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Mapping genes for double podding and other morphological traits in chickpea

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Summary

Seed traits are important considerations for improving yield and product quality of chickpea (*Cicer arietinum* L.). The purpose of this study was to construct an intraspecific genetic linkage map and determine map positions of genes that confer double podding and seed traits using a population of 76 F_{10} derived recombinant inbred lines (RILs) from the cross of 'ICCV-2' (large seeds and single pods) × 'JG-62' (small seeds and double podded). We used 55 sequence-tagged microsatellite sites (STMS), 20 random amplified polymorphic DNAs (RAPDs), 3 inter-simple sequence repeats (ISSR) and 2 phenotypic markers to develop a genetic map that comprised 14 linkage groups covering 297.5 cM. The gene for double podding (*s*) was mapped to linkage group 6 and linked to Tr44 and Tr35 at a distance of 7.8 cM and 11.5 cM, respectively. The major gene for pigmentation, *C*, was mapped to linkage group 8 and was loosely linked to Tr33 at a distance of 13.5 cM. Four QTLs for 100 seed weight (located on LG4 and LG9), seed number plant^{-1} (LG4), days to 50% flower (LG3) were identified. This intraspecific map of cultivated chickpea is the first that includes genes for important morphological traits. Synteny relationships among STMS markers appeared to be conserved on six linkage groups when our map was compared to the interspecific map presented by Winter et al. (2000).

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinating diploid annual (2n = 2x = 16) and the world's third most important food legume (FAO, 2000). The crop is widely grown in the Indian sub-continent, the Middle East, eastern Africa, North America and the Mediterranean region. It is widely used as a rotational crop to improve soil nutritional status through symbiotic nitrogen fixation. The world mean seed yield of about 0.8 t ha^{-1} is small compared to other major legume crops. Biotic stresses such as fusarium wilt (caused by *Fusarium oxysporum* f.sp. *ciceri*), ascochyta blight (caused by *Ascochyta rabiei*), and pod borer (*Heliocoverpa armigera*) and abiotic stresses such as drought and cold are considered responsible for low seed yields in many areas (Ryan, 1997).

Mapping the chickpea genome has been of interest to identify genomic locations of important traits and particularly disease resistance (Simon & Muehlbauer, 1997; Winter et al., 1999; Winter et al., 2000). Genetic resistance to fusarium wilt and ascochyta blight has stabilized chickpea seed yields; however, low yield potential is still a major problem. Furthermore, economically important traits such as seed size and seed number per plant are quantitatively inherited and have low heritability (Muehlbauer & Singh, 1987) that slows progress in breeding. Even though good progress has been made in developing methods to study the genetics of quantitative traits in other crops, it is difficult to apply those methods to chickpea because of limited genetic poly-

morphism (Muehlbauer & Singh, 1987; Kazan et al., 1993; Simon & Muehlbauer, 1997).

Skeletal linkage maps of the chickpea genome were constructed by Ahmad & Slinkard (1992) and Kazan et al. (1993). A more extensive linkage map that comprised 91 RAPD, isozyme, RFLP and morphological markers was constructed by Simon & Muehlbauer (1997) using an interspecific cross of *C. arietinum* × *C. reticulatum*. The development of sequence-tagged microsatellite sites (STMS) that consistently identify polymorphism among cultivated chickpea lines has helped increase map density (Hüttel et al., 1999; Winter et al., 1999; Winter et al., 2000). Santra et al. (2000) developed a map from an interspecific cross of *C. arietinum* × *C. reticulatum* that comprised 90 RAPD, 9 isozyme, 17 inter-simple-sequence-repeats, and 1 morphological marker to identify three quantitative trait loci (QTL) for ascochyta blight resistance. Relative positions of genes of interest in the chickpea genome became possible based on these maps; however, only a small degree of synteny was shown among these maps possibly due to the limited number of molecular markers, differences in marker types and the populations used for the mapping.

Many genetic studies have been carried out concerning the inheritance of morphological traits in chickpea. A review of chickpea genetics by Muehlbauer & Singh (1987) discussed single major gene control of pigmentation of flowers, stems and seeds as well as genes for other morphological traits. Simon & Muehlbauer (1997) mapped the flower color gene. Kazan et al. (1993) also studied the inheritance and linkage relationships of the pigmentation gene using interspecific crosses of *C. reticulatum* × *C. arietinum* and *C. arietinum* × *C. echinospermum*. In his study, Kazan et al. (1993) used four other genes (*p* for flower color, *Gst* for epicotyl color, T^3 for seed coat coloration, and *Rs* for seed surface) and mapped them to the same linkage group along with several isozyme markers. Genetic studies of double podding in chickpea showed that a single recessive gene, *s*, conferred the trait (Khan & Akhtar, 1934; Ahmad, 1964). Recently, Kumar et al. (2000) studied the inheritance of *s* using F_{10} derived recombinant inbred lines (RILs) from a cross of ICCV-2 x JG-62. The gene *s* reportedly increases seed yield by 10–18% under moisture-limiting conditions (Sheldrake et al., 1979; Kumar et al., 2000). Seed size is a component of seed yield and one or two genes explain substantial genetic variation for this trait (Niknejad et al., 1971). Ghatge (1993) identified two additive genes '*Bsd*' and '*Smsd*' for seed size in chickpea. Kumar & Singh (1995) reported that small seed size was partially dominant to large size, while Malhotra et al. (1997) suggested that recessive genes controlled large seed size.

Days to flowering is also an important trait for crop adaptation and productivity especially when grown under environmental conditions of late season drought and high temperatures. Kumar & van Rheenen (2000) identified a gene '*efl-1*' for flowering time using F_6 derived RILs from the cross of ICCV-2 × JG-62. A recessive gene '*ppd*' for early flowering was identified in a cross of late flowering cultivar 'Hadas' and early flowering line ICC-5810 (Or et al., 1999). The *efl-1* of Kumar & van Rheenen (2000) may be allelic to the *ppd* gene of Or et al. (1999). Knowing the map position of these genes would be helpful for breeding purposes. Also, allelism tests of *efl-1* and *ppd* are needed to determine if they represent the same locus.

Our goal in this study was to develop a molecular marker map of chickpea that includes genes for important morphological traits and associated molecular markers that might be used for marker-assisted selection in a breeding program.

Materials and methods

Plant materials and morphological trait evaluation

A cross between two chickpea cultivars, ICCV-2 and JG-62, was made at ICRISAT, Patancheru, India in 1993. Table 1 shows the comparison of two parental lines for 10 morphological traits. 76 F_{10} derived RILs were developed from the F_2 population using single-seed-descent. Field experiments comprised the 76 RILs, two parents and four control cultivars. The experiment to measure quantitative traits was conducted using a α lattice design (11x12) with three replications and was planted at ICRISAT (17°N). Individual plots were single rows 4 m long and spaced 60 cm apart with plants spaced 20 cm apart within rows. The crop was grown without irrigation during the post-rainy season. Weed and insect control was carried out as needed. Quantitative data for individual traits were collected as the mean of 15 competitive plants per RIL from three replications.

Each RIL was phenotyped for pigmentation of flowers, stems, and seeds, days to first flower, days to 50% flower, seed shape, and pod number per peduncle by the comparison to the parental lines during

Table 1. Comparison of ICCV-2 and JG-62 for 10 morphological traits

Parent	Seed type	100 seed wt. (g)	Seed color	Flower color	Stem color	Pod number	Average leaf size (g)	Early growth vigor	Crude fiber concentration (%)	Days to flowering
ICCV-2	Kabuli, round edged	28.9	light pink	white	non-pigmented	single	29.6	rapid	4.1	34.9
JG-62	Desi, sharp edged	16.5	light yellow	pink	pigmented	double	17.3	slow	9.8	51.2

Table 2. Chi square tests for goodness of fit to expected segregation ratios for four morphological traits in a RIL population from the cross of ICCV-2 × JG-62

Traits	Number of RILs				Expected ratio	p (χ^2)
	Total	ICCV-2 type	Intermediate	JG-62 type		
Flower color	74	43	0	31	1: 1	0.1630
Pod number per node	76	45	0	31	1: 1	0.1083
Seed shape	76	16	45	15	1: 2: 1	0.2718
Days to first flower	76	37	0	39	1: 1	0.8185

the 1997/98 and 1998/99 growing seasons. Quantitative data for days to first and 50% flower were also collected for QTL analysis. Quantitative trait data of crude fiber concentration of seed, seed number plant^{-1} and 100 seed weight (g) were collected during the same period. Crude fiber concentration of seed was measured following the procedure used by Prosky et al. (1988).

Molecular marker analysis

DNA was extracted from young leaves of F_{10} seedlings grown in the greenhouse using the CTAB protocol (Weising et al., 1995). We used 400 UBC (University of British Columbia) RAPD primers, 100 ISSR primers, and 186 STMS primers to analyze polymorphism in 76 RILs. PCR amplification of each STMS marker followed the method of Hüttel et al. (1999). Primers that amplified clear polymorphism were used as genetic markers for linkage analysis.

Genetic mapping and QTL analysis

Each segregating marker was tested for goodness of fit to the expected 1: 1 ratio by χ^2 test ($p < 0.05$). All markers including those with distorted distribution were used for linkage mapping. Distorted markers were rescored to confirm the segregation ratios. Linkage mapping was carried out at the LOD score of 3.0 with a maximum distance of 20 cm between any two loci using MAPMAKER 3.0 (Lander et al., 1987). QTL analyses were performed by simple interval mapping for each linkage group using Qgene (Nelson, 1997) at a LOD score of 3.0. Single point regression analysis function was used to identify markers that were significantly associated to each QTL. To help generate a consensus map of the *Cicer* genome we assigned the same linkage group designations as those of Winter et al. (1999, 2000) where synteny between genetic maps was shown.

Results

Morphological trait evaluation

The two parents (ICCV-2 and JG-62) of the cross that was used to develop the F_{10} derived RILs were distinctly different for all 10 morphological traits that were scored (Table 1). The 76 RILs were evaluated for seven morphological traits including seed shape, seed weight, seed number plant^{-1}, pigmentation of flowers, stems, and seeds, double podding, crude fiber concentration, and days to first and 50% flowering. As expected, two of the traits (pigmentation and double podding) appeared to be controlled by single major genes and clearly fit a 1: 1 segregation ratio ($p > 0.05$)

(Table 2). Frequency distributions of RILs for each quantitative trait evaluated were plotted and are shown in Figure 1. In the case of days to 50% flowering, the data could be classified into one of the two parental types even though the RILs had wide variation typical of a quantitatively inherited trait (Figure 1C). Data for days to 50% flowering were analyzed for linkage and were used for QTL mapping. Seed shape segregated in a 1 angular: 2 intermediate: 1 ratio for seed shape among RILs and appeared to be controlled by at least two genes.

Molecular marker analysis

Polymorphic bands between the two parental lines were found for 68 STMS, 34 RAPD, and 4 ISSR markers. Therefore, 5 morphological and 106 molecular markers were available for linkage analysis and mapping. Ninety-four of 111 markers fit the expected 1: 1 segregation ratio ($p > 0.05$).

Linkage analysis

Eighty (55 STMS, 20 RAPDs, 3 ISSR, and 2 morphological markers) of the 111 markers were mapped to 14 linkage groups (Figure 2) that covered 297 cM with an average distance of 3.7 cM between markers. The gene for double podding (*s*) was located on linkage group 6 at a distance of 7.8 cM from Tr44 and 11.5 cM from Tr35. Genes for pigmentation mapped to linkage group 8. The pigmentation gene was designated as *c*, which imparts stem and seed coat coloration as well as seed pigmentation (Kumar et al., 2000). One STMS marker, Tr33, was located on linkage group 8 and 13.5 cM from *c*, the gene for pigmentation. Days to first flower and days to 50% flower did not show linkage to any of molecular markers.

QTL analysis

Four QTLs were identified on the current intraspecific linkage map. Using interval mapping, a QTL for 100 seed weight was identified between UBC465 and Ga137 on linkage group 4 (LOD score = 11.7) (Figure 3). Single point interval regression analysis showed that Ta130s, within this QTL, was significantly associated with 100 seed weight and accounted for 52% of total phenotypic variation ($r^2 = 0.521$). A QTL for seed number plant^{-1} was also identified in the same region on linkage group 4 with a LOD score of 5.7. Ta130s was also significantly associated with seed number plant^{-1} and accounted for 31% of the variation ($r^2 = 0.309$). An additional relatively weak QTL for 100 seed weight was identified on linkage group 9 which comprised only two markers, UBC465 and UBC96a (LOD score = 2.88). UBC96a was associated with 100 seed weight with an r^2 of 0.277. One QTL for days to 50% flower was identified between Ts57 and Ta127 on linkage group 3 with a LOD score of 3.03. A loosely linked QTL for days to first flower was also found on the same location with a LOD score

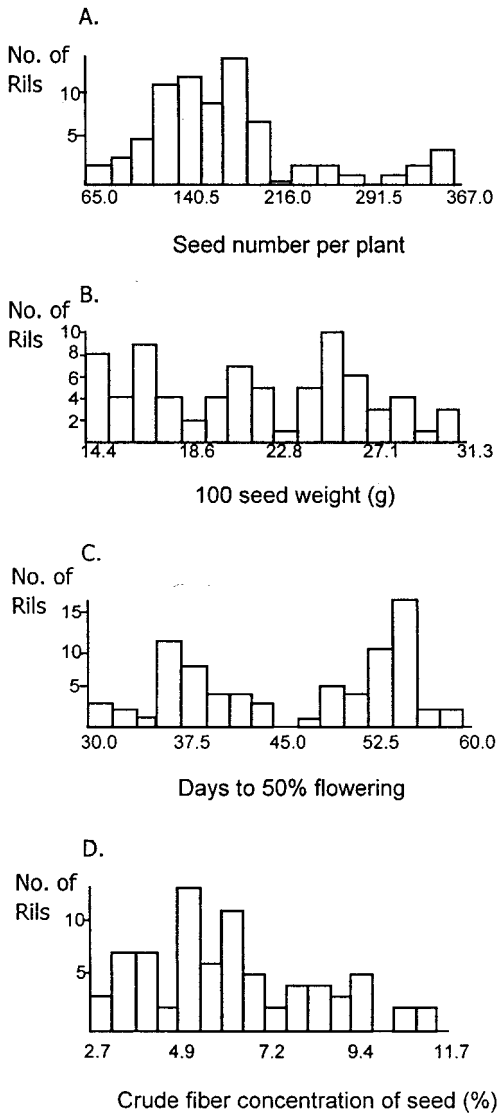

Figure 1. Frequency distributions of RILs for four quantitative traits in chickpea (A. seed number plant^{-1}; B. 100 seed weight; C. days to 50% flowring; D. crude fiber concentration in seed).

of only 2.34. A QTL for crude fiber concentration was not identified on any of the linkage groups.

Genomic synteny

Twenty-six STMS markers were common on 6 linkage groups between the current map and the most recent interspecific map published by Winter et al. (2000). Expected genomic locations of two genes, *Foc 4* and *Foc 5*, could be assigned on linkage group 2 of the current linkage map by reciprocal comparison to the interspecific map published by Winter et al. (2000) as shown in Figure 1. Genomic locations for two QTLs for seed number per plant and seed weight on LG 4 and a gene for double podding, *s*, on LG 6 could also be postulated on the interspecific map of Winter et al. (2000) (Figure 1).

Discussion

The use of STMS markers facilitated the construction of an intraspecific linkage map that includes 5 morphological markers and 2 QTLs and allowed the comparison to the interspecific map of Winter et al. (2000). Previous limitations of the lack of sufficient polymorphism in chickpea were circumvented with the STMS markers. In comparison to allelic distortion observed by Winter et al. (2000), allelic distortion in the RILs from the intraspecific cross that we used was minimal. This result was an advantage for using an intraspecific cross for mapping. However, clustering of STMS markers in certain linkage groups was similar to that observed for the interspecific linkage map of Winter et al. (1999). This may be due to the uneven distribution of microsatellite sequences within the chickpea genome as was reported in other plant species (Schmit & Heslop-Harrison, 1996; Areshchenkova & Ganal, 1999) and the limited number of molecular markers on our map. In spite of the codominant nature of microsatellites and their frequency of polymorphism, the mostly clustered distribution of STMS markers remained. Considering the fact that STMS markers used in this study were generated from a genomic library representing only 18% of the chickpea genome (Winter et al., 1999), the application of additional STMS markers and the generation of additional markers to cover remaining regions of the genome is needed. The application of other types of markers may span other genomic regions and provide information in associations with markers on the current chickpea genome maps.

Morphological traits that segregated in the cross of ICCV-2 × JG-62 were mapped as single genes. Pigmentation of flowers, stems and seeds cosegregated in the progenies as was expected based on the report by Muehlbauer & Singh (1987). We mapped the major pigmentation gene, *c*, to linkage group 8. The gene for double podding was mapped to linkage group 6 and was the only morphological marker closely linked to a molecular marker. Even though the RILs were phenotyped for days to first flower and days to 50% flower as was done by Kumar & van Rheenen (2000), no linkage to other molecular markers was found. This may have been due to the quantitative nature of the phenotypic data even though the data could be categorized into either of the two parental types (Figure 1). However, analysis of quantitative data for the two traits revealed a QTL for days to 50% flower on linkage group 3. An additional weak QTL for days to first flower could also be found on the same location with low LOD score of 2.34. Weak QTLs for flowering were located on five other linkage groups with low LOD scores (1.14–1.96). Based on these findings, it is apparent that several unknown factors confer time to flowering in chickpea even though segregation for a major flowering gene was observed in this study (Figure 1C). QTLs for 100 seed weight and seed number plant^{-1} were found on linkage group 4 (Figure 3) with LOD scores of 6.93 and 5.66, respectively. These two traits were negatively correlated (r = –0.476). Close association in the genome seems to indicate pleiotropic action of a single QTL. It is also possible that two or more tightly linked genes control the two traits simultaneously. In case of crude fiber concentration of seed, even a weak linkage of crude fiber concentration with any of the genes for seed traits was expected because of its tight association with seed type in general. However, no linkage was identified on the current linkage map. When seed shape data, ignoring the intermediate shape, were compared to crude fiber concentration, a high correlation between seed shape and crude fiber concentration was observed. Identification of genomic locations associated with seed shape is required in order to assign QTLs for crude fiber concentration through further genomic study. Mapping additional molecular markers closely linked to these traits will also enable identification of QTLs for crude fiber concentration in the chickpea genome.

Synteny relationships among STMS markers appeared to be conserved on six linkage groups when our map was compared to the map of Winter et al. (2000). The overall distribution of STMS markers on our map

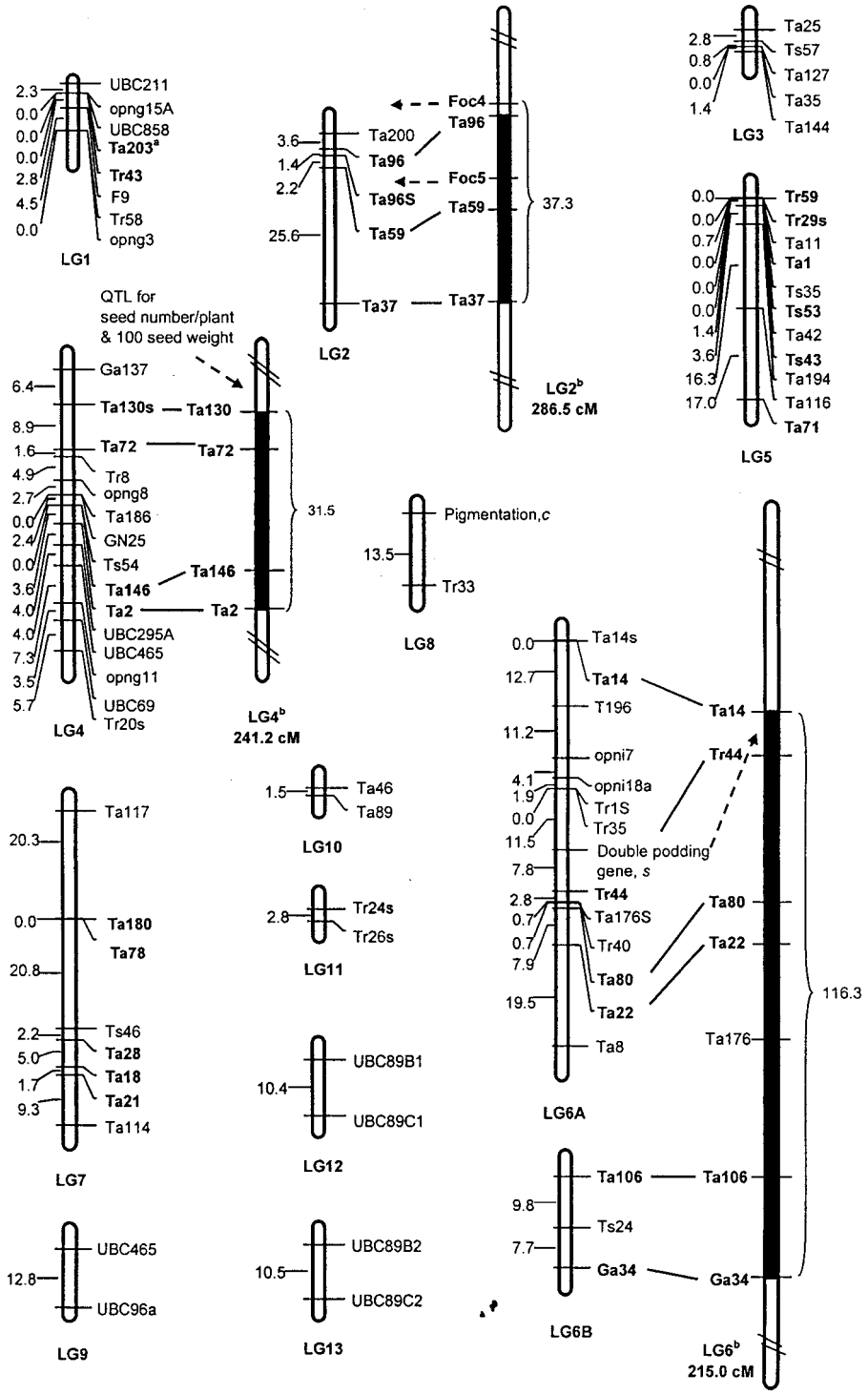

Figure 2. Linkage map of the *Cicer* genome based on morphological traits, ISSR, RAPD and STMS markers with LOD score of 3 and a maximum distance between markers of 20cM. [a] The markers in bold letters are those in common with the map published by Winter et al. (2000). [b] Partial consensus mapping of important trait loci in the chickpea genome by reciprocal comparison of syntenous linkage groups between the current intraspecific map and interspecific map by Winter et al. (2000).

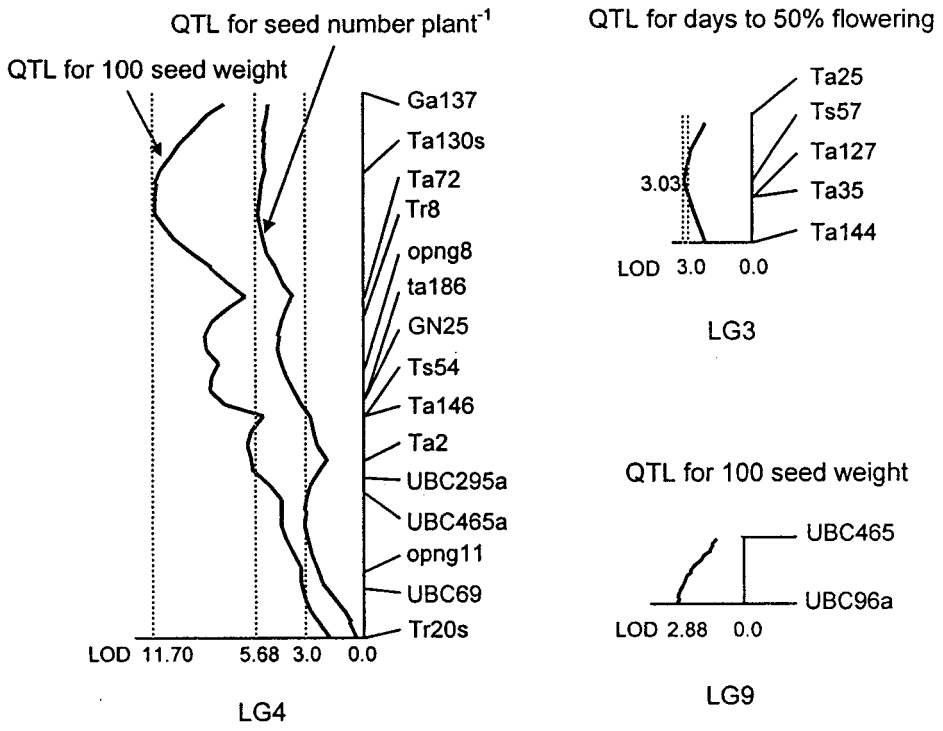

Figure 3. QTLs for 100 seed weight and seed number plant^{-1} on linkage group 4.

was similar to that of Winter et al. (2000) even though the order of STMS markers did not exactly match for some loci. A partial consensus map constructed by reciprocal comparison between the two linkage maps enabled the approximate identification five loci of the important traits on the corresponding genomic regions of the two linkage maps (Figure 2). Construction of a consensus map based on synteny between the several linkage maps will make it possible to expand the chickpea genetic map and increase marker density.

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