Genome Mapping of Soybean Cyst Nematode Resistance Genes in 'Peking', PI 90763, and PI 88788 Using DNA Markers

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ABSTRACT

Several sources of soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) resistance have been identified in soybean [*Glycine max* (L.) Merrill]; yet, the genetic basis of this economically important trait remains poorly characterized. This study was conducted to identify quantitative trait loci that control disease response in three commonly used sources of SCN resistance. Using genetic markers, we analyzed three segregating soybean F_2 populations ['Evans' × 'Peking', Evans × Plant Introduction (PI) 90763, and Evans × PI 88788] and compared the results with those of a previous study involving PI 209332. For the Peking, PI 90763, and PI 209332 populations, races 1, 3, and 6 were used as SCN inoculum; for the PI 88788 population, only races 3 and 6 were used. To uncover putative resistance loci, F_2 DNA marker genotypes at between 63 and 99 loci in each population were contrasted with cyst indices averaged from 12 $F_{2:3}$ progeny individuals. Four independent partial SCN resistance loci were uncovered at $P < 0.0002$ (probability per locus). One of these loci, located at the top of linkage group 'G' near RFLP locus C006V, was significant at $P < 0.0001$ in all populations and races tested. Other significant loci included one near RFLP A378H at the opposite end of linkage group 'G' from C006V, another on linkage group 'J' near marker B032V-1, and a fourth on linkage group 'N' near marker A280Hae-1. Comparisons between different SCN races indicated that some of the putative resistance loci behave in a race-specific manner. These results may serve as a resource for SCN researchers and soybean breeders by summarizing a wide range of genetic data on the soybean-SCN interaction.

Soon after the discovery of cyst nematode on soybean in the USA, sources of resistance were identified by Ross and Brim (1957). Some of these lines, such as 'Peking' and Plant Introduction (PI) PI 88788, were quickly incorporated into breeding programs. Peking became the most widely used source of resistance because of a lack of agronomically undesirable traits (Caviness, 1992), with 'Pickett' as the first SCN resistant cultivar released (Brim and Ross, 1966). Soon it became apparent that certain SCN populations could overcome resistant soybean cultivars, leading to the establishment of a race classification scheme (Golden et al., 1970). Extensive screening for additional sources of resistance to the new races of SCN was performed, resulting in the discovery of resistant genotypes like PI 90763 (Epps and Hartwig, 1972; Thomas et al., 1975), PI 209332 (Anand and Brar, 1983; Anand and Gallo, 1984), and PI 437654 (Anand et al., 1985, 1988).

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Throughout this time, researchers studied the inheritance of SCN resistance in order to understand this complex plant-pathogen interaction. In the process, classical genetic studies demonstrated that inheritance of SCN resistance is oligogenic and complex (Anand and Rao-Arelli, 1989; Caviness, 1992). The studies indicated that genes for resistance may be linked or that multiple alleles at a single locus could be involved (Anand and Rao-Arelli, 1989; Hancock et al., 1987; Hartwig, 1985). Analysis of genetic relationships also indicated that resistance genes may be shared among different sources of resistance (Rao-Arelli and Anand, 1988). Despite these many attempts to understand SCN resistance by conventional genetic strategies, results have remained confusing and ambiguous because of the complex nature of both host and pathogen. Consequently, our knowledge about this complex host-parasite interaction remains incomplete.

Recently, molecular mapping has been used to enhance our understanding of SCN resistance in soybean (Concibido et al., 1994, 1996a,b; Mahalingam and Skorupska, 1995; Webb et al., 1995). By means of this approach, a major partial SCN resistance locus on linkage group 'G' of the soybean molecular linkage map was identified in PI 209332 (Concibido et al., 1994, 1996b) and PI 437654 (Webb et al., 1995), as well as in other loci found elsewhere on the soybean genome (Concibido et al., 1994, 1996a; Mahalingam and Skorupska, 1995; Webb et al., 1995).

We have conducted an extensive search for quantitative trait loci (QTL) underlying resistance to three SCN race isolates (1, 3, and 6) in Peking, PI 90763, and PI 88788 (along with results of an earlier study with PI 209332). This paper describes the use of DNA markers to identify QTL conferring resistance to SCN and provides information for SCN researchers and soybean breeders who may wish to use marker-assisted selection in developing SCN resistant varieties.

MATERIALS AND METHODS

Plant Materials

Three F_2 populations segregating for SCN resistance were used in the study, each constructed by crossing the maternal parent, 'Evans' (an SCN-susceptible variety), with one of the following sources of SCN resistance as a male parent: 'Peking' (Ross and Brim, 1957), PI 90763 (Epps and Hartwig, 1972; Thomas et al., 1975), or PI 88788 (Ross and Brim, 1957). The Evans × Peking population consisted of 110 lines, Evans × PI 90763 consisted of 115 lines, and Evans × PI 88788 consisted of 106.

Abbreviations: LOD, $\log_{10}$ of the odds ratio; PI, plant introduction; QTL, quantitative trait locus (loci); RFLP, restriction fragment length polymorphism; SCN, soybean cyst nematode.

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 (Chase et al., 1990; Keim et al., 1989; Menancio-Hautea et al., 1993: Nodari et al., 1993). Soybean probes were provided by R. Shoemaker (USDA-ARS, Iowa State University, Ames, IA) and common bean probes were provided by E. Vallejos (University of Florida, Gainesville, FL) and P. Gepts (University of California-Davis, Davis, CA). Probe names are based on the recommendations of the 1995 Soybean Genetics Committee Report (Pfeiffer et al., 1995).

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 individually digested with eight different restriction enzymes: *Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Taq*I, and *Xba*I (New England Biolabs, Beverly, MA; Promega, Madison WI; Gibco/BRL, Gaithersburg, MD). Digested DNA was electrophoresed on 10 g L^{-1} agarose gels and transferred onto Hybond N+ membranes (Amersham Corporation, Arlington Heights, IL) by a method adapted from Southern (1975), including an alkaline transfer in 0.5 *M* NaOH and 0.5 *M* NaCl. The clone-enzyme combination that gave the clearest band length polymorphism 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 "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 to 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 to 24 hr at 60°C in a hybridization solution of 5× saline sodium citrate (SSC; 1× SSC is 0.15 *M* NaCl plus 0.015 *M* sodium citrate), 0.1 *M* phosphate buffer, pH 7.5, 1× Denhardt's solution (20 mL L^{-1} of Ficoll, 20 g L^{-1} polyvinylpyrrolidone, and 20 mL L^{-1} bovine serum albumin fraction V), 1 g L^{-1} SDS, and 50 g L^{-1} dextran sulfate. After incubation, blots were washed at medium stringency (2.0 ×, 1.0 ×, and 0.5 × SSC with 1 g L^{-1} SDS) three times for 15 min each at 60°C. After washing, blots were placed against Kodak X-AR film and stored at −80°C for 1 to 7 d to produce autoradiographs. Blots were re-used as many as seven times.

Soybean Cyst Nematode Assay

A total of 10 620 SCN assays were performed over the course of analyzing the three mapping populations and three SCN race isolates used in this study. Twelve F_3 seeds from each F_2 plant were individually germinated in sand-filled Ray Leach cone-tainers (Stuewe & Sons, Inc., Portland, OR) placed in sand-filled 20-L buckets in a water bath. Seedlings were inoculated with 2000 eggs 3 d after germination in separate assays with one of three SCN isolates that behaved as either race 1, 3, or 6 (Riggs and Schmitt, 1988). Inocula for each race, which were originally collected from fields in Minnesota, were maintained on susceptible checks, Evans or 'Essex', and appropriate susceptible differential soybean lines. For example, the race 1 isolate, which can successfully develop on PI 88788, was maintained on PI 88788 and the susceptible genotypes, Essex or Evans; the race 3 isolate was maintained on Essex or Evans only; and the race 6 isolate on 'Pickett' and Evans. Cysts and white females from each isolate were collected every 35 d, stored in sand at 4°C, and later used for inoculations. Race determination tests were regularly conducted 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 the inoculum was standardized to 2000 eggs per plant with a hemocytometer. The parental lines, soybean differential lines (Peking, 'Pickett', PI 90763, PI 88788, as well as PI 209332), and susceptible checks (Evans and Essex) were included in all tests. Water bath temperatures were maintained at 28°C with 16-h daylength for 28 d. On the 28th day, plants were uprooted, randomly bulked into groups of three to simplify processing, and the cysts were collected by washing the roots with pressurized water over wire mesh sieves. For each F_2 line, the SCN index was estimated by dividing the average number of cysts per plant for all 12 F_3 plants by the average number of cysts recovered from the susceptible checks.

Linkage Mapping

A total of 287 genomic clones were analyzed by hybridization with "parental survey" blots and of these, 63 were analyzed by segregation analysis in PI 90763 and PI 88788 and 99 were analyzed in Peking. Segregation patterns for each clone were coded into a numeric form and analyzed by two-way contingency table analysis with Statview II (Abacus Concepts, Berkeley, CA) and maximum likelihood analysis with 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. A LOD score is the $\log_{10}$ of the ratio between the odds of one hypothesis (the markers are linked) against an alternative hypothesis (unlinked). 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 seed coat color locus, *i* (Matson and Williams, 1965), was also included in the linkage analysis.

Whenever possible, linkage blocks identified in our study were anchored to the published linkage groups of Shoemaker and Olsen (1993) by linkage to an "anchor" marker locus – RFLP loci that are known to map to specific positions on linkage groups based on restriction enzyme used and molecular weight (Shoemaker and Olson, 1993). In some cases, the assignment of certain RFLPs to specific linkage groups was tentative because of a lack of polymorphic anchor probes. Whenever we found two or more RFLP markers linked in our mapping population(s) that also mapped to same linkage group in Shoemaker and Olson (1993), then we presumed that those markers mapped to their published locations, even in the absence of an anchor probe.

Analyzing Quantitative Resistance Loci

To uncover genomic regions associated with SCN resistance, F_2 genotypic classes for each DNA marker were contrasted with the mean SCN disease response of $F_{2:3}$ progeny using single factor regression analysis and one-way analysis of variance (ANOVA). These analyses were performed using Statview-II and SuperAnova (Abacus Concepts, Berkeley, CA). A significance level of $P \leq 0.0002$ was used to uncover potential associations between DNA markers and SCN disease response. Detailed analysis of interactions among putative loci was not

performed in this study since the population sizes were too small to conduct meaningful tests.

Since deviations from normality were observed in the distribution of SCN indices for all three races in all three populations (data not shown), statistical analyses using arc-sin transformation and non-parametric procedures (Kruskal-Wallis, Spearman Rank Correlation) were also performed. However, no meaningful differences were observed among the results obtained from various analyses, so the data presented are all based on untransformed data.

To assess race specificity, a locus was tentatively inferred to be race specific if it showed a significant association ($P \leq 0.0002$) in SCN disease response against one or more SCN race isolates, but was non-significant ($P > 0.0002$) against any other SCN race tested.

RESULTS AND DISCUSSION

Status of the DNA Linkage Maps

The published soybean molecular map contains 490 RFLP and random amplified polymorphic DNA (Williams et al., 1992) markers placed onto 24 molecular linkage groups spanning a recombinational distance of about 3000 centimorgans (cM) (Shoemaker and Olson, 1993). In the present study, a total of 287 DNA markers were surveyed among all the mapping populations. In the Peking population, 40.3% polymorphism was observed, 36.2% polymorphism in PI 90763, and 38.3% polymorphism in PI 88788. In Peking, a total of 99 marker loci were mapped, comprising 24 linkage blocks and 12 unlinked marker loci (Fig. 1). In this map, 15 of the linkage groups described by Shoemaker and Olson (1993) contained one or more RFLP markers. We estimate that approximately 78% of the soybean genome could be assayed for QTL associated with SCN resistance in this mapping population (based on total map distance, number of linkage groups, and number of unlinked markers in our population). In the 90763 population, a total of 63 marker loci were mapped, comprising 15 linkage blocks and 18 unlinked markers (data not shown). In this map,

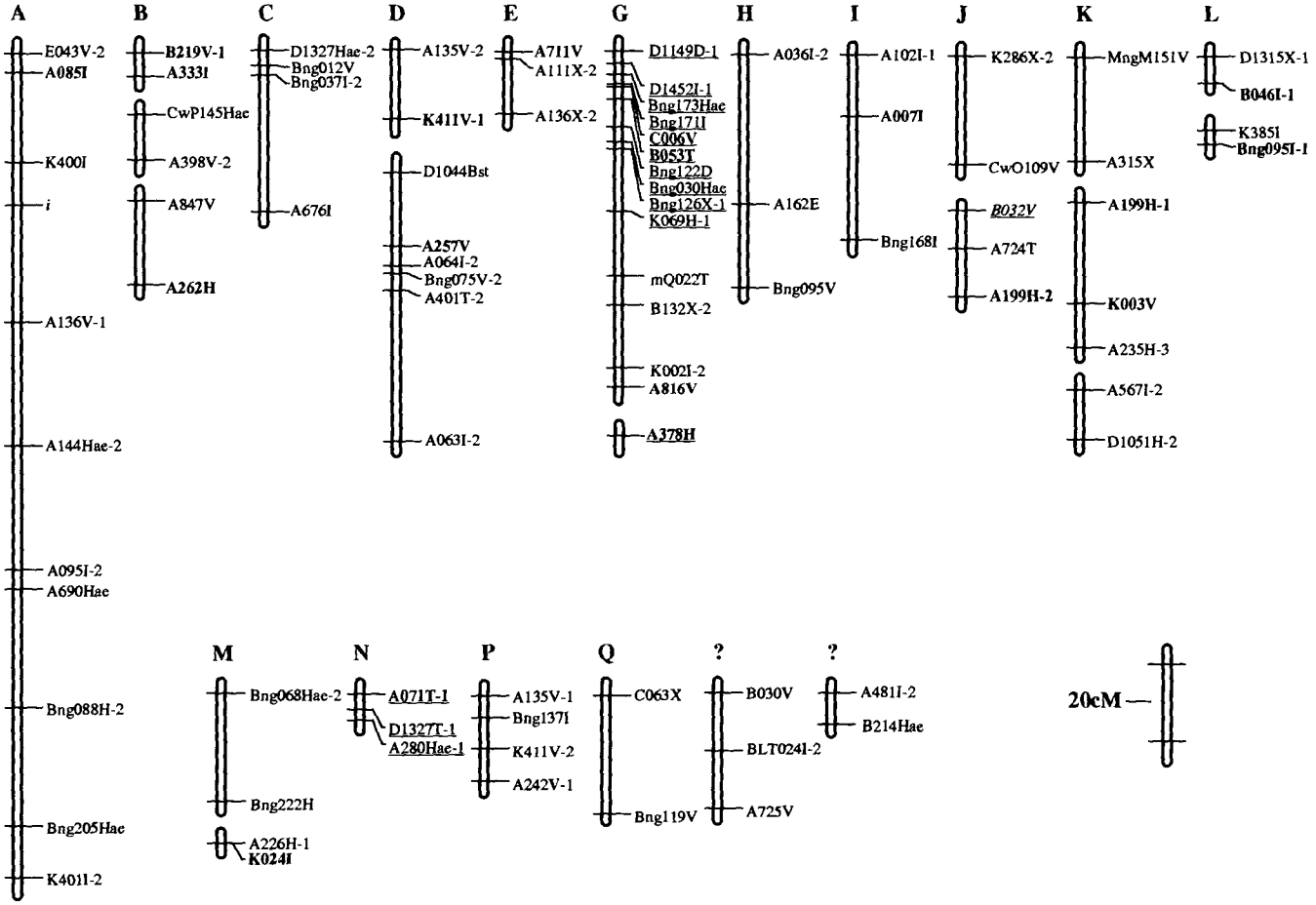

Fig. 1. Soybean linkage map from the Evans × Peking F_2 population. Long vertical lines represent linkage groups and short horizontal tick marks represent RFLP marker loci. Marker names in bold are "anchored" to the map of Shoemaker and Olsen (1993); underlined markers exhibited association with SCN resistance at $P \leq 0.002$. Locus names are based on the recommendations of the 1995 Soybean Genetics Committee Report (Pfeiffer et al., 1995). Also included on the map are the seed coat color gene, *i* (Matson and Williams, 1965), and RFLP marker B032V, which was not mapped in the Evans × Peking population but is still included in the figure based on its map position in Shoemaker and Olsen (1993); these loci are italicized. Markers with prefixes A, B, BLT, C, or K are from soybean, markers with prefixes Mng and MQ are from mungbean, markers with the prefixes CwP and CwO are from cowpea, and markers with prefixes Bng or D are from common bean. Non-anchored RFLPs that were unlinked to any other in the Peking mapping population, as well as RFLPs mapped only in the PI 90763 or PI 88788 populations, are not shown. Two linkage blocks whose linkage group designation could not be determined are labeled with a "?".

14 of the linkage groups described by Shoemaker and Olson (1993) had one or more RFLP markers and approximately 52% of the soybean genome could be assayed for QTL associated with SCN resistance. In PI 88788, a total of 63 marker loci were mapped, comprising 18 linkage blocks and 14 unlinked markers (data not shown). In this map, 15 of the linkage groups described by Shoemaker and Olson (1993) had one or more RFLPs, but only 46% of the soybean genome could be assayed for QTL associated with SCN resistance.

QTL for SCN Resistance

A total of four genomic regions showed significant associations with SCN resistance at $P \leq 0.0002$ (Table 1). One of these genomic regions, defined by RFLP C006V near the top of linkage group 'G', was common to all mapping populations and races tested and it consistently explained the largest proportion of phenotypic variation (almost 45% in PI 90763). A total of nine contiguous RFLP markers on linkage group 'G' were significant at $P \leq 0.002$, going from K069H-1 to D1149D-1 (Fig. 1). In a separate study, qualitative mapping and Mapmaker-QTL (Lander and Botstein, 1989) analysis indicated that this putative resistance locus is found in the interval between C006V and Bng122D (Concibido et al, 1996b). Moreover, this same genomic regions has been shown to be associated with SCN resistance in PI 209332 (Concibido et al., 1994, 1996a) and PI 437654 (Webb et al., 1995).

Three other genomic regions were highly significant in our analysis. A genomic region on linkage group 'G' near RFLP marker A398H was significant in Peking against race 1 and 6 isolates (Table 1; Fig. 1) and approached statistical significance against race 3 ($P = 0.0011$). However, there was no indication of significance in any of the other SCN resistance sources. Because this region is on the opposite end of linkage group 'G' and did not link to the genomic block containing C006V, we presume that it represents a distinct SCN resistance locus on the same chromosome. A third highly significant genomic region was found on linkage group 'J' near RFLP marker B032V-1 in PI 90763 against the race 3 isolate (Table 1). This region has also been found to be significant in PI 209332 (Concibido et al., 1994, 1996a). In the original report of this putative resistance locus, B032V was mistakenly placed on linkage group 'K' (Concibido et al., 1994). However, subsequent mapping with anchor loci A724T and A199X-2 has confirmed it placement on 'J' (Fig. 1). A fourth highly significant genomic region was found on linkage group 'N' near marker A280Hae-1. This genomic region also includes two linked marker locus, A071T-1 and D1327T-1, significant at $P \leq 0.002$ (Fig. 1). This region was only significant in Peking against the race 6 isolate, though it approached significance in PI 209332 against the race 6 isolate ($P = 0.0093$) and in PI 90763 against the race 1 isolate ($P = 0.0026$).

Contrary to our previous findings (Concibido et al., 1994) and those of others (Mahalingam and Skorupska, 1995; Webb et al., 1994), there was little evidence for the presence of a resistance locus on linkage group 'A', even though RFLP markers were placed in this genomic region (Fig. 1). Moreover, the seed coat color locus *i*, which has been found to be tightly linked to the SCN resistance locus *Rhg1* (Caldwell et al., 1960; Matson and Williams, 1965), was assayed in all our mapping populations. In the Peking mapping population, RFLP marker A400 on 'A' did show a weak association with resistance to race 3 ($P = 0.0110$) and race 6 ($P = 0.0115$). It is possible that the sources of SCN resistance we analyzed in the current study differed from those used in previous research. There appear to be several

Table 1. RFLP marker loci associated with SCN resistance organized according to linkage group, including loci identified in PI 209332 in a previous study (Concibido et al., 1966a).

Linkage group	Linked marker	Resistant parent	Effective against races	Percentage of variation	*P*-value	Slope†	d/a‡
G_1§	C006V	Peking	1	18.0	<0.0001	−0.102	−0.362
G_1	C006V	Peking	3	26.2	<0.0001	−0.160	−0.622
G_1	C006V	Peking	6	17.8	<0.0001	−0.188	−0.743
G_1	C006V	PI 90763	1	13.6	<0.0001	−0.101	−0.156
G_1	C006V	PI 90763	3	44.8	<0.0001	−0.252	−0.093
G_1	C006V	PI 90763	6	42.0	<0.0001	−0.235	−0.149
G_1	C006V	PI 88788	3	36.3	<0.0001	−0.179	−0.553
G_1	C006V	PI 88788	6	26.2	<0.0001	−0.201	−0.529
G_1	C006V	PI 209332	1	34.8	<0.0001	−0.191	−0.131
G_1	C006V	PI 209332	3	49.9	<0.0001	−0.197	−0.278
G_1	C006V	PI 209332	6	50.9	<0.0001	−0.183	−0.206
G_2	A378H	Peking	1	14.1	0.0002	−0.091	−0.354
G_2¶	A378H	Peking	3	10.3	0.0011	−0.152	−0.254
G_2	A378H	Peking	6	17.6	<0.0001	−0.170	−0.056
J	B032V-1	PI 90763	3	18.8	<0.0001	−0.130	0.862
J#	B032V-1	PI 209332	3	34.9	<0.0001	−0.238	0.076
N	A280Hae-1	Peking	6	14.3	<0.0001	−0.189	0.052

† Slope of the regression line between the genomic region and SCN disease response, a positive slope indicates that the resistance allele is found in the susceptible parent (Evans), a negative slope indicates that the resistance allele is found in the resistant parent.
‡ Dominance-to-additive ratio, 1 = completely dominant, 0 = completely additive, −1 = completely recessive.
§ Numerical subscript following the linkage group indicates distinct genomic regions on a single linkage group.
¶ Data on this locus-race combination are included for illustrative purposes, although the locus did not reach the level of statistical significance established in this study.
# Detected only in lines selected for the absence of the resistant allele at RFLP loci C006V and Bng122D, Concibido et al. (1996a).

different soybean genotypes that are all known as "Peking", even though each is genetically distinct (Skorupska et al., 1994). It is also possible that the SCN isolates used in our study lacked the appropriate virulence genes for the resistance locus on linkage group 'A'.

One concern in our mapping analysis might have been that a genomic region that was found to be associated with SCN resistance in one population might have lacked suitable RFLPs in another. If so, our results might have falsely indicated that a genomic region was associated with SCN resistance in one of the soybean genotypes tested, but not another. However, this was not the case because one or more RFLP marker loci mapped in the regions of interest in each of the mapping populations analyzed. Another source of concern is the relatively small populations sizes analyzed (106–115 individuals per mapping population). Given the many samples that already needed to be processed (three mapping populations, three SCN race isolates, 12 greenhouse assays per F2 line), larger populations would have been unrealistic. Nonetheless, computer simulations described by Beavis (1995) indicate that some real QTL can be overlooked and that the effects of others can be overestimated in small mapping populations. Finally, some QTL for resistance may simply have been overlooked because the fraction of the soybean genome analyzed by the markers in our studies were too low. This would be most likely in the PI 88788 population where only 46% of the soybean genome could be tested. This may help to explain why only a single genomic region was found to be associated with resistance in PI 88788 in our analysis (Table 1). Clearly, the four SCN resistance loci uncovered in the present study should be considered tentative. Some of the putative resistance loci that we identified could, indeed, be false positives, and there is certainly the possibility of additional resistance loci not uncovered in our study.

Race Specificity of SCN Resistance Loci

In this study, a resistance locus was inferred to be race specific if it was found to be significant against one or more of the SCN race isolates tested, but non-significant against any other. Although this definition is somewhat arbitrary, it provides a framework for analyzing race specificity of resistance QTL. By this definition, the putative resistance locus near C006V was race non-specific in all of the resistance sources (Table 1). Though statistically significant against all races, this locus did explain differing proportions of total variation with the three race isolates tested. In PI 90763, for example, the resistance locus near C006V explained more than three times as much total phenotypic variation against race 3 than against race 1 (Table 1). Such variability could have been due to the effects of genetic background, variability among SCN isolates tested, or simply an artifact of the small population sizes analyzed. The putative resistance locus on linkage group 'G' near A378H was also significant against both race 1 and 6 isolates and approached statistical significance against race 3. Although not strictly race non-specific by the definition used in this study, this locus appears to be important in Peking resistance to several different SCN races.

The other two putative resistance loci uncovered in this study both behaved in a race-specific manner. The region on linkage group 'J' near B032V-1 was only significant against race 3 in both PI 209332 and PI 90763 (Table 1). This region did approach significance against race 6 in PI 209332, but was clearly non-significant against race 1 in all populations tested (data not shown). The region on linkage group 'N' near A280Hae-1 was highly significant against race 6 in Peking (Table 1) and approached significance against race 6 in PI 209332 and race 1 in PI 90763, but was not significant against the race 3 isolate in any population (data not shown). These results suggest that some SCN resistance loci behave in a race-specific manner. Race specificity among partial resistance loci has also been found in potato (*Solanum tuberosum* L.) against *Phytophthora infestans* (Leonards-Schippers et al., 1994) and in tomato (*Lycopersicum esculentum* L. Mill.) against *Pseudomonas solanacearum* E.F. Smith (Danesh and Young, 1994).

Perspectives on SCN Resistance

Using DNA markers, we have identified four loci associated with SCN resistance. Among these resistance loci, the locus on linkage group 'G' near C006V was definitely the most important and was common among all sources of resistance, explaining up to 50% of total phenotypic variation in SCN disease response. Efforts are underway to clone this major partial resistance gene through the development of a high resolution map and qualitative scoring of SCN disease response in recombinant inbred populations (Concibido et al., 1996b). This research has already placed the resistance locus in the genomic interval between C006V and Bng122D very near the RFLP marker, B053T (Fig. 1). The fact that this putative resistance locus controls such a large proportion of phenotypic variation, is shared by all sources of SCN resistance analyzed to date, and is not race specific suggests that selection for this genomic region would be important in any SCN breeding effort.

It is interesting that all of the SCN resistance loci uncovered in this study map to clusters of genes involved in plant-microbe interactions. Previous studies have shown that the region near B032V-1 contains genes for *Rj2* nodulation in *Bradyrhizobium japonicum* Kirchner, *Rmd* resistance to *Microsphaera diffusa* Cooke & Peck, and *Rps2* resistance to *Phytophthora sojae* Kaufmann & Gerdemann (Polzin et al., 1995). Similarly, a resistance gene to sudden death syndrome [*Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Synd. & Hans.] has been shown to map in the genomic region on linkage group 'G' near C006V (Chang et al., 1996), while another sudden death syndrome resistance gene maps to the genomic region on linkage group 'N' near A280Hae-1 (Hnetkovsky et al., 1996) as does *Rps*1, another resistance gene to *P. sojae*. Likewise, *Rps*4 maps near A378H on linkage group 'G' (Diers et al., 1992). Thus, the results of our study add to the growing list of plant disease resistance genes that seem to be clustered (Martin et al., 1993; Islam and Shepherd, 1991; Sudupak et al., 1993; Paran et al., 1991).

In many ways, our results corroborate those of classical genetic studies of SCN resistance. First and foremost, our results clearly show that many resistance genes are shared among the different sources of SCN resistance (Rao-Arelli and Anand, 1988; Rao-Arelli et al., 1992). Moreover, the total number of resistance loci identified in our study using DNA markers (four) is similar to previous estimates (Rao-Arelli and Anand, 1988; Rao-Arelli et al., 1992). Classical analysis indicated there should be one dominant resistance gene (near the seed color gene, *i*) and three recessive genes conferring resistance to race 3 in Peking and PI 90763 (Rao-Arelli et al., 1992). In our results, a total of three distinct resistance loci for race 3 were uncovered near marker loci C006V, A378H, and B032V-1. The putative resistance loci near C006V and A378H both behaved in a partially recessive manner (as indicated by the d/a ratios in Table 1), while the locus near B032V-1 behaved in an additive to partially dominant manner. However, there was no evidence of a dominant resistance locus on linkage group 'A' near the *i* gene in our experiments. Also in contrast to the results of classical studies (Rao-Arelli and Anand, 1988; Rao-Arelli et al., 1992), not all of the resistance loci uncovered in our study were common between Peking and PI 90763 (Table 1). Specifically, Peking was found to carry a resistance locus near A378H, while there was no indication of statistical significance for this locus in PI 90763. Similarly, PI 90763 (along with PI 209332) was found to carry a resistance locus for race 3 near B032V-1, while there was no indication of a resistance locus in this region in Peking. It should be noted, however, that we were unable to test Peking directly with the marker B032V-1, as this probe was not polymorphic in the Evans × Peking cross. Instead, two linked RFLP marker loci (A199H-2 and A724T) were tested and these RFLPs are approximately 10 to 16 cM away from B032V-1. As noted above, there are several different lines that are all referred to as "Peking" (Skorupska et al., 1995); so it is also possible that we tested a different one than previous researchers.

Finally, classical studies indicated that PI 88788 carries one dominant and two recessive genes for race 3 and that two of these genes are common with PI 90763 and Peking (Rao-Arelli et al., 1992). Our experiments only uncovered a single major locus in PI 88788 near C006V and this is probably one of the common loci identified previously. The fact that our analyses were performed with small mapping populations, less than complete genome coverage, and non-inbred SCN isolates that were different from those used by the previous researchers might explain some of these discrepancies.

Dong and Opperman (1997, personal communication) have found that virulence is controlled by a single dominant gene in a highly inbred SCN isolate capable of infecting PI 88788. Virulence is also controlled by single (recessive) genes in two other SCN inbreds, one capable of infecting Peking and another capable of infecting PI 90763. Genetic mapping has demonstrated that these loci are all distinct (K. Dong and C.H. Opperman, 1996, personal communication). Now that specific loci involved in SCN resistance have been tagged with DNA markers in the plant host and comparable loci have been tagged in the pathogen, it may be possible to explore the possibility of gene-for-gene (Flor, 1955) relationships between these genes. Essential for these studies will be soybean lines with defined sets of resistance genes challenged with SCN inbreds that carry defined sets of virulence genes. Such genetic stocks can now be developed through the use of marker-based selection. Considering how complicated the genetics of the soybean-SCN interaction has always seemed in the past, the possiblity of studying this phenomenon through the use of DNA markers is very attractive.

A major goal of DNA marker analysis is to provide a foundation for marker-assisted breeding. In this regard, the fact that several important genes for SCN resistance have been tagged with nearby RFLP markers may aid the work of soybean breeders and geneticists. Two examples of marker-assisted breeding strategies with particular relevance in this system might be breaking linkage drag to increase yield (Anand and Koenning, 1986) and deterministic development of novel SCN resistance genotypes. Preliminary field experiments indicate that the genomic region proximal to the resistance locus on linkage group 'G' (in the region between C006 and K069H-1; Fig. 1) harbors a QTL for depressed yield (Mudge et al., in press). Results from 2 yr of field trials indicate that the alleles from PI 209332 in this region are associated with yield losses between 138 and 294 kg/ha. Thus, marker-assisted selection offers the potential for breaking this apparently undesirable linkage (assuming the yield depression is not due to pleiotropism at the SCN resistance locus itself). An example of a novel SCN resistance genotype that could be developed through the use of marker-based selection might be a line that carries a recombinant linkage group 'G' with the Peking allele near A378H and an allele from a different SCN resistance source at C006V. In a similar vein, it may be possible to prolong the useful life of SCN resistance genes by developing varieties with different gene combinations coupled with spatial and temporal gene deployment (Anand and Koenning, 1986). Race shifts in SCN field populations might then be countered by deploying novel resistance gene combinations.

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