RFLP Mapping of Resistance to Southern Root-Knot Nematode in Soybean

J. P. Tamulonis,* B. M. Luzzi, R. S. Hussey, W. A. Parrott, and H. R. Boerma

ABSTRACT

In the USA, the southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] (Mi) is a serious pathogen of soybean [*Glycine max* (L.) Merrill]. The objectives of this study were the following: (i) to use restriction fragment length polymorphism (RFLP) to identify markers associated with quantitative trait loci (QTL) conferring Mi resistance, (ii) to estimate the relative contribution to resistance of each QTL, and (iii) to locate each QTL on the molecular map of the soybean genome. PI96354, a plant introduction with a high level of resistance to Mi, was crossed with 'Bossier', a highly susceptible cultivar, and 110 F_2 plants were produced and mapped with 121 RFLP markers. A greenhouse screening procedure was used to determine the level of galling for the $F_{2:3}$ lines. Data were analyzed using single-factor and two-factor analysis of variance and interval mapping. Two QTL were identified which mapped to LG-O and LG-G of the USDA/ARS-Iowa State Univ. (USDA/ARS-ISU) soybean RFLP Map. Results showed a major resistance QTL (R^2 = 31%) linked to marker G248A-1 on LG-O. The second QTL (R^2 = 14%) was located on LG-G in the interval from K493H-1 to Cs008D-1 and was dominant with respect to resistance. The two QTL explained 39% of the variation in Mi galling in a multiple QTL model. Marker G248A-1 may be closely linked to the *Rmi*1 gene. When galling data was classified into three distinct classes, it mapped distally to G248A-1 on LG-O. Mi resistance also was linked to G248A-1 through simulated marker analysis. The root-knot nematode resistance QTL identified were found in duplicated DNA segments.

ROOT-KNOT NEMOTODES, *Meloidogyne* spp., rank among the principal pathogens affecting the world's food supply (Sasser, 1980). Their distribution is worldwide, they have extensive host ranges, and they interact with fungi and bacteria in disease complexes. In the USA, the distribution of *M. incognita* (Mi), *M. arenaria* (Neal) Chitwood (Ma), and *M. javanica* (Treub) Chitwood (Mj) is concentrated in the southern states and in states along the east and west coasts (Walters and Barker, 1994). These three main species of *Meloidogyne*, Mi, Ma, and Mj, parasitize soybean, and are responsible for significant yield loss of soybean in the southern USA (Mulrooney, 1986). Soybean growers annually lose $25 to $30 million of the soybean crop as a result of root-knot nematode damage (Sciumbato, 1993). In a Louisiana field heavily infested with Mi, soybean yield of a susceptible cultivar was suppressed by 85% (Harville et al., 1985).

The development and use of resistant soybean cultivars in combination with crop rotation are currently the most effective method of reducing root-knot nematode damage. Greenhouse screening procedures (Hussey and Boerma, 1981) have provided an effective method to identify new sources of resistance. Considerable effort has been directed toward the development of root-knot nematode-resistant soybean cultivars (Boerma and Hussey, 1992).

New sources of resistance were identified in the Southern Soybean Germplasm Collection (Luzzi et al., 1987). PI96354 was identified with the highest level of resistance to Mi for both gall formation and nematode reproduction. Using PI96354, Moura et al. (1993) showed that most second stage Mi juveniles in the roots emigrated ten days after inoculation. Nematodes parasitizing roots of PI96354 had the slowest developmental rate and produced 99% fewer eggs per egg mass than females on either 'Forrest' or Bossier (Moura et al., 1993). In addition, Herman et al. (1991) showed that differential emigration of second stage juveniles was responsible for the low number of nematodes in roots of highly resistant PI96354 rather than fewer invasion sites. PI96354 possesses genes that confer near complete resistance to Mi, and can provide a higher level of resistance than currently exists in modern soybean cultivars.

Soybean inheritance studies showed that the variance component heritability estimates for resistance to Mi, Ma, and Mj ranged from moderate to high, (h^2 = 0.60–0.90), (Luzzi et al., 1994b, 1995a,b; Boquet et al., 1976). In a cross with Forrest and Bossier, partial resistance to Mi in Forrest was identified as a single additive resistance gene, *Rmi*1 (Luzzi et al., 1994a).

Several types of molecular markers have made it possible to map entire genomes of various species of plants and animals (NIH/CEPH Collaborative Mapping Group, 1992; O'Brien, 1993). Molecular markers have allowed the dissection of quantitatively inherited traits with low heritabilities (Paterson et al., 1988). Many quantitative traits have been studied in many crop species including soybean (Brummer et al., 1996; Lee et al., 1996a,b,c; Mansur et al., 1993; Diers et al., 1992; Keim et al., 1990). Genetic markers have been used for mapping qualitative and quantitatively inherited disease resistance in soybean. For example, DNA markers associated with resistance to *Phytophthora soja* Auf. and Gender. (Diers et al., 1992) soybean mosaic virus (Yu et al., 1994), soybean cyst nematode (*Heterodera glycines* Ichinohe), (Concibido et al., 1994; Webb et al., 1995) and soybean sudden death syndrome [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans., Type A FSA] (Hnetkovsky et al., 1996) have been identified. In addition, *M. javanica* (Tamulonis et al., 1997a) and *M. arenaria* (Tamulonis et al., 1997b) resistance QTL have been mapped in soybean.

Interval mapping has led to the placement of many

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Published in Crop Sci. 37:1903–1909 (1997).

Abbreviations: cM, centimorgan; LG, linkage group; LOD, log likelihood of the odds; Mi, *Meloidogyne incognita*; Mj, *Meloidogyne javanica*; Ma, *Meloidogyne arenaria*; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; RKN, root-knot nematode.

QTL on molecular maps from various species. However, due to low marker number and lack of polymorphism, sometimes only markers that flank a QTL on one side are found. Procedures have been developed to simulate markers based on existing flanking marker data to ascertain whether a search for more markers is warranted (Mark Daly, 1996, personal communication, Whitehead Institute for Biomedical Research, Cambridge, MA). Results from these simulated marker experiments may justify attempts to place additional markers on a targeted region of a map.

Placement of QTL on the genetic map is a prerequisite for many fundamental studies and applied breeding applications. The objectives of this study were the following: (i) to use RFLP markers to identify quantitative trait loci (QTL) conferring Mi resistance, (ii) to determine the relative contribution to resistance of each QTL, and (iii) locate each QTL on the molecular map of soybean.

MATERIALS AND METHODS

An F_2 population of 110 individuals developed from the cross of Mi-resistant PI96354 (Luzzi et al., 1987) with the Mi-susceptible cultivar, Bossier, was grown at the Plant Sciences Farm near Athens, GA, in 1993. Resistance to Mi was mapped by means of gall number per plant from 101 of the 110 $F_{2:3}$ lines; the remaining nine $F_{2:3}$ lines produced insufficient seed. One hundred-one $F_{2:3}$ lines, the parental genotypes, and checks were evaluated for galling in the greenhouse in a randomized complete block design with two replications. Each $F_{2:3}$ line was represented by seven plants per plot and $r = 2$, and for each parental genotype ten plants also with seven plants per plot, were grown. Details for preparing inoculum (Hussey and Barker 1973), the inoculation procedure, and the Mi assay are described elsewhere (Luzzi et al., 1987). Each $F_{2:3}$ plant in the experiment was inoculated with 3000 Mi eggs per plant. Thirty days post inoculation after galls developed on the roots of Bossier, soil was washed from each root and the galls on each root system were counted.

Most of the probes used in this study were provided by Dr. R. Shoemaker, USDA-ARS and Iowa State Univ. (Keim and Shoemaker, 1988b). A storage protein gene in plasmid pGEMGy4H was provided by Niels Nielsen, Purdue Univ., West Lafayette, IN. We designed primers using Oligo software and the pGEMGy4H plasmid was used as a template to amplify the storage protein gene coding region, "pG248". Several common bean (*Phaseolus vulgaris* L.) genomic clones, and peanut (*Arachis hypogea* L.) cDNA clones were also mapped in this study.

Soybean DNA was extracted from individual parental genotypes and individual field-grown F_2 plants according to previously published procedures (Keim et al., 1988a). Soybean DNA was quantified by spectrophotometric analysis and 10 μg were digested to completion overnight. The five enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Taq*I with which the USDA/ARS-ISU soybean map (Shoemaker and Specht, 1995) was initially constructed were employed. Twenty-two additional enzymes also were investigated to search for polymorphism for probes pG248 and pA081 (a *Hae*III digest was found to be polymorphic to map pG248). Digested DNA was electrophoresed (22 V) for 16 h on 0.8% (w/v) agarose in 10- by 20-cm gels and transferred onto GeneScreen membranes (Dupont, Wilmington, DE) by capillarity (Southern, 1975). Multiple sets of parental survey Southern blots were made to identify restriction fragment length polymorphism.

Lithium chloride mini prep plasmids lysed from DH5α bacterial overnight cultures were prepared (Kochert et al., 1991). Cloned DNA inserts were amplified from these mini preps by the polymerase chain reaction. Hybridization conditions were the same as in a previous study (Lee et al., 1996a). Procedures for nomenclature of polymorphic RFLP markers set forth by (Cregan et al., 1995) were followed. If a probe detected several polymorphic fragments, then individual fragments were designated by the same name but distinguished by a dash and a number (e.g., B219V-1). In order to anchor markers to the USDA/ARS-ISU map, all bands on autoradiograms produced were sized and were systematically compared with and matched with hybridization images downloaded from the soybean database (SoyBase, 1995). All named LGs had at least one anchored marker. All dominant markers were designated with an 'n' (n for null) after the probe name (e.g., A053H-1n).

Multipoint linkage analysis was performed using MAPMAKER-EXP (Lander et al., 1987; Lincoln et al., 1992b) with 110 F_2 individuals from the cross of PI96354 and Bossier (resistance to Mi was mapped using gall number per plant from 101 of the 110 $F_{2:3}$ lines). Linkage data were calculated to assign markers to linkage groups with criteria of recombination frequency ≤ 37 cM (centimorgan, Kosambi mapping function) and log likelihood of the odds (LOD) ≥ 3.0. A broad sense heritability estimate based on variance component was calculated on a family mean basis (selection unit: seven plants per family and two replications) with the following formula: $h^2 = \sigma^2_{F2:3} / [\sigma^2_{F2:3} + (\sigma^2_e / r)]$ (Fehr, 1987), where $\sigma^2_{F2:3}$ is the genotypic variance component for gall number per plant among $F_{2:3}$ families, σ^2_e is the error variance, and r is the number of replications. Because no significant association between family means and variance, a transformation of the raw gall number data was found unnecessary.

Two methods of analysis were employed to identify markers associated with Mi resistance. Data were analyzed by a general linear model using marker genotypic class (e.g., A_1A_1, A_1A_2, A_2A_2) as the predictor variable and gall number as the response variable (SAS, 1988). The coefficient of determination (R^2) served as a measure of the magnitude of the marker association. To search for epistasis, a two-way analysis of variance was performed on all significant markers with all other markers in the data set. Markers and their interaction term were included in the model. The interaction term was dropped if it was not significant at $P = 0.01$.

Interval mapping, which employs the maximum likelihood method, was used as the second type of analysis. Interval mapping of QTL was accomplished with MAPMAKER-QTL (Lincoln et al., 1992a). The minimum LOD value for significance was 2.2. Tests for each mode of inheritance (additive, dominant, or recessive) for QTL were conducted (Paterson et al., 1991). A LOD decrease of 1.0 for any constrained mode of inheritance was considered adequate to exclude specific modes of inheritance for the QTL. If a QTL position was identified, its position was fixed and the map was again scanned to look for additional significant markers. Weights (the effect of allele substitution) were obtained from the map and scan commands and reported with respect to the male parent, Bossier (Lincoln et al., 1992a). Predicted means were obtained from the effect of allele substitution.

RESULTS AND DISCUSSION

Four hundred forty-five probes were tested with the DNA of the two parental genotypes. With at least one

restriction enzyme, a polymorphism was detected in 187 (42%) of the markers. This frequency of polymorphism was similar to levels of polymorphism observed in other soybean studies (Boutin et al., 1995; Chen et al., 1996; Skorupska et al., 1993; Tamulonis et al., 1997a,b).

As determined by the Lande (1992) formula, a minimum of 101 markers is required to detect substantial linkage disequilibria with QTL. One hundred twenty-seven markers were mapped with 110 F_2 plants. The 127 markers converged into 25 linkage groups encompassing a total of 1214 cM with an average of 10 cM between markers. Seventeen markers remained unlinked which potentially contributed an additional 340 cM to the map length (10 cM on each side of each unlinked marker) (Danesh et al., 1994), for a total length of 1554 cM. Fifteen linkage groups contained three or more markers, and 11 linkage groups contained only two markers. For most linkage groups, the order was identical and distances between markers were similar to the USDA-ARS/ISU map distances (Shoemaker and Specht, 1995).

Bossier averaged 94 ± 9.2 galls per plant compared with 3 ± 0.1 galls per plant for PI96354. The means of each $F_{2:3}$ lines ranged from 0.4 to 135 galls per plant (Fig. 1). Among the lines, 23 had less than 10 galls per plant. A transformation of the data was unnecessary as no association was found between family means and variances. The variance component heritability estimate on a family mean basis was 69% suggesting a major genetic component for Mi resistance as measured by gall number (selection unit: seven plants per family and two replications). This estimate of heritability was similar to variance component heritabilities previously reported for Mi (Luzzi et al., 1994b), Ma (Luzzi et al., 1995a; Tamulonis et al., 1997b) and Mj (Luzzi et al., 1995b; Tamulonis et al., 1997b).

Resistance to Mi was mapped by gall number per plant from 101 of the 110 $F_{2:3}$ lines. Two QTL were identified as indicated by LOD score peaks. A major resistance QTL was detected (LOD = 6.9) at marker G248A-1 on LG-O and explained 31% of the variation in gall number (Fig. 2). However, no marker distal to G248A-1 was available to determine whether the true maximum LOD was observed. The LOD score for the additive model was significantly higher than for the dominant model. The main effect for the QTL on LG-O was +24 galls, and the predicted mean was 16 galls per plant when the marker was homozygous for the PI96354 alleles (Table 1). The other QTL (LOD = 2.4) was detected in the interval from K493H-1 to Cs008D-1 on LG-G, and the peak LOD was positioned 5.4 cM from marker K493H-1 (Fig. 2, Table 1). The dominant model was significantly different (LOD ≥ 1.0) than the additive model for the QTL position K493H-1 + 5.4 cM. The main effect for the QTL on LG-G was +12 galls per plant, and the resistance for this QTL also was derived from the PI96354 parent. The predicted mean was 30 galls per plant when the QTL was either homozygous or heterozygous for the PI96354 allele. The predicted mean gall number per plant was 13 when the two QTL were fixed at G248A-1 + 0 cM (LG-O) and K493H-1 + 5.4 cM (LG-G). The overall effect of the two QTL predicted a level of galling that approached the level of galling detected in PI96354, and 39% of the variation in gall number was explained in a two QTL model (Fig. 3).

Results from analysis of variance were similar to results found with MAPMAKER-QTL analyses. Four RFLP markers, two on LG-O and two on LG-G, were associated with Mi gall number (Table 2). Marker G248A-1 on LG-O accounted for the greatest variation in gall number (31%), while K493H-1 and A890V-1 on LG-G each accounted for 11% of the variation in gall number. The lines that had marker alleles derived from PI96354 had fewer galls per plant for all four markers. No cases of significant two-way epistasis were observed

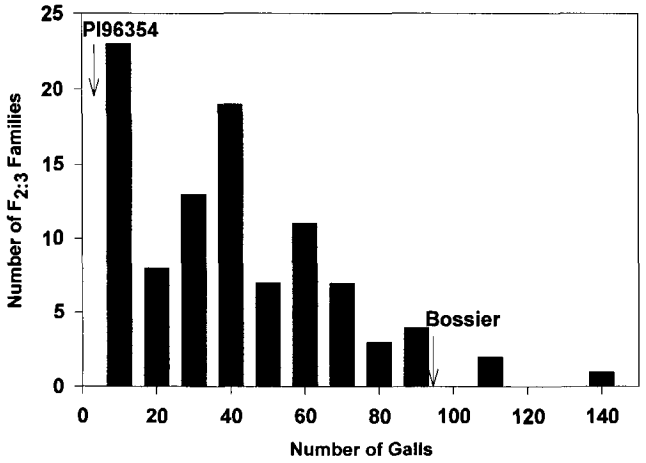

Fig. 1. Frequency distribution of number of $F_{2:3}$ lines from the PI96354 × Bossier population for the mean number of *Meloidogyne incognita* galls per plant. The mean parental values for number per plant are shown with arrows.

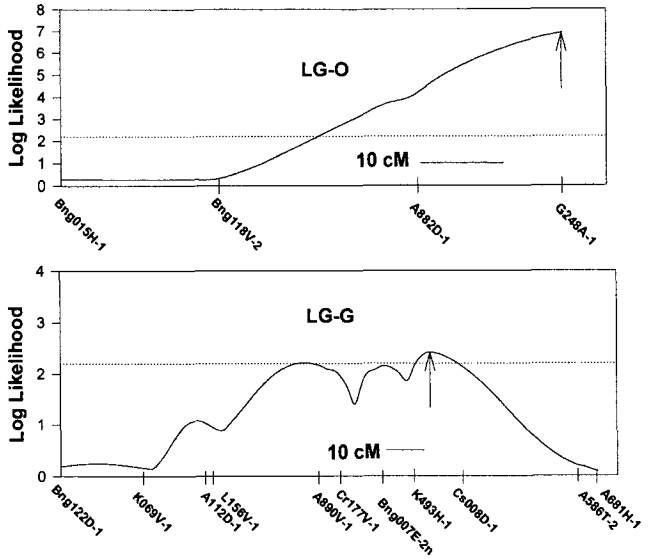

Fig. 2. QTL likelihood plots indicating LOD scores for mean number of *Meloidogyne incognita* galls per plant for $F_{2:3}$ lines on LG-O and LG-G. The QTL peaks are shown by the upward pointing arrows and the bars represent 10 cM. Note that the scale for the upper and lower graphs are different. The horizontal dotted line at LOD of 2.2 represents the minimum LOD required for significance as determined by MAPMAKER-QTL (Lincoln et al., 1992a).

Table 1. QTL associated with *Meloidogyne incognita* gall number based on MAPMAKER-QTL analysis (Lincoln et al., 1992a).

Linkage group	Interval	Length	QTL position†	R^2	LOD	Effect	Predicted means¶ PI/PI‡	PI/B	B/B
				%			galls per plant		
O	A882D-1 to G248A-1	13.3	13.3	31.0	6.9	24	16	40	64
G	K493H-1 to Cs008D-1	8.2	5.4	14.4	2.4	12	30	30	42

† Indicates the most likely QTL position represented by the distance in cM from the left marker of the interval, and corresponds to the LOD score peak.
‡ PI = PI96354.
¶ Effects of each marker were calculated to obtain predicted marker means based on additive genetics for the interval from A882D-1 to G248A-1, and dominant genetics for the interval from K493H-1 to Cs008D-1. The gene action for the interval from K493H-1 to Cs008D-1 was dominant with respect to resistance derived from PI96354.

for a given significant marker and any other marker ($P = 0.01$) (data not shown).

The gall number heritability estimate can be used as an upper limit for the amount of phenotypic variation (R^2) that can be explained in a QTL model (Knapp et al., 1990). The variation in gall number explained by the two QTL was 39% and the heritability estimate was 69%. Therefore, only 57% (39/69) of the genetic variation was explained by this two QTL model.

The mean gall number for each $F_{2:3}$ line was classified into three genetic classes with bounds defined by the parental ranges. Specifically, lines with mean gall number from 0 to 10 were classified as homozygous PI96354, and lines with mean gall number greater than 49 were classified as homozygous Bossier. All other means were classified as heterozygous. The three classes were designated 'A' (homozygous PI96354), 'B' (homozygous Bossier), and 'H' (heterozygous) then mapped in MAPMAKER-EXP (Lander et al., 1987; Lincoln et al., 1992b). This classification also was analyzed as a trait with MAPMAKER-QTL (Lincoln et al., 1992a). Using this classification scheme, it mapped 27 cM distal to G248A-1 on LG-O. When analyzed as a QTL (LOD peak = 30.7), the classification explained most of the variation (R^2 = 80.4%) in gall number and the gene action was additive (d/a = 0.22). This method of analysis also was used to map blight resistance QTL in pea (*Pisum sativum* L.) (Dirlewanger et al., 1994), and K^+/Na^+ discrimination in wheat [*Triticum aestivum* (L.) em. Thell.] (Dubcovsky et al., 1996).

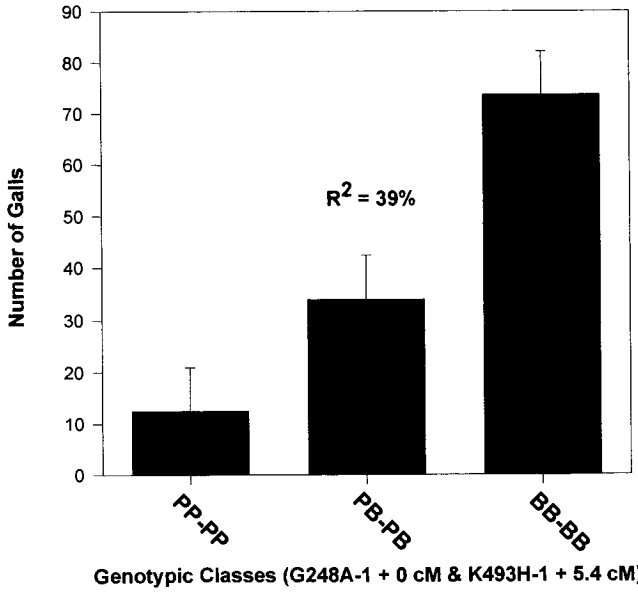

Fig. 3. Predicted mean gall number per plant across three selected genotypic classes for the combined effects of two fixed QTL as determined by MAPMAKER-QTL (Lincoln et al., 1992a). PP-PP and BB-BB are homozygous for QTL at G248A-1 (LG-O) and K493H-1 + 5.4 cM (LG-G) for PI96354 (P) and Bossier (B), respectively. Standard error bars are shown above each bar.

Our initial strategy was to find markers distal to A882D-1 by surveying 27 enzymes to detect polymorphisms in pG248 and pA081. Using this strategy, we were able to map pG248 distal to A882D-1 by hybridization of pG248 with soybean DNA cut with *Hae*III enzyme, G248A-1 (see Fig. 2 and 4). However, no polymorphism was found for pA081 and the QTL mapped on LG-O remained without a flanking marker. Simulated markers were formulated to position the QTL on LG-O. Several markers were devised to simulate interval mapping of a QTL found on LG-O. Similar analysis has been employed in human mapping studies to position disease genes (Mark Daly, 1996, personal communication, Whitehead Institute for Biomedical Research, Cambridge, MA). Through trial and error, markers were created by determining in which lines recombination events would be required to occur to produce a marker that would link '*x*' cM distal to an existing RFLP marker (G248A-1 in this study). Simulated markers were mapped with MAPMAKER-EXP and their effects were determined with MAPMAKER-QTL.

The formulated markers mapped 5, 10, and 15 cM distal to G248A-1, and explained 47, 53, and 60% of the variation, respectively, (maximum LOD = 16.2). Based on the simulated markers, the most likely position of the QTL was 10 to 15 cM distal to G248A-1. It is likely that most of the remaining variation in gall number that was not explained by the two QTL model (Fig. 3) would

Table 2. RFLP markers significantly ($P \leq 0.008$) associated with *Meloidogyne incognita* gall number based on analysis of variance.

RFLP marker	P	Linkage group	R^2	Marker allelic means PI/PI†	PI/B‡	B/B
			%	galls plant^{-1}		
A882D-1	0.0001	O	20	20	41	52
G248A-1	0.0001	O	31	17	37	64
A890V-1	0.0070	G	11	29	31	51
K493H-1	0.0080	G	11	36	28	51

† PI = PI96354.
‡ B = Bossier.

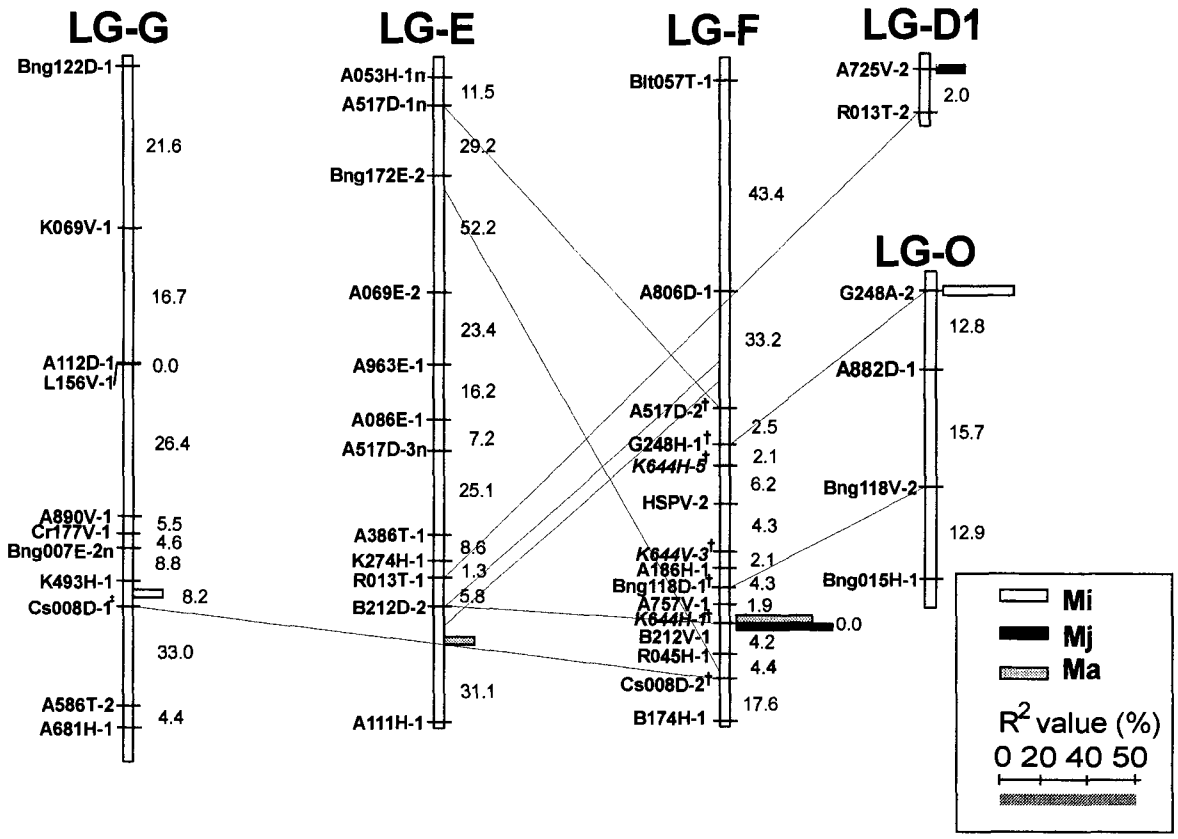

Fig. 4. Linkage groups containing resistance QTL for *M. incognita*, white bars, *M. arenaria*, black bars, and *M. javanica*, grey bars, and for which homoelogy was exhibited. Distances (cM) between RFLP markers are shown to the right of each linkage group. The magnitude of the resistance QTL, R^2, is indicated by the length of the bar, and was calculated by interval analysis. Locations of root-knot nematode resistance QTL (marker positions and distances) were derived from three independent studies (this study and Tamulonis et al., 1997a,b). Markers in italics indicate duplication in *cis*. For simplicity, not all markers or duplicated segments are shown. Map locations for these markers were derived from Shoemaker et al. (1996). ‡ Only one duplicated marker was found on LG-G.

have been explained if a marker had been mapped distally to G248A-1.

The inheritance of resistance to Mi was determined from a cross of Bossier (susceptible) and Forrest (partially resistant) (Luzzi et al., 1994a). Analysis of the data suggested that Forrest possessed a major Mi resistance gene (*Rmi*1). With respect to resistance to Mi the following comments can be made: (i) the major QTL on LG-O (G248A-1) explained 31% of the variation in gall number and acted additively, (ii) gall number data when classified as a single codominant gene was 27 cM from the QTL, and (iii) the level of resistance observed in Forrest, 26 galls, (Luzzi et al., 1994b) is similar to the level of resistance observed in lines that were homozygous for the major QTL (G248A-1) derived from PI96354 and homozygous for the minor QTL (K493H-1 + 5.4 cM) derived from Bossier, 27 galls. (The last observation assumes genetic background effects and differences due to experiments conducted at different times were minimal, and all significant Mi resistance QTL were identified.) In addition, Forrest is partially resistant to Mi in relation to PI96354 (Luzzi et al., 1994a), and segregation for gall number per plant was observed in a cross between Forrest and PI96354 (Luzzi et al., 1994b). Moura et al. (1993) observed total Mi egg production on Forrest was intermediate between egg production on Bossier and PI96354. From these observations we can speculate that the major resistance QTL located on LG-O is the *Rmi*1 gene that was found in Forrest (Luzzi et al., 1994a) and that Forrest may lack the minor QTL mapped on LG-G in this study. Alternatively, additional QTL may have gone undetected in this study.

Data from Shoemaker et al. (1996), comparative mapping of soybean, common bean, and mungbean (*Vigna radiata* L.) (Boutin et al., 1995), mapping Ma and Mj resistance QTL (Tamulonis et al., 1997a,b), and data from this study was used to determine if root-knot nematode resistance QTL were found within duplicated segments. Concordance of QTL positions within duplicated segments was determined with the same criteria as Shoemaker et al. (1996). Shoemaker et al. (1996) considered QTL homoelogous if they were found within clearly delineated homoelogous segments as defined by three or more common markers.

Major QTL for resistance to *M. javanica* (Tamulonis et al., 1997a) and *M. arenaria* (Tamulonis et al., 1997b) resistance mapped to the same location at marker B212V-1 (LG-F). Minor QTL were mapped to LG-D1, *M. javanica*, and LG-E, *M. arenaria*. In this study, Mi resistance QTL were found on LGs O and G (Fig. 4). With respect to the Mi resistance QTL on LG-O, com-

parative mapping (Boutin et al., 1995) showed a region on LG-A of the common bean map (Bng015 is completely linked to Bng118) conserved on LG-F and is also duplicated on LG-O of the RFLP map (Shoemaker and Specht, 1995). In addition, A081 (located on the distal end of LG-O on the soybean map) is linked 25 cM from Bng118 on LG-4 of the mungbean map (Boutin et al., 1995). From these observations we conclude that root-knot nematode resistance QTL are located on duplicated segments found on LGs F E, D1, O, and G (Fig. 4). QTL for soybean seed protein and oil showed correspondence across homeologous chromosomal regions, and the data suggested that the genes or gene families retained similar functions throughout the evolution of the chromosomes (Shoemaker et al., 1996). Mi, Ma, and Mj data suggest that QTL conferring resistance to three root-knot nematode species may have been duplicated during evolution of the soybean as was found for protein and oil QTL. During this process, the duplicated QTL have retained similar functions, and presumably were tempered by coevolutionary processes of host-pathogen interactions.

Orthologous seed-weight QTL in soybean and cowpea [*Vigna unguiculata* (L.) Walp.] (Maughan et al., 1996) and in mungbean and cowpea (Fatokun et al., 1992) have been reported. Orthologous root-knot nematode resistance QTL may be found in common bean and mungbean. A dense cluster of eight resistance QTL has been found on 10 cM region on LG-F (cited in Tamulonis et al., 1997a) which includes the Ma and Mj resistance QTL. Using degenerate oligonucleotide primers for the nucleotide-binding site region from cloned resistance genes, Yu et al. (1996) reported that some of the 11 classes of PCR products mapped closely to Ma and Mj resistance QTL as well as other known disease resistance genes. A similar cluster of resistance genes (resistances to viral, fungal, and bacterial pathogens) has been found in common bean (Miklas et al., 1997). The soybean and common bean resistance gene clusters may be orthologous.

Using both analysis of variance and maximum likelihood methods of analysis, we report the identification and mapping of two QTL derived from PI96354 conferring resistance to Mi in soybean. A QTL was found at marker G248A-1 (LG-O) and explained 31% of the variation in gall number. Gall number data, when classified into three classes and mapped as a codominant marker, was linked 27 cM from the major QTL identified at marker G248A-1 on LG-O. Additionally, evidence was discovered to suggest that marker G248A-1 is linked to the *Rmi*1 gene found in the cultivar Forrest (Luzzi et al., 1994a). An independent QTL, found within the interval from K493H-1 to Cs008D-1 on LG-G, explained 14% of the variation. The gene action for G248A-1 was additive, whereas the gene action for QTL on LG-G was dominant with respect to resistance. Thirty-nine percent of the variation in gall number was accounted for in a two QTL model. Based on analysis of the data, we conclude that one major gene linked 10 to 15 cM distal from G248A-1, and a minor gene within the interval from K493H-1 to Cs008D-1 on LG-G, together confer resistance to Mi in soybean. The root-knot nematode resistance QTL may have been duplicated during evolution and their overall function conserved, yet moderated by coevolutionary processes of host-pathogen interactions.

ACKNOWLEDGMENTS

We thank Randy Shoemaker, USDA-Iowa State Univ., Ames, IA, Gordon Lark, Univ. of Utah, Niels Nielsen, Purdue University, West Lafayette, IN, Gary Kochert and Ron Nagao, Univ. of Georgia, Athens, GA, for DNA clones. We thank Barbara Stewart for technical assistance.

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