DNA Marker Analysis of Loci underlying Resistance to Soybean Cyst Nematode (*Heterodera glycines* Ichinohe)

V. C. Concibido, R. L. Denny, S. R. Boutin, R. Hautea, J. H. Orf, and N. D. Young*

ABSTRACT

Inheritance of resistance to soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is oligogenic and complex. DNA markers, such as restriction fragment length polymorphisms (RFLPs), can be used to identify loci associated with complex genetic characters, including SCN resistance. To uncover these associations, 56 F_2 lines from a cross between a susceptible (M83–15) and a resistant (M85–1430) soybean [*Glycine max* (L.) Merr.] inbred line were mapped for DNA marker polymorphisms. F_3 lines derived from these F_2 individuals were assayed for SCN disease response in the greenhouse to a field isolate of SCN from Minnesota that tested as Race 3. The F_2 genotypic classes for each of 43 DNA markers were then contrasted with SCN disease response to identify marker loci associated with SCN resistance. Two unlinked RFLP markers, pA85 and pB32, were found to be significantly associated with SCN disease response, together accounting for 51.7% of total phenotypic variation. Based on RFLP analysis, individual $F_{2:3}$ lines that retained either one or both resistance loci were identified. Lines that possess both resistance loci could be valuable in developing resistant soybean lines free of linkage drag, while lines that have just one of the resistance loci may be useful as single-gene differentials for SCN race determination.

THE SOYBEAN CYST NEMATODE is one of the most destructive pests of soybean. To date, host plant resistance has been the most effective control measure, but it is oligogenic in nature and genetically complex. Classical genetic studies indicate that resistance is conditioned by at least five major genes: one dominant and four recessive, each with multiple alleles (Caldwell et al., 1980; Matson and Williams, 1965; Ross and Brim, 1957; Thomas et al., 1975). In particular, resistance against Race 3 appears to be controlled by one dominant and two recessive genes in soybean lines 'Peking' and PI 90763 (Rao-Arelli et al., 1992). Analysis of genetic relationships has revealed that resistance genes may be shared among sources of resistance to SCN Race 3 (Rao-Arelli and Anand, 1988). Breeding for SCN resistant soybean lines is further complicated by the high level of genetic variability that exists among field populations of SCN, along with the difficulty involved in screening soybean germplasm and in SCN race determination. Thus, conventional breeding for SCN resistance is time consuming and difficult.

Advances in biotechnology now make it possible to map agriculturally important genes, including SCN resistance genes, with DNA markers such as restriction fragment length polymorphisms (RFLPs). With DNA markers, it will be possible to rapidly identify resistant soybean lines based on genotype instead of phenotype. This would eliminate actual nematode tests and may enable the development of lines with a minimum of linkage drag. Potentially, individual lines containing a combination of different types of SCN resistance genes could also be created.

In the past, the use of DNA markers to study quantitative trait loci (QTL) has primarily focused on agronomic and morphological characters in plants. RFLPs have been utilized in locating and determining effects of QTLs associated with fruit size, pH, soluble solids (Paterson et al., 1990), and water-use efficiency (Martin et al., 1989) in tomato (*Lycopersicum lycopersicon* L.). Quantitative trait loci contributing to heterosis in several quantitative traits have been mapped in maize (*Zea mays* L.) (Stuber et al., 1992), as have QTLs for heat tolerance (Ottaviano et al., 1991) and morphological characters distinguishing maize from teosinte (*Zea mays* ssp. *mexicana*) (Doebley et al., 1992). In soybean, QTLs underlying several reproductive and morphological traits (Keim et al., 1990b) and hard-seededness (Keim et al., 1990a) have also been identified by molecular markers.

However, in studying disease resistance in plants, the use of DNA genetic markers has been primarily focused on single locus resistance genes such as *Tm2* (tomato mosaic virus), *Pto* (*Pseudomonas syringae* pv. *tomato* Okabe), and *I2* (*Fusarium oxysporium* Schlect f. sp. *lycopersici* Sacc.) resistance loci in tomato (Young et al., 1988; Martin et al., 1991; Sarfatti et al., 1989), *Dm* (*Bremia lactucae* Regel) resistance loci in lettuce (*Lactuca sativa* L.) (Michelmore et al., 1991), leaf blast (*Magnaporthe grisea* Barr) resistance in rice (*Oryza sativa L.) (Yu et al., 1991),* and the *rps* loci for *Phytophthora sojae* (Kauf. and Gende.) resistance in soybean (Diers et al., 1992). Similarly, single gene resistances for nematode pests have been identified, such as *Mi* resistance gene to the nematode *Melodogyne incognita* (Kofoid and White) in tomato (Messeguer et al., 1991), *Heterodera schactii* (Schmidt) in sugar beets (*Beta vulgare* L.) (Jung et al., 1990), and *Globodera rostochiensis* (Woll) in potato (*Solanum tuberosum* L.) (Barone et al., 1990).

The use of DNA markers has not been widely utilized in studies of oligogenic or polygenic forms of resistance in plants. A few of these studies that have been carried out include: powdery mildew (*Erysiphe polygoni* D.C.) resistance in mungbean (*Vigna radiata* L. Wilczek) (Young et al., 1993), glandular trichomes, an insect resistance trait in tomato (Nienhuis et al., 1987), and maize dwarf mosaic virus resistance in maize (Romero-Severson et al., 1989).

In this paper, we demonstrate the utility of RFLP genetic markers in identifying genomic regions contributing partial resistance to SCN and consider the implications

V.C. Concibido, R.L. Denny, S.R. Boutin, and N.D. Young, Dep. of Plant Pathology, Univ. of Minnesota, St. Paul, MN 55108; R. Hautea, Univ. of the Philippines at Los Baños, College, Laguna, Philippines; and J.H. Orf, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108. Contribution from the Minnesota Agricultural Experiment Stn. Journal series no. 20,438 on research conducted under Project 015, supported by G.A.R. funds. This paper was also supported, in part, by Minnesota Soybean Research and Promotion Council Grant no. 36–93–2. Received 6 April 1993. *Corresponding author.

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Abbreviations: dNTPS, deoxynucleotide triphosphates; cM, centimorgan; LOD, $\log_{10}$ of the odds ratio; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SCN, soybean cyst nematode.

of DNA markers in genetic dissection and marker-associated breeding for SCN resistance in soybean.

MATERIALS AND METHODS

Plant Materials

The segregating F_2 population was constructed by crossing two contrasting soybean lines, M85–1430, carrying resistance originally from PI 209332 (Anand and Brar, 1983; Anand and Gallo, 1984), and the susceptible line, M83–15, which has no known resistance in its background, and advancing the cross to the F_2 generation. Relative to 'Essex', the susceptible parent in this cross had nearly the same level of susceptibility (Table 1). Briefly, M85–1430 is a selection from L77–906 (a selection from 'Williams' × PI 209332) × L78–189 (a selection from 'Corsoy' (8) × 'Kingwa'). The susceptible parent, M83–15, was derived from the cross between 'A2' and 'Hodgson 78'. Fifty-six F_2 individuals, together with the parents, were grown in a greenhouse in St. Paul, MN, and used as source of leaf tissue for DNA extraction and DNA marker analysis. Plants were allowed to recover and set F_3 seeds, which were saved for SCN disease assay. Data were also collected from each F_2 plant for mean 30-seed weight and hilum color.

DNA Clones

The source of putative RFLP markers was a soybean genomic library, prepared by digestion with the methylation-sensitive restriction enzyme, *Pst*I, and ligated to the bacterial plasmid, pBS+ (Stratagene, La Jolla, CA). This library was provided by Dr. Randy Shoemaker (Iowa State University, Ames, IA).

Plant DNA Extractions, Restriction Digestion, Electrophoresis and Blotting

Plant DNA was extracted by the modified method of Dellaporta et al. (1983) and analyzed following the technique of Young et al. (1992). First, the best restriction enzyme for each DNA clone was determined. Individual clones were probed against *Parental survey* blots, which consisted of parental DNA digested with fifteen different restriction enzymes (*Ban*I, *Bam*HI, *Bcl*I, *Bstn*I, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hind*III, *Hinf*I, *Rsa*I, *Sca*I, *Taq*I, and *Xba*I), followed by 10 g L^{-1} agarose gel electrophoresis, and transfer onto Hybond N+ membranes (Amersham Corporation, Arlington Heights, IL) using a method adapted from Southern (1975). The best clone/enzyme combination was used for segregation analysis. This involved probing *F_2 progeny blots* containing digested DNA from all 56 F_2 individuals with the corresponding DNA clones.

DNA Hybridizations

Cloned DNA inserts were amplified by the polymerase chain reaction (Saiki et al., 1990) for use in radiolabeling reactions and nucleic acid hybridization as described by Young et al. (1992). Briefly, lysates from colonies of individual DNA clones were prepared from individual bacterial colonies and a sample of 2 μl of the supernatant was then used in a polymerase chain reaction to amplify the insert sequence using oligonucleotide primers flanking the insert sequence. About 40 to 50 ng of the amplified product was then radiolabeled by the random hexamer reaction (Feinberg and Vogelstein, 1983) and incubated with either a Parental survey or F_2 progeny blot as described by Young et al. (1992).

Random Amplified Polymorphic DNA Analysis

Random amplified polymorphic DNA (RAPD) analysis was carried out by a method adapted from Williams et al. (1990). First, primers that gave clear, polymorphic, and unambiguous bands were identified. This involved amplifying 25 ng of DNA from the parents and five F_2 lines in a 25 μL solution of 1 m*M* dNTPs, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.5% TWEEN-20, 15 ng of a 10-base primer (Operon Technologies, Alameda, CA) and 1.25 units *Taq* DNA Polymerase (Promega, Madison, WI) per reaction. Individual reactions were thoroughly mixed, overlaid with mineral oil and placed in a Coy thermal cycler. The thermal cycle profile consisted of an initial soak at 94 °C (45 s) and then followed by 45 thermal cycles of 94 °C (45 s), 35 °C (1 min) and 72 °C (90 s). The RAPD products were resolved on 14 g L^{-1} agarose gels, stained with ethidium bromide and visualized with ultraviolet light. The best primers were then used to amplify DNA samples for all 56 individuals to generate segregation data for mapping analysis.

Table 1. Race determination tests by means of host differentials using the same source of inoculum for both the F_2 greenhouse assay and for seven experiments conducted on separate test dates.

Differential Cultivars	Host Reaction (% of susceptible check)†	
	Differential Test 1‡	Differential Test 2§
Pickett	3.66 ± 0.89	7.21 ± 1.40
Peking	0.80 ± 0.76	0.54 ± 0.34
PI 88788	2.84 ± 0.52	3.24 ± 0.71
PI 90763	1.34 ± 0.38	2.77 ± 0.63
PI 209332	2.81 ± 0.50	—
Essex	100.00	100.00
M83-15	—	102.34 ± 12.84
M85-1430	—	10.74 ± 3.34

† Differential host reaction is expressed as percentage of the number of females and cysts removed on the susceptible check 'Essex' (Riggs and Schmitt, 1989).
‡ Means of differential cultivars based on seven greenhouse experiments conducted on seven separate test dates.
§ Means of differential cultivars assayed simultaneously with the F_2 segregating population in the greenhouse.

Soybean Cyst Nematode Assay

F_3 seedlings were germinated and transplanted either to pots on a greenhouse bench or to Ray Leach Cone-tainers (Stuewe & Sons, Inc., Portland, OR) in a waterbath. A total of 25 F_3 seedlings for each F_2 line was assayed, 13 by the bench method and 12 in the waterbath. Each plant was inoculated with 1000 eggs at transplanting and again three days later with an SCN isolate originally isolated in a field in southern Minnesota in the summer of 1990. This isolate was maintained in 6-inch pots with soil that was continuously planted to susceptible checks, 'Evans' and Essex. Cysts and white females were then collected every 28 d, stored in sand at 4 °C and later used for inoculations.

Race determination tests were regularly conducted on this isolate to check for the possibility of race shifts. In a series of eight experiments done on the isolate, including the F_2 population nematode assay, the isolate was confirmed to be Race 3 in all experiments (Table 1). According to the present race determination scheme, a susceptible host reaction (+) is declared if the number of females and cysts recovered is greater than or equal to 10% on the susceptible check; a resistant reaction (−) if the number of females and cysts recovered is less than 10% on the number on the susceptible check (Riggs and Schmitt, 1989).

For this study, the parents, as well as the soybean differential lines (Peking, 'Pickett', PI 90763 and PI 88788), and susceptible checks ('Evans' and Essex) were included in both the bench and waterbath tests. The experiment was laid out in a completely randomized design. Soil temperatures were maintained at 28 °C with 16-h daylength for 28 d. On Day 28, individual plants were uprooted and cysts were collected by blasting the roots with pressurized water and collecting the

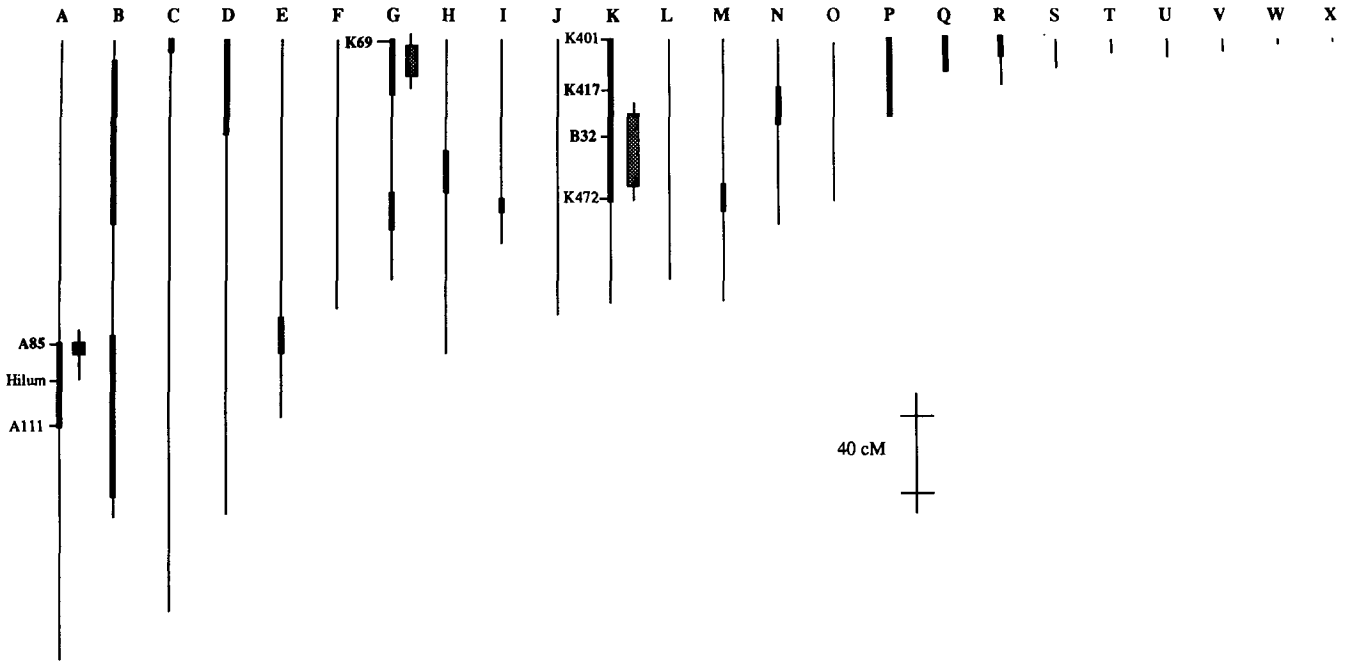

Fig. 1. An RFLP map of soybean (Keim et al., 1990b) showing locations of putative soybean cyst nematode (SCN) resistance loci. Introgressed regions that have been retained from the resistant ancestor (PI 209332) in this cross are shown as thick lines in the chromosomes. Locations of putative SCN resistance loci corresponding to intervals of LOD scores within 1.0 and 2.0 log units of the peak are indicated by broad and intermediate bars adjacent to chromosomes, respectively. RFLP markers showing an association significant at $P < 0.002$ (LOD > 2.5) are in bold letters, while markers indicated by an asterisk have been previously suspected to be associated with SCN resistance, but did not reach the cut off for significance in this study.

cysts on sieves. The total number of cysts from individual plants was counted under a dissecting microscope and converted to an index by dividing this number by the average total number of cysts on the susceptible parent. For each F_2 line, SCN indices of the 25 F3 plants were averaged to estimate mean SCN index for that line.

Mapping Quantitative Resistance Loci

To uncover genomic regions associated with SCN resistance, the F_2 genotypic classes for each DNA marker were contrasted with SCN disease response using regression analysis and analysis of variance (ANOVA). These analyses were performed with Statview-II and SuperAnova (Abacus Concepts, Berkeley, CA). A significant association between a DNA marker and SCN disease response was declared if the probability was equal or less than 0.002 to minimize the detection of false positives experiment-wide (Lander and Botstein, 1989). Data were also analyzed by Mapmaker-QTL (Lander et al., 1987). Putative QTLs were inferred whenever the LOD ($\log_{10}$ of the odds ratio) score exceeded 2.5. Statview-II and SuperAnova were also used to perform two-factor analysis of variance of all marker loci tested in this study in order to determine if there were significant interaction among loci. A significance level of $P < 0.001$ was used to uncover potential interactions.

RESULTS AND DISCUSSION

Status of Soybean RFLP Linkage Map

A total of 126 DNA clones and 60 RAPD primers were surveyed for polymorphisms, but only 36 DNA clones gave clear restriction fragment length polymorphisms and seven RAPD primers gave polymorphic amplified products in the segregating population. Thirty-two RFLP and five RAPD markers mapped to 14 coherent linkage groups (Fig. 1) based on the existing soybean RFLP map (Keim et al., 1990b). In theory, the polymorphisms that were observed should either correspond to linkage blocks of introgressed DNA material from PI 209332 or polymorphisms between M83–15 (the susceptible parent) and the cultivated soybean background of M83–1430. Assuming that all the observed DNA polymorphisms were due to introgressed material from PI 209332, an estimate of the amount of introgressed regions from PI 209332 into M85–1430 was approximately 20% (Fig. 1). After two crosses away from the ancestral donor parent (PI 209332) to generate M85–1430, it is estimated that about 25% of the donor parent's genome should have been retained in this line (Briggs and Knowles, 1967).

Frequency Distribution of SCN Disease Response among F_2 Lines

The SCN disease response among F_2 plants showed an approximately normal, continuous distribution, although there is some indication of peaks at both extremes (Fig. 2). Negative transgressive segregation was observed among F_2 plants based on mean SCN index (Fig. 2). A number of positive transgressive segregants were also observed based on individual F_3 mean SCN indices. However, since these individuals represent only single F_3 plants, it is difficult to determine if the observed variations are genetic in nature or due to environmental effects. Nonetheless, we are investigating the possibility of identifying positive transgressive segregants from this population. If such lines are identified, they may be useful as starting points for developing resistant soybean cultivars.

Correlation analysis showed a significant association

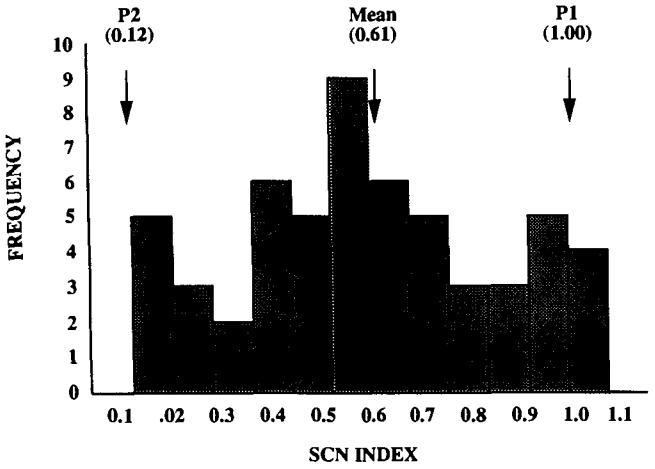

Fig. 2. Frequency distribution of 56 F2 lines for mean SCN disease response, averaged from results of bench and waterbath methods. Vertical axis shows the number of individuals for a given disease score. Horizontal axis represents the corresponding SCN indices for all 56 lines. The mean SCN index of all F2 lines, as well as the two parents, are also shown.

between the bench and the waterbath method ($r = 0.79$, $P < 0.0001$), indicating a high degree of homogeneity between the two tests, allowing us to pool the SCN indices for both. This was further confirmed by tests of homogeneity (paired *t*-test, ANOVA) between data sets to compare the effects of inoculation method on SCN disease response, which were found to be not significant (data not shown).

DNA Markers Associated with SCN Disease Response

The strategy used in locating potential partial resistance loci to SCN in this study was to detect statistically significant associations between F_2 genotypic classes for each DNA marker and corresponding SCN disease responses. A significant association between a DNA marker and SCN disease response was declared only if the probability was equal or less than 0.002 to minimize the detection of false positives experiment-wide (Lander and Botstein, 1989). Fifty-six plants is a relatively small population to potentially uncover all genes controlling SCN disease response. With a population of this size, only those partial resistance loci with moderately high effects would be expected to be uncovered (Lander and Botstein, 1989). Thus, minor loci involved in SCN response were probably overlooked in this study.

Two unlinked RFLP loci, pA85 and pB32, showed significant effects on SCN disease response. Together, these two loci explained 51.7% of the total variation in SCN disease response. The combined effects of the two RFLP markers are illustrated in Fig. 3a and 3b. In Fig. 3a, the segregation patterns and corresponding genotypic scores for the parental lines and 16 randomly chosen F_2 individuals are shown for pA85 and pB32. In Fig. 3b, a bar chart showing the corresponding SCN indices for the same F_2 individuals is shown. Note that individuals that retained both resistant (M85–1430) alleles for both markers generally had considerably lower SCN indices than individuals that have retained only one or none of the resistant alleles. By contrast, individuals with both the susceptible alleles for pA85 and pB32 had significantly higher SCN indices.

The RFLP marker pA85 alone accounted for 15% of the total variation, with $P = 0.0015$. Homozygotes and heterozygotes for the M85–1430 allele for this marker were, on average, 32% more resistant than homozygous individuals for the M83–15 allele. Since there was no significant difference between homozygotes and heterozygotes for the M85–1430 alleles, pA85 appeared to be completely dominant in effect (Table 1, Fig. 4).

The RFLP marker pA85 was tentatively located to linkage group A of the soybean RFLP map and was also linked to hilum color at a distance of 10.9 centimorgans (cM) (Fig. 1). Previously, this character was reported to be tightly linked to a dominant SCN resistance gene, called *Rhg4* (Matson and Williams, 1965; Weiss, 1970). Additional RFLP markers reported to map on linkage group A were also surveyed for polymorphisms. Only pA111 was polymorphic and linked to pA85 at a distance of 29.6 cM (Fig. 1). However, pA111 did not show a significant effect on SCN disease response, although it was also linked to hilum color at a distance of 18.7 cM. Hilum color was also weakly associated with SCN response, although at a level that did not reach statistical significance ($r^2 = 0.10$, $P = 0.013$) (data not shown).

It is possible that the putative resistance locus uncovered on linkage group A in the current study is the same as the locus described by Weismann et al. (1992). In their study, they found two molecular markers, pBLT24 and pBLT65, to be associated with SCN resistance gene *Rhg4*, although they did not perform a direct assay for nematode resistance and the association was based solely on tight linkage of the two markers to the *i* locus (seed coat color).

A second marker, pB32, tentatively located on linkage group K of the soybean RFLP map, showed a stronger influence on SCN disease response. This RFLP marker accounted for 38% of the total variation ($P < 0.0001$). Homozygotes and heterozygotes for the M85–1430 allele for pB32 were 48 and 34% more resistant than the homozygous individuals for the M83–15 allele, respectively. Thus, pB32 appears to be partially additive in its effect (Table 2, Fig. 4). The genomic region where pB32 was located was also defined by three other RFLP markers, pK472, pK401, and pK417 (Fig. 1). Of these, pK417 also showed a significant effect on SCN response ($r^2 = 0.16$; $P = 0.0048$).

The assignment of pB32 to linkage group K is tentative because this RFLP was previously mapped to two different soybean linkage groups (J and K) (Keim et al., 1990b). However, one of the other RFLPs it was linked to in our study, pK401, has previously been mapped to linkage group K (Keim et al., 1990b). Moreover, only a single pair of hybridizing bands were observed on the autoradiograph used to map pK401, strongly suggesting it maps to a single locus only. The other RFLP markers in this linkage block, pK417 and pK472, were previously mapped to different soybean linkage groups (Keim et al., 1990b). However, both of these RFLPs hybridized to multiple bands and it is likely, therefore, that we mapped alternative loci on linkage group K for these RFLP probes.

Mapmaker-QTL analysis (Lander and Botstein, 1989) identified the same genomic regions (near markers pA85

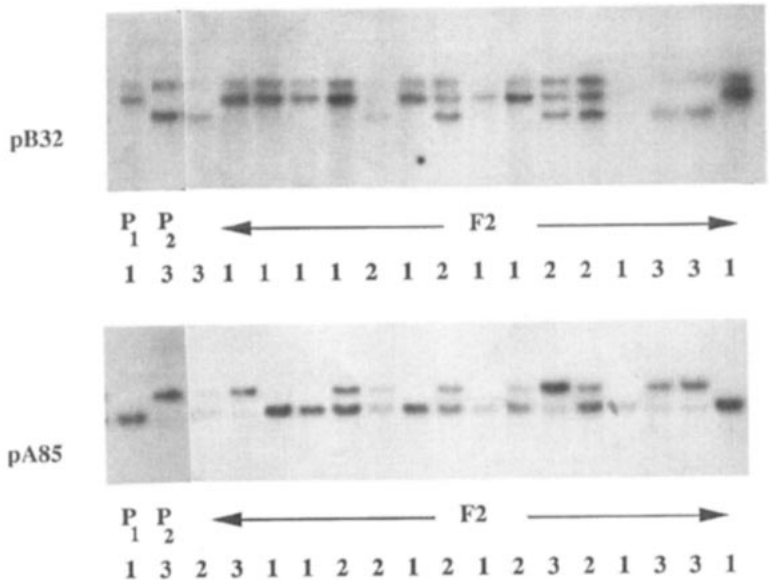

Fig. 3a. F2 segregation analysis of RFLP markers pB32 and pA85. Fifty-six F2 progeny, derived from a cross between a resistant (M85–1430) and a susceptible (M83–15) soybean inbred line, were analyzed for DNA marker polymorphism. On the left, autoradiographs of the two parents showing the RFLP patterns for markers pB32 and pA85 are shown; on the right, the RFLP patterns for 16 F2 progeny plants. Below the RFLP patterns, the corresponding genotypic score for each plant is shown. "1" indicates homozygous for M83–15 (susceptible parent) alleles; "2" indicates heterozygous; and "3" indicates homozygous for M85–1430 (resistant parent) alleles.

and pB32) as containing major QTLs for SCN resistance, explaining 21.4 and 40.2% of the total SCN disease response, respectively (Table 1). Two-way ANOVA did not indicate any interactions observed between the partial resistance loci or between the loci and any of the other DNA markers analyzed in this study (data not shown).

In addition, a third genomic region defined by RFLP pK69 was found to be associated with SCN resistance ($r = 0.16$, $P = 0.0026$), but at a significance level slightly over the cut-off of $P = 0.002$ used in this study. The RFLP pK69 was previously reported to be associated with SCN resistance in a study using isogenic soybean lines (Boutin et al., 1992). Based on the current soybean genetic map, this pK69 locus appears to be located on linkage group G (Randy Shoemaker, 1992, personal communication).

Implications for Genetic Dissection and Marker-Assisted Breeding

Now that we have demonstrated the utility of DNA markers in characterizing partial resistance loci for SCN resistance, lines that have retained all the major resistance loci with minimum linkage drag can be identified by DNA marker genotype. This will not only accelerate the breeding of SCN-resistant soybean lines, but may also minimize the need for nematode tests, which are tedious and very difficult. These putative partial resis-

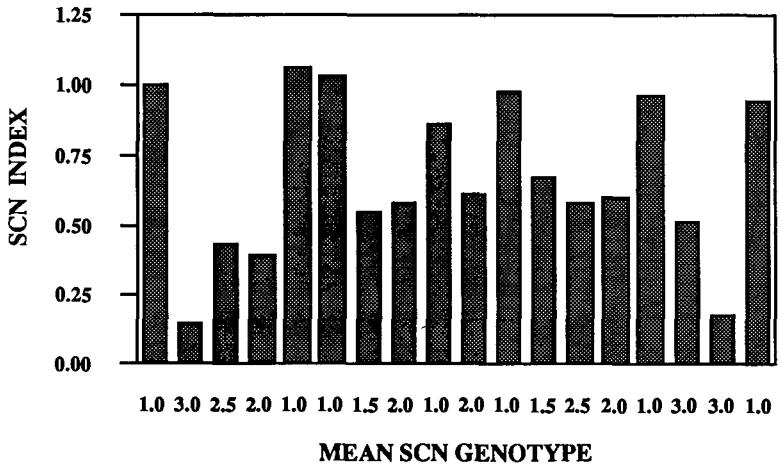

Fig. 3b. Bar chart illustrating the combined effects of RFLP markers pB32 and pA85 on the mean SCN indices of the 16 F2 progeny shown in Figure 3a. Vertical axis shows the mean SCN index for each individual plant. The first two bars on the left of the horizontal axis correspond to the two parents. The remaining bars correspond to the 16 F2 progeny. The average genotypic scores (based on the results of RFLP markers pB32 and pA85) is shown below the bars.

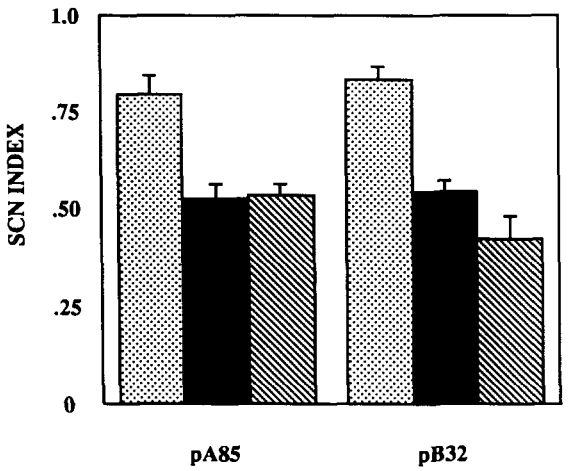

Fig. 4. Comparison of mean SCN indices for different genotypic classes of RFLP markers pB32 and pA85. Average SCN indices for the three genotypic classes [(▨) M83–15/M83–15, (■) M83–15/M85–1430, and (▨) M85–1430/M85–1430], including the standard errors of means, are shown.

Table 2. RFLP markers and linkage groups showing significant associations with SCN disease response.

Locus	Linkage Group	*r* (p-value)	*F*-test (p-value)	LOD† score	d/a‡	% variation
A85	A	0.15 (0.0041)	7.42 (0.0015)	2.88	1.1	21.4
B32	K	0.38 (0.0001)	17.80 (0.0001)	6.07	0.4	40.2

† LOD is the $\log_{10}$ of the odds ratio that supports evidence for the presence of a QTL at the locus.
‡ is the ratio of dominance to additivity, 0 indicates complete additivity, 1 indicates complete dominance.

tance loci can also be used to determine the genetic relationships among different sources of resistance. In the past, genetic relationships of known sources of SCN resistance were estimated using classical genetic approaches (Rao-Arelli and Anand, 1988; Anand and Rao-Arelli, 1989). With DNA markers, it may be possible to determine the genetic relationships among different sources of SCN resistance with greater accuracy by comparing genomic regions defined by linked DNA markers.

Through genetic dissection, it is also possible to evaluate the effects of individual resistance locus in detail. With DNA markers, lines that are homozygous for all putative resistance loci except one can be identified or developed. For example a line that is segregating for pB32 alleles and fixed for resistance in all other markers can be challenged with a SCN race. The resistance locus in question would then be allowed to segregate and its individual effect on SCN disease response evaluated without the influence of the other putative resistance loci. Experiments with this goal in mind are now underway for the genomic regions around RFLP markers pB32 and pA85.

The high level of genetic variability that exists among SCN field populations complicates breeding SCN resistant soybean cultivars (Cloud et al., 1988). Therefore, it is imperative to have a reliable race determination test. At present, race determination is based on parasitism of a given isolate on a set of four soybean differentials and is not entirely clear cut. However, in our F_2 population, we have identified two lines that are homozygous resistant for just one of the two major genomic regions described in this study. For example, one of the lines that we identified is homozygous for the resistance alleles of pB32, and homozygous for the susceptibility alleles of pA85. It will now be possible to evaluation the ability of this line to differentiate known races of SCN. Hence, we may potentially develop single-gene differentials that can be utilized in SCN race determination tests. Lastly, the use of DNA markers in genotypic selection offers the potential to combine different sources of SCN resistance into a common SCN background by pyramiding genes.

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