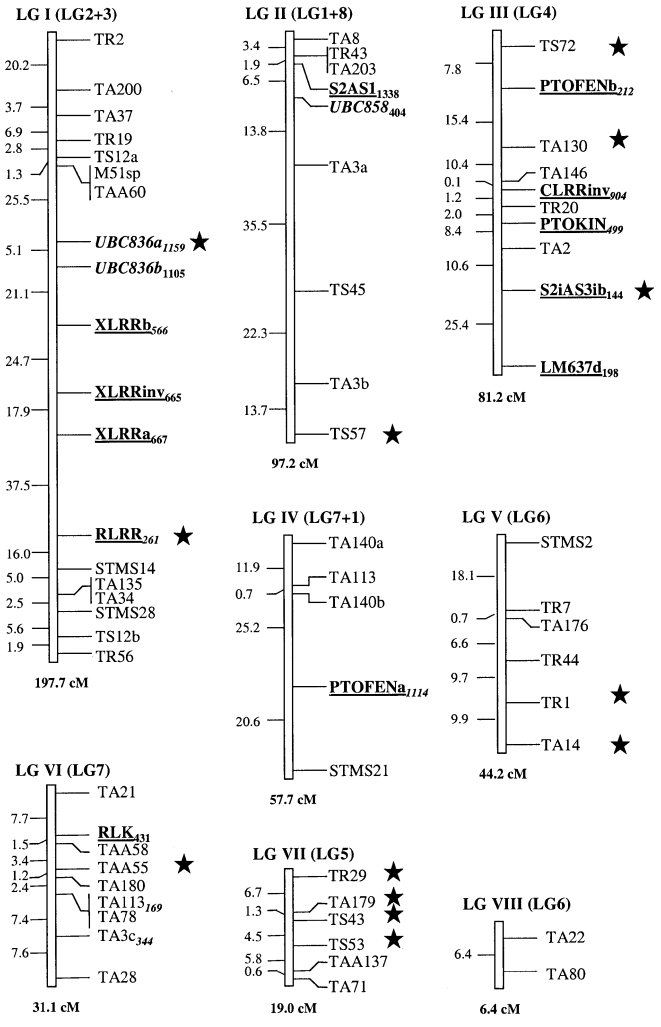
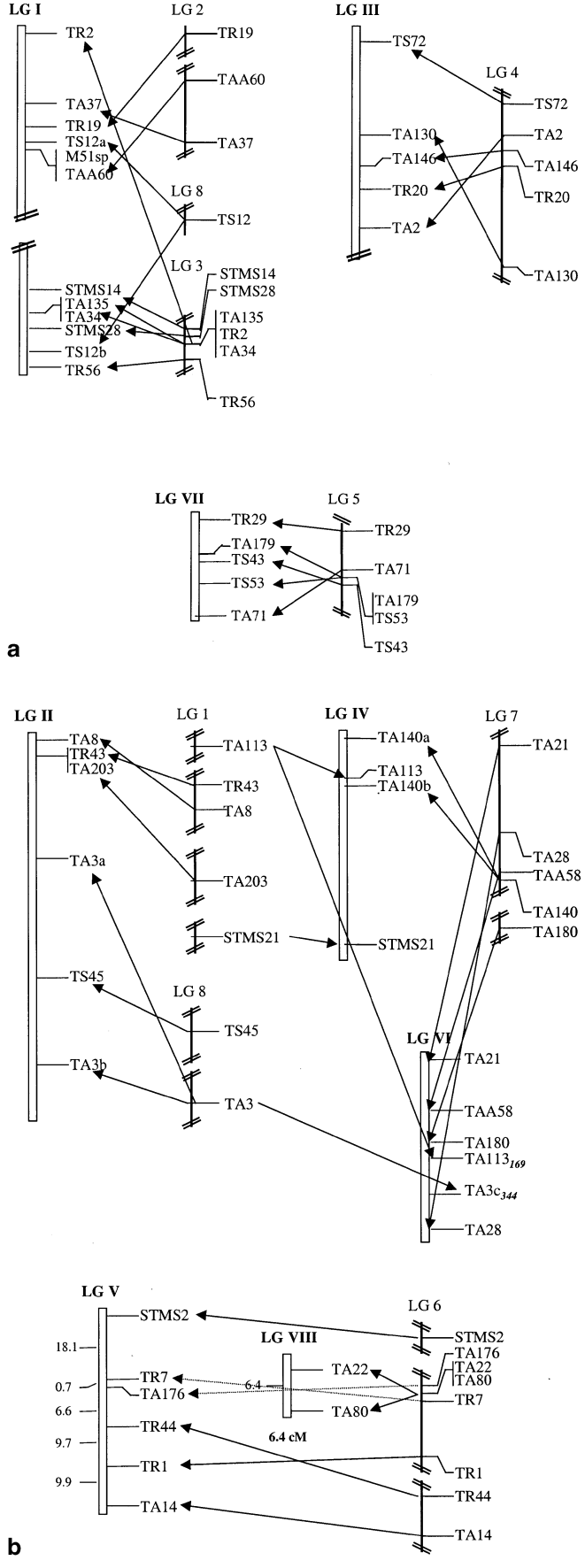


Fig. 1 Intraspecific map of the chickpea genome. Marker distance was set in cM by the Kosambi function (Lander et al. 1987), with 1 cM–1.4 Mbp. Loci that showed aberrant segregation are marked with *stars*. The linkage groups are numbered in Roman numerals (*LG I to VIII*) to differentiate them from the Arabic numbering of the previous *C. arietinum* × *C. reticulatum* map (*LG 1 to LG 8*; Winter et al. 2000). STMS markers are described as that of Winter et al. (1999 and 2000), except for the dominant marker and heterologous locus resolved from PCR-multiplexing. ISSR markers are *italicised* and resistance gene analog markers *underlined*

Markers that showed segregation distortions were indicated in the linkage map (Fig. 1). Regions or loci with aberrant segregations were located on almost all linkage groups (Table 4). Interestingly, several distorted markers were clustered on LG VII. Four out of six mapped chickpea-STMS markers on LG VII had aberrant segregations in the F_2 mapping population.



Linkage and order of chickpea-STMS markers in comparison with the previous interspecific map

There was reasonable synteny between at least three linkage groups (LG I and LG 3; LG III and LG 4; LG VII and LG 5) of the chickpea map and the *C. arietinum* × *C. reticulatum* genome mapped by Winter et al. (2000; Fig. 2A). Markers on LG VII were also colinear with markers on LG 5 of the interspecific linkage map. These highly conserved regions spanned a total of approximately 79.2 cM. Except for the LG 2-distal end of LG I, only minor inversions were observed with the other non-linear markers (Figs. 2A and B). Intra- and inter-linkage group translocation of markers were detected on the LG 2-distal end of LG I. Furthermore, three linkage groups from the interspecific map were merged in the chickpea map, while three split into separate linkage groups (Figs. 2A and B).

Discussion

An intraspecific linkage map of the chickpea genome was established using an F_2 population. This is the first reported linkage map for chickpea within the cultivated gene pool (Galvez et al. 2002). Although far from marker saturation, the map comprised eight linkage groups of the genome, upon which anchor markers were distributed at an informative marker density. These linkage groups may have corresponded to the chromosome number of chickpea (*C. arietinum*, 2n = 16) however, more markers would have to be mapped to make the correlation between linkage groups and chromosomes certain. The linkage map was predominantly constructed using chickpea-STMS markers that were strategically chosen from the eight large linkage groups of the *C. arietinum* × *C. reticulatum* map (Winter et al. 2000). Because of the availability of genome-wide anchor markers and stringent linkage criteria (r = 20 cM), linkage groups were established at LOD-score of 2.0. At a higher LOD-score, more than two linkage groups from the interspecific map were linked together, with few spurious linkages (r > 50 cM) and the previous eight linkage groups were not reconstructed. Nonetheless, a strict LOD threshold of 4.0 was set as a multipoint criteria parameter when markers were ordered in each linkage group by multipoint analysis. In potato, two backcross-linkage maps were constructed at a LOD-score of 2.0 using tomato RFLP markers based on homoeology of the potato and tomato genomes (Bonierbale et al. 1988). Whereas in mungbean and cowpea, the best orders of markers were determined at LOD ≥ 2.0 (Menancio-Hautea et al. 1993), although LOD thresholds were set at 2.5 and 3.0 during the

Fig. 2A, B Comparative mapping of the chickpea map (*double line* chromosome representation) with the *C. arietinum* × *C. reticulatum* map of Winter et al. (2000; *thin line*). Linkage conservation was observed in at least three linkage groups (**A**), whereas inversion of DNA sequences and chromosomal translocations were apparent in LG II, IV, V, VI and VIII (**B**)

preceding two-point and three-point analyses, respectively.

The intraspecific linkage map consisted of 66 (predominantly chickpea STMS) markers, which covered 534.5 cM at an average marker density of 8.1 cM. Relative to the estimated physical size of the chickpea genome (750 Mbp; Arumuganathan and Earle 1991), 1-cM distance in the map is approximately 1.4 Mbp (1,400 Kbp). This marker density is almost twice as sparse as the 750 Kbp/cM high-density map of tomato (Tanksley et al. 1992). This means that another 66 molecular markers may be evenly added into the linkage map to approximate the high-density linkage map of the tomato genome.

There was a tendency for the RGA markers to locate at specific regions in the chickpea linkage map. In particular, the XLRRs and $RLRR_{261}$ markers amplified by the RGA primers designed from the leucine-rich repeat (LRR) regions of rice R-genes to *Xanthomonas compestris* pv *oryzae* (Chen et al. 1998) mapped in the central region of LG I. As well, RGA markers clustered on LG III, which may suggest that this region contains a conserved repertoire of different but functionally related R-genes of chickpea. Genetic linkage and the clustering of R-genes have been classically established (Pryor 1987) and confirmed by RGA-PCR and linkage analyses in major crops, including oat (Rayapati et al. 1994), barley (Mahadevappa et al. 1994), flax (Ellis et al. 1995), soybean (Kanazin et al. 1996), lettuce (Maisonneuve et al. 1994) and corn (Faris et al. 1999). The clustering of R-genes has been suggested to be due to a common evolutionary mechanism (Sudapak et al. 1993). Indeed, the mapping of the ISSR markers associated with ascochyta blight resistance in an interspecific chickpea population (Santra et al. 2000) adjacent to a RGA marker or cluster may indicate the location of ascochyta blight resistance genes in the region. However, since the ISSR markers are dominant markers, there is no certainty that the loci mapped in this study were the same as those mapped by Santra et al. (2000).

The parental genotypes used in this study were 44.5% polymorphic at 110 microsatellite sites. Hüttel et al. (1999) also observed 41% polymorphism using STMS markers among three chickpea accessions. A higher level of polymorphism/genetic diversity (48% to 94%) was detected using microsatellite markers in studies that compared a larger number of chickpea cultivars (Weising et al. 1992; Sharma et al. 1995; Hüttel et al. 1999; Sant et al. 1999; Winter et al. 1999), thereby increasing the informativeness of each marker. The level of DNA polymorphism within chickpea is quite low for high-density linkage mapping in the *C. arietinum* genome. In the chickpea intraspecific map, a genetic distance of 1 cM to 1.4 Mbp, requires at least 107 evenly distributed markers to resolve a marker density of 5 cM, which is the upper limit required for marker-assisted pyramiding of genes (Winter 1997). Consequently, at least 240 microsatellite sequences should be screened in chickpea to generate this number of markers. However, there have been only 174 microsatellite loci characterized so far in chickpea (Winter et al. 1999). Efforts should be directed to characterize more microsatellite loci that are distributed across the whole genome, especially for TAA motifs. In the present study, only the TAA motifs revealed polymorphism between the parents of the mapping population (data not shown), which was in agreement with that of Winter et al. (1999).

The observed segregation distortion of the chickpea-STMS markers (20.4%) was comparable to the average marker distortion (16.4 ± 7%) found in F_2 populations of other related legumes such as *Glycine* spp. (Keim et al. 1990), *Phaseolus vulgaris* (Nodari et al. 1993), *Arachis* spp. (Halward et al. 1993), *Vigna radiata* and *Vigna unguiculata* (Menancio-Hautea et al. 1993). This is in contrast to the segregation distortion of 39.8% found in the *Cicer* interspecific linkage map (Winter et al. 2000). The high number of distorted loci in the interspecific population may have been due to recombination suppression at meiosis caused by a considerable degree of non-/or partial-homology between the chromosomes of *C. arietinum* and *C. reticulatum*, and the use of RIL as a mapping population. Even if the anomalous recombination was minimal, segregation distortion would still be accumulated in the population with the progressive selfing or cycles of meiosis undergone in the development of the RIL. In tomato, Paran et al. (1995) reported a significant increase in the number of loci that deviated from the expected Mendelian inheritance from F_2 to F_7 generations. They accounted this increase to a cumulative effect of selection against alleles of one of the parents during the propagation of the RIL.

All the distorted STMS markers were skewed in favor of the maternal allele types. Almost all of these distorted markers (ten out of 11) were mapped in the genome, and clustered mostly into one linkage group (LG VII). This finding suggests that LG VII may harbour the genetic factor(s) responsible for the preferential transmission of the maternal alleles in the progeny. In rice, Xu et al. (1997) detected a cluster of distorted markers in six out of 11 gametophyte gene regions and five out of seven sterility regions. Although physiological and environmental factors have been shown to affect segregation distortion (Yang et al. 1983; Graner et al. 1991), there should be a corresponding genetic component that governs the degree and magnitude of distortion in response to varying levels of physiological and environmental effects. This is especially true when the heritability of the genetic component is high, which is the case of the locus suggested with this finding. LG VII was also identified as a cluster of distorted markers in the interspecific RIL map (Winter et al. 1999). With the number of distorted markers in the cluster and high heritability, LG VII is a likely major locus for a gene that governs the segregation distortion in chickpea. Even if a very small percentage of outcrossing occurs within chickpea (0–1%; Smithson et al. 1985; Singh 1987), and probably with *C. reticulatum* because of their inter-crossability (Singh and Ocampo 1993), the preferential transmission of maternal alleles

and predominant self-pollination reproduction could explain why the genetic diversity of the crop has remained very narrow.

Preliminary alignment of the intraspecific linkage map of chickpea with the interspecific linkage map (Winter et al. 2000) revealed high linkage conservation in at least three linkage groups and colinearity in one linkage group. These conserved regions spanned a total of about 79.2 cM, which could be approximated to be 15% of the total genome size of chickpea based on the recombination rate of the intraspecific linkage map (1.4 Mbp/cM). In a recent rudimentary map alignment, Tekeoglu et al. (2002) reported conservation of markers in seven linkage groups between their *C. arietinum* × *C. reticulatum* linkage map with that of Winter et al. (2000) and a partial-genomic *C. arietinum* × *C. arietinum* linkage map. However, only five and three skeletal-linkage groups of the other interspecific and intraspecific maps respectively, were compared with their map. The three intraspecific linkage groups were also conserved in the present chickpea genome map, based on few common STMS markers.

Further comparative mapping revealed that chickpea may have evolved from *C. reticulatum*, possibly via inversions and minor intra- and inter-chromosomal translocations of DNA sequences. If confirmed by high-density mapping, this could provide molecular evidence to support the observed rearrangement of the chromosome karyotype between *C. reticulatum* and particular lines of chickpea. Using in-situ hybridization coupled with C-banding techniques, Galasso et al. (1996) demonstrated that the distal ends of the long arms of the satellite chromosomes A and B were shortened in chickpea. The missing segment was postulated to have been translocated and/or eliminated during evolution. However, there was only one accession of each *Cicer* species evaluated and no interspecific hybridization was made to check for cross-ability. In the cytological and breeding studies of Ladizinsky and Adler (1976) involving an accession of *C. reticulatum* and five lines of *C. arietinum*, meiosis of four hybrid combinations were normal. Pollen fertility and seed set values were also similar to those of the parental species. However, one particular *C. arietinum* line produced irregular chromosome pairing with *C. reticulatum* indicating translocation and paracentric inversion (Ladizinsky and Adler 1976). Pollen fertility in this cross-combination was also very low and with no single seed formed.

The splitting of LG 6 of the interspecific linkage map into two linkage groups, LG V and VIII, could partly clarify the evolutionary enigma described above. LG VIII was a translocated segment from the central region of LG 6. The translocation of this small segment into a separate linkage group was unlikely due to error in linkage analysis because the flanking markers (TR7 and TA176) in the break point were conserved on LG V. In the interspecific map, no recombination was determined between the markers TA22 and TA80 of the translocated segment, while a recombination distance of 6.4 cM was revealed in the intraspecific map (LG VIII). The presence of homology and consequent recombination between the markers in the intraspecies cross could be the reason for this disparity in recombination distance. Likewise, the merging of some linkage groups from the interspecific map in the intraspecific map (LG I, II and IV) may also not be evolutionary, but rather due to the homology and thus resolution of the sequences that joined the linkage groups in the intraspecies mapping population. Although not very conclusive, results of this preliminary comparative mapping have provided a molecular insight of the likely chromosomal rearrangements that led to the speciation of the cultivated chickpea from *C. reticulatum*. An intraspecific map of *C. reticulatum* with the same set of STMS markers as anchor markers should be available to validate this evolutionary inference. Comparative mapping between intraspecific linkage maps of *C. arietinum* and *C. reticulatum* would be conclusive because bias estimation of recombination distance due to the unbalanced formation of gametes is not prevalent in an intraspecies cross compared with a wide cross of genetically diverged genomes.

The chickpea intraspecific linkage map developed in this study will serve as a core map in the mapping and tagging of genes for disease resistance, particularly ascochyta blight resistance, in chickpea. As the map becomes saturated with markers, more complex traits known to limit the production potential of chickpea could be dissected and utilized more effectively in national and international breeding programs. Finally, the use of chickpea-STMS markers as anchor markers has provided a molecular insight of the genetic evolution of chickpea, which is a logical starting point towards intra-genera comparative mapping in *Cicer*.

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