Association of Loci Underlying Field Resistance to Soybean Sudden Death Syndrome (SDS) and Cyst Nematode (SCN) Race 3

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ABSTRACT

Coinheritance of field resistance of soybean [*Glycine max* (L.) Merr.] to sudden death syndrome (SDS) [caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans.] and soybean cyst nematode (SCN) race 3 (caused by *Heterodera glycines* Ichinohe) sometimes occurs in crosses among adapted cultivars. Our objective was to characterize the loci underlying this coinheritance. One hundred thirty DNA markers were compared with SDS disease response and SCN score among 100 recombinant inbred lines (RILs) derived from a cross between SDS and SCN resistant 'Forrest' and SDS and SCN susceptible 'Essex'. SDS disease incidence (DI) was determined in replicated sites during 4 yr encompassing five locations. The SCN score was determined in the greenhouse from naturally infested field soil samples. Two separate genomic regions identified by random amplified polymorphic DNA (RAPD) markers $OI03_{450}$ and $OW15_{400}$ were associated with mean SCN score ($P = 0.0001$) and jointly accounted for about 47% of variability in SCN score. $OI03_{450}$ identified a QTL for resistance to SCN ($R^2 = 14\%$) within a genomic region that was strongly associated with SDS DI ($R^2 = 20\%$), partly explaining the coinheritance of the two traits. This locus could be assigned to the region of linkage group G already known to encompass the major SCN resistance locus.

An association between partial resistance to SCN race 3 and partial resistance to SDS has been observed among more than 1000 soybean cultivars tested in the field (Rupe et al., 1991, Gibson et al., 1994), wherein the association between susceptibility to SCN race 3 and SDS susceptibility is most clear. Inheritance studies with two populations derived from crosses between two different SDS and SCN race 3 resistant cultivars and two different SDS and SCN susceptible cultivars both showed this association can be highly heritable. SCN race 3 response accounted for about 50% of phenotypic variability in the response to SDS in those populations (Matthews et al., 1991; Njiti et al., 1996). However, both cultivar trials (Gibson et al., 1994) and inheritance studies (Njiti et al., 1996) show the beneficial effect of resistance to SCN on resistance to SDS is often environmentally sensitive. Therefore, selection of the SCN resistant phenotype does not assure SDS resistance. In addition, some cultivars that are resistant to SCN race 3 are quite SDS susceptible suggesting the association may be broken by recombination quite frequently.

Paradoxically, most of the original sources of SCN race 3 resistance, Peking, PI88.788, PI 90.763, and PI437.654 (but not PI209.332), are also very susceptible to SDS (Gibson et al., 1994). This suggests that the sources of SCN resistance lack the beneficial alleles at the loci contributing partial resistance to SDS or, since several loci are involved in resistance to SDS (Hnetkovsky et al., 1996; Chang et al., 1996), they contain more susceptibility alleles than resistance alleles. In either case, genetic association between loci underlying resistance to SDS and loci underlying resistance to SCN may contribute significantly to their co-inheritance in adapted germplasm.

Four loci conditioning SCN race 3 resistance have been defined (Rao Arelli and Anand, 1988). Three resistance loci have been located by genetic mapping as QTL (Concibido et al. 1994; Webb et al., 1995; Mahalingam and Skorupska, 1995), allowing their direct selection with DNA markers. Four genes for resistance to SDS have been located by genetic mapping as QTL (Hnetkovsky et al., 1996; Chang et al., 1996). Therefore, by means of DNA markers, the loci underlying the coinheritance of resistance to SDS and SCN may be identified.

In this paper, we show the utility of DNA genetic markers for identifying genomic segments underlying the coinheritance of partial resistance to a field population of SCN race 3 and to SDS. The implications of using DNA markers for improved selection of dual resistance to SDS and SCN in soybeans are considered.

MATERIALS AND METHODS

Plant Material

The cross of Essex (Smith and Camper, 1973) by Forrest (Hartwig and Epps, 1973) (E×F) was made and an F_5 derived population of 100 RILs generated. During the studies described herein the RILs were advanced to the $F_{5:11}$ generation. Residual heterogeneity was theoretically 6.25%; in fact, it was about

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Abbreviations: DAF, DNA amplification fingerprint; DI, SDS disease incidence; DS, SDS disease severity; DX, SDS disease index; IP, SCN index of parasitism; MG, maturity group; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; SCN, soybean cyst nematode; SDS, soybean sudden death syndrome.

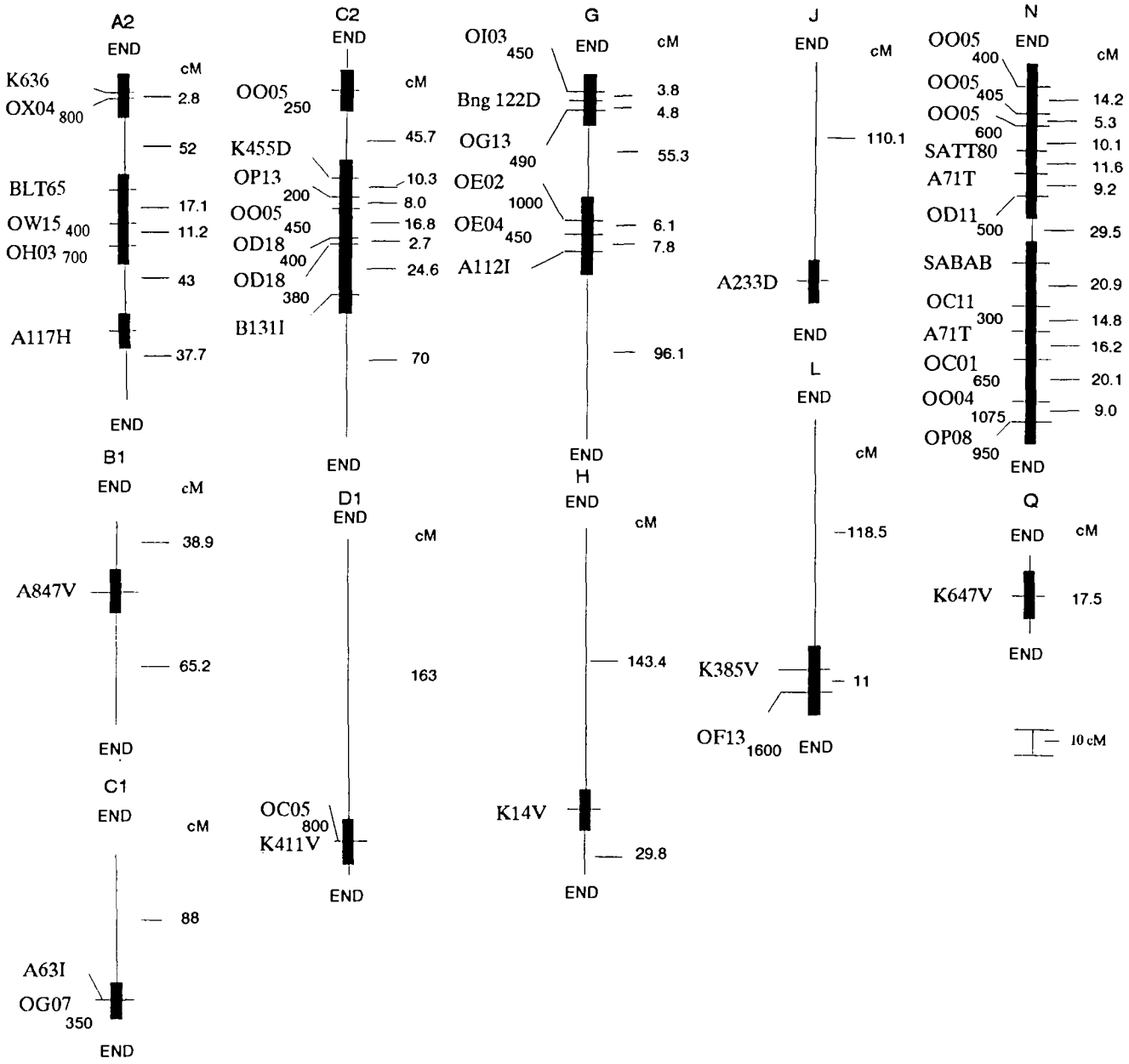

Fig. 1. Soybean linkage map from the Essex by Forrest RIL population. The map was generated by the computer program Mapmaker using locus segregation data for flower color, 27 RFLPs, 91 RAPD bands, 10 DAF bands, and three microsatellites. Loci for which linkage was not detected are not shown unless the locus was identified by an RFLP marker that was mapped with the same enzyme and the same band sizes as in Shoemaker and Specht (1995). These anchored RFLP markers were used to identify linkage groups where possible (e.g., A), groups with no such markers were numbered arbitrarily (e.g., SIU1), or tentatively in parentheses [e.g., SIU4 (D, J, or K)]. Marker loci are identified by a horizontal bar and a probe designation after Cregan et al. (1995). Two subscipts by a RAPD primer name indicates apparently codominant RAPD loci. Distances are given as Haldane centimorgans (cM). END indicates the likely position of the telomeres on linkage groups. Thick vertical bars represent regions of the genome less than 10 cM from the nearest marker. Thin vertical bars represent regions of the genome further than 10 cM from the nearest marker. Panel A: linkage groups A2, B1, C1, C2, D1, G, and H; Panel B: linkage groups J, L, N, and Q; Panel C: linkage groups SIU 1-12.

8% as detected by codominant markers (Hnetkovsky et al., 1996; Chang et al., 1996). Essex is susceptible to both SDS and SCN, while Forrest is resistant to both SDS and SCN race 3 (Fig. 1; Gibson et al., 1994). The potential for unintentional natural selection for SDS or resistance to SCN was reduced by all inbreeding being carried out in fields with low incidences of SCN and no history of SDS.

SDS Disease Scoring

The methods for SDS scoring of the E×F lines have been described in detail (Matthews et al., 1991; Hnetkovsky et al., 1996). Five field experiments were conducted during 4 yr in southern Illinois (Hnetkovsky et al., 1996). All fields were selected based on a history of visually uniform SDS incidences. Disease was rated weekly and the last score before and the first score after R6 (full pod) were used to standardize DI (1–100%) and DS (1–9) to the R6 stage. Trait data were used for QTL analysis both directly (Hnetkovsky et al., 1996) and after being normalized by serial arc sine transformation and back-transformation (Little and Hills, 1978, p. 139–164.) to test the effect of the trait distribution normality on the QTL and their interactions (Chang et al., 1996).

Table 1. Means of back-transformed disease incidences (DI) and disease severities (DS) of Essex, Forrest and 100 F_5 derived lines from the cross Essex × Forrest grouped based on reaction to SCN.

Gen.†	*n*	SDS DI	SDS DS [1-9]
		%	
Essex	8	58.3 d‡	1.56 d
Forrest	8	13.0 a	1.20 a
SCN-R	24	25.0 b	1.29 b
SCN-I	8	39.1 bc	1.35 bc
SCN-S	68	58.2 d	1.52 d
Mean	100	48.4 c	1.45 cd
h (%)	100	90	78

† Genotype or SCN class, R is resistant, I is segregating, S is susceptible.
‡ Means in a column followed by a common letter are not significantly different by pairwise *t*-tests ($P < 0.05$).

SCN Score Determination

The methods for SCN scoring of the E×F lines have been described in detail (Matthews et al., 1991; Njiti et al., 1996). Briefly, the lines were characterized for their resistance to SCN race 3 in the greenhouse by their reaction in soil containing a field population of *H. glycines* collected from Elkville, IL, in 1990. The race characterization of the soil was based on SCN disease reactions of standard differentials compared with Essex (Rao-Arelli and Anand, 1988). The pots were placed on top of a heating pad to regulate temperature, the temperature in the soil ranged from 20 to 23°C. The index of parasitism (IP) of each $F_{5:7}$ derived line was calculated from the average cyst count of six single-plant replications compared to Essex, 36 d after planting. Using six plant replications allowed us to detect segregation in lines with residual heterogeneity in genomic regions encompassing SCN resistance genes. Lines with an mean IP greater than eight and less than 25 were screened again but with 18 single plant replications. The group with resistance to SCN (Table 1) was defined as those lines with IP less than 10 (SCN score < 1.0). The group of lines defined as susceptible to SCN showed a IP of 10 or greater (SCN score ≥ 1.0). Lines segregating for resistance to SCN were identified by equal numbers of plants within the line with contrasting IPs. SCN IP was converted to a SCN score on a 0 to 5 scale to ease scoring and reduce within genotype variability associated with the assay method and the unselected field SCN population used. SCN score was defined as 0 = 0 IP; 1 = 1 to 10 IP; 2 = 11 to 20 IP; 3 = 21 to 60 IP; 4 = 61 to 100; 5 > 100 IP. Trait data were used for QTL analysis both directly (Concibido et al., 1994; Webb et al., 1995) and after being partially normalized by square root transformation (Thomas et al., 1975; Mansur et al., 1993) to test the effect of distribution normality on the QTL and their interactions.

Restriction Fragment Length Polymorphism (RFLP) Markers

Bacterial strains containing cloned soybean *PstI* genomic DNA inserts were obtained from Dr. R. Shoemaker, USDA-ARS, Ames, IA (Shoemaker and Specht, 1995). Polymorphic loci were detected and screened as described by Chang et al. (1996). Polymorphic RFLP loci were referred to after Cregan et al. (1995).

Microsatellite Markers

Microsatellite markers were generated and scored by 60 g L^{-1} denaturing polyacrylamide gel electrophoresis exactly as described by Akkaya et al. (1995).

Random Amplified Polymorphic DNA Markers

The amplification reactions were done after Williams et al. (1990) with 380 separate primers from kits A, B, C, D, E, F, G, H, I, J, L, M, N, O, R, S, W, and X from Operon Technologies, Inc. (Alameda, CA). DNA was amplified as described previously (Hnetkovsky et al., 1996; Chang et al., 1996). Most of the RAPD markers reported were amplified with Stoffel fragment including $OG13_{490}$, $OI03_{450}$, $OE02_{1000}$, $OE04_{450}$, $OR10_{390}$, and $OW15_{400}$. RAPD markers associated with resistance to SDS were amplified independently on three or more separate occasions to assure reproducibility. Polymorphic RAPD loci were referred to after Cregan et al. (1995).

DNA Amplification Fingerprint (DAF) Markers

DAF markers were bands generated by 8-mer primers exactly as described by Prabhu et al. (1994).

Mapping Quantitative Resistance Loci

To detect genomic regions associated with SCN score and resistance to SDS, the RILs were classified as Essex type or Forrest type for each marker. The heterogenous marker-line combinations identified by RFLP (about 8%) were excluded. Markers were compared with SDS disease response scores by the *F*-test in analysis of variance (ANOVA) done with SAS (SAS Institute Inc., Cary, NC, 1988). The probability of association of each marker with each trait was determined and a significant association was declared if $P \leq 0.005$ (unless noted otherwise in the text) to reduce the detection of false associations (Lander and Botstein, 1989).

Selected pairs of markers were analyzed by the two-way ANOVA PROC GLM procedure to detect non-additive interactions between the unlinked QTL (Lark et al., 1995; Chang et al., 1996). Non additive interactions between markers which were significantly associated with SDS response were excluded when $P \geq 0.005$. Selected groups of markers were analyzed by multi-way ANOVA to estimate joint heritabilities for traits associated with multiple QTL. Joint heritability was determined from the R^2 term for the joint model in multi-way ANOVA.

Mapmaker-EXP 3.0 (Lander et al., 1987) was used to calculate map distances (cM, Haldane units) between linked markers and to construct a linkage map. The RIL (ri-self) genetic model was used. The $\log_{10}$ of the odds ratio (LOD) for grouping markers was set at 2.0, maximum distance was 30 cM. Conflicts were resolved in favor of the highest LOD score after checking the raw data for errors. Marker order within groups was determined by comparing the likelihood of many map orders. A maximum likelihood map was computed with error detection. Groups were assigned to linkage groups by anchored RFLP markers (Shoemaker and Specht, 1995), where possible.

To identify the approximate position of QTL within intervals governing SDS response, the marker map and disease data were simultaneously analyzed with Mapmaker/QTL 1.1 (Paterson et al., 1988) using the F_2-backcross genetic model for trait segregation (after Webb et al., 1995; Hnetkovsky et al., 1996, Chang et al., 1996). Putative QTL were inferred when LOD scores exceeded 2.0 at some point in each interval, since this value was empiracally determined to be equivalent (but not equal) to a single marker $P < 0.005$, the criterium used in one-way ANOVA. The position of the QTL were inferred from the LOD peaks at individual loci detected by maximum likelihood tests at positions every 2 cM between adjacent linked markers.

RESULTS AND DISCUSSION

Polymorphism and Linkage

The present report summarizes the data from a total of 131 loci, including 106 unique loci and 25 loci that were less than 1 cM from a second marked locus, and therefore functionally one locus. Twenty-seven loci were identified by RFLP markers, 90 loci by RAPD bands, 10 loci by DAF bands, three loci by microsatellite markers, and one locus by a morphological marker (flower color). In total, 74 discrete loci were mapped to 23 linkage groups (Fig. 1). The mean interval was about 18 cM. Since major QTL for SDS resistance can be detected by markers 10 to 15 cM from the QTL peak ($P < 0.005$ or LOD > 2.0) (Hnetkovsky et al., 1996; Chang et al., 1996) the linkage groups encompass about 1102 to 1332 cM for the purpose of QTL detection. These linkage groups together with 31 unlinked markers would allow detection of associations with resistance to SDS QTL spanning about 1722 to 2262 cM. This compares with a recombination distance of more than 3000 cM for the soybean genome within 25 linkage groups (Shoemaker and Specht, 1995).

Frequency Distribution of SCN Score

Based on untransformed data, the mean SCN score was 2.72 and the standard deviation among progeny line means was 1.8. The distribution showed an abnormal ($W = 0.88$, $P = 0.0001$) bimodal, noncontinuous distribution with significant kurtosis (-1.25, $P < 0.01$) (Fig. 2). Square root transformation of the SCN score data failed to normalize the data ($W = 0.85$, $P = 0.0001$), with significant kurtosis (-1.10) and significant skewness evident (-0.51, $P < 0.01$). Therefore, the bimodal distribution was not simple.

DNA Markers Associated with Resistance to Cyst Nematode Race 3

Associations between markers and SCN score were made with both square root transformed and untransformed data in both ANOVA and Mapmaker QTL. Since the nature of the associations did not change substantially, the results with untransformed data are presented.

The locus identified by RAPD marker $OI03_{450}$ accounted for 14% of total variation in SCN score, with $P = 0.0001$ (Table 2). The RFLP marker Bng122D-1 was also strongly associated with SCN score ($P = 0.0001$) but was so closely linked (3.8 cM) that statistical values were similar to those for $OI03_{450}$. However, the linked (8.6 cM) RAPD marker, $OG13_{490}$ was not significantly associated with SCN score ($R^2 = 0.14$, $P = 0.056$). The beneficial allele for resistance to SCN race 3 was derived from Forrest, the resistant parent. Analysis by Mapmaker QTL suggested the QTL was associated with $OI03_{450}$ within the same genomic segment as a QTL with a major contribution to resistance to SDS locus (Fig. 3). Linkage to the anchored RFLP marker Bng122D-1 allowed the QTL to be putatively assigned to linkage group G and to be identified as the major QTL for resistance to SCN (Concibido et al., 1994; Webb et al., 1995; Concibido et al., 1995, 1997).

The locus identified by RAPD marker $OW15_{400}$ accounted for 16% of total variation in SCN score, with $P = 0.0001$ (Table 2). The linked (17.1 cM) RFLP marker BLT65 was also associated with SCN score ($P = 0.0001$, $R^2 = 0.24$). Therefore, they map to the region located on linkage group A2 associated with resistance to SCN race 3 (Webb et al., 1995; Mahalingham and Skorupska, 1995) (Fig. 1).

Multi-way ANOVA with both QTL jointly explained just 47% of SCN score variability. Interactions occurred ($P = 0.0068$) that explained an additional 10% of the variability. The QTL did not behave additively. With the Forrest (resistance) allele at $OI03_{450}$ but Essex (susceptibility) allele at $OW15_{400}$ (SCN scores 3.7 ± 0.3) or with the Forrest allele at $OW15_{400}$ and the Essex allele at $OI03_{450}$ (3.4 ± 0.3) mean resistance to SCN was not different from genotypes with two Essex alleles (4.0 ± 0.2). Genotypes with both Forrest alleles were significantly more resistant than the other allelic classes (SCN score = 1.4 ± 0.4); however, not all genotypes with both Forrest alleles at the loci were SCN resistant (SCN score <1.0). This suggests recombination between the DNA markers and their associated QTL or that one or two additional genomic regions affecting SCN race 3 resistance may remain to be discovered in this population (Webb et al., 1995; Concibido et al., 1995). However, the variability explained by the two QTL may be underestimated since neither QTL was delimited with two satisfactory flanking markers.

Comparative Genome Analysis for the QTL for Resistance to SCN

Of the loci strongly associated with resistance to SCN race 3 in other reports (Concibido et al., 1994; Mahalingam and Skorupska, 1995; Webb et al., 1995; Concibido et al., 1997; Vierling et al., 1996) both Bng122D-1 on linkage group G and BLT65 on linkage group A2 have been unequivocally identified in the E×F population. The Forrest QTL on linkage group G explains less variation (14%) than the equivalent QTL in PI437,654 (22%; Webb et al., 1995), in Peking (about 26%), in PI90,763 (45%), in PI88,788 (36%), or in PI209,332 (50%) (Concibido et al., 1997). This and other differences may derive from our use of a field population of *H. glycines* for SCN scoring rather than the single cyst isolates used in the other studies. Alternatively, the difference may derive from our lack of delimiting markers or that $OI03_{450}$ is not as close to the SCN resistance QTL as B53 or Bng30 (Concibido et al., 1995, 1997). The effect of the cyst source heterogeneity was noted by Vierling et al. (1996) using a fourth-generation SCN inbred for QTL mapping in PI437,654. We conclude that field resistance to SCN is more complex and may require more QTL of

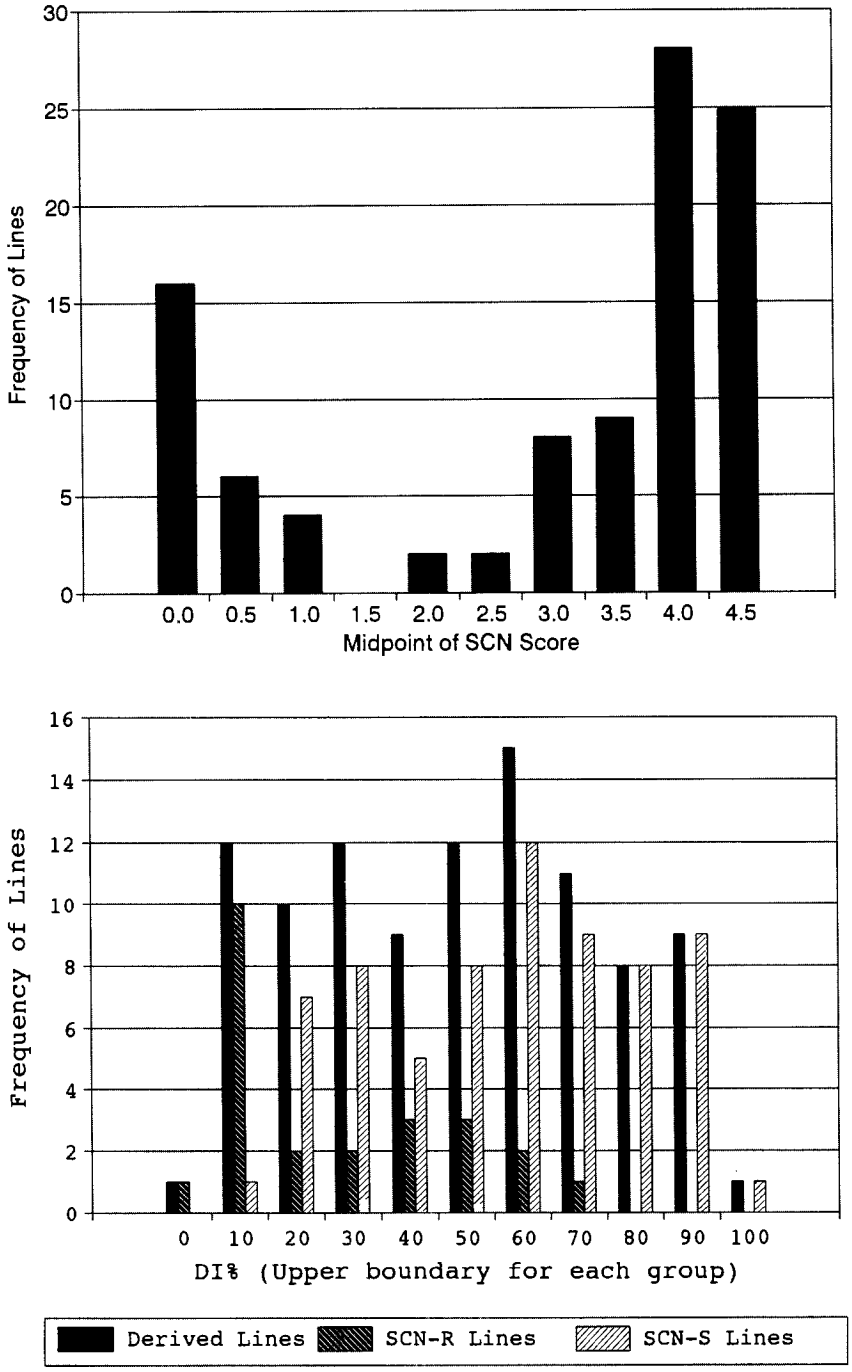

Fig. 2. Panel A: SCN race 3 score frequency distribution among 100 recombinant inbred lines (RILs). The mean SCN score for Essex was 4.0 (IP = 100) and for Forrest was 0.2 (IP = 2). The 24 lines with scores less than 1.0 (IP < 10) were classed as SCN race 3 resistant. Panel B: SDS DI frequency distribution of mean scores across five field locations among 100 recombinant inbred lines (RILs). The RILs were separated by SCN response class. The mean DI scores for each parent in the same environments are shown in Table 1.

smaller effect than the resistance to *H. glycines* measured with cyst populations containing reduced genetic diversity has indicated to date.

Further map integration by scoring additional RFLP markers (Shoemaker and Specht, 1994), converting RAPD bands to RFLP markers or sequence characterized amplified regions (Witsenboer, 1995) and the discovery of microsatellite markers (Akkaya et al., 1995) is needed to fully integrate the QTL maps for resistance to SCN.

Analysis of SDS Response within SCN Class

The population means for SDS DI based on the combined progeny line means and the progeny line means separated by the resistance to SCN class are shown in Table 1. Resistance to SCN race 3 is clearly associated with lower mean DI and DS in *F. solani* infested environments. The association occurred where cyst counts were low (1–10 per 100 cm^3 of soil) and moderate (Villa Ridge 1990, 150 per 100 cm^3 of soil) (Njiti et al., 1996).

Table 2. Markers showing significant ($P \geq 0.005$) associations with mean SCN score.

DNA Marker	Linkage group†	R^2	$P > F$	LOD‡	QTL var.§	SCN score for RILs with alleles from Essex	SCN score for RILs with alleles from Forrest
$OG13_{490}$	G	0.04	0.056	1.2	4.2	3.4 ± 0.2	2.7 ± 0.3
Bng122D	G	0.12	0.0002	2.5	11.3	3.7 ± 0.2	2.6 ± 0.3
$OI03_{450}$	G	0.14	0.0001	2.7	12.9	3.8 ± 0.2	2.5 ± 0.3
$OW15_{400}$	A2	0.16	0.0001	2.8	15.1	3.9 ± 0.2	2.3 ± 0.3
BLT65	A2	0.24	0.0001	5.1	23.2	3.9 ± 0.2	2.2 ± 0.3

† G was assigned from Concibido et al., (1995; 1996), based on Shoemaker and Specht (1995). A2 was assigned from Webb et al. (1995).

‡ LOD is the $\log_{10}$ of the odds ratio that supports evidence for the presence of the QTL at the locus from Mapmaker/QTL 1.1. nd is not determined because no flanking marker was available.

§ The percent variation associated with the interval from Mapmaker/QTL 1.1. nd is not determined because no flanking marker was available.

A Locus underlying the Coinheritance of Resistance to SDS and SCN

The region of linkage group G that contains a QTL for resistance to SCN race 3 and was strongly associated with resistance to SDS (Fig. 3) may partly explain the association between resistance to SDS and resistance to SCN in inheritance studies (Matthews et al., 1991; Njiti et al., 1996) and cultivar trials (Rupe et al., 1991; Gibson et al., 1994). The locus near $OI03_{450}$ explains about 20% of SDS DI and about 14% of SCN response (about 24% with interaction). This is still less than the 50% of phenotypic variability for SDS response coinherited with SCN response in Essex by Forrest (Matthews et al., 1991; Gibson et al., 1994).

We cannot distinguish between close linkage of the SDS QTL and the SCN QTL and pleiotropy of a single QTL by this analysis (Darvasi et al., 1993). Fine mapping and QTL introgression will be employed to distinguish these two hypotheses. Cross protection by a single gene product to pathogens from two separate kingdoms is not without precedent (Ward et al., 1991) but resistance gene clustering is common (Witsenboer et al., 1995).

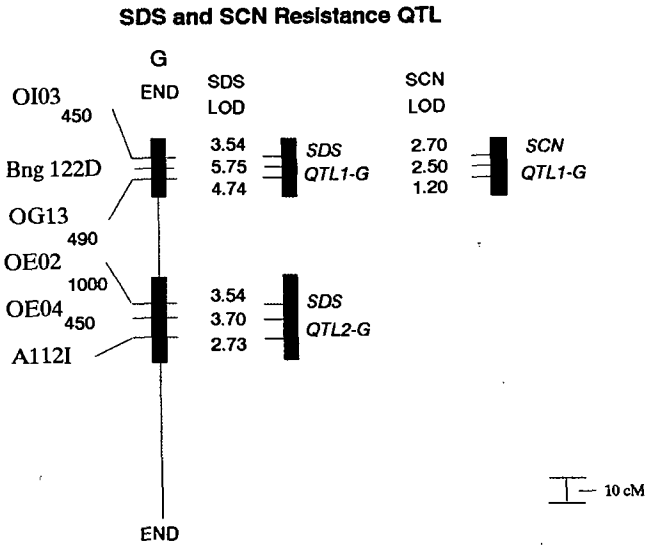

Fig. 3. Locations of two QTL conditioning SDS DI and one conditioning SCN race 3 resistance within a genomic region. The QTL were putatively assigned to linkage group G on the soybean genetic map by anchored RFLP (Shoemaker and Specht, 1995; Concibido et al., 1995). END shows the likely position of the telomere on a linkage group. The thin vertical bar indicates the rest of the linkage group. Marker names are given on the left and marker-QTL LOD scores are given on the right of each linkage group. MAPMAKER/QTL 1.1 generated LOD scores at markers from single-locus analyses of additive gene effects. MAPMAKER/QTL 1.1 was used to estimated the position of the QTL shown based on 2-cM interval mapping. Boxes indicate the region with greater than 1 – LOD (10 fold) likelihood intervals.

Interactions between SDS QTL and Resistance to SCN

Two-way ANOVA among markers identifying the QTL underlying SDS response (Hnetkovsky et al., 1996, Chang et al., 1996) and markers identifying the QTL underlying SCN response (Table 2) did not detect significant interactions ($P > 0.005$). In addition, two-way ANOVA between the two SCN resistance classes and markers identifying the QTL for resistance to SDS showed no interactions ($P < 0.005$). Therefore, epistatic interactions between QTL for resistance are not likely to contribute significantly to the coinheritance of resistance to SDS and SCN.

SUMMARY

Two of the soybean loci that influence resistance to field populations of SCN race 3 are reported here. The locus on linkage group G mapped to the same region of the genome as a major QTL conditioning resistance to SDS. The QTL might reflect the prescence of clustered genes for resistance in coupling in Forrest (see Witsenboer et al., 1995, and references therein) or the pleiotropic effect of a single gene (McLean and Lawrence 1993a, b). Most likely the genes underlying the QTL detected reflect the existance of a gene cluster.

The origin of the putative gene cluster in Forrest is of interest. SCN resistance in Forrest was derived from Peking by a modified backcross strategy (Hartwig and Epps, 1973) through Lee and Dyer. Peking [DX = 28.2 ± 4.4, maturity group (MG) 4 late] and Lee (DX = 25.6 ± 6.1, MG6) are very suceptible to SDS in replicated field tests (Gibson et al., 1994). Bragg the SCN susceptible parent of Forrest was also SDS susceptible (DX = 19.6 ± 7.1, MG6). However, Forrest is durably SDS resistant (DX = 9.3 ± 6.1, MG5) as is Forrest's SCN resistant parent, Dyer (DX = 9.3 ± 5.6, MG5). Critically, Dyer's SCN susceptible parent, Hill, is SDS resistant (DX = 11.0 ± 3.5, MG5) indicating the major SDS resistance loci in Forrest are derived from Hill not from Peking. SDS resistance in Hill may have arisen by recombination since all Hill's progenitors, CNS, S100, Dunfield (PI 36846), and Haberlandt (PI 6396), are SDS susceptible (DX > 20). Microsatellite markers and DNA sequence of markers tightly linked to the genes underlying the clustered QTL will be used to test these hypotheses.

Fine mapping of the QTL in near isogeneic line populations derived from the eight lines that segregated for SCN resistance (Table 1) is ongoing. This may ease map based cloning of the gene cluster underlying the coinheritance of resistance to SDS and SCN and lead to an understanding of its molecular basis.

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