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DNA marker analysis of loci conferring resistance to peanut root-knot nematode in soybean

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Abstract Peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood] (Ma) is a serious pathogen of soybean, *Glycine max* L. Merrill, in the southern USA. Breeding for root-knot nematode resistance is an important objective in many plant breeding programs. The inheritance of soybean resistance to Ma is quantitative and has a moderate-to-high variance-component heritability on a family mean basis. The objectives of the present study were to use restriction fragment length polymorphism (RFLP) markers to identify quantitative trait loci (QTLs) conferring resistance to Ma and to determine the genomic location and the relative contribution to resistance of each QTL. An F_2 population from a cross between PI200538 (Ma resistant) and 'CNS' (Ma susceptible) was mapped with 130 RFLPs. The 130 markers converged on 20 linkage groups spanning a total of 1766 cM. One hundred and five $F_{2:3}$ families were grown in the greenhouse and inoculated with Ma Race 2. Two QTLs conferring resistance to Ma were identified and PI200538 contributed the alleles for resistance at both QTLs. One QTL was mapped at 0-cM recombination with marker B212V-1 on linkage group-F (LG-F) of the USDA/ARS-Iowa State University RFLP map, and accounted for 32% of the variation in gall number. Another QTL was mapped in the interval from B212D-2 to A111H-2 on LG-E, and accounted for 16% of the variation in gall number. Gene action for the QTL located on LG-F was additive to partially dominant, whereas the gene action for the QTL on LG-E was dominant with respect to resistance. The two QTLs, when fixed on the framework map, accounted for 51% of the variation in gall number in a two-QTL model. The two QTLs for Ma resistance were found in duplicated regions of the soybean genome, and the major QTL for Ma resistance on LG-F is positioned within a cluster of eight diverse disease-resistance loci.

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Introduction

Plant parasitic nematodes cause damage resulting in an estimated $8 billion/year crop loss to US growers, and a $78 billion/year crop loss on a global scale (Sasser and Freckman 1987; Barker et al. 1994). Of these, root-knot nematodes (*Meloidogyne* spp.) cause the most serious damage to many crops worldwide (Sasser 1977; Mulrooney 1986). The three main species of *Meloidogyne* that cause damage in soybean are *M. incognita* (Kofoid and White) Chitwood (Mi), *M. arenaria*, and *M. javanica* (Treub) Chitwood (Mj) (Riggs and Schmitt 1987). In the southern USA, soybean growers annually lose an estimated $30 million due to plant damage caused by root-knot nematodes (Sciumbato 1993).

The development and deployment of root-knot nematode-resistant soybean cultivars, in combination with crop rotation, is currently the most effective control measure to reduce root-knot nematode damage.

Considerable effort has been directed toward developing root-knot nematode-resistant soybean cultivars (Mai 1985; Boerma and Hussey 1992; Roberts 1992). PI200538 was identified in the Southern Soybean Germplasm Collection with the highest level of resistance to Ma Race 2 for gall formation and nematode reproduction (Luzzi et al. 1987). PI200538 provides a higher level of resistance to Ma than currently exists in modern soybean cultivars. Genetic studies conducted in soybean show that resistance to Ma (Luzzi et al. 1995 a), Mi (Luzzi et al. 1994b) and Mj (Luzzi et al. 1995 b) is quantitative, with moderate to high heritability. One exception was reported in which the resistance of 'Forrest' to *M. incognita* was controlled by a single gene, *Rmi1* (Luzzi et al. 1994 a). Heritability estimates for Ma range from 0.74 to 0.83 on a mean basis. Soybean genotypes (PI200538 and PI230977) with different Ma resistance genes have been identified (Luzzi et al. 1995 a).

Two host races of Ma have been defined based on their ability (Race 1) or inability (Race 2) to reproduce on the peanut (*Arachis hypogea* L) cultivar Florunner (Sasser 1954). Among six soybean cultivars, population Govan (Race 2) gave the highest root-gall index. Soybean yields were reduced by 90% in susceptible cultivars grown in field micro-plots after the second year (Carpenter and Lewis 1991). In field micro-plots infested with Ma Race 2, PI200538 had less damage than other resistant genotypes (Pedrosa et al. 1994). Pedrosa et al. (1996 b) observed that Ma Race 2 was more pathogenic than Race 1 on susceptible cultivars. This was attributed to differences in the nutrient status of the nematode feeding sites. The number of nuclei per giant cell observed for race 2 was higher than race 1 on resistant, susceptible, and partially resistant cultivars (Pedrosa et al. 1996 a).

The advent of molecular markers (Lander and Botstein 1989; Botstein et al. 1980) has facilitated the genetic dissection of quantitatively inherited traits in plants (Paterson et al. 1988). Many studies using DNA markers have concentrated on QTLs that control important agronomic traits. In soybean, RFLPs were used to locate and determine effects of QTLs associated with seed protein and oil content (Diers et al. 1992 a; Lee et al. 1996 b; Brummer et al. 1997), plant height, lodging and maturity (Lee et al. 1996 a, 1996 c; Mansur et al. 1993), pod dehiscence (Bailey et al. 1996), hard seededness (Keim et al. 1990 b), and various other traits (Keim et al. 1990 a; Mansur et al. 1993).

Genetic markers also have been used for mapping disease genes in soybean. *Phytopthora sojae* Kauf. and Gende. resistance (Diers et al. 1992 b) and soybean mosaic virus resistance QTLs (Yu et al. 1994) have been identified. Using information from mapping soybean mosaic virus resistance (LG-F) (Yu et al. 1994), an effective marker-assisted screening for sources of *Rsv1* resistance was conducted in 67 soybean genotypes (Yu et al. 1996). In addition, DNA markers have been used to dissect quantitatively inherited resistance (Melchinger 1990) including resistance to the soybean cyst nematode, *Heterodera glycines* Ichinohe, (Concibido et al. 1994; Webb et al. 1995) and the soybean sudden death syndrome produced by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans., type A FSA, (Hnetkovsky et al. 1996).

The placement of QTLs on the genetic map is a prerequisite for many fundamental studies and for applied breeding applications. The objectives of the present study were to use RFLP markers to identify QTLs conferring resistance to Ma and to determine the genomic location and magnitude to resistance of each QTL.

Materials and methods

An F_2 population of 142 individuals was developed from the cross of Ma-resistant PI200538 (Luzzi et al. 1987) with the Ma-susceptible cultivar, CNS. To obtain leaf material for DNA extraction, 105 of the 142 plants were grown at the Plant Sciences Farm near Athens, Ga., and 37 lines were grown in the greenhouse. Parental genotypes and only 105 of the 142 $F_{2:3}$ families were evaluated for Ma in a randomized complete block design with two replications in the greenhouse using the screening procedures described by Luzzi et al. (1987) and Hussey and Barker (1973). Each plant in the experiment was inoculated with 3500 Ma eggs/plant^{-1}. Thirty days post-inoculation after galls developed on the roots of the susceptible parent, soil was washed from each root and the galls on each root system were counted.

Most of the RFLP markers used in this study were provided by Randy Shoemaker, USDA/ARS and Iowa State University. Several common bean, *Phaseolus vulgaris* L., genomic clones, and peanut, *A. hypogea* L., cDNA clones were mapped. In addition, a heat-shock-protein gene (*Gm*HSP-L176) (Nagao et al. 1985) provided by Dr. Ron Nagao (University of Georgia, Athens, Ga.) was mapped.

Soybean DNA was extracted from individual parental genotypes and individual F_2 plants according to previously published procedures (Keim et al. 1988). Soybean DNA was quantified by spectrophotometric analysis, and 10 µg were digested to completion overnight. The same five enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Taq*I, with which the USDA/ARS-ISU soybean map (Shoemaker and Specht 1995) was initially constructed, were used for this study. Digested DNA was electrophoresed (22 V) for 16 h using 0.8% agarose 10×20-cm gels and transferred onto GeneScreen membranes (Dupont, Wilmington, Del.) by capillarity (Southern 1975). Multiple sets of parental survey Southern blots were made to identify restriction fragment 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. 1996 a). The procedures for the nomenclature of polymorphic RFLP markers established by Cregan et al. (1995) were followed. If a probe detected multiple polymorphic fragments, then individual fragments were designated by the same name but distinguished by a dash and a number while the enzyme abbreviations used in probe designation are as follows: *Dra*I (D), *Eco*RI (E), *Eco*RV (V), *Hind*III (H) and *Taq*I (T) (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 to, and matched with, hybridization images down-loaded 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). Polymorphic fragments determined from the hybridization of probes are referred to as markers throughout this paper.

To dissociate $F_{2:3}$ family means and variances, a $\log_{10}(\times+1)$ transformation was used on the number of galls plant^{-1}. All values presented for gall number are the antilog of the mean minus one. Broad-sense heritability based on variance-component estimates was calculated on a mean basis with the following formula: $H^2 = \sigma^2_{F2:3}/[\sigma^2_{F2:3} + \sigma^2_e/r]$, where σ^2_{F3} is the genotypic variance for transformed gall number plant^{-1} among $F_{2:3}$ families, σ^2_e is the error variance, and r is the number of replications (r = 2 for this experiment).

Two methods of analysis were employed to identify markers associated with Mi resistance. Data were analyzed by a general linear model using marker genotypic classes (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. In order 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 $P = 0.01$. Significant differences among $F_{2:3}$ lines and significant differences among marker classes were determined with a least-significant-difference test ($P \leq 0.001$).

Multi-point linkage analysis was performed using MAPMAKER-EXP (Lander et al. 1987; Lincoln et al. 1992 a) with 142 F_2 individuals from the cross of PI200538 and CNS. Interval mapping, which employs the maximum-likelihood method, was used as the second type of analysis. Interval mapping of QTLs was accomplished with MAPMAKER-QTL (Lincoln et al. 1992 b). The minimum LOD value for significance was 2.2. Tests for each mode of inheritance (additive, dominant, or recessive) for QTLs were performed (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. 1992 b). Predicted means were obtained from the effect of allele substitution.

Results and discussion

DNA extracts of the two parents were screened against 444 RFLP probes and polymorphisms were detected with 178 (40%) of the probes. This level of polymorphism was similar to levels observed in other soybean studies (Skorupska et al. 1993; Boutin et al. 1995; Chen et al. 1996; Lee et al. 1996 a; Tamulonis et al. 1997 a, b).

One hundred and thirty informative markers were mapped with 142 F_2 plants (all 142 F_2 plants were used to create the map, however only 105 F_2 plants were screened for Ma resistance). The 130 markers converged into 20 linkage groups spanning a total of 1366 cM with an average of 13 cM between markers. Twenty markers remained unlinked which potentially could contribute an additional 400 cM to the map length (10 cM on each side of each unlinked marker) (Danesh et al. 1994) for a total length of 1766 cM. Fifteen linkage groups contained three or more markers, and five 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).

CNS averaged 117 ± 16 galls plant^{-1}, compared with 9 ± 2 galls/plant^{-1} for PI200538. The means of the $F_{2:3}$ families ranged from 6 to 128 galls plant^{-1}. A $\log_{10}(x+1)$ transformation, of gall-number data was found to be necessary to disassociate family means and variances and resulted in a continuous distribution of the $F_{2:3}$ lines (data not shown). The variance-component heritability estimate on a family mean basis (seven plants family^{-1} and two replications) was 55%. This was consistent with a previous estimate (Luzzi et al. 1995 a) and was similar to the variance-component heritability estimated for Mj (Luzzi et al. 1995 b; Tamulonis et al. 1997 a).

Resistance to Ma was mapped using gall number plant^{-1} from the mean of 105 $F_{2:3}$ lines and analyzed using MAPMAKER-QTL (Lincoln et al. 1992 b). A QTL (LOD = 8.6) was detected at marker B212V-1 on LG-F (Fig. 1, and see Table 2). The 10-to-1 confidence interval for the QTL was 11 cM. The dominance-to-additive ratio (d/a) was 0.36 for B212V-1, indicative of additive-to-partial dominant gene action (Stuber et al. 1987). There was no significant difference between

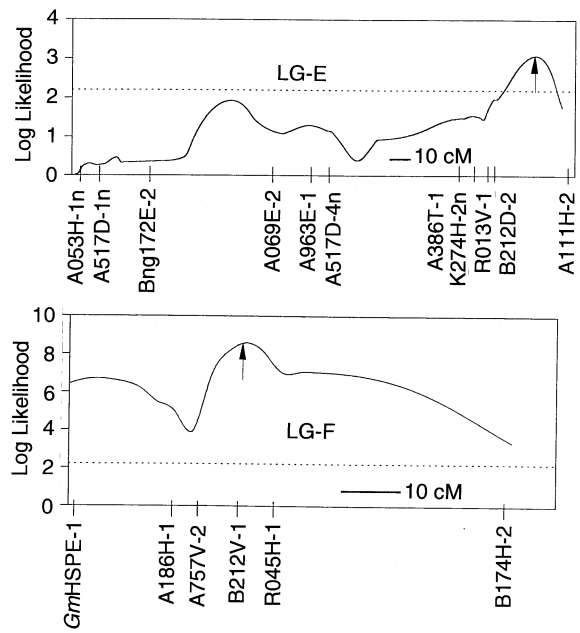

Fig. 1 QTL likelihood plots indicating LOD scores for *M. arenaria* galls/plant^{-1} using a ($\log_{10}$ gall number + 1) transformation for $F_{2:3}$ lines on LG-F and LG-E. The most likely position of the QTLs is shown by the *upward pointing arrow* for the peak LOD scores. The *thick bar* represents 10 cM. The *horizontal dotted line* at LOD of 2.2 represents the minimum LOD required for significance (Lincoln et al. 1992 b). Note that the scale for the upper and lower graphs is not the same

LOD scores for the additive (8.0) and dominant (7.1) models. The main effect for B212V-1 was +18 galls, and the predicted mean was 29 galls plant^{-1} when the QTL was homozygous PI200538 (Table 1). Another QTL (LOD = 2.4) was detected in the interval from B212D-2 to A111H-2 on LG-E, and was positioned 20 cM from B212D-2 (Fig. 1). For B212D-2, the dominant model for resistance had a significantly higher LOD (LOD $\geq$ 2.0) than the additive model. The main effect for B212D-2 +20 cM was +14 galls plant^{-1} and the predicted mean was 45 galls plant^{-1} (Table 2). In a two-QTL model (B212V-1 + 0 cM and B212D-2 + 20 cM) 51% of the variation in gall number was explained. The average gall number was reduced to 25 galls plant^{-1} for lines with alleles derived from PI200538 at both QTLs (Fig. 2). The mean gall number observed for lines with QTLs derived from PI200538 at both QTLs (B212V-1 + 0 cM and B212D-2 + 20 cM) did not differ significantly ($P \leq 0.001$) from the mean gall number observed in the resistant parent PI200538.

Results from ANOVA revealed that five markers ($P \leq 0.001$), all on LG-F, were associated with Ma gall number (Table 1). Among the five markers, B212V-1 accounted for the greatest variation in gall number (32%), while the lines with marker alleles derived from the resistant parent PI200538 had consistently fewer galls plant^{-1}. These five markers span a total distance of 19 cM on LG-F. The R^2 values ranged from 17% (A757V-2) to 32% (B212V-1). No cases of significant epistasis were observed using a two-way ANOVA ($P = 0.001$) (data not shown). The outcome of ANOVA and interval mapping differed. The position of the QTL on LG-E found by MAPMAKER-QTL was midway in the 31-cM interval between markers B212D-2 and A111H-2. The minor QTL on LG-E would not have been detected if only ANOVA had been used to analyze the data.

In addition to the Ma-resistance QTL on LG-F reported in this study, seven other disease-resistance QTLs are known to reside in the same 10-cM region of LG-F (cited in Tamulonis et al. 1997 a). These clustered QTLs confer resistance to a diverse group of pathogens from three kingdoms (animal, monera, and fungi) and two viruses. Also, Mj-resistance QTLs were mapped to LGs-F and -D1 (Tamulonis et al. 1997 a), Mi-resistance QTLs were mapped to LGs-O and -G (Tamulonis et al. 1997 b), and in the present study Ma-resistance QTLs were mapped to LGs-F and -E. Mi, Ma, and Mj data suggest that QTLs conferring resistance to the three root-knot nematode species may have been duplicated in the evolution of soybean. During this process, the duplicated QTLs have retained similar functions, and presumably were tempered by co-evolutionary

Table 1 RFLP markers significantly ($P = 0.001$) associated with *M. arenaria* gall number based on an analysis of variance

RFLP marker	Linkage group	R^2	Marker allelic means (galls plant^{-1})		
			PI/PI[a]	PI/CNS	CNS/CNS
		%			
*Gm*HSP	F[b]	25	29	55	63
A186H-1	F	27	34	57	69
A757V-2	F	17	37	55	64
B212V-1	F	32	29	54	69
R045H-1	F	29	31	52	78

[a] PI = PI200538
[b] Markers are shown in the order in which they were mapped on linkage group F (see Fig. 1)

Table 2 RFLP markers associated with *M. arenaria* gall number based on MAPMAKER-QTL analysis (Lincoln et al. 1992 b)

Linkage group	Interval	Length	QTL position[a]	R^2	LOD	d/a[b]	Predicted means[d] (galls plant^{-1})		
							PI/PI	PI/CNS	CNS/CNS
				%					
F	B212V-1-R045H-1	3.5	0.0	32	8.6	0.36	29	54	69
E	B212D-2-A111H-2	31	20.0	16	2.4	—	45	45	74

[a] The most likely QTL position, corresponding to the LOD score peak, that represents the distance from the left marker of the interval
[b] d/a is the dominance to additive ratio
[c] PI = PI200538
[d] Effects of each marker were used to obtain predicted marker means based on unconstrained genetics (Lincoln et al. 1992 b) for B212V-1 and dominant genetics for B212D-2. The gene action for the interval from B212D-2 to A111-2 was dominant with respect to the resistance derived from PI200538

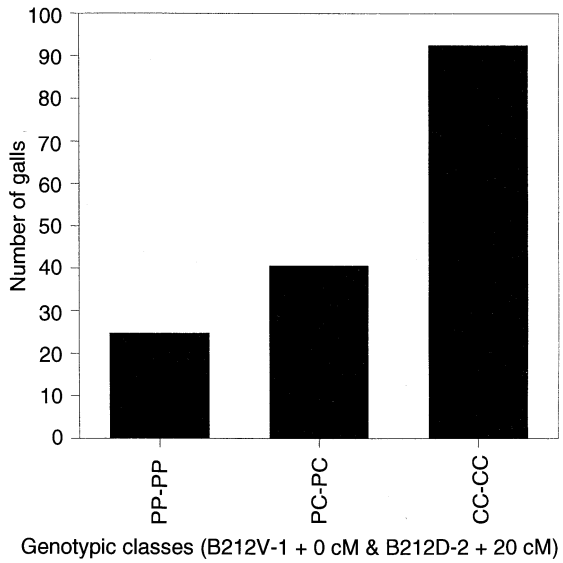

Fig. 2 Predicted mean gall number/plant^{-1} (antilog of the mean minus one) across three selected genotypic classes for the combined effects of the two fixed QTLs. Effects were calculated using MAPMAKER-QTL (Lincoln et al. 1992 b). PP-PP and CC-CC are homozygous for the QTLs that were detected at B212V-1 on LG-F and B212D-2 + 20 cM on LG-E for PI200538 (*P*) and CNS (*C*), respectively

processes of host-pathogen interactions (Tamulonis et al. 1997 b).

The heritability estimate for gall number (55%) is the theoretical limit for the amount of phenotypic variation that can be accounted for by QTLs (Knapp et al. 1990). The variation in gall number explained by the two QTLs was 51% (Fig. 3). Therefore, 93% (51/55) of the genetic variation was explained by the two-QTL model.

We can speculate on the existence of co-evolutionary significance between the results of the RFLP mapping of Ma (found in this study) and Mj (Tamulonis et al. 1997 a) and the phylogenetic analysis of *Meloidogyne* spp. Phylogenetic analyses show a closer relationship between Ma and Mj than between Ma and Mi (Hyman and Powers 1991; Baum et al. 1994). *M. hapla* was found to be the most distant from Ma, Mj, and Mi. Results from the RFLP mapping of Ma and Mj species identified the same major QTL on LG-F and different minor QTLs. Different minor-resistance QTLs for Ma and Mj resistance would support the segregation in gall number observed in the cross of PI200538 × PI230977 when inoculated with Ma (Luzzi et al. 1995 a). PI200538 and PI230977 are resistant to both Mj and Ma (Pedrosa et al. 1994; Luzzi et al. 1987). RFLP mapping of Mj resistance (Tamulonis et al. 1997 a) and Ma resistance in the present study reveals that the major resistance QTL for Ma and Mj could be the same gene. Additionally, the locations of the major and minor QTLs for Mi resistance (Tamulonis et al. 1997 b) were not in common for the QTL locations of Ma and Mj. Therefore, with respect to the co-evolution of pathogenicity of Ma/Mj and soybean, the major QTL controlling resistance to both species may reflect phylogenetic similarities found between Ma and Mj, whereas the different minor-resistance QTLs may reflect the phylogenetic uniqueness between Ma and Mj. Alternatively, the major QTLs on LG-F could be tightly linked and combined in combination with different minor QTLs conferring resistance to Mj and Ma.

Using both maximum-likelihood and analysis of variance methods of analysis, we report the identification and mapping of two QTLs from the resistant PI200538 conferring resistance to Ma. A QTL was identified at marker B212V-1 (LG-F) and explained the greatest amount of variation for gall number (32%). An independent QTL, B212D-2 + 20 cM on LG-E, accounted for 16% of the variation. The gene action for the QTL on LG-F (linked to B212V-1) was additive to partially dominant whereas the gene action for the QTL on LG-E (marker B212D-2 + 20 cM) was dominant with respect to resistance. When fixed on the map, the QTLs accounted for 51% of the variation in gall number in a two-QTL model. Using the variance-component heritability estimate on a mean basis ($H^2 = 55\%$) as a measure of the total genetic variation in gall number for this population, the model explained 93% (51/55) of the variation in gall number. From the amount of variation explained by each QTL, we conclude that one major gene, tightly linked to B212V-1 on LG-F, and a minor gene, within the interval that was bounded by B212D-2 and A111H-2 on LG-E, together control the resistance to Ma in soybean. The major-resistance QTL on LG-F controlling resistance to both Ma and Mj root-knot nematode species may reflect phylogenetic similarities found between Ma and Mj, whereas the different minor-resistance QTLs may reflect the phylogenetic uniqueness between Ma and Mj.

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