Theor Appl Genet (1996) 93: 234–241

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Targeted comparative genome analysis and qualitative mapping of a major partial-resistance gene to the soybean cyst nematode

Received: 10 November 1995 / Accepted: 15 March 1996

Abstract A major partial-resistance locus to the soybean cyst nematode (*Heterodera glycines Ichinohe*; SCN) was identified on linkage group 'G' of soybean [*Glycine max* (L.) Merr.] using restriction fragment length polymorphisms (RFLPs). This locus explained 51.4% (LOD=10.35) of the total phenotypic variation in disease response in soybean Plant Introduction (PI) 209332, 52.7% (LOD=15.58) in PI 90763, 40.0% (LOD=10.50) in PI 88788, and 28.1% (LOD=6.94) in 'Peking'. Initially, the region around this major resistance locus was poorly populated with DNA markers. To increase marker density in this genomic region, first random, and later targeted, comparative mapping with RFLPs from mungbean [*Vigna radiata* (L.) R. Wilcz.] and common bean (*Phaseolus vulgaris* L.) was performed, eventually leading to one RFLP marker every 2.6 centimorgans (cM). Even with this marker density, the inability to resolve SCN disease response into discrete Mendelian categories posed a major limitation to mapping. Thus, qualitative scoring of SCN disease response was carried out in an $F_{5:6}$ recombinant inbred population derived from 'Evans'×PI 209332 using a 30% disease index cut-off for resistance. Using the computer program *JoinMap*, an integrated map of the region of interest was created, placing the SCN resistance locus 4.6 cM from RFLP marker B53 and 2.8 cM from Bng30. This study demonstrates how a combination of molecular-mapping strategies, including comparative genome analysis, join mapping, and qualitative scoring of a quantitative trait, potentially provide the necessary tools for high-resolution mapping around a quantitative-trait locus.

Key words Quantitative trait locus · QTL · Disease resistance · Polygenic

Communicated by G. Wenzel

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Introduction

Most economically important traits in plants, including many forms of disease resistance, are quantitative in nature (Geiger and Huen 1989; Tanksley 1993). These traits are characterized by complex inheritance patterns, environmental interactions, and continuous distribution of phenotypes in segregating populations. For these reasons, breeding for quantitative traits can be complicated and analyzing the underlying genes extremely difficult. DNA markers, such as restriction fragment length polymorphisms (RFLPs), allow for the resolution of multigenic traits into individual Mendelian components (Paterson et al. 1988), paving the way for analyzing, mapping, and eventually cloning, such genes.

Previously, we used RFLPs to map several loci conferring partial-resistance to the soybean cyst nematode, *Heterodera glycines* Ichinohe (SCN), the most serious pest of soybean (Concibido et al. 1994). This work provided preliminary information about the map locations of SCN resistance loci, including one on linkage group 'G' that was especially significant in effect (Concibido 1995; Concibido et al. 1995). However, efforts at developing a high-density genetic map near this resistance locus were hampered by relatively low levels of DNA polymorphism in soybean (Keim et al. 1989), few markers in the region of interest (Shoemaker and Olson 1993), and the inability to characterize SCN disease response as a qualitative trait.

Several powerful DNA marker applications, such as bulked segregant analysis (Michelmore et al. 1991), comparative genome (Tanksley et al. 1988) and integrated mapping (Stam 1993), provide methods for increasing marker density in genomic regions of interest. Comparative genome mapping is a strategy that utilizes mapping information from one taxon to predict linkage relationships in related taxa. Likewise, integrated or "Join" mapping (Stam

1993) integrates linkage maps that were developed in independent populations. Genes and markers that do not segregate in different mapping populations can thus be placed onto a joint map by combining information from multiple mapping populations.

Here, we describe the use of comparative genome analysis and join mapping, in combination with the resolution of a partial-resistance gene as a qualitative character, to develop a medium-density genetic map near the most important partial SCN resistance locus. The results demonstrate how a combination of mapping strategies can provide the tools for eventual high-resolution mapping around quantitative-trait loci.

Materials and methods

Mapping populations

Four segregating F_2 populations and an $F_{5:6}$ recombinant inbred-line (RIL) population were used in the study. The F_2 populations were constructed by crossing the maternal parent 'Evans' (a SCN-susceptible variety) with the following sources of SCN race-3 resistance as male parents: PI 209332 (Anand and Brar 1983; Anand and Gallo 1984), PI 90763 (Anand et al. 1985; Hartwig 1985), PI 88788 (Anand et al. 1985; Hartwig 1985) and 'Peking' (Anand et al. 1985; Hartwig 1985). There were 76 lines in the Evans PI 209332 F_2 mapping population, 115 lines in the Evans × PI 90763 population, 110 lines in the Evans×Peking population, and 106 in the Evans×PI 88788 population.

The $F_{5:6}$ RIL population was constructed by advancing the 76 lines from the Evans × PI 209332 F_2 population plus an additional 22 sibling lines (98 lines total) to the F_5 generation by single-seed descent. Seventy five seeds from each line were row-planted in Rosemount, Minn, and used as a source of bulk leaf tissue for DNA extraction and analysis. Plants were allowed to recover and set F_6 seeds, which were bulked and saved for SCN disease assay.

RFLP probes

Cloned DNA fragments from soybean, mungbean [*Vigna radiata* (L.) R. Wilcz.], cowpea [*V. unguiculata* (L.) Walp.], and common bean (*Phaseolus vulgaris* L.) were used as probes for RFLP analysis. These probes have all been described previously (Keim et al. 1989; Chase et al. 1990; Young et al. 1992; Nodari et al. 1993).

DNA analysis

Plant DNA was extracted by a modified method of Dellaporta et al. (1983) and analyzed by the technique of Young et al. (1992). Individual clones were probed against "Parental survey" blots containing parental DNA digested with up to 15 different restriction enzymes: *Ban*I, *Bam*HI, *Bcl*I, *Bst*NI, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hind*III, *Hinf*I, *Rsa*I, *Sca*I, *Taq*I, and *Xba*I (New England Biolabs, Beverly, Mass.; Promega, Madison Wis.; Gibco/BRL, Gaithersburg, Md.). Digested DNA was electrophoresed on 1% agarose gels and transferred onto Hybond N+ membranes (Amersham Corporation, Arlington Heights, Ill.) using a method adapted from Southern (1975), including an alkaline transfer in 0.5 M NaOH and 0.5 M NaCl. The best clone/enzyme combination was then used in segregation analysis. DNA samples from all the progeny in a population were digested and blotted as described above to produce F_2 and F_5 "progeny" blots.

Cloned DNA inserts were amplified by the polymerase chain reaction (Saiki et al. 1988) for use in radiolabeling reactions and nucleic-acid hybridization as described by Young et al. (1992). About 40–50 ng of the amplified product was radiolabeled by the random hexamer reaction (Feinberg and Vogelstein 1983). The radiolabeled sequence was then incubated with either a parental survey or progeny blot for 16–24 h at 60°C in a hybridization solution of 5× saline sodium citrate, 0.1 M phosphate buffer, pH 7.5, 1× Denhardt's solution (2% each of Ficoll, polyvinylpyrrolidone and bovine serum albumin fraction V), 0.1% SDS, and 5% dextran sulfate. After incubation, blots were washed three times for 15 min each at 60°C at medium stringency (2.0×, 1.0× and 0.5× saline sodium citrate with 0.1% SDS). After washing, blots were placed against Kodak X-AR film and stored at –80°C for 1–7 days to produce autoradiographs. Blots were re-used up to seven times.

Soybean cyst nematode assay

Ten F_3 seeds from all F_2 individuals, as well as ten F_6 seed from all F_5 lines, were germinated in sand-filled Ray Leach cone-tainers (Stuewe and Sons, Inc., Portland, Ore.) placed in sand filled 20-l buckets in a waterbath. Each seedling was then inoculated with 2000 eggs 3 days after germination with SCN race 3. The SCN race-3 isolate used in the assays was maintained in cone-tainers planted with susceptible soybean hosts 'Essex' or Evans. Cysts and white females were then collected every 28 days, stored in sand at 4°C, and later used for inoculations. Race determination tests (Golden et al. 1970) were regularly conducted on the SCN isolate to check for the possibility of race shifts.

At the time of inoculation, cysts and white females were recovered from sand, ground in a tissue grinder to release eggs, and standardized using a hemocytometer. In each assay, the parental lines, the soybean differential cultivars (Peking, Pickett, PI 90763 and PI 88788), and the susceptible check, Essex, were included. Waterbath temperatures were maintained at 28°C with 16-h daylength for 28 days. On the 28th day, individual plants were uprooted and cysts were collected by blasting the roots with pressurized water over sieves. The total number of cysts from individual plants was counted under a dissecting microscope and converted to an index by dividing the count by the average total number of cysts on the susceptible parent. For each F_2 or RIL line, SCN indices of the ten F_3 or F_6 plants were averaged to estimate the mean SCN index for that line.

Linkage analysis and QTL mapping

A comprehensive RFLP map was constructed by linkage analysis in the Evans×PI 209332 population where most mapping information was available. A total of 258 genomic clones were analyzed by hybridization with "parental survey" blots and 133 were subsequently hybridized against F_2 and F_5 progeny blots for segregation analysis. Segregation data for each clone were analyzed by two-way contingency table analysis with Statview II (Abacus Concepts, Berkeley, Calif.) and Mapmaker-II (Lander et al. 1987). Linkage between RFLP markers was inferred if the probability of observing a chi-square value was less than 0.001, or if the "LOD" score exceeded 3.0. To determine the order of markers, a multipoint analysis was used in which the favored order of markers exceeded other possible orders by a LOD of 2.0 or greater. The resulting RFLP map contained 98 RFLP markers distributed throughout 16 of soybean's 22 published linkage groups (Shoemaker and Olson 1993).

At the same time, a search for genomic regions associated with SCN resistance was conducted. Briefly, F_2 and F_5 genotypic classes for each DNA marker were contrasted with the mean SCN disease response by regression analysis and an analysis of variance (ANOVA) using Statview-II and SuperAnova (Abacus Concepts, Berkeley, Calif.). A significance level of $P<0.002$ was used to uncover putative association between a DNA marker and SCN disease response. Data were also analyzed by Mapmaker-QTL (Lander and Botstein 1989). A putative QTL was inferred when the LOD score exceeded 2.7. Interactions among loci were not evaluated in this study since population sizes were too small to conduct meaningful tests.

"Join" mapping

JoinMap (Stam 1993), a computer program that allows the integration of linkage maps from distinct populations, was used to create a consensus map of linkage group 'G' based on mapping information from the RIL and four F_2 populations. Briefly, the JoinMap software pooled segregation data of the five mapping populations, re-calculated the pairwise recombination frequencies and LOD scores, and created a joint linkage map giving the best-fitting arrangement with a LOD of 3.0 (Hauge et al. 1993; Stam 1993). Qualitative scoring of SCN disease response (see below) was included among the loci in the integrated map.

Qualitative analysis of SCN disease response

To resolve SCN disease response into a Mendelian factor, "qualitative" mapping of SCN was conducted in the RIL population. Briefly, lines that had SCN indices greater than 40% were rated "susceptible" and plants with indices less than or equal to 30% were rated "resistant". Lines with indices between 30 and 40% were not included in the analysis since they could not be classified with a high degree of certainty. This range was chosen because the SCN distribution of the 98 RIL lines split into two distinct distributions (Fig. 1) and because a 30% cut-off is commonly used to score SCN resistance by plant pathologists (Schmitt and Shannon 1992). The extra caution in leaving out individual lines falling between 30 and 40% was taken because misclassification of these lines would drastically bias estimations of map distances between flanking markers and the SCN resistance locus. Assigning qualitative disease scores was only possible in the RIL population, as the distribution of SCN indices was not sufficiently discrete in the F_2 mapping populations (data not shown).

Results

Identification of a major partial SCN resistance locus

Previously, we identified several genomic regions associated with partial SCN resistance in PI 209332 using RFLP markers (Boutin et al. 1992; Concibido et al. 1994). In subsequent experiments, the region on linkage group 'G' bounded by RFLPs C6 and Bng122 has been found to be most important (Concibido 1995; Concibido et al 1995). In a derivative of PI 209332 (MN85-1430), RFLPs in this region explained 43.2% of the total phenotypic variation in disease response and, in the present study, up to 51.4% of the total variation could be explained by markers in this genomic region in PI 209332 (Table 1). This same genomic region has also been found to be significant in other sources of SCN resistance. In PI 90763, the putative locus on 'G' accounted for 52.7% in SCN disease response, as well as 40% of the variation in SCN response in PI 88788 and 28% in Peking (Table 1). In separate studies, Webb et al. (1995) identified a putative SCN resistance locus in the same genomic region in the resistant soybean genotype, PI 437654.

To determine whether the genetic effects of the putative locus on 'G' were largely additive, dominant, or recessive, the relative likelihood of models restricting it to specific types of gene action were evaluated by the method of Paterson et al. (1991). Briefly, the locus on 'G' was tested for fitness to a purely additive, dominant , or recessive model.

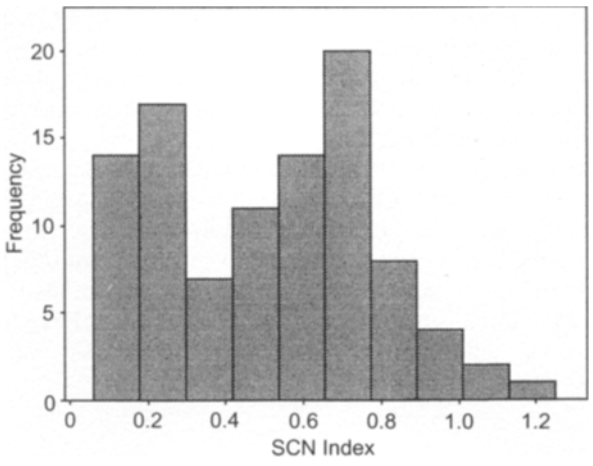

Fig. 1 Frequency distribution of 98 $F_{5:6}$ RIL lines from the Evans×PI 209332 cross for mean SCN disease response to race 3. The *vertical axis* shows the number of individuals for a given disease index. The *horizontal axis* represents the corresponding SCN indices for all 98 lines

Table 1 Percent of variation, LOD scores, and dominance to additivity ratio (d/a) in SCN disease response explained at the peak of the resistance locus on linkage group 'G' in four mapping populations

Population	Approximate location[a]	Percent of variation	LOD Score[b]	d/a[c]
Evans × PI 209332	C6 – Bng122	51.4	10.35	–0.095
Evans × PI 90763	C6 – Bng122	52.7	15.58	–0.073
Evans × PI 88788	C6 – Bng122	40.0	10.50	–0.422
Evans × Peking	C6 – Bng122	28.1	6.94	–0.506

[a] Interval with LOD score peak
[b] Log_{10} of the odds ratio that supports evidence for the presence of a QTL at the locus
[c] The ratio of dominance over additivity: 0 indicates complete additivity, +1 indicates complete dominance, –1 indicates completely recessive

If one model was at least one LOD unit (ten-fold) more likely than the others, that model was considered the predominant type of gene action. With this form of analysis, the resistance locus on 'G' was found to be predominantly additive in gene action in all populations except for the Evans×Peking population, which was compatible with both additive and recessive gene actions (Table 1).

Increased marker density by comparative genome mapping

Initially, the soybean linkage map had a low marker density in the genomic region near the major partial SCN resistance gene (Fig. 2). Within the nearly 70-centimorgan (cM) region now known to span the SCN resistance locus, only two RFLPs were available for mapping when this study began (Shoemaker and Olson 1993). Of the two

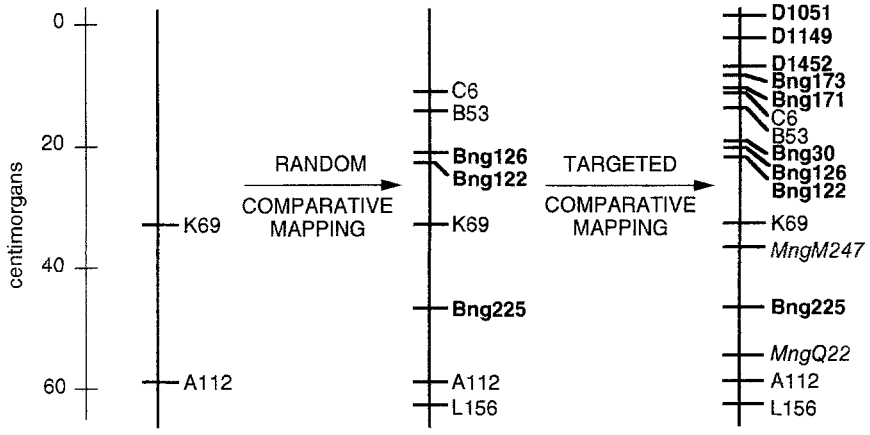

Fig. 2 Increase in marker density near the SCN resistance locus by mapping clones from *P. vulgaris* and *V. radiata* in *G. max*. *Vertical lines* represent the 'G' linkage group, *horizontal tick marks* represent the locations of marker loci. Soybean clones are in *plain text*, *P. vulgaris* markers are in *bold*, and *V. radiata* markers are in *italics*. Distances are given in Kosambi (1944) units

markers, RFLP K69 was most strongly associated with the resistance locus based on Mapmaker-QTL and ANOVA analysis (Concibido et al. 1994; Young et al., unpublished observations).

In parallel experiments, *P. vulgaris* and *V. radiata* clones were randomly tested as RFLP probes in soybean to compare legume genome organization and increase marker density (Boutin et al. 1995). Using this approach, three new *P. vulgaris* clones (Bng122, Bng126 and Bng225) were found to map in the region of interest on linkage group 'G' distal to K69. At the same time, R. Shoemaker and associates independently identified three new soybean RFLP marker loci (L156, B53 and C6) in this same genomic region (R. Shoemaker, Iowa State University, personal communication).

With this information, genomic blocks in *P. vulgaris* and *V. radiata* that were likely to be conserved with the genomic region near the SCN resistance locus could be discerned and used to provide additional RFLP markers as candidates for study. Specifically, comparative mapping indicated that linkage group 'H' of the *P. vulgaris* map, developed by E. Vallejos and associates (Vallejos et al. 1992), as well as linkage group 'D1' of the *P. vulgaris* map, developed by P. Gepts and associates (Nodari et al. 1993; P. Gepts, personal communication), were colinear with the top of linkage group 'G' of soybean. Likewise, comparative mapping indicated that linkage groups 2 and 7 of *V. radiata* (Menancio-Hautea et al. 1993) probably contained probes that would map to soybean linkage group 'G'.

With this approach, a total of eight new RFLP markers from *V. radiata* and *P. vulgaris* were found to map in the region of interest on linkage group 'G' of soybean, including six from *P. vulgaris* (D1051, D1149, D1452, Bng171, Bng173 and Bng30) and two from *V. radiata* (MngM247 and MngQ22) (Fig. 2). In the region most strongly associated with SCN resistance (the interval between D1051 and Bng122) the final marker density increased to one RFLP marker every 2.6 cM, demonstrating how conservation among these three related species can be used to accelerate genome mapping research in any one of them.

Integrated linkage map near the SCN resistance locus

Despite the availability of 16 potential RFLP marker loci in the region of interest on linkage group 'G' (including the 11 uncovered by comparative mapping), there was a notable lack of DNA polymorphism between parents in any one of the five mapping populations. This impeded progress towards the development of a high-resolution map. Out of the 16 possible RFLP marker loci, 13 were polymorphic in the Peking population, 11 in the PI 209332 population, and eight in the PI 90763 and PI 88788 populations. Markers D1149, D1452, Bng173, C6, Bng122, K69 and L156 were polymorphic in all populations tested. RFLPs Bng171, B53, Bng30 and Q22 were polymorphic in the Peking population only, while RFLPs MngM247 and A112 were only polymorphic in the PI 209332 populations

Using the information from the five mapping populations, a consensus map with all 16 polymorphic markers was generated by *JoinMap* analysis. Since markers that were common among populations were mapped using the same restriction enzymes, the final placement and order of markers on the joint map of linkage group 'G' were similar to the maps of each individual mapping population (Fig. 3). Although not encountered in this study, differing enzyme-probe combinations can pose difficulties in determining whether a segregating marker in one mapping population is identical with the corresponding RFLP marker locus in a separate mapping population (Shoemaker and Specht 1995). However, integration of the RIL population into the JoinMap analysis flipped the order of Bng30–Bng126–Bng122 relative to flanking markers on linkage group 'G' (Fig. 2 and 3) when compared to the join map generated by the four F_2 populations alone. Nonetheless, the development of a consensus map of linkage group 'G' now provides a moderately high-density map around the SCN resistance region.

Qualitative scoring of SCN resistance

In an attempt to precisely locate the SCN disease resistance locus on linkage group 'G', a qualitative analysis of SCN

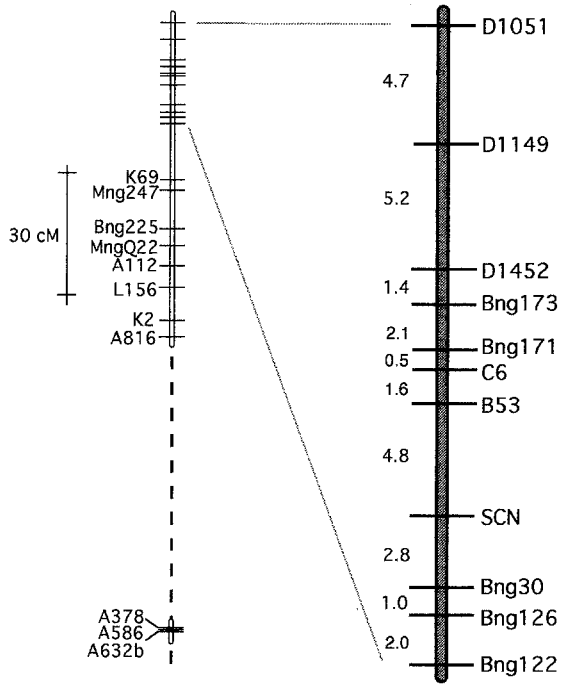

Fig. 3 Map showing the distal region of linkage group 'G' containing a major partial SCN resistance locus. The figure on the left represents the entire linkage group 'G' of the soybean RFLP map (Shoemaker and Olson 1993). The *vertical line* represents the linkage group, the *horizontal tick* marks represent mapped marker loci, and the *vertical dashed line* indicates a genomic region with no markers analyzed in this study. The figure on the right shows details of the region containing the major partial SCN resistance locus. Markers preceeded by *Bng* are *P. vulgaris* clones; markers preceeded by the *letters A, B, K, L* and *P* are *G. max* clones; and markers preceded by *Mng* are *V. radiata* clones. Distances are given in Kosambi (1944) units

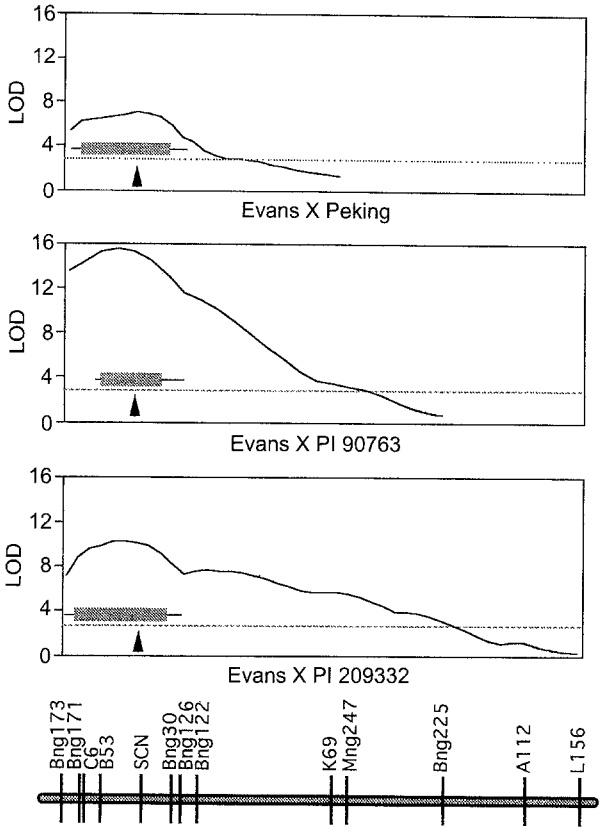

Fig. 4 Mapmaker-QTL scans of the genomic region on linkage group 'G' containing a major partial SCN resistance locus in three mapping populations. *Horizontal axes* correspond to the genetic map for linkage group 'G' (shown at the bottom); *vertical axes* represents LOD scores for the presence of a QTL at the corresponding position on the linkage group. The *dotted line* at LOD=2.7 indicates the required significance level for declaring the presence of a QTL. Near the bottom of each graph, a *horizontal line* indicates the most likely location for a QTL, with the *thicker portion* indicating the genomic segment where the LOD score drops one unit from the peak of the curve and the *thinner portion* indicating the segment where LOD scores drop by two units. The vertical arrows underneath correspond to the map location of the SCN resistance locus based on qualitative analysis. Markers D1051, D1149, and D1452 were not included in the analysis

response was conducted in the RIL population. Figure 1 shows the bimodal distribution of SCN indices for the RIL population, indicative of an approach to homozygosity at both extremes. Moreover at the F_2 stage, a means comparison of SCN resistance indicated that plants homozygous for the resistance (PI 209332) allele in the C6/Bng122 interval had a cyst index of 0.26±0.023 (standard error of the mean) on a scale of 0.00 to 1.00, while plants homozygous for the susceptible (Evans) allele had a cyst index of 0.67±0.027. Thus we could reasonably expect that in fixed RIL lines, the two homozygous classes would be clearly distinguished by disease phenotypes. The qualitative scoring strategy was based on the use of a modified 30% cut-off for resistance, a level commonly used by plant pathologists and breeders (Schmitt and Shannon 1992). Consequently, the SCN indices could be categorically scored either as susceptible (Evans) or resistant (PI 209332) in the RIL population and then treated like any other qualitative gene or marker locus. With this approach, the SCN resistance locus could be definitely placed in the genomic interval between markers C6 and Bng122 in the RIL population, facilitated by the identification of recombination events between the SCN locus and these two flanking markers. Eight recombinants were uncovered between C6 and the SCN resistance locus and ten recombinants between Bng122 and the resistance locus. These recombinants will be especially valuable in future studies in which additional markers near the SCN resistance locus are identified.

Using the qualitative SCN scoring obtained from the RIL population, in combination with the consensus mapping information from the four F_2 populations, an integrated map of linkage group 'G' spanning 69 cM was created by *JoinMap*. Based on this joint map, the SCN resistance locus was placed 4.6 cM from B53 and 2.8 cM from Bng30 (Fig. 3). However, neither of these RFLPs were polymorphic in the RIL population, so recombinants between these marker loci and the SCN resistance locus could not be identified. A new RIL population, derived

from the Peking Evans F2 population, is currently under development. This population should enable us to identify lines with recombination between B53, Bng30, and the SCN locus, as well as clarifying the real marker order for RFLPs Bng30, Bng126 and Bng122.

Mapmaker-QTL scans of the region around the major partial SCN resistance in PI 209332, PI 90763, and Peking populations indicated that the "qualitative" scoring of SCN was within a one-LOD unit of the peak in all three populations analyzed (Fig. 4). In the PI 209332 population the qualitative SCN resistance locus was placed within 2 cM of the corresponding LOD peak, while in the Peking and PI 90763 populations, the SCN resistance locus was within 1 cM of the peaks (Fig. 4). Since there were only three markers mapped in the PI 88788 population, the Mapmaker-QTL scan for this population was not analyzed in detail.

Discussion

Targeted comparative mapping and marker saturation

Through a combination of targeted comparative genomic and integrated linkage mapping, we have increased marker density significantly in the genomic region surrounding a major SCN resistance locus in *G. max*. A previous attempt to target this genomic region with markers through bulked segregation analysis (Michelmore et al. 1991) and random amplified polymorphic DNAs (Williams et al. 1990), showed only limited success (Ferreira et al. 1994). By contrast, markers identified through conserved linkage with *P. vulgaris* and *V. radiata* increased the number of RFLPs in the 25.6-cM region nearest the SCN resistance locus from two to ten, leading to a density of one RFLP marker every 2.6 cM. Recent studies have demonstrated that many related plant taxa have substantial regions of genome conservation (Bonierbale et al. 1988; Song et al. 1990; Weeden et al. 1992; Helentjaris 1993; Pereira et al. 1994). The present study takes the next logical step – using information about conserved marker linkages to increase marker density in regions around genes of interest. While newer types of DNA markers, such as amplified fragment length polymorphisms (Vos et al. 1995) in combination with bulked segregant analysis, might eventually provide enhanced marker density in genomic regions of interest, orthologous markers based on comparative mapping provide an essentially "off the shelf" source of marker loci in regions of interest. Indeed, conserved linkages at a physical level are also possible and might provide shorter distances to traverse in chromosome landing (Tanksley et al. 1995). This would be especially relevant to *G. max* since its genome is nearly twice the size of the genomes of *P. vulgaris* and *V. radiata* (Arumuganathan and Earle 1991). In fact, two *P. vulgaris* RFLPs that were not polymorphic among our mapping populations, but known by comparative mapping to be located in the region of interest (Bng113 and Bng189), have subsequently been shown to be physically linked to the RFLPs described in this study through the use of pulsed-field gel electrophoresis (Danesh et al. 1995).

Qualitative mapping of the partial SCN resistance locus

This study demonstrates the usefulness of RIL populations in qualitative mapping of loci underlying traits exhibiting continuous phenotypic distribution in early segregating generations. An especially valuable aspect of this result was the ability to uncover individuals with crossovers very near to the SCN resistance locus. Such recombinant individuals will be essential for high-resolution mapping in the future.

Clearly, quantitative traits controlled by numerous minor genes will not be easily resolved into discrete loci simply by analysis in an RIL population. However, some quantitative traits showing continuous distributions of phenotype have recently been shown to be under the control of one or two major loci plus a larger number of minor genes (Doebley and Stec 1991; Fatokun et al. 1992; Phillips et al. 1993; Danesh et al. 1994), a situation highly analogous to SCN resistance. Given the need for precise mapping as a basis for marker-assisted breeding and positional gene cloning, our results indicate that such QTLs may be amenable to qualitative mapping.

We recognize that the strategy described in this study is not the only, or even necessarily the best, approach to quantitative scoring of partial-resistance loci (Paterson et al. 1990). An alternative would be to develop lines in which the entire genome is homozygous except for the major partial SCN resistance locus and its flanking markers on linkage group 'G'. The progeny of such lines would segregate for the major partial resistance locus in a truly Mendelian fashion and the individual effect of this locus on SCN disease response could be evaluated without genetic variance due to other SCN resistance loci. The process of identifying recombinants between flanking markers and the SCN resistance locus could also be accelerated by first genotyping, and later phenotyping, only recombinant progeny. This would dramatically reduce the number of plants that need to be assayed for SCN response. A similar strategy was used by DeScenzo et al. (1994) in developing a high-resolution map around the *Mla* resistance locus on chromosome 5S in barley.

Possibility of resistance gene clustering

The major resistance locus on linkage group 'G' identified in this study was assumed to be one and the same in all mapping populations in order to place the locus on a unified map of linkage group 'G'. However, it is possible that this genomic interval contains distinct, but tightly linked, SCN resistance genes in the populations evaluated. Since the existence of resistance gene clusters has been documented in several plant/pathogen systems (Islam and Shepherd 1991; Martin et al. 1993; Paran et al. 1991; Sudupak et al. 1993), together with the fact that four independent

and presumably unrelated soybean accessions have all been found to have SCN resistance genes in the same genomic region, it is possible that a gene cluster does exist. If so, the consensus map shown in Fig. 3 would actually show the most likely location for the PI 209332 SCN resistance locus relative to an integrated linkage map of DNA marker loci collectively inferred from all five mapping populations.

Acknowledgments We thank Ronald Phillips and Jim Groth for their suggestions and a critical review of the manuscript. We are also grateful to Paul Gepts, Randy Shoemaker, and Eduardo Vallejos for providing DNA clones. This paper is published as contribution no. 22,167 of the Minnesota Agricultural Experiment Station Journal Series, on research conducted under Project 015, supported by G.A.R. funds. This work was also supported, in part, by Minnesota Soybean Research and Promotion Council grants 23-94 and 13-95 and USDA grant 95-37300-1593.

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