Use of random amplified polymorphic DNA markers for mapping the chickpea genome

H. BANERJEE[1]*, R.A. PAI*, J.P. MOSS** and R.P. SHARMA*

*NRC on Plant Biotechnology, IARI, New Delhi 110012, India**
*Legume Cell Biology Unit, ICRISAT, Patancheru 502324, Andhra Pradesh, India***

Abstract

Three interspecific crosses were developed using *Cicer arietinum* (ICC 4918) as the female parent and wild *Cicer* species [*C. reticulatum* - JM 2100, JM 2106 and *C. echinospermum* - ICCW 44] as the male parent. *Cicer arietinum* (ICC 4918) × *C. reticulatum* (JM 2100) cross produced the largest number of F_2 plants and was chosen for linkage mapping using Random Amplified Polymorphic DNA (RAPD) primers. A partial linkage map was constructed based upon the segregation of 36 RAPD markers obtained by amplification using 35 primers. The linkage map consists of two linkage groups with 17 linked markers covering a total of 464.9 cM. Analyses also revealed association of three morphological traits with linked RAPD markers. Out of seven morphological traits tested for association with linked markers in the segregating plants, four Quantitative trait loci (QTL) were detected for the trait leaf length and three QTLs each for the traits leaf width and erect plant habit.

Additional key words: *Cicer arietinum*, *Cicer reticulatum*, *Cicer echinospermum*, RAPD, molecular linkage map, quantitative trait loci.

Introduction

Chickpea (*Cicer arietinum* L.) is a self pollinated diploid (2n = 2x = 16) annual grain legume and is traditionally grown in many parts of the world. Analysis of genetic polymorphism in cultivars of chickpea based on morphological traits, seed protein profile and isozyme studies (Muehlbauer and Singh 1987, Gaur and Slinkard 1990a,b, 1991, Kazan and Muehlbauer 1991, Kazan *et al.* 1993, Ahmad *et al.* 1992) have shown few polymorphic loci. Low levels of usable polymorphic loci in this crop has limited the linkage analysis of the various genes and assignment of important traits in linkage map of chickpea. However, wild species of *Cicer* are a treasure of resistance genes to a variety of biotic and abiotic stresses (Singh and Saxena 1992). The wild annual and few perennial species of *Cicer* were examined using isozymes and have shown many polymorphic isozyme loci useful for studying the genetic relationships among them (Ahmad *et al.* 1992, Kazan and Muehlbauer 1991, Kazan *et al.* 1993, Tayyar and Waines 1996). Interspecific hybridisations were also performed between the cultivated accessions and various wild *Cicer* species including *C. reticulatum*, the presumed progenitor of modern day chickpea (Sheila *et al.* 1992, Ladizinsky and Adler 1976a,b) in order to introgress various disease resistance genes into chickpea cultivars. Gaur and Slinkard (1990a,b) developed the linkage map of 13 isozyme loci on 4 linkage group based on an interspecific cross. The development of molecular marker techniques like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and microsatellites have helped to identify useful polymorphic loci in various crop species (Tanksley *et al.* 1989, Williams *et al.* 1990, Winter and Kahl 1995).

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Abbreviations: RAPD - random amplified polymorphic DNA; QTL - quantitative trait loci.
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[1]Present address: MCD Biology, University of Colorado at Boulder, Boulder, CO, 80309-0347, USA; fax: (+1 303) 492 7744; e-mail: hiren.banerjee@colorado.edu

RFLP analysis in chickpea accessions has detected very low level of polymorphism and concluded a narrow genetic base in this important grain legume (Udupa *et al.* 1993, Van Rheenen 1992, Banerjee *et al.* 1999). However, oligonucleotide fingerprinting with short microsatellite motifs have revealed considerable variation in chickpea at the interspecific level (Weising *et al.* 1992, Sharma *et al.* 1995). Using a PCR based technique that uses sequences flanking the microsatellites as primers, size differences in the amplification products was generated due to variations in the number of tandem repeats within microsatellite thus generating the Sequence Tagged Microsatellite Sequences or STMS markers (Litt and Luty 1989). Winter *et al.* (1999) have mapped STMS markers on 90 recombinant inbred lines from an interspecific cross between *C. reticulatum* and chickpea cultivar IC 4958 and developed a chickpea map comprising 11 linkage groups covering 613 cM of the genome.

In this paper we report the effectiveness of RAPD markers for mapping the chickpea genome using an F_2 population derived from *C. arietinum* × *C. reticulatum* cross and analyse association of few morphological traits with linked RAPD markers.

Materials and methods

Plants: Seeds of chickpea (*Cicer arietinum* L.) accession ICC 4918 and wild *Cicer* species of *C. reticulatum* Ladizinsky (JM 2100 and JM 2106), *C. echinospermum* P.H. Davis (ICCW 44) were obtained from ICRISAT, Patancheru, India and ICARDA, Aleppo, Syria. A single plant of ICC 4918 was used as a female parent and a single plant of wild *Cicer* sp. accessions were used as a male parent to generate three wide crosses (ICC 4918 × JM 2100; ICC 4918 × JM 2106, and ICC 4918 × ICCW 44). These three crosses were developed during 1992 - 1993 at ICRISAT, Patancheru. F_1 plants were selected from each of these crosses and self mated to produce the segregating F_2 population. The size of F_2 segregating population varied for each of the crosses. ICC 4918 × JM 2100 cross produced a total of 264 segregating plants out of which 86 plants were selected for linkage mapping. ICC 4918 × JM 2106 cross produced only 45 segregating plants while ICC 4918 × ICCW 44 cross produced 25 segregating plants that were not used in linkage analysis. The segregating F_2 population showed variation for a number of qualitative and quantitative traits [number of leaflets, leaf length, leaf width, leaf area, plant habit (erect, semi-spreading), plant height, plant width, number of pods per plant, number of seeds per plant and seed mass]. The parents and their segregating progenies were grown in field at ICRISAT, Patancheru and the various genetic traits were scored when the plants reached maturity.

DNA extraction: Plant DNA was extracted from the young leaves (5 g) using the CTAB method (Saghai-Maroof *et al.* 1984) after incorporating the modifications proposed by Doyle and Doyle (1987).

Primers and RAPD analysis: Random decamer primers (*OPERON Tech. Inc.*, Alameda, USA) were dissolved in sterile distilled water at a concentration of 0.015 mg cm^{-3}. 240 primers belonging to *OPERON kits* - OPA to OPL were used for RAPD analysis. PCR amplification and RAPD analysis was performed according to Banerjee *et al.* (1999).

Scoring data and linkage analysis: The segregation of polymorphic DNA band was scored among the amplified DNA samples of the F_2 plants and were denoted by "A" (presence) and "C" (absence) when the polymorphic band is present in parent 1 (ICC 4918) and absent in parent 2 (JM 2100); while presence of DNA band was denoted as "D" and absence as "B" when the polymorphic band is present in parent 2 (JM 2100) and absent in parent 1 (ICC 4918). This nomenclature is followed according to Lander *et al.* (1987). Segregating bands which were difficult to score in some lanes or were weakly amplified were recorded as missing data.

Linkage analysis of the segregating marker alleles among the F_2 plants was performed as described by Lander *et al.* (1987) and Lander and Botstein (1989) using *MAPMAKER version 2.0*. The entire set of markers was processed using two point and multipoint analysis to determine the order and recombination function for the markers. A maximum recombination value of 50 and a log likelihood ratio (LOD) score of 3.0 was used for pairwise linkage analysis. Recombination distances were converted to centimorgan (cM) by mapping function of Kosambi (1944). Goodness of fit to a 3:1 Mendelian ratio of the segregating RAPD markers was tested by χ^2 analysis at 5 % significance level (Strickberger 1985). The nomenclature for individual RAPD marker allele is described as first the brand of primer "OP" (*OPERON Tech.*) followed by kit, *e.g.*, A, B, C..., primer number 1, 2, 3... and lastly approximate base pair size of the DNA band (marker). If any particular primer amplified more than one segregating RAPD marker then the alleles are represented by smaller letter alphabets (a, b, c...) following the designated marker [OPD $15a_{(1800)}$].

Analysis of various statistical parameters based on the genetic traits data scored on F_2 plants were performed using a basic statistical analysis software. Quantitative trait loci (QTL) analysis was performed for the seven quantitative traits [leaf characteristics - number of leaflets, leaf length, leaf width; plant habit (erect or semi-spreading); plant width and 100 seed mass] using *MAPMAKER/QTL version 1.1* program. The trait data

scored on F_2 plants were analysed along with the segregating RAPD marker data in *MAPMAKER/QTL* software. Further analysis of association of any RAPD marker with a specific trait was done by comparing a single trait with all the segregating marker alleles to locate the tightly linked marker.

Results and discussion

Degree of RAPD variation among the parents: A set of 240 primers was used for screening the DNA samples of ICC 4918, JM 2100 and JM 2106 accessions while a second set of 100 primers was used for screening polymorphism between ICC 4918 and ICCW 44, a more distant wild relative of chickpea. Using the optimised PCR conditions the number of detectable RAPD amplification products ranged from 4-6 with a maximum of 10 amplified DNA bands ranging in size from 0.3 to 2 kb. The association between the primer sequences, their guanosine + cytosine content and the yield of amplification products or the number of polymorphic DNA bands were not found. A total of 98 primers among the 240 primers used for screening were found to amplify DNA bands from ICC 4918, JM 2100 and JM 2106 accessions that could be reliably scored for polymorphism. ICC 4918 and JM 2100 accessions could be distinguished by 40 % of the primers while only

Table 1. Segregation pattern of 36 RAPD marker loci in the F_2 population of ICC 4918 x JM 2100 cross (* - significant at 5 % level).

Marker locus	Number of allele A	Number of allele B	Total number of F_2 plants	Expected # allele A	Expected # allele B	χ^2 (3:1)
OPA $4_{(800)}$	49	37	86	64.5	21.5	13.4
OPA $8_{(400)}$	53	33	86	64.5	21.5	7.4
OPA $9_{(900)}$	60	24	84	63.0	21.0	0.75*
OPA 12 $a_{(1000)}$	54	31	85	63.75	21.25	5.37
OPA 12 $b_{(800)}$	53	32	85	63.75	21.25	6.58
OPA 12 $c_{(600)}$	71	14	85	63.75	21.25	2.85*
OPB $1_{(600)}$	49	36	85	63.75	21.25	12.68
OPB $10_{(1000)}$	56	30	86	63.75	21.25	4.75
OPB $12_{(950)}$	56	26	82	61.5	20.5	2.16*
OPB 17 $a_{(564)}$	52	26	78	58.5	19.5	3.12*
OPB 17 $b_{(310)}$	60	13	73	54.75	18.25	1.65*
OPB $18_{(500)}$	54	27	81	60.75	20.25	2.57*
OPC $16_{(880)}$	61	25	86	64.5	21.5	0.54*
OPD $13_{(400)}$	43	43	86	64.5	21.5	27.3
OPD 15 $a_{(1800)}$	54	30	84	63.0	21.0	4.54
OPD 15 $b_{(1260)}$	54	29	83	62.25	20.75	3.86*
OPD $18_{(1246)}$	46	32	78	58.5	19.5	3.12*
OPE $19_{(1600)}$	50	36	86	64.5	21.5	12.1
OPF $1_{(900)}$	49	34	83	62.25	20.75	10.4
OPF $3_{(950)}$	61	25	86	64.5	21.5	0.85*
OPF $6_{(750)}$	45	41	86	64.5	21.5	24.1
OPF $9_{(890)}$	56	28	84	63.0	21.0	2.67*
OPF $14_{(900)}$	54	32	86	64.5	21.5	7.1
OPF $16_{(950)}$	54	32	86	64.5	21.5	7.1
OPF $20_{(950)}$	54	32	86	64.5	21.5	7.1
OPG $4_{(378)}$	62	24	86	64.5	21.5	0.48*
OPH 7 $a_{(1120)}$	60	25	85	63.75	21.25	0.65*
OPH 7 $b_{(473)}$	58	28	86	64.5	21.5	2.22*
OPI $8_{(582)}$	70	16	86	64.5	21.5	1.72*
OPI $16_{(600)}$	41	45	86	64.5	21.5	34.9
OPI $19_{(550)}$	58	26	84	63.0	21.0	1.28*
OPJ $7_{(641)}$	58	25	83	62.25	20.75	0.89*
OPK $1_{(1230)}$	55	31	86	64.5	21.5	5.01
OPK 17 $a_{(936)}$	53	33	86	64.5	21.5	7.47
OPK 17 $b_{(835)}$	67	19	86	64.0	21.5	0.24*
OPL $19_{(900)}$	48	37	85	63.75	21.25	14.5

21 % of the primers detected polymorphism between accessions ICC 4918 and JM 2106. Screening ICC 4918 and ICCW 44 for polymorphism showed that 45 % of the primers are useful for detecting polymorphism.

The RAPD analysis of the chickpea (*C. arietinum* L.) and wild *Cicer* species (*C. reticulatum* and *C. echinospermum*) accessions has shown more polymorphism between *C. arietinum* and *C. echinospermum* accessions compared to that between *C. arietinum* and *C. reticulatum* accessions. This is not entirely unexpected since *C. reticulatum* has been confirmed to be the progenitor of modern day cultivated chickpea (Ohri and Pal 1991, Tayyar and Waines 1996) while *C. echinospermum* differs from the cultivated chickpea accessions in various morphological traits and have larger genetic distance compared to *C. reticulatum* (Singh *et al.* 1991, Kazan and Muehlbauer 1991, Tayyar and Waines 1996). In our wide hybridisation studies, a greater number of F_2 progenies were recovered from *C. arietinum* × *C. reticulatum* cross compared to the *C. arietinum* × *C. echinospermum* cross.

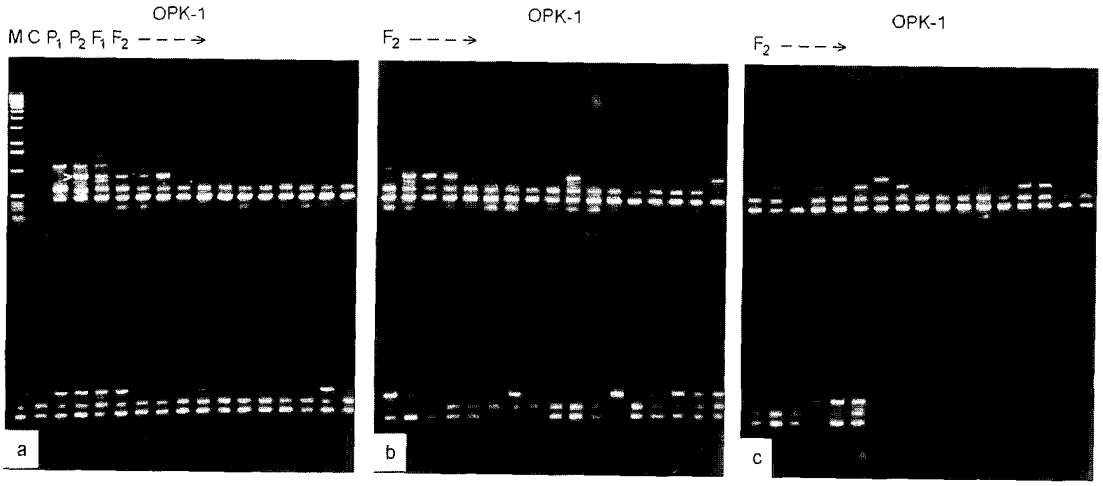

Fig. 1. RAPD profile obtained by amplification of DNA from chickpea accessions and their F_1 and F_2 progeny plants. *Lane M*: λ *Eco* RI and *Hind* III digest. *Lane C*: Amplification without DNA. *Lane P1, P2*: ICC 4918, JM 2100. *Lane F1*: F1 hybrid obtained from ICC 4918 x JM 2100 cross. *Lane F2*: F2 progeny plants derived from the ICC 4918 x JM 2100 cross.

Linkage mapping: Based on initial screening of parents (ICC 4918, JM 2100, JM 2106 and ICCW 44) for RAPD variation, the accessions ICC 4918 and JM 2100 were found to reveal more polymorphic DNA bands differentiating them compared to JM 2106. The largest size of F_2 progeny plants also was derived from ICC 4918 × JM 2100 cross and hence this population was found suitable for linkage mapping of various genetic traits. A total of 35 primers out of 69 primers that showed polymorphism between accessions ICC 4918 and JM 2100 were selected for RAPD analysis of segregating F_2 plants since their strongly amplified DNA bands could be reliably scored for linkage analysis. Five primers (OPA 5, OPB 15, OPD 16, OPE 11 and OPE 12,) were found to produce polymorphic DNA bands that were present in 90 % of the F_2 plants and their segregation was highly skewed. Thus, a total of 30 primers were finally used for analysing 86 F_2 plants. Out of these 30 primers 25 detected only one segregating locus, 4 (OPB 17, OPD 15, OPH 7 and OPK 17) primers detected 2 segregating loci while only one (OPA 12) primer detected 3 segregating loci, thereby 36 marker alleles could be detected in the segregating plants (Table 1, Fig. 1). Of the 36 marker alleles detected, 18 markers [OPD $15a_{(1800)}$, OPK $17a_{(936)}$, OPK $1_{(1230)}$, OPL $19_{(900)}$, OPE $19_{(1600)}$, OPD $13_{(400)}$, OPA $4_{(800)}$, OPA $8_{(400)}$, OPA 12 $a_{(1000)}$, OPA $12b_{(800)}$, OPB $1_{(600)}$, OPB $10_{(1000)}$, OPF $1_{(900)}$, OPF $6_{(750)}$, OPF $14_{(900)}$, OPF 16 $_{(950)}$, OPF $20_{(950)}$ and OPI $16_{(600)}$]showed significant deviation from the expected 3:1 segregation ratio. The remaining 18 markers [OPD $15b_{(1260)}$, OPK $17b_{(835)}$, OPJ $7_{(641)}$, OPH $7a_{(1120)}$, OPH $7b_{(473)}$, OPC $16_{(880)}$, OPA $9_{(900)}$, OPA $12c_{(600)}$, OPB $12_{(950)}$, OPB $17a_{(564)}$, OPB $17b_{(310)}$, OPB $18_{(500)}$, OPF $3_{(950)}$, OPF $9_{(890)}$, OPD $18_{(1246)}$, OPG $4_{(378)}$, OPI $19_{(550)}$ and OPI $8_{(582)}$] showed expected 3:1 Mendelian segregation. The phenomenon of segregation distortion of marker alleles has been reported in several studies where RAPD markers were used for mapping in alfalfa (Echt *et al.* 1992), *Picea glauca* (Carlson *et al.* 1991), *Pseudotsuga menziensii* (Jermstad *et al.* 1994), and lettuce (Kesseli *et al.* 1994). The F_2 population size, number and quality of genetically altered loci (heterozygosity) in F_1 generation determine the extent of distorted segregation ratio of the marker alleles (Kiss *et al.* 1993). Close proximity of the marker allele to one or more genes that have lost their function (selective damage or mutation) and nonhomologous / homologous pairing of the chromosomes of non-allelic regions producing thereby abnormal chromosomes also could eventually lead to lethality and distorted segregation of markers.

Table 2. LOD score and cM distance for mapped RAPD markers to Linkage group I (* - values on top is cM distance and bottom is LOD score for each RAPD marker locus).

	OPD13 (400)	OPI 16 (600)	OPI 19 (550)	OPB 1 (600)	OPA12c (600)	OPD 15b (1260)	OPC 16 (880)	OPI 8 (582)	OPH 7a (1120)	OPE 19 (1600)	OPK 1 (1230)	OPF 20 (950)	OPK17a (936)	OPD15a (1800)
OPI 16 (600)	45.4 1.64													
OPI 19 (550)	46 1.79	26.7 6.68												
OPB 1 (600)	34.5 3.14	46.9 1.33												
OPA 12c (600)	52.3 1.09	34.7 3.82	26.4 7.22	44.6 1.72										
OPD 15b (1260)	41.6 2.33	39.6 2.71	28.7 6.24	38.1 2.71	30.1 5.44									
OPC 16 (880)	50.3 1.37	27.7 6.5	23 9.52	57.2 0.77	30.2 5.93	29.6 6.09								
OPI 8 (582)	33.6 4.73	35.3 4.3	24.2 9.45	44.8 2.05	32.4 5.49	25 8.76	19.3 13.16							
OPH 7a (1120)	38.6 3.05	54.7 1	33.4 4.85	47.3 1.56	42.2 2.61	33.3 4.77	26.9 7.64	18 13.96						
OPE 19 (1600)	- 0.56	59.1 0.51	62.1 1.43	- 2.82	- 2.96	47.5 3.14	39.1 -	39.6 -	37.2 -					
OPK 1 (1230)	- 4.18	- 1.8	35.3 2.75	44.4 5.26	40.9 7.1	31.1 7.13	27.6 7.34	28.7 2.18	26.4 4.32	41.8 -	33.3 -			
OPF 20 (950)	48.5 1.35	39.8 2.5	40.9 2.57	48.6 1.22	46 1.78	31.8 4.64	26.3 7.05	28 6.76	36.3 3.64	39.5 2.41	31.5 5.16	23.4 8.05		
OPK 17a (936)	41.1 2.43	50.5 1.27	35 4.14	45.6 1.62	45.8 1.92	35 4.06	28 6.81	27.2 7.7	29.4 6.19	36.4 3.1	30.9 5.31	39.6 2.69	32.4 4.8	
OPD 15a (1800)	52.8 1.07	36.6 3.34	30.8 5.48	44.8 1.69	36.6 3.61	26.8 6.92	29.5 6.14	20.5 11.62	36.3 3.93	56.6 0.74	61.1 0.55	53.3 0.88	40.7 2.34	42.4 2.11
OPA 4 (800)	51.5 0.97	37.9 2.59	36.3 3.29	- 0.68	57.8 1.61	46.2 1.67	46.7 0.77	58.8 1.09	52.3 -					

In cases where the segregating population is derived from wide hybridisation as has been in this study there could possibly be two reasons for the observed segregation distortion of the marker alleles in the F_2 plants. First, being a wide cross, the number of genetically altered loci is higher in F_1 generation and during the meiosis stage the altered loci [alleles from wild parent (*C. reticulatum*)] were eliminated thus resulting in a strong gametophytic selection. Second, deleterious mutations of genes located in chromosomal regions containing markers could have inhibited their onward transmission to F_2 progenies. Both the above processes could have led to distorted segregation of marker alleles.

Linkage analysis using 36 segregating marker alleles resulted in mapping 17 markers to two linkage groups while the remaining 19 markers were found unlinked at LOD 3.0 and a recombination value of 0.5. The first linkage group contained 15 marker while the second group contained only two markers and a total of 464.9 cM of the genome was covered by these 17 markers (Table 2, Fig. 2). Duplicate loci OPB $17a_{(564)}$ and OPB $17b_{(310)}$ are located in linkage group I and II respectively while in case of other duplicate loci derived from amplification products from primers OPD 15, OPA 12, OPH 7 and OPK 17 were found located in group I by either or both of their duplicated loci. The linkage map produced from this analysis shows clustering of large number of marker alleles to linkage group I and thus group I may contain more than required number of marker loci. Large cM distance of 52.5 cM between the marker alleles OPB $1_{(600)}$ and OPI $19_{(550)}$ may help to break linkage group I and yield another new linkage group consisting of three markers [OPI $19_{(550)}$, OPI $16_{(600)}$ and OPD $13_{(400)}$]. The linkage map produced is partial due to clustering of marker alleles to mainly linkage group I that may be due to a large number of markers showing distorted segregation and the skewed segregation observed for many quantitative traits in the F_2 population.

Variation of quantitative traits in segregating plants: The most obvious trait that can differentiate a cultivated chickpea and a *Cicer reticulatum* accession is the erect plant habit, absence of anthocyanin pigment in leaves and young stem, large pod and seed size in cultivated accessions. The F_2 segregating population derived from this wide cross has produced a range of traits which are a hybrid of traits possessed by their parents – plants with both erect and semi-spreading branches, range of

anthocyanin pigmentation in mature leaves (very highly pigmented to weak pigmentation), plants with few leaves and branches to plants with many branches and pods. Out of the ten quantitative traits that has been studied in 86 F_2 plants, five traits (leaf area, plant height, plant width, plant habit and seed mass) exhibited normal distribution at 95 % confidence level (data not shown). The distribution of other six quantitative traits were found skewed – a larger number of plants showed decrease in number of their leaflets, increased size of their leaf length and leaf width, increased size of pods and number of seeds per plant. The traits that followed normal distribution suggest that they are controlled by multiple genes each contributing its share and thus suitable for mapping of quantitative trait loci (QTL) involved.

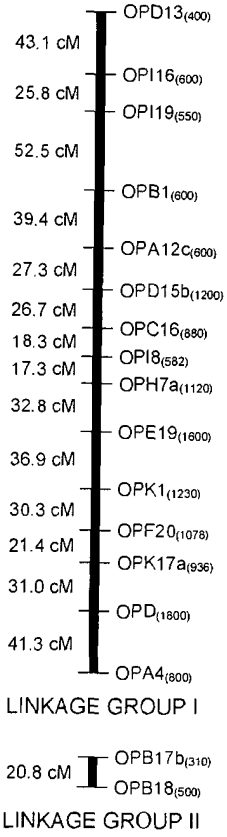

Fig. 2. Linkage map of chickpea based on RAPD markers.

RAPD marker and QTL association: QTL analysis revealed three quantitative traits that were found putatively linked to various mapped RAPD markers in linkage group I (Table 3). For leaf length, four QTLs were detected. The regions in the linkage group I flanked by RAPD markers OPA $12c_{(600)}$ and OPD $15b_{(1260)}$; OPD $15b_{(1260)}$ and OPC $16_{(880)}$; OPE $19_{(1600)}$ and OPK $1_{(1230)}$ and OPK $17a_{(936)}$ and OPA $4_{(800)}$ were found linked to genes controlling leaf length. In case of quantitative trait leaf width, three QTLs were found. In linkage group I, flanking RAPD markers OPI $19_{(550)}$ and OPB $1_{(600)}$; OPB $1_{(600)}$ and OPA $12c_{(600)}$; OPK $17a_{(936)}$ and OPA $4_{(800)}$ were found linked to genes controlling leaf width. For erect plant habit four QTL were detected. The regions in linkage group I flanked by RAPD markers OPD $13_{(400)}$ and OPI $16_{(600)}$; OPI $16_{(600)}$ and OPI $19_{(550)}$; OPI $19_{(550)}$ and OPB $1_{(600)}$; OPK $17a_{(936)}$ and OPA $4_{(800)}$ were found linked to genes controlling plant habit. The *MAPMAKER/QTL* analysis has revealed few markers that are linked with multiple quantitative traits - the region in linkage group I flanked by markers OPK $17a_{(936)}$ and OPA $4_{(800)}$ was found linked to all the three quantitative traits, while the region flanked by markers OPB $1_{(600)}$ and OPI $19_{(550)}$ was found linked to both leaf width and plant habit. All these associations of RAPD markers with the QTLs are putative and saturating the linkage map with more markers will help to determine the tightly linked marker allele with a given genetic trait. This analysis also revealed that allelic effects of "B" allele contributed by wild male parent helped to increase in leaf length and leaf width while the allelic effects of "A" allele contributed

Table 3. Summary of putative QTL controlling morphological traits.

Trait	Linkage group	Flanking markers	[cM]	LOD score	Additive	Dominance	Variance [%]	Minimum QTL
Leaf length	I	OPA $12c_{(600)}$ & OPD $15b_{(1260)}$	34.4	3.47	0.0124	-3.0971	73.7	1
	I	OPC $15b_{(1260)}$ & OPC $16_{(880)}$	33.5	4.10	-0.1881	-2.8907	72.9	1
	I	OPE $19_{(1600)}$ & OPK $1_{(1230)}$	49.6	2.70	-0.6893	3.1310	75.8	1
	I	OPK $17a_{(936)}$ & OPA $4_{(800)}$	54.5	33.38	0.1549	-3.0944	74.5	1
Leaf width	I	OPI $19_{(550)}$ & OPB $1_{(600)}$	76.2	3.59	-0.0136	0.1809	71.0	1
	I	OPB $1_{(600)}$ & OPA $12c_{(600)}$	53.5	3.59	-0.0170	0.1824	70.7	1
	I	OPK $17a_{(936)}$ & OPA $4_{(800)}$	54.5	4.96	0.0004	-0.1822	74.4	1
Erect plant habit	I	OPD $13_{(400)}$ & OPI $16_{(600)}$	59.7	91.49	0.000	-1.000	99.5	1
	I	OPI $16_{(600)}$ & OPI $19_{(550)}$	32.2	88.55	0.000	-1.000	99.5	1
	I	OPI $19_{(550)}$ & OPB $1_{(600)}$	76.2	91.44	0.000	-1.000	99.5	1
	I	OPK $17a_{(936)}$ & OPA $4_{(800)}$	54.5	3.11	0.3591	0.6306	58.9	1

by female parent helped in erect plant habit. However, for QTLs having minor effects both the parental alleles might have contributed to increased trait expression. A correlation was noted between the traits leaf length and leaf width based on the present analysis using two linkage groups containing 17 RAPD markers and the data derived from field analysis of morphoagronomic traits.

The preliminary results obtained from this study demonstrate the efficiency of RAPD markers in distinguishing the components controlling a few morphoagronomic traits studied in chickpea. Genome mapping using more polymerase chain reaction (PCR) based markers may help to produce a saturated linkage map of chickpea and expedite the characterisation of the QTLs controlling various agronomic traits in this important grain legume.

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