Theor Appl Genet (2001) 103:1167–1173

Z. Li · L. Jakkula · R.S. Hussey · J.P. Tamulonis
H.R. Boerma

SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode

Received: 29 September 2000 / Accepted: 17 April 2001

Abstract Root-knot nematodes (*Meloidogyne* spp.) can cause severe yield loss of soybean [*Glycine max* (L.) Merr.] in the southern production region of the USA. Planting root-knot nematode-resistant cultivars is the most effective method of preventing yield loss. DNA marker-assisted breeding may accelerate the development of root-knot nematode-resistant cultivars. RFLP markers have previously been used to identify quantitative trait loci (QTLs) conferring resistance to southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] (Mi) in a $F_{2:3}$ soybean population created by crossing the resistant PI96354 and the susceptible 'Bossier.' A major QTL on linkage group (LG) O conditioning 31% of the variation in Mi gall number and a minor QTL on LG-G conditioning 14% of the gall variation were reported. With the development of SSR markers for soybean improvement, a higher level of mapping resolution and semi-automated detection has become possible. The objectives of this research were: (1) to increase the marker density in the genomic regions of the QTLs for Mi resistance on LG-O and LG-G with SSR markers; and (2) to confirm the effect of the QTLs in a second population and a different genetic background. With SSR markers, the QTL on LG-O was flanked by Satt492 and Satt358, and on LG-G by Satt012 and Satt505. Utilizing SSR markers flanking the two QTLs, marker-assisted selection was performed in a second $F_{2:3}$ population of PI96354× Bossier. Results confirmed the effectiveness of marker-assisted selection to predict the Mi phenotypes. By screening the BC_2F_2 population of Prichard (3)×G93–9009 we confirmed that selection for the minor QTL on LG-G with flanking SSR markers would enhance the resistance of lines containing the major QTL (which is most-likely *Rmi1*).

Keywords Marker-assisted selection · Quantitative trait loci · Simple sequence repeats · *Meloidogyne incognita* · *Glycine max*

Communicated by M.A. Saghai Maroof

Z. Li · L. Jakkula · H.R. Boerma (✉)
Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602, USA
e-mail: rboerma@arches.uga.edu
Fax: +1-(706) 542-0560

R.S. Hussey
Department of Plant Pathology, University of Georgia, Athens, GA 30602, USA

J.P. Tamulonis
Monsanto Company, 634 E. Lincoln Way, Ames, IA 50010, USA

Introduction

Southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White)] (Mi) is a major soybean [*Glycine max* (L.) Merrill] pest that causes severe yield losses in the southeastern USA. Considerable effort has been directed toward the development of root-knot nematode-resistant soybean cultivars (Boerma and Hussey 1992). Although 28% of the cultivars released in the southeastern USA showed high levels of resistance to Mi, no current soybean cultivar exhibited complete resistance to Mi gall formation (Hussey et al. 1991). Among the resistant sources found through the screening of 2370 soybean accessions in Maturity Groups V,VI, VII and VIII from the USDA Soybean Germplasm Collection (Urbana, Ill.), the highest level of resistance to Mi was found in PI96354 (Luzzi et al. 1987). Studies on the inheritance of Mi resistance in soybean indicated that few genes condition Mi resistance. 'Forrest', a moderately resistant source, contained a single gene, *Rmi1*, conferring resistance to Mi (Luzzi et al. 1994a), which differed from PI96354 in Mi resistance by at least one gene (Luzzi et al. 1994b). Tamulonis et al. (1997) indicated that this major gene might reside on linkage group (LG) O of the public soybean genetic linkage map (Cregan et al. 1999).

DNA marker technology has been developed and integrated into soybean improvement programs (Boerma and Mian 1999). Marker-assisted breeding may accelerate the development of nematode-resistant cultivars. Using RFLP markers, Tamulonis et al. (1997) mapped

two QTLs for Mi resistance in a $F_{2:3}$ population derived from the cross of highly resistant PI96354 and susceptible Bossier. A major QTL on LG-O explained 31% of the total variation in gall number, and a minor QTL on LG-G accounted for 14% of variation. Tamulonis et al. (1997) suggested that the lack of polymorphic RFLP markers on LG-O prevented more precise mapping of the genomic location of this QTL and the study's inability to account for a greater percentage of the variation in Mi gall number. Microsatellites or simple sequence repeat (SSR) markers that have recently been developed in soybean are highly polymorphic (Maughan et al. 1995; Rongwen et al. 1995), abundant, and distributed throughout the genome (Cregan et al. 1999). They are highly amenable for automation and allele sizing, which allows their high-throughput application (Diwan and Cregan 1997; Mitchell et al. 1997). With the development and public release of SSR primers, SSR markers have become available on LG-O and LG-G in the regions of the major and minor QTLs for Mi resistance (Cregan et al. 1999).

Verification and confirmation of the QTLs identified with DNA markers are important aspects for the selection of those QTLs with DNA markers in a practical breeding program (Boerma and Mian 1999). Based on our experience, phenotypic selection for Mi resistance is highly effective in identifying the resistance allele at the major QTL and relatively inefficient in selecting the resistance allele at the minor QTL. Phenotypic selection for the minor QTL requires high levels of Mi inoculum, extensive replication, and optimum environmental conditions (Luzzi et al. 1994b, 1996). Selection of the minor QTL on LG-G with DNA markers would improve the efficiency of trait selection and enhance the resistance of the major QTL (Tamulonis et al. 1997). In this study, we report the mapping of SSR markers in the region of the major (LG-O) and minor (LG-G) QTLs for Mi resistance in the population of PI96354× Bossier. We confirm the effectiveness of those SSR markers to identify Mi-resistant plants and validate the effect of the minor QTL in a different genetic background.

Materials and methods

SSR mapping of the QTL for Mi resistance

Mapping population and Mi screening

One hundred and ten F_2 plants from the cross of PI96354× Bossier were used in this study. This population was utilized in the initial RFLP mapping of the QTL conferring Mi resistance by Tamulonis et al. (1997). The collection of phenotypic data has been previously described by Tamulonis et al. (1997). Briefly, one hundred and one $F_{2:3}$ lines were evaluated for Mi galling in the greenhouse. The lines and both parents were arranged in a randomized complete block design with two replications. An experimental unit of each $F_{2:3}$ line consisted of seven plants, and each plant was inoculated with 3000 Mi eggs. The Mi galls that developed on each plant were counted 30 days after inoculation.

SSR marker data collection

SSR markers on LG-G and LG-O were chosen to achieve a higher marker density in the regions previously identified by Tamulonis et al. (1997). DNA was extracted using the modified CTAB procedure of Keim et al. (1988), quantified using a TKO 100 Fluorameter (Hoffer Scientific Instruments, San Francisco, Calif.) or a UV/VIS Spectrometer (Pekin Elmer), and diluted to 20 ng/µl. PCR reactions were prepared based on the protocol of Diwan and Cregan (1997) with slight modifications. The reactions were performed in a 384-well ABI 877 robotic thermal cycler or a dual 384-well and 96-well GeneAmp PCR System 9700 (PE-ABI, Foster City, Calif.). Fluorescence dye-labeled primers were synthesized by PE-ABI using phosphoramidite chemistry. The 10-µl PCR reaction mix contained 2 µl of 40-ng template DNA, 1.0× PCR buffer, 2.5 mM of $MgCl_2$, 100 µM of each dNTP, 0.2 µM each of forward and reverse primers, and 0.5 unit of *Taq* DNA polymerase. AmpliTaq Gold DNA polymerase (PE-ABI, Foster City, Calif.) was used in the PCR reactions for the ABI 877 robotic thermal cycler. The amplification program consisted of 1 min at 95°C, followed by 32 cycles of 25 s for denaturation at 94°C, 25 s of annealing at 46°C, and 25 s of extension at 68°C. At the end of amplification, the reaction mixtures were held at 4°C. With an ABI 877 robotic thermal cycler, 10 min of the enzyme activation step at 95°C, followed by 32 cycles of amplification was used.

Electrophoresis was run with 12-cm plates on an ABI-Prism 377 DNA Sequencer (PE-ABI, Foster City, Calif.) at 750 V for 2 h. A loading sample for each lane was prepared with 2.5 µl of deionized formamide, 1.5 µl of loading buffer, 0.2 µl of ROX-500, and 2 to 3 µl of the pooled PCR products. The mixture was denatured at 95°C for 2 min, and an approximately 1.0-µl vol was loaded for each lane on a 4.8% acrylamide: bisacrylamide (19:1) gel with KLOEHN micro-syringes (Kloehn Ltd., Las Vegas, Nevada). Marker data were collected with PE ABI 377–96 DNA Sequencer Data Collection software (Version 2.5, PE-ABI, Foster City, Calif.). With ABI PRISM 377 filter set D, FAM was visualized as blue, NED as yellow, HEX as green, and ROX as red. The DNA fragments were analyzed with GeneScan (Version 3.0) and scored with Genotyper (version 2.1).

Mapping and statistical analysis

The SSR marker data were combined with the previously collected RFLP data to estimate genetic linkage using the Kosambi mapping function (Kosambi 1944) of MAPMAKER/EXP (Lander et al. 1987, Lincoln et al. 1992a). The markers were assigned to linkage groups with the criteria of LOD ≥3.0 and a maximum distance ≤ 37.2 centimorgan (cM). The positions of QTLs were estimated with interval mapping of MAPMAKER/QTL (Lincoln et al. 1992b). A minimum LOD score of 3.0 was used for the determination of significance. The percentage of variation explained by each QTL and the additive and dominant effects were estimated at the maximum-likelihood QTL position.

Single-factor analysis of variance was also used to determine the significance between SSR genotypic class-means using the General Linear Model (GLM) (SAS Institute 1989). To detect epistasis, two-factor analysis of variance was performed on all pairs of significant markers. A multiple regression model was used for the identification of independent markers linked to the QTL within a single linkage group (SLG-reg) and among multiple linkage groups (MLG-reg) (SAS Institute 1989).

The QTL Cartographer Windows version (version 1.01) was also used to identify QTLs using the output files from MAPMAKER/EXP (Basten et al. 1994; Wang et al. 1999). Composite interval mapping was done with the Zmapqtl Model 6 and a forward regression method. In the Zmapqtl model 6, five control markers and a 10-cM window size were selected.

QTL confirmation and marker-assisted selection

Marker-assisted selection

A second population of $F_{2:3}$ lines derived from the cross of PI96354× Bossier was created. Ninety six lines were randomly se-

lected, and seeds from each line along with their parents were planted in the greenhouse. Young trifoliolate leaves from ten plants of each line were sampled for DNA extraction. SSR makers were assayed based on the above procedure. Based on the SSR mapping results a SSR marker near the major QTL on LG-O (Satt538) and a SSR marker near the minor QTL on LG-G (Satt012) were selected. Five lines homozygous for the PI96354 allele (PIPI/PIPI) and one line homozygous for the Bossier allele (BB/BB) at both Satt358 (LG-O) and Satt012 (LG-G) markers were selected from 96 $F_{2:3}$ lines. Four entries of PI96354 and three entries of Bossier were also included in the experiment. All entries were planted in three replications of a randomized complete block design in the greenhouse. Individual seeds of each entry were planted in Ray-Leach Cone-Tainers (3.8-cm diameter and 14.0 cm depth). The experimental unit consisted of six plants of each entry (one plant per Cone-Tainer). Twelve days after planting, each plant was inoculated with 3000 Mi eggs according to the procedures of Luzzi et al. (1987). Thirty eight days after inoculation, the numbers of Mi galls were counted on the roots of each plant.

Confirmation

To verify the effect of the minor QTL from PI96354 in a different genetic background, a BC_2F_2 population derived from the cross of 'Prichard'(3)× G93–9009 was constructed. This population consisted of 136 plants. G93–9009 (Luzzi et al. 1996) has PI96354 and Forrest in its pedigree, and Prichard (Boerma et al. 2001) has Forrest in its pedigree (Fig. 1). In each backcross-generation F_2 plants were phenotypically screened in a greenhouse nematode assay and F_2 plants with the PI96354 level of resistance were used for backcrossing with Prichard. The 136 plants from the BC_2F_2 population, along with parents, grandparents and the susceptible check Bossier, were evaluated for Mi resistance in a greenhouse nematode assay. Each BC_2F_2 seed was planted in a one Ray-Leach Cone-Tainer (see above). Ten days after planting, each plant was inoculated with 12,000 Mi eggs. Forty days after inoculation the number of Mi galls were counted on the roots of each plant. Leaf samples were taken from a unifoliolate leaf of each individual BC_2F_2 plant for DNA exaction. Data on three polymorphic SSR makers flanking the minor QTL on LG-G were collected based on the above procedures. Marker data from BC_2F_2 plants were collected until 25 plants homozygous for Prichard alleles at all three loci and 25 plants homozygous for G93–9009 alleles at all three loci were identified.

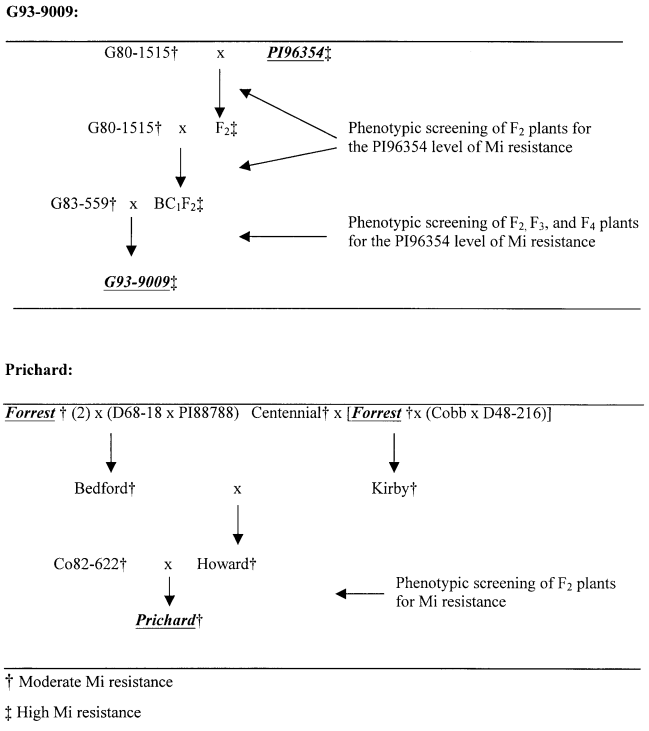

Fig. 1 The pedigrees of G93-9009 and Prichard

Results and discussion

QTLs conditioning Mi resistance

Based on the integrated soybean genetic linkage map (Cregan et al. 1999), we selected 25 SSR markers that had the potential to map near the QTL for Mi resistance identified by Tamulonis et al. (1997). Twenty of these twenty five were polymorphic between PI96354 and Bossier. Among those markers, ten SSR markers were expected to map on LG-O and ten on LG-G. These markers mapped onto LG-O or LG-G in the PI96354× Bossier population and their locations were in close agreement with their positions on the public genetic map (Figs. 2 and 3) (Cregan et al. 1999).

When the marker and phenotypic data from the PI96354× Bossier population were analyzed with MAPMAKER/QTL, a major QTL was indicated in the Satt492 to Satt358 interval (Table 1; Fig. 2). Its most-likely location was 3.1 cM from Satt492. Tamulonis et

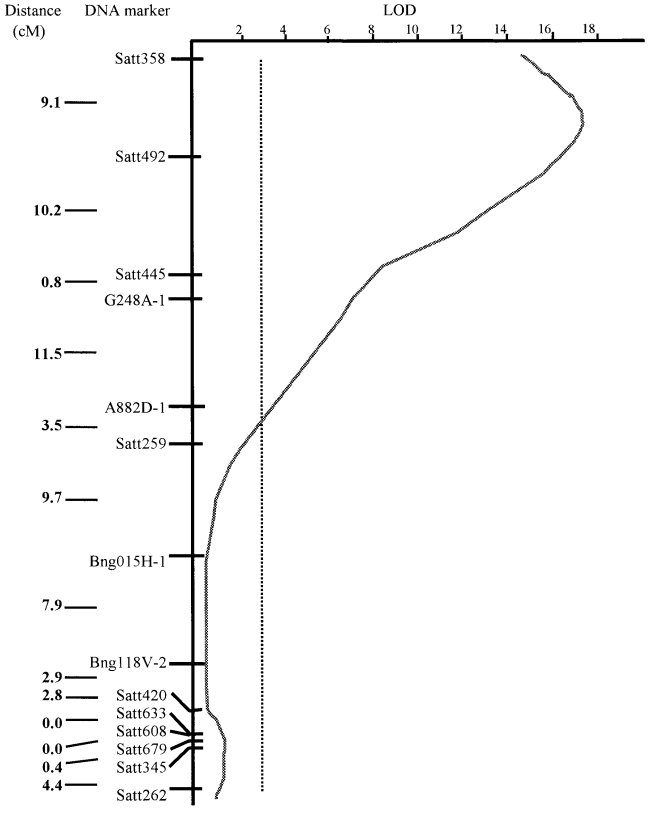

Fig. 2 Linkage map of SSR and RFLP markers in the region of the major QTL and the QTL likelihood plot for the Mi gall number on Linkage Group O

Table 1 QTLs associated with Mi gall numbers on soybean roots in the population of PI96354× Bossier based on MAPMAKER/QTL analysis

Linkage group	Interval	Length (cM)	QTL position[a] (cM)	R^2 (%)	LOD	Effect[b]	Predicted mean		
							PI/PI[c]	PI/B	B/B
						Galls/plant			
O	Satt492–Satt358	9.1	3.1	55.8	16.3	27	9	36	61
G	Satt012–Satt505	8.2	4.0	17.7	3.6	12	27	26	51

[a] The distance from the left marker of the interval
[b] Effect: average change in galls/plant resulting from a QTL allele substitution
[c] PI/PI: homozygous for marker allele from PI96354;
PI/B: heterozygous for marker alleles from PI96354 and Bossier; and
B/B: homozygous for marker allele from Bossier

al. (1997) created simulated markers to predict the most-likely location of this QTL. The simulated markers located the QTL 10–15-cM distal to RFLP marker G248A-1. In the current study the addition of SSR markers on LG-O, map this QTL 14 cM from G248A-1. The LOD score for the presence of this QTL 3.1-cM distal to Satt492 was 16.3 compared to a LOD of 6.9 for the most-likely location in the interval between RFLP markers A882D-1 and G248A-1. The QTL located in the Satt492 to Satt358 interval explained 56% of the variation in gall number (Table 1).

After mapping the ten SSR markers onto LG-G, the MAPMAKER/QTL analysis suggested the location of a minor QTL within the 8.2-cM interval between Satt012 and Satt505 (Table 1; Fig. 3). The most-likely site of this QTL was 4 cM from Satt012. The LOD score for the presence of a QTL conditioning gall number at its most-likely location in this interval was 3.6. This compares with the LOD score of 2.4 for the presence of a minor QTL for gall numbers on LG-G in the previously reported K493H-1 to Cs008D-1 interval (Tamulonis et al. 1997). At its most-likely location, the QTL in the Satt012 to Satt505 interval explained 18% of the phenotypic variation in gall number (Table 1).

Multiple regression analysis can be useful for determining the most-important markers within and among linkage groups and resolving whether multiple peaks generated with interval mapping are caused by single or multiple QTLs on same linkage group. Two peaks were observed from interval mapping on LG-G. The result obtained from a SLG-reg for all significant markers on LG-G showed that only Satt012 was retained in the model (Table 2). This result indicated the presence of a QTL near the marker Satt012 and agreed with the result from MAPMAKER/QTL analysis (Table 1). Two markers, Satt492 and Satt358, on LG-O were retained in a SLG-reg, which suggested that both markers are essential for the marker-assisted selection of major QTL on LG-O (Table 2). However, relative to Satt492, Satt358 only accounted for a small portion (3%) of the variation in gall number (Table 2). The coefficient of determination (R^2) obtained from multiple regression models among linkage groups represents the contributions of the significant markers to the phenotypic variation. By including all significant markers from LG-O and LG-G in

Table 2 Results from multiple regression analysis of the DNA markers on LG-O and LG-G

Markers	Linkage group	SLG-reg[a]		MLG-reg[b]	
		P value	Partial R^2 (%)	*P* value	Partial R^2 (%)
Satt492	O	<0.001	50	<0.001	52
Satt358	O	0.019	3	0.029	2
Satt012	G	0.002	10	<0.001	7
				MLG-Reg R^2	61

[a] Multiple regression analysis within a single linkage group
[b] Multiple regression analysis across linkage groups

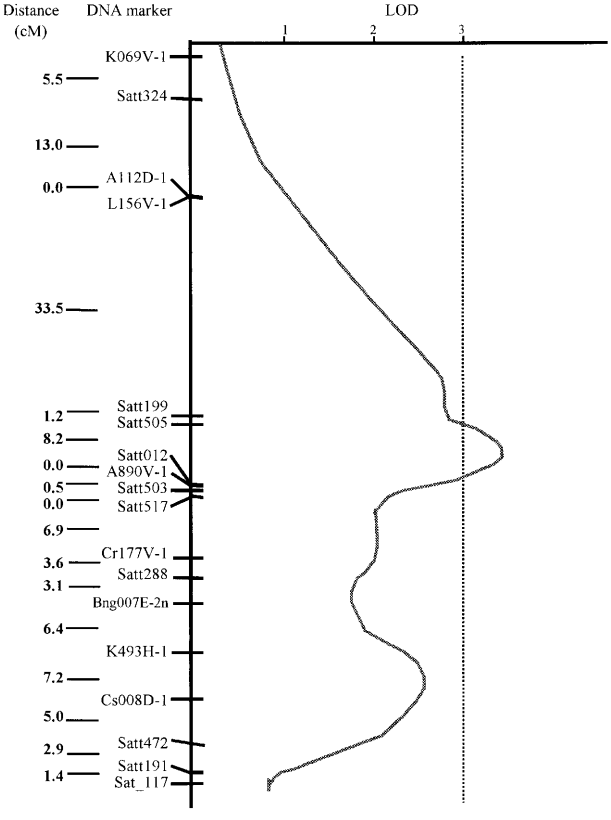

Fig. 3 Linkage map of SSR and RFLP markers in the region of the minor QTL and the QTL likelihood plot for the Mi gall number on Linkage Group G

Table 3 Mean number of Mi galls on soybean roots for nine marker genotypes at Satt492 (LG-O) and Satt012 (LG-G)

Satt492	Satt012			Mean
	PI/PI[a]	PI/B	B/B	
	No. of galls			
PI/PI	6.5 (10)[b]	8.7 (21)	21.5 (6)	10.2
PI/B	39.0 (10)	35.6 (25)	48.8 (12)	39.7
B/B	42.2 (5)	68.4 (5)	71.2 (8)	68.5
Mean	26.6	27.7	49.4	

[a] PI/PI: homozygous allele from PI96354;
PI/B: homozygous allele from Bossier; and
B/B: heterozygous alleles from PI96354 and Bossier
[b] Number of plants in each genotypic class is shown in parenthesis

MLG-reg, three markers were retained in the model, and accounted for 61% of total variation (Table 2). This is very close to the heritability estimate of 69% for gall number in this population (Tamulonis et al., 1997).

Composite interval mapping combines the interval mapping with multiple regression (Zeng 1994). The composite interval mapping with QTL Cartographer also identified two QTLs, one on LG-O, and other on LG-G in this population (data not shown). The peak positions of the two QTLs identified by composite interval mapping were similar to those identified by MAPMAKER/QTL and indicated by multiple regression with SAS programs. The consistency of results across procedures supported the presence of a single QTL on LG-O and one on LG-G.

The significant shift in map location of the QTL on LG-G (approximately 25–30 cM) from the Tamulonis et al. (1997) study is not totally unexpected upon close examination of the original results. In the work of Tamulonis et al. (1997), two peaks were observed on LG-G (one peak in the K493H-1 to Cs008D-1 interval and another in the L156V-1 to A890V-1 interval). Because of lack of DNA markers in the interval of L156V-1 to A890V-1, the actual effect of the potential QTL in this interval was unable to be resolved. After adding more SSR markers on LG-G in this study, the effect of the QTL in the interval of Satt012 to Satt505 (which is embedded within the L156V-1 to A890V-1 interval) became larger than that in the K493H-1 to Cs008D-1 interval (Fig. 3). Thus, it appears the increased marker density on LG-G has provided a better resolution of this QTL's location.

The resistance alleles for both the major QTL on LG-O and the minor QTL on LG-G were inherited from PI96354 (Table 1). Based on the results of this study from MAPMAKER/QTL and the analysis of variance with SSR markers, the major QTL on LG-O showed additive gene action, and the minor QTL on LG-G showed dominant gene action. Two-factor analysis of variance demonstrated that there was no significant (P=0.05) interaction between markers Satt492 and Satt012. These results are consistent with the findings of Tamulonis et al. (1997). When these two markers were evaluated in a multiple regression model, both were significant (P=0.05) and together explained 57% of the phenotypic variation in gall number (data not shown). With the reported heritability estimate of 69% for the gall number (Tamulonis et al. 1997), the two markers together could predict 83% (57/69=83%) of the genotypic variation.

The ten 10 $F_{2:3}$ lines from the PI96354× Bossier mapping population that were homozygous for the PI96354 alleles at both Satt492 and Satt012 averaged 6.5 galls per plant, and individual $F_{2:3}$ lines ranged from 0.7 to 22 galls per plant (Table 3). By comparison, eight lines homozygous for Bossier alleles at both markers averaged 71.2 galls per plant and ranged from 34.5 to 135.3 galls per plant. In general, the mean gall number of nine genotypic classes for these two SSR markers indicated the lack of interaction between the two markers (Table 3). Lines heterozygous at Satt492 and homozygous for PI96354 marker alleles at Satt012 produced more galls than predicted by additive gene action for the major QTL, but the expected number of galls is within the range of the mean ±SD. The results are consistent with those of Tamulonis et al. (1997).

Marker-assisted selection for Mi resistance in PI96354× Bossier

Prior to the utilization of DNA markers in a marker-assisted breeding program, it is a good strategy to confirm the association between DNA markers and QTLs (Boerma and Mian 1999). In order to confirm the association of Satt358 and Satt012 with the QTL for Mi resistance, 96 $F_{2:3}$ lines from an independent population of PI96354× Bossier (independent from the original population) were screened for their genotypes with these two markers. Before development and mapping of Satt492, Satt358 was considered as the closest marker linked to the major QTL on LG-O, and was used in this experiment.

Five families homozygous for the PI96354 alleles and one family homozygous for the Bossier alleles at both Satt358 and Satt012 were selected and evaluated for Mi gall formation in the greenhouse. The five $F_{2:3}$ lines homozygous for the PI96354 alleles (PIPI/PIPI) averaged 1.1±0.3 galls per plant and the line homozygous for the Bossier alleles (BB/BB) averaged 22.3±3.0 galls. The parents, PI96354 and Bossier averaged 0.0±0 and 32.8±2.1 galls/plant, respectively. Among the five PIPI/PIPI lines, one line produced three galls per plant, while the other four averaged only 0.3 galls per plant. Compared to previous studies (Luzzi et al. 1994b; Tamulonis et al. 1997), there was considerably lower gall development on the susceptible Bossier in this experiment. Regardless of the level of gall development in the present study, the selection of five $F_{2:3}$ lines based on the presence of PI96354 alleles at both Satt358 and Satt012 identified four $F_{2:3}$ lines with Mi resistance equal to PI96354 and one line with only 13% of the galls of the

Table 4 Comparison of band sizes of polymorphic SSR markers from the region of the minor QTL on Linkage Group G for G93–9009, its ancestors, and Prichard

SSR marker	PI96354	G93–9009	G83–559	G80–1515	Forrest	Prichard
	bp					
Satt303	256	256	222	222	222	222
Satt501	341	341	347	347	347	347
Satt138	212	212	298	298	298	298
Satt199	200	200	159	159	159	159
Satt505	237	237	234	234	234	234
Satt012	144	144	163	163	163	163
Satt503	260	260	263	263	263	263
Satt517	272	272	279	279	279	279
Satt288	249	249	246	249	249	249
Satt472	225	225	209	204	204	204
Satt191	193	193	210	207	207	207

Mi-susceptible Bossier. These results strongly support the association of these markers with the QTL for Mi resistance.

Results from this study indicated that Satt358 on LG-O and Satt012 on LG-G should permit marker-assisted selection for initial screening of Mi resistance in soybean. Further mapping of both regions with recently developed SSR markers (developed after the initiation of the present study) suggested that the combination of flanking markers Satt492 and Satt358 on LG-O and Satt012 and Satt505 on LG-G should be more efficient in marker-assisted selection for Mi resistance. According to the public soybean linkage map (Cregan et al. 1999), four additional SSR markers, Sat_132, Satt445, Satt500 and Satt487, that were monomorphic in this mapping population are in the region of the major QTL on LG-O. These markers could also be useful in the initial screening of the major QTL on LG-O if they are polymorphic in the targeted populations.

Confirmation of the minor QTL from PI96354 in a different genetic background

In a practical breeding program, it is often difficult to select the minor genes (QTLs) through conventional phenotypic screening. These minor QTLs usually have small effects on the phenotypes and are often affected by the environment. In spite of the difficulties in selection, the pyramiding of minor genes with major resistance genes should enhance the level of resistance. Luzzi et al. (1994) reported that one gene, *Rmi1*, conditioned Mi resistance in Forrest. Based on the segregation data for Mi resistance in a cross of Forrest× PI96534, Tamulonis et al. (1997) suggested that PI96354 possessed the *Rmi1* allele for resistance. It is likely that the *Rmi1* locus is located at the QTL we have mapped 3.1-cM distal to Satt492 on LG-O. In this study, a BC_2F_2 population of Prichard (3)× G93–9009 was constructed in which Prichard was used as a recurrent parent. G93–9009 has both PI96354 and Forrest in its pedigree, while Prichard has Forrest in its pedigree (Fig. 1). We have assumed that the *Rmi1* allele for Mi resistance is present in both Prichard and G93–9009. If this assumption is correct, the minor QTL on LG-G should segregate in the BC_2F_2 population and condition the variation in gall number among the BC_2F_2 plants.

G93–9009 and its parents were screened with 11 polymorphic SSR markers in the region of the minor QTL on LG-G (Table 4). At ten of these marker loci G93–9009 was homozygous for the marker loci from PI96354. These results indicate that the phenotypic selection for the PI96354 level of Mi resistance in the development of G93–9009 retained these PI96354 SSR marker alleles on LG-G. Among these SSR markers, eight markers, Satt303, Satt505, Satt138, Satt199, Satt505, Satt012, Satt503 and Satt517, segregated in the BC_2F_2 population of Prichard (3)× G93–9009. The high ratio of segregating markers in the region of the minor QTL on LG-G in this BC_2F_2 population also suggest that the minor resistance locus is located on LG-G as identified in the mapping population

In the Mi greenhouse screening, G93–9009 (similar to PI96354 in resistance) averaged 0.5±0.36 galls per plant, and Prichard (similar to Forrest in resistance) produced 5.4±0.61 galls per plant. The susceptible check, Bossier, averaged greater than 86 galls per plant. Although a lower gall development on Forrest was observed in this experiment compared with previous studies (Luzzi et al. 1994b; Tamulonis et al. 1997), the difference between PI96354 and Forrest in Mi gall number was significant ($P<0.01$). No differences in gall number were observed between PI96354 and G93–9009 or between Prichard and Forrest.

The BC_2F_2 plants were classified based on the presence of homozygous alleles at Satt505, Satt012 and Satt503 in the region of the minor QTL on LG-G. Twenty five BC_2F_2 plants homozygous for the G93–9009 marker alleles produced 0 galls. By comparison, the 25 plants that were homozygous for the marker alleles from Prichard averaged 3.3±0.9 galls/plant with a range of 0 to 14 galls/plant. These results indicated that marker-based selection for the minor QTL in a population that was already fixed for the major QTL was effective in selecting plants with the PI96354 levels of resistance. Because the greenhouse Mi assay was based on a single BC_2F_2 plant, some escapes of susceptible genotypes (moderately resistant) may have occurred. Regardless,

selection of individuals homozygous for G93–9009 alleles at these three SSR markers resulted in plants without galls. These results should facilitate marker-assisted selection for the root-knot nematode resistance.

Acknowledgements The authors thank Jennie Alvernaz, Dale Wood, Steven Finnerty and Jarrod Griner for their technical assistance, and Bruce Luzzi for collecting the Mi data on the initial mapping population. Funding for this research was provided by the Georgia Agricultural Commodity Commission for Soybeans, the United Soybean Board, and the Georgia Agricultural Experimental Station.

References

Basten CJ, Weir BS, Zeng, ZB (1994) Zmap-a QTL cartographer. In: Smith C, Gavora JS, Benkel B, Chesnais J, Fairfull W, Gibson JP, Kennedy BW, Burnside EB (eds). Proc 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software. Volume 22. Guelph, Ontario, Canada, pp 65–66

Boerma HR, Hussey RS (1992) Breeding plants for resistance to nematodes. J Nematol 24:242–252

Boerma HR, Mian MA (1999) Soybean quantitative trait loci and marker-assisted breeding. World Soybean Res Conf VI Proc, Chicago, Illinois, pp 68–83

Boerma HR, Hussey RS, Phillips DV, Wood ED, Rowan GB, Finnerty SL, Griner JT (2001) Registration of 'Prichard' Soybean. Crop Sci (in press)

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, VanToai TT, Lohnes DG, Chung J, Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Sci 39:1464–1490

Diwan N, Cregan PB (1997) Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor Appl Genet 95:723–733

Hussey RS, Boerma HR, Raymer PL, Luzzi BM (1991) Resistance in maturity groups V–VIII soybean cultivars to soybean cyst and root-knot nematodes. J Nematol 23:576–583

Keim P, Olson TC, Shoemaker RC (1988) A rapid protocol for isolating soybean DNA. Soybean Genet Newslett 15:150–152

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Licoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lincoln SE, Daly MJ, Lander E (1992a) Constructing genetic maps with MAPMAKER/EXP 3.0. 3rd edn, Whitehead Institute Technical Report

Lincoln SE, Daly MJ, Lander E (1992b) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. 3rd edn, Whitehead Institute Technical Report

Luzzi BM, Boerma HR, Hussey RS (1987) Resistance to three species of root-knot nematode in soybean. Crop Sci 27:258–262

Luzzi BM, Boerma HR, Hussey RS (1994a) A gene for resistance to the southern root-knot nematode in soybean. J Hered 85: 484–486

Luzzi BM, Boerma HR, Hussey RS (1994b) Inheritance of resistance to the southern root-knot nematode in soybean. Crop Sci 34:1240–1243

Luzzi BM, Boerma HR, Hussey RS, Phillips DV, Tamulonis JP, Finnerty SL, Wood ED (1996) Registration of southern root-knot nematode-resistant soybean germplasm line G93–9009. Crop Sci 36:823

Maughan PJ, Saghai-Maroof MA, Buss GR (1995) Microsatellite and amplified sequence length polymorphisms in cultivated and wild soybean. Genome 38:715–723

Mitchell SE, Kresovich S, Jester CA, Hernandez CJ, Szewc-McFadden AK (1997) Application of multiplex PCR and fluorescence-based, semi-automated allele sizing technology for genotyping plant genetic resources. Crop Sci 37:617–624

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90:43–48

SAS Institute (1989) SAS/STAT User's Guide, Version 6, 4th edn, Vol 1. SAS Institute, Inc., Cary, North Carolina

Tamulonis JP, Luzzi BM, Hussey RS, Parrott WA, Boerma HR (1997) RFLP mapping of resistance to southern root-knot nematode in soybean. Crop Sci 37:1903–1909

Wang SC, Basten CJ, Weir BS, Zeng ZB (1999) QTL cartographer for Windows version 1.01. (ftp://statgen.ncsu.edu/pub/qtlcart; verified Sept. 25, 2000)

Zeng Z (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468