Theor Appl Genet (1999) 99:1131–1142

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Clustering among loci underlying soybean resistance to *Fusarium solani*, SDS and SCN in near-isogenic lines

Received: 22 September 1998 / Accepted: 12 May 1999

Abstract In the soybean [*Glycine max* (L.) Merr.] cultivar 'Forrest' a single chromosomal region underlies co-inheritance of field resistance of the sudden-death syndrome (SDS), caused by the fungus *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* (Burk.) Snyd. & Hans. and soybean cyst nematode (SCN) race 3 (caused by *Heterodera glycines* Ichinohe). Our objectives were to verify that co-inheritance was derived from a single chromosomal region in near-isogenic lines and to separate component gene clusters. DNA markers were compared with a SDS leaf-scorch index (DX), *F. solani* root-infection severity (IS) and a SCN index of parasitism (IP) among 80 near-isogenic lines (NILs). The genomic region identified by the RFLP marker Bng122D was strongly associated ($0.0004 \leq P \leq 0.006$) with mean SDS DX ($R^2 >$ 16–38%) and IS ($R^2 >$ 38–73%), but only marginally associated with resistance to SCN. However, the linked (4.3–7.4 cM) microsatellite marker SATT309 was strongly associated with both resistance to SCN ($0.0001 \leq P \leq 0.0003$; $R^2 >$ 24–97%) and mean leaf DX ($0.0001 \leq P \leq 0.0003$; $R^2 >$ 25–63%), but not root IS. Recombination events among markers and traits enabled separation of the qualitative loci underlying resistance to SDS and SCN. Our data showed that resistance to SDS DX, SDS IS and SCN IP in Forrest may be caused by four genes in a cluster with two pairs in close linkage or by a two-gene cluster with each gene displaying pleiotropy, one conditioning SDS IS and DX and the other SCN IP and SDS DX.

Key words Near-isogeneic lines · Qualitative mapping · QTL · Disease resistance · Co-inheritance · Gene cluster · Pleiotropy

Communicated by M.A. Saghai Maroof

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Introduction

The continuous variation in phenotype observed for many important traits in agriculture is caused by the segregation of independent polygenes of small effect (Paterson et al. 1990). Polygenes can be detected and mapped within 10–20-cM intervals as quantitative trait loci, particularly with DNA markers. Such localization is sufficient for some aspects of their study and manipulation. However, highly accurate estimates of the QTL map location, within less than 1–2 cM, are necessary for effective marker-assisted selection (Prabhu et al. 1999), the dissection of genes within complex loci (Chase et al. 1997; Graham et al. 1997), and the physical mapping of genes underlying QTLs (Meksem et al. 1998).

Fine mapping of QTLs cannot be achieved in F_2 or RIL populations (Darvasi et al. 1993; Darvasi and Soller 1995). Errors in phenotype scores and the effects of loci that alter the trait independently of the QTLs for which the map is generated preclude accurate fine mapping of QTLs in such populations (Kearsey and Farquhar 1998). Fine-mapping relies on the analysis of derived sub-populations in which the QTLs can be further localized. Such methods include: substitution mapping in BC_2F_1 lines (Paterson et al. 1990); mapping in advanced intercross lines (Darvasi and Soller 1995); mapping in recombinant backcross inbred lines (Eshed and Zamir 1995); and mapping in re-

Table 1 Overview of the populations and QTL segregation in Essex × Forrest-derived RIL and NIL populations

Population	Type	Alleles[a] and linkage groups of						
		n	SDS QTLs				SCN QTLs	
			IG	2G	C2	N	1G	A2
ExF	RIL	100	Ha	H	H	H	H	H
ExF11	NIL	40	H	F	F	E	H	F
ExF34	NIL	40	H	E	E	H	H	F
Qualitative genes inferred		*rfs1*	nd	nd	nd	*rhg1*	*Rhg4*	

[a] Alleles at the major QTLs detected in Chang et al. 1997 are designated: E is the allele derived from Essex, F is the allele derived from Forrest and H indicates the population is heterogeneous across the interval containing that QTL

combinant inbred sub-line populations (Haley et al. 1994). Each of these related methods involves isolation of the QTLs to a 30–40-cM region using molecular markers, followed by the analysis of recombination events within this region in an otherwise homogeneous genetic background. A single cycle of recombination analysis can place QTLs within intervals less than 1 cM. Subsequent cycles generated by intercrossing novel recombination events will reduce the interval size still further, given a sufficient marker density and population size.

The degree of localization necessary for individual QTLs is likely to vary widely with the trait and locus studied. Both marker-assisted selection and separation of a beneficial QTL from a closely linked deleterious QTL will often be effective, with localization to a 1-cM interval. However, for gene isolation smaller intervals may be required since regions of the plant genoe vary widely in their recombination frequency per Mbp of DNA (Funke et al. 1993; Martin et al. 1993 a, b). The soybean (Xiaozhu et al. 1994) and the region of linkage group G studied herein are known to range from 70 kbp to 1000 kbp per cM (Danesh et al. 1998; Meksem et al. 1999). Therefore, the degree of marker saturation and the extent of fine mapping required for each QTL will vary.

The heritability of traits underlying QTLs can vary widely (Kearsey and Farquhar 1998). However, methods for the fine mapping and genetic dissection of QTLs can be applied to field-trait data where heritability of near unity is seldom achieved (Edwards et al. 1996; Njiti et al. 1998). In soybean, both the sudden-death-syndrome (SDS) resistance trait and the cyst-nematode (SCN) reistance trait are much more heritable (90–100%) than many field traits. Resistance to SDS should provide a model for detailed analyses of field-trait QTLs.

Resistance to SDS appears to be partly derived from a locus on linkage group G conferring rate-reducing resistance to colonization of the taproot by *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* form. nov., as well as loci on linkage groups C2, G and N that reduce foliar symptoms but not root infection (Hnetkovsky et al. 1996; Chang et al. 1997; Njiti et al. 1998). Resistance to SCN race 3 (caused by *Heterodera glycines* Ichinohe), whilst race-cultivar specific, is also incomplete and quantitative. It is largely derived from a locus in the same region of linkage group G and sometimes a second locus on linkage group A2 (Concibido et al. 1994, 1996; Webb et al. 1995). Analysis of both regions could be improved by the use of near-isogenic lines where genetic heterogeneity in other loci affecting the traits is reduced.

We show here the isolation and separation of two closely linked QTLs on linkage group G that underlie resistance to SDS and SCN, and identify the underlying gene clusters in near-isogenic lines.

Materials and methods

Plant material

The F_5-derived population of 100 RILs (Hnetkovsky et al. 1996; Chang et al. 1997) generated from the cross of 'Essex' (Smith and Camper 1973) by 'Forrest' (Hartwig and Epps 1973), (ExF), was advanced to the $F_{5:13}$ generation from never less than 300 plants per RIL per generation during these studies. Essex is susceptible to both SDS and SCN, while Forrest is resistant to both SDS and SCN race 3 (Gibson et al. 1994; Hnetkovsky et al. 196; Chang et al. 1997).

Two RILs, ExF11 and ExF34 were used to derive NIL populations (after Haley et al. 1994) of 40 sublines at the $F_{5:9}$ generation by seed-to-row descent (Njiti et al. 1998). From the $F_{5:9:11}$ to the $F_{5:9:13}$ generations the NIL populations were used to test for resistance to SDS and SCN.

Assays of resistance of SDS

The soybean NILs Essex and Forrest were planted in a randomized complete block design in two-row plots, three replications, and four Southern Illinois locations [Villa Ridge 1994 (V94), Ridgway 1995 (R95), Ullin 1995 (U95), and Ridgway 1996 (R96)]. The Ridgway soil type was Bonnie silt loam, fine-silty mix, acid, mesic Typic Fluvaquents; the Villa Ridge soil type was Belknap Silt Loam, coarse-silty, mixed, acid, mesic typic fluquavents; and the Ullin soil type was Patton silty clay loam, fine-silty mix, mesic, Typic Haplaquolls. Experiments were planted between 15 May and 15 June each year. Rows were 0.75-m wide and 3.0-m long, with about 17 plants/m. At Ridgway 1996 we assayed *F. solani* infection severity (IS), but DX was too low (Essex DX < 2.0) to distinguish resistant and suceptible cultivars or NILs. At V94, U95 and R95 we assayed DX where SDS was moderate to severe (Essex DX was 3.0–12; Njiti et al. 1998), but not IS since this method was not invented until 1996 (Njiti et al. 1997).

SDS disease scoring

The methods for SDS scoring of the ExF lines have been described in detail (Matthews et al. 1991; Hnetkovsky et al. 1996; Njiti et al. 1996). From the onset of disease, plots were rated weekly for disease incidence (DI; 0–100%), disease severity (DS; 1–9) and the reproductive stage (R; R0–R8) (Fehr et al. 1971). The disease index (DX, 0–100%) was calculated as DI*DS/9 based on the closest score to the R6 (full pod).

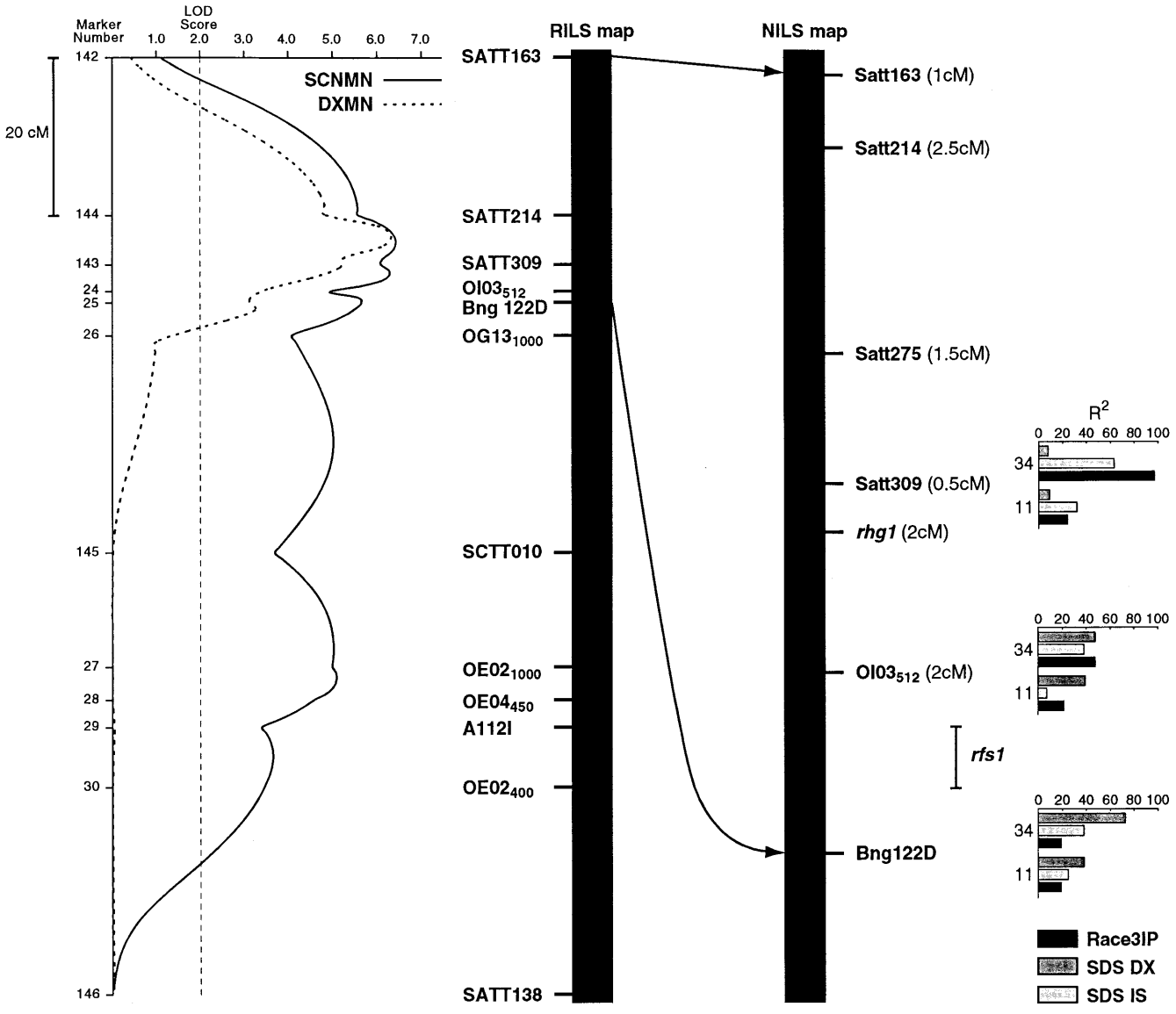


Fig. 1 Estimated positions of the SDS and SCN resistance genes on linkage group G, relative to SATT309, Bng122D and $OI03_{512}$, by quantitative and qualitative mapping in NILs compared to quantitative mapping in RILs. To the left is the LOD plot for the RIL population (n = 100) and the supporting map (Chang et al. 1996, 1997). *Numbers* correspond to the markrs on the RILs map in the center. To the right is the map from the combined NIL populations (n = 80) derived from ExF34 and ExF11. *Arrows* indicate the relative positions of flanking markers that are heterogeneous within the NIL populations. The map distances shown for the NIL populations have been reduced by 5-fold, rather than the normal 2-fold, to compensate for the increased recombination in NILs compared to RILs. This empirical adjustment is based on the effect of an increased frequency of heterozygous NILs from the F5:9 to F5:13. On the bar charts to the right the *dark bars* indicate the relative R^2 term from ANOVA at the marker for resistance to SCN (IP2) conditioned by the *rhg1* gene. The *dark gray bars* indicate the relative R^2 term form ANOVA at the marker for resistance to SDS leaf scorch (DX). The *light gray bars* indicate the relative R^2 term from ANOVA at the marker for resistance to root infection severity (*IS*). *Discontinuous lines* indicate longer distances. From qualitative gene mapping the position of the gene for resistance to SCN is shown as *rhg1*; the position of the *rfs1* gene for resitance to *F. solani* is shown as an *interval* to reflect the inaccuracy of scoring for this field trait

Root colonization by *F. solani*

The sample was taken at R8 (harvest maturity) from Ridgway in 1996. Following Njiti et al. (1997, 1998) five plants per plot were randomly harvested, recovering at least 15 cm of the taproot. Roots from sampled plants were transported on ice to the laboratory where they were stored at 4°C (1–7 days). Fifteen slices of taproot per sample were cultured on a restrictive medium at room temperature for 14 days. Pure colonies of all slow-growing fungi from each segment were transferred onto fresh medium and allowed to incubate at room temperature for 14 days. The percentage of plants yielding blue *F. solani* from at least one segment was determined as the infection frequency (IF). The percentage of segments yielding blue *F. solani* from all sampled plants of each plot was determined the infection severity (IS).

SCN index of parasitism (IP) determination

Two SCN indexes of parasitism were determined for each population by comparing the number of white female cysts on each genotype to the number of white female cysts on a susceptble check (Rao-Arelli and Anand 1988). The first index of parasitism (IP1) was determined on seedlings in the greenhouse at Southern Illinois University, Carbondale, 36 days after planting in soil from a field

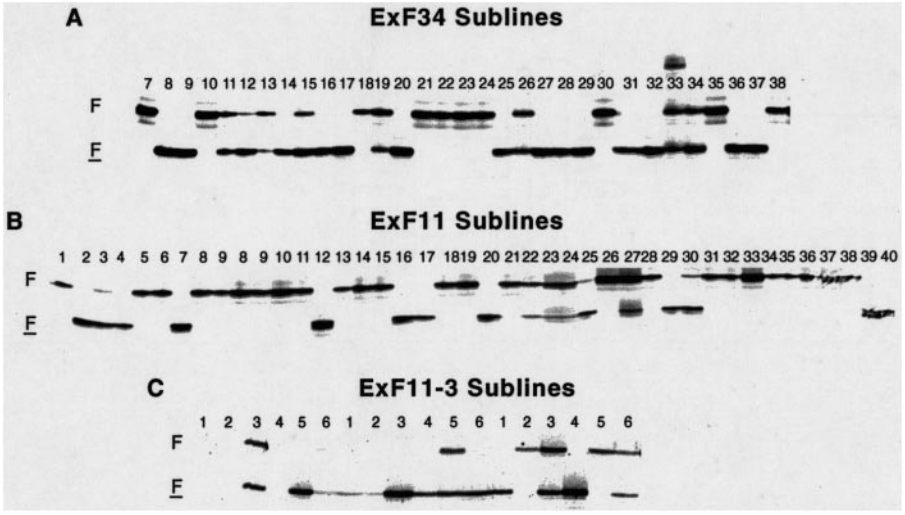


Fig. 2A–C DNA marker analyses in ExF34- and ExF11-derived near-isogenic line populations showing the high frequency of heterogeneous lines. Panel **B** ExF34 NILs 7–34 amplified with SATT309. Panel **B** ExF11 NILs 1–40 amplified with SATT309. Panel **C** ExF11–3 subline-derived single-plant subsubline populations amplified with SATT309

infested with a heterogeneous field population of *H. glycines*, race 3. Temperature was regulated by a heating pad and Essex was the susceptible check (Chang et al. 1997). The second index of parasitism (IP2) was determined on seedlings at the University of Missouri Research Center by inoculating the genotypes with 2000 ± 25 eggs from a homogeneous isolate of *H. glycines.*Temperature was regulated in a water bath and 'Hutcheson', (Rao-Arelli et al. 1992; Webb et al. 1995) was used as the susceptible check. All experiments used five single-plant replications per NIL. The mean number of white female cysts on each genotype and the susceptible check were determined, and IP was calculated as the ratio of the mean number of cysts on each genotype to the mean number of cysts on the susceptible check.

DNA markers

Bacterial strains contained cloned soybean *Pst*I genomic-DNA inserts were obtained from Dr. R. Shoemaker, USDA ARS, Ames, Iowa (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 were generated and scored by 6% (w/v) denaturing PAGE exactly as described by Akkaya et al. (1995). All markers used in this study were BARC markers except SIUCSAT122. The primer sequences are reported in Mudge et al. (1997) and/or are available through Soybase.

For RAPD-PCR markers the amplification reactions were after Williams et al. (1990) as described previously (Chang et al. 1996, 1997; Hnetkovsky et al. 1996).

Mapping resistance loci

To detect genomic regions associated with SCN IP and resistance to SDS the RILs were classified as Essex type or Forrest type for each marker. Markers were compared with SDS disease-respone scores by the *F*-test in an analysis of variance (ANOVA) done with SAS (SAS Institute Inc., Cary, N.C.). The probability of association of each marker with each trait was determined and a significant association was declared if $P \leq 0.05$ (unless noted otherwise in the text) since the detection of false associations is reduced in isogenic lines (Lander and Botstein 1989; Paterson et al. 1990).

Mapmaker-EXP 3.0 (Lander et al. 1987) was used to calculate the map distances (cM, Haldane units) between linked markers and to construct a linkage map including traits as genes. The RIL (ri-self) and F_3-self genetic models were used. The $\log_{10}$ of the odds ratio (LOD) for grouping markers was set at 2.0; the 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. Trait data were used for QTL analysis in Mapmaker/QTL 1.1 (Webb et al. 1995; Chang et al. 1997).

Mean comparison

The data were subjected to ANOVA (SAS Institute Inc., Cary, N.C.), with mean separation by LSD. Graphs were constructed by Quattro Pro version 5.0 (Novell Inc., Orem, Utah).

Results and discussion

Polymorphism and linkage

The present report summarizes the data from seven polymorphic marker loci. One locus was identified by an RFLP marker, two loci by RAPD bands and four loci by microsatellite markers. Markers that were not polymorphic in the near-isogenic-line population included the 74 discrete loci previously mapped to 23 linkage groups (Chang et al. 1997).

The ExF34-derived near-isogenic-line population was shown to be polymorphic in the region of linkage group G associated with resistance to SDS (Njiti et al. 1998) and SCN (Chang et al. 1997). The heterogeneous region of linkage group G encompassed about 10-27 cM. The DNA markers SATT163, SATT214, SATT275, SATT309, Bng122D and $OI03_{512}$, but not $OG13_{490}$ (Chang et al. 1997), segregated within this population see (Figs. 1, 2). Among the 40 $F_{5:9:13}$ NILs two recombination events were detected between Bng122D and $IO03_{512}$, eight between Bng122D and SATT309 and six between SATT309 and $OI03_{512}$. The number of recombination events was surprising in view of the close linkage (3.2 ± 1.0 cM) of these three markers reported in the $F_{5:9}$ RIL populations (Fig. 1; Chang et al. 1996, 1997). This distance represented six recombination events among 100 recombinant inbred lines between Bng122D and SATT309.

Table 2 Substitution mapping among recombination-event lines in the ExF 34-derived population either resistant or susceptible based on marker and disease scores

Line no.	SATT309 allele	SCN[a]		SDS[b]		$OI03_{512}$[c] allele	*F. solani*[d]		Bng122D allele
		IP1/IP2	Allele	Allele	DX		IS	allele	
3	H	0.23/0.81	E	E	24.8	E	56	E	E
9	E	0.77/1.08	E	E	14.6	F	31	F	F
13	H	0.31/0.56	H	E	10.3	E	nd	nd	E
17	E	0.48/1.10	E	E	19.5	F	30	F	F
19	H	0.34/0.28	H	E	15.4	F	19	F	E
23	F	0.00/0.02	F	F	1.4	F	60	E	E
26	H	0.11/0.72	H	F	5.9	F	53	E	F
28	E	0.50/1.38	E	E	11.5	F	36	F	F

[a] An SCN IP1 of less than 0.23 and more than 0.46 were the critical scores used to distinguish between resistant and susceptible lines. An SCN IP2 of less than 0.03 and more than 0.1 were the critical scores used to distinguish between resistant and susceptible lines. Heterogeneous lines (H) were identified by individual plants within a line being resistant or susceptible

[b] A DX of 5.9 or less and 10.3 or more were the critical values used to distinguish between resistant and susceptible lines for disease index

[c] $OI03_{512}$ is a dominant marker with a visible E allele; scores for the F allele were inferred by the absence of the E allele. The E allele could not be distinguished from the H state

[d] An infection severity of 36 or less and 54 or more were the critical values used to distinguish between NILs resistant and susceptible to *F. solani*

In addition, an unexpectedly ($P < 0.001$) large number (4) of heterogeneous NILs were identified with the microsatellite marker SATT309 (Fig. 2). Either sampling error has occurred where one heterozygous F5:9 genotype was sampled four times, or some NILs were mixed unintentionally, or some genetic region has been isolated in the NIL that selects for heterozygous progeny. Sampling error is unlikely because not all the heterogenous lines are identical by genotype or phenotype (Table 2) and not all satellite markers identify the same lines as heterogeneous (data not shown). Unintentional mixing of the NILs is equally unlikely since the RFLP probe Bng122D detected no heterogenous NILs (see below). These heterogeneous NILs must be derived from RIL plants that were still heterozygous at the F5:9. Errors in marker scores were further excluded by replications (4) from independent DNA preparations by independent researchers (3) for all three markers. Further, for $OI03_{512}$ the scores were confirmed by Southern hybridization to the RAPD profile with the cloned 512-bp band as a probe. To further confirm Bng122D scores we have isolated a BAC, identified a microsatellite marker (SIUC-SAT122) and scored it twice in the lines (Meksem et al. 1998, 1999). Heterogeneous NILs were confirmed by analysis of individuals within a single NIL (Fig. 2 C). Finally, we have generated a very large NIL population from ExF34 by crossing contrasting NILs. In this population we have detected a high frequency of recombination between SAT309 and Bng122 (Meksem et al. 1999 and seed is being prepared for trait scoring. Therefore, we conclude that these scores are accurate and do not represent the doubling of map distance per 3% marker miss-scores (Kearsey and Farquhar 1998). Rather, ExF34 may contain a genetic element that selects against the homozygous state in this region in NILs but not RILs. This genetic element promotes the maintenance of the heterozygous state in this region, causing recombination to be increased about 3-fold over the four generations between line-extraction (F_5) and subline-extraction (F_9).

The ExF11-derived near-isogenic-line population was shown to be polymorphic in the region of linkage group G associated with resitance to SDS. The heterogeneous region of about 10–17 cM again encompassed SATT163, SATT214, SATT275, SATT309, Bng122D and $OI03_{512}$, but not $OG13_{490}$ (Chang et al. 1997) which segregated within this population. Among the 40 $F_{5:9:13}$ NILs at least 13 recombination events were detected between Bng122D and $OI03_{512}$, 14 between Bng122D and SATT309, and at least two between SATT309 and $OI03_{512}$ (Table 3). The total number of recombination events (17) was surprising in view of the close linkage (2.0–4.2 cM) of these three markers reported in the $F_{5:9}$ RIL populations (Fig. 1; Chang et al. 1996; 1997) and greater than that seen in the ExF34 NIL population.

Unexpectedly ($P < 0.001$) large numbers (5–6) of heterogeneous NILs were identified with SATT309 (Fig. 2) and Bng122D (Table 3). Only one line was heterogeneous with both markers (# 3) and this NIL was shown to contain heterozygous plants at the F_{13} (Fig. 2, panel C). This plant has been used to generate a very large population of F_2 individuals and recombination has been shown to be elevated (data not shown). Clearly, sampling error has not occurred or NILs mixed unintentionally, and scores have been exhaustively verified as above. Therefore, we conclude that ExF11 and ExF34 both contain a genetic element that selects against the homozygous state in this region. This locus promotes the maintenance of the heterozygous state in this region and recombination is increased about 5-fold over the four generations between line-extraction (F_5) and subline-extraction (F_9).

Since the region is rich in recombination events it should be possible to develop a map of the resistance traits that are associated with this region as QTLs and as qualitative genes.

Table 3 Substitution mapping among recombination-event lines in the ExF 11-derived population either resistant or susceptible based on marker and disease scores

Line no.	SATT309 allele	SCN[a]		SDS[b]		$OI03_{512}$[c] allele	*F. solani*[b]		Bng122D allele
		IP1/IP2	Allele	DX	Allele		IS	allele	
17	F	0.11/0.01	F	1.9	F	E	nd	nd	H
14	E	0.38/0.26	H	4.1	E	F	nd	nd	H
33	F	0.03/0.45	H	3.7	E	F	nd	nd	H
28	F	0.18/0.01	F	4.5	E	F	nd	nd	H
36	F	0.18/0.00	F	1.2	F	F	22	F	H
3	H	0.08/0.02	F	1.6	F	F	nd	nd	H
1	F	0.08/0.01	F	5.9	E	F	50	E	E
5	F	0.18/0.75	E	4.9	E	F	12	F	E
35	F	0.35/1.09	E	2.6	E	F	10	F	E
39	E	1.11/0.92	E	4.1	E	E	23	F	F
13	F	0.26/0.01	F	5.3	E	F	29	F	F
19	E	0.45/0.68	E	2.6	E	E	nd	nd	F
18	E	0.30/1.06	E	4.0	E	E	nd	nd	F
15	F	0.10/0.02	F	3.0	E	E	nd	nd	F
10	F	0.07/0.02	F	1.5	F	F	55	E	E
16	F	0.11/0.01	F	0.9	F	E	56	E	E
4	E	0.37/0.60	H	5.6	E	E	nd	nd	E

[a] An SCN IP1 of less than 0.23 and more than 0.46 were the critical scores used to distinguish between resistant and susceptible lines. An SCN IP2 of less than 0.03 and more than 0.1 were the critical scores used to distinguish between resistant and susceptible lines. Heterogeneous lines (H) were identified by individual plants within a line being resistant or susceptible

[b] A DX of 5.9 or less and 10.3 or more were the critical values used to distinguish between resistant and susceptible lines for disease index

[c] $OI03_{512}$ is a dominant marker with a visible E allele; scores for the F allele were inferred by the absence of the E allele. The E allele could not be distinguished from the H state

[d] An infection severity of 36 or less and 54 or more were the critical values used to distinguish between NILs resistant and susceptible to *F. solani*

Frequency distributions of traits

Mean DX, IS, IP1 and IP2 did not show approximately normal ($P > 0.3$), continuous distributions. There is evidence for biphasic distribution in each trait although the separation is clear only for IP1. The other traits, representing field data, will include more error variance that is equalized, but not removed, by replication. Heritabilities were high for DX (> 70%), IS (> 78%) IP1 (> 75%) and IP2 (97%).

Correlations between traits

For both near-isogenic-line populations the traits of IP1, IP2 and SDS DX are significantly correlated ($0.36 < r < 0.97$; Table 4). However, SDS IS is not significantly correlated with the other traits ($0.09 < r < 0.24$). IS was previously shown to be significantly correlated with SDS DX in cultivar trials ($0.29 < r < 0.37$; Njiti et al. 1998). Such correlations were not improved by fixation of the genetic background in NILs. However, removing recombinant NILs from the correlations increases significance and *r* values, and some correlations while IS are significant ($0.45 < r < 0.66$). Therefore, the poor correlation between IS and the other traits may reflect the action of separate genes or loci.

DNA markers associated with SDS disease index and infection severity

To detect loci conditioning partial resistance to SDS, we tested associations between $F_{5:9:13}$ genotypic classes for each DNA marker and the corresponding mean SDS IS and DX. Significant ($P < 0.005$) effects on both IS and DX (Tables 5 and 6) were noted for the region on linkage group G (Chang et al. 1996, 1997; Njiti et al. 1998).

In the ExF34-derived population the associations with mean IS were highly significant. The RFLP marker Bng122D was associated with mean IS ($R^2 > 0.73$, $P < 0.0004$). The linked RAPD, $OI03_{512}$, accounted for 47% of the total variation in mean IS, with $P < 0.0017$ (Table 5). The microsatellite marker SATT309 was not significantly associated with mean IS.

The marker Bng122D was also associated with mean DX ($R^2 > 38\%$, $P < 0.006$) and marker $OI03_{512}$ accounted for 38% of total variation in mean DX, with $P < 0.0004$ (Table 5). The association was significant ($P \leq 0.01$) in all three locations. Surprisingly, given the IS data, SATT309 was strongly associated with DX ($R^2 > 68\%$, $P < 0.0001$). The data imply two QTLs in this region; that identified by SATT309 reduces DX but not IS, whereas that associated with Bng122D reduces IS and DX. Separate QTL positions are inferred from the eight NILs carrying recombination between Bng122D and SATT309 (Table 2).

Again in the ExF11-derived population, Bng122D and $OI03_{512}$, but not SATT309, were strongly associated

Table 4 Correlation between disease measures among near-isogenic lines (NILs); Trait-1, *n* = 40 (including recombinants); Trait-2 *n* = 32 or *n* = 23 (excluding recombinants). H IP1 was determined at Southern Illinois University at Carbondale using a predominantly race-3 field population of SCN. I IP2 was determined at the University of Missouri at Columbia using a homogeneous population of SCN race 3

Trait	SDS disease severity (DS)	SDS disease index (DX)	SCN index of parasitism (IP1)	SCN index of parasitism (IP2)	SDS infection severity (IS)
ExF34 NILs					
DI-1	0.86***	0.97***	0.57***	0.76***	0.18
DI-2	0.85***	0.97***	0.61***	0.78***	0.30
DS-1		0.95***	0.57***	0.66***	0.12
DS-2		0.94***	0.61***	0.72***	0.20
DX-1			0.58***	0.72***	0.17
DX-2			0.63***	0.77***	0.25
IP1-1				0.70***	0.04
IP1-2				0.71***	0.20
IP2					0.17
IP2					0.32
ExF11					
DI-1	0.66***	0.94***	0.36*	0.58***	0.19
DI-2	0.67***	0.95***	0.42*	0.65***	0.58*
DS-1		0.84***	0.50***	0.57***	0.09
DS-2		0.84***	0.61***	0.53**	0.49*
DX-1			0.47**	0.63***	0.22
DX-2			0.58**	0.66***	0.66**
IP1-1				0.44**	0.24
IP1-2				0.41*	0.62*
IP2-1					0.13
IP2-2					0.45

*, **, *** Significant at *P* = 0.05, 0.01, and 0.001 respectively

Table 5 Markers associated with disease resistance in the ExF34-derived near-isogenic line population

Trait	Marker								
	Bng122D			$OI03_{450}$			SATT309		
	P	R^2	Allelic mean ± SEM	P	R^2	Allelic mean ± SEM	P	R^2	Allelic mean ± SEM
SCN IP1[a]	0.050	23%	E 0.44 ± 0.19 F 0.19 ± 0.18	0.006	30%	E 0.55 ± 0.12 F 0.24 ± 0.11	0.0001	42%	E 0.44 ± 0.06 F 0.16 ± 0.05
SCN IP2[b]	0.073	19%	E 0.89 ± 0.14 F 0.44 ± 0.19	0.0001	47%	E 1.10 ± 0.06 F 0.37 ± 0.14	0.0001	97%	E 0.51 ±0.03 F 0.02 ± 0.02
V94DX[c]	0.046	23%	E 8.5 ± 1.40 F 4.5 ± 0.98	0.014	21%	E 9.5 ± 0.98 F 5.90 ± 0.95	0.0005	49%	E 9.66 ± 0.92 F 3.99 ± 0.93
U95DX	0.003	38%	E 8.0 ± 2.05 F 0.8 ± 0.18	0.001	35%	E 8.73 ± 1.67 F 2.03 ± 0.83	0.002	41%	E 7.01 ± 1.48 F 0.91 ± 0.42
R95DX	0.008	36%	E 27.9 ± 4.17 F 12.2 ± 2.54	0.0006	37%	E 31.2 ± 2.84 F 16.9 ± 2.33	0.0001	62%	E 30.11 ± 2.45 F 10.92 ± 2.42
MeanDX	0.006	38%	E 14.8 ± 2.36 F 5.7 ± 1.12	0.0004	38%	E 16.5 ± 1.63 F 8.29 ± 1.29	0.0001	63%	E 15.65 ± 1.37 F 5.28 ± 1.15
IS (R96)	0.0004	73%	E 56.3 ± 5.1 F 20.9 ± 4.5	0.0017	47%	E 51.0 ± 4.3 F 26.00 ± 4.9	0.0797	8%	E 48.43 ±4.93 F 45.80 ± 8.80

[a] SCN IP1 = SCN index of parasitism, measured in the greenhouse in SIUC, and was based on the number of cysts on the susceptible check, Essex

[b] SCN IP2 was calculated in the same manner as IP1 except that the data were collected after infection with SCN eggs at the University of Missouri research station compared to Hutcheson

[c] R94, Ridgway 1994; U95, Ullin 1995; R95, Ridgway 1995. Disease index at R6 stage; DX is DI*DS/9. IS is the infection severity by *F. solani*. E s the allele derived from Essex, F is the allele derived from Forrest

with mean IS. Bng122D accounted for 38% of the total variation in mean IS, with $P < 0.0039$ (Table 6). The RAPD marker $OI03_{512}$ was also associated with mean IS ($R^2 > 0.39$, $P < 0.017$).

The marker locus Bng122D accounted for 32% of the total variation in mean DX, with $P < 0.002$ (Table 6). The association was significant ($P \leq 0.05$) in all three locations. The RAPD marker $OI03_{512}$ was not associated with mean DX ($R^2 > 0.07$, $P < 0.09$), surprisingly, given the IS data. Again SATT309 was strongly associated with DX ($R^2 > 25\%$, $P < 0.002$) supporting the hypothesis of two QTLs in this region. Separate QTL positions are inferred from trait scores of the 16 NILs carrying recombination events between Bng122D and SATT309 (Table 3).

The associations between markers and QTLs tend to be weaker in the ExF11 population compared to the

Table 6 Markers associated with disease resistance in the ExF11 sub-line-derived near-isogenic line population

Trait	Marker Bng122D			$OI03_{450}$			SATT309		
	P	R^2	Allelic mean ± SEM	P	R^2	Allelic mean ± SEM	P	R^2	Allelic mean ± SEM
SCN IP1 [a]	0.42	3%	E 0.40 ± 0.04 F 0.24 ± 0.10	0.12	6%	E 0.42 ± 0.20 F 0.20 ± 0.05	0.003	24%	E 0.63 ± 0.24 F 0.14 ± 0.02
SCN IP 2[b]	0.02	19%	E 0.60 ± 0.12 F 0.21 ± 0.11	0.003	21%	F 0.62 ± 0.10 F 0.17 ± 0.09	0.002	25%	E 63.00 ± 12 F 17.00 ± 11
V 94 DX[c]	0.03	28%	E 6.68 ± 1.06 F 3.43 ± 0.86	0.21	9%	E 5.56 ± 0.94 F 3.69 ± 0.98	0.09	17%	E 5.92 ± 1.07 F 3.28 ± 0.91
U95DX	0.046	16%	E 2.19 ± 0.86 F 0.41 ± 0.19	0.24	4%	E 1.77 ± 0.59 F 0.94 ± 0.40	0.03	14%	E 2.70 ± 1.01 F 0.80 ± 0.35
R95DX	0.002	34%	E 9.58 ± 1.60 F 3.76 ± 0.72	0.11	7%	E 7.57 ±1.24 F 4.95 ±1.02	0.003	27%	E 9.47 ± 1.34 F 4.20 ±0.85
Mean DX	0.002	2%	E 5.91 ± 0.98 F 2.34 ± 0.42	0.09	7%	E 4.75 ± 0.77 F 3.04 ± 0.60	0.002	25%	E 6.11 ± 0.97 F 2.64 ± 0.53
Mean IS	0.0039	38%	E 43.2 ± 6.40 F 21.1 ± 1.80	0.0017	39%	E 42.9± 5.60 F 21.3 ± 3.20	0.183	9%	E 35.6 ± 7.6 F 25.2 ± 3.4

[a] SCN IP1 = SCN index of parasitism, measured in the greenhouse in SIUC, and was based on the number of cysts on the susceptible check, Essex

[b] SCN IP2 was calculated in the same manner as IP1 except that the data were collected after infection with SCN eggs at the University of Missouri research station compared to Hutcheson

[c] R94, Ridgway 1994; U95, Ullin 1995; R95, Ridgway 1995. Disease index at R6 stage; DX is DI*DS/9. IS is the infection severity by *F. solani*. E s the allele derived from Essex, F is the allele derived from Forrest

ExF34 population. Associations would be weakened by the many heterogeneous lines in the ExF11 population if gene action was codominant or recessive. Dominance relations are unknown for ficus resistance to SDS. For SDS scores (but not SCN scores, see below) associations may be weaker because greater resistance to SDS tends to increase the standard deviation as a proportion of the mean (Njiti et al. 1996, 1998). The greater resistance to SDS in ExF11 compared to ExF34 is derived from the second QTL on linkage group G for resistance to SDS identified by A112I (Chang et al. 1997; Fig. 1; Table 1). However, in both the ExF11- and ExF34-derived populations the QTLs for resistance to SDS and SCN, predicted to be closely linked to Bng122D and $OI03_{512}$ in RIL populations (Chang et al. 1996, 1997), are segregating in a second test population. The effect of higher-frequency recombination does not prevent detection of the QTLs. In fact the QTLs that were not separated in the RIL population analysis (Chang et al. 1997) can be distinguished by the analysis of NIL populations due to the high numbers of recombination events.

DNA markers associated with resistane to cyst nematode race 3

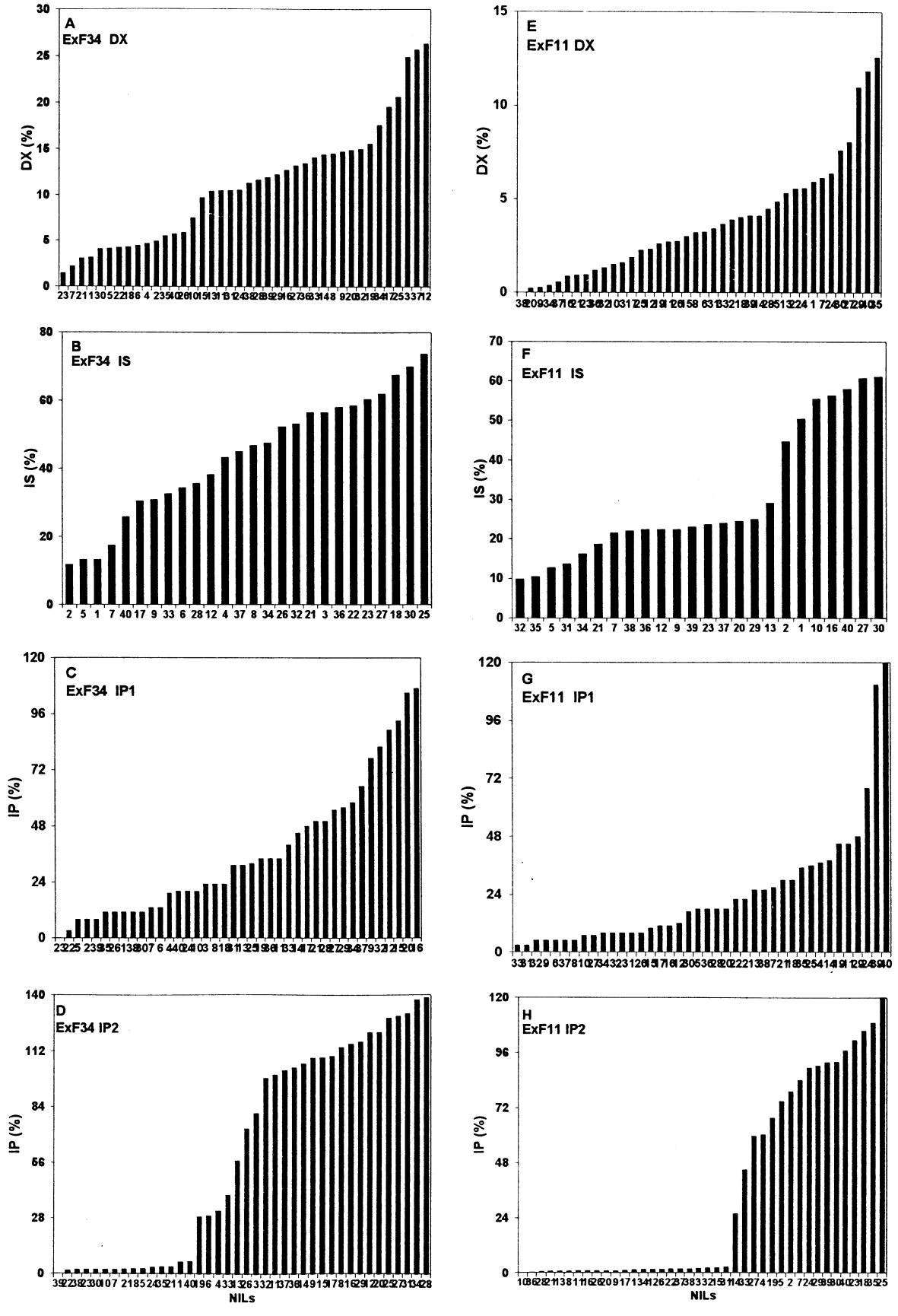
Associations between markers and SCN IP were determined by a one-way ANOVA. Only one chromosomal region had significant ($P < 0.005$) effects on resistance to SCN, the region identified on linkage group G reported previously (Chang et al. 1996, 1997).

In the ExF34-derived population the marker locus SATT309 accounted for 42% ($P < 0.0001$) of the total variation in mean SCN IP1 and 97% ($P < 0.0001$) of the total variation in IP2. RAPD marker $OI03_{512}$, accounted for 30% ($P < 0.006$) of the total variation in mean SCN IP1 and 47% ($P < 0.0001$) of the total variation in IP2. However, Bng122D accounted for only 23% and 19% of the total variation in IP1 and IP2 respectively (Table 5).

In the ExF11-derived population the locus identified by SATT309 accounted for 24% ($P < 0.002$) of the total variation in mean SCN IP1 and 25% ($P < 0.003$) of the total variation in IP2. RAPD marker $OI03_{512}$ accounted for 6% ($P < 0.12$) of the total variation in mean SCN IP1 and 21% ($P < 0.003$) of the total variation in IP2. However, Bng122D accounted for only 3% and 19% of the total variation in IP1 and IP2 respectively (Table 6).

The associations between markers and QTLs tend to be weaker in the ExF11 population compared to the ExF34 population. Associations would be weakened by the many heterogeneous lines in the ExF11 population if gene action was codominant or recessive, as predicted for resistance to SCN (Rao-Arelli et al. 1988; Concibido et al. 1994). However, in both the ExF11- and ExF34-derived NIL populations the QTLs for resistance to SDS and SCN, detected in RIL populations (Chang et al. 1996, 1997), are segregating in a second and third test population. The effect of a higher frequency of recombination does not prevent detection of the QTLs. In fact

Fig. 3A–H Distributions of disease resistance traits with NIL populations arranged by trait score. The ExF34-derived NIL population (**A–D**) and the ExF11-derived NIL population (**E–H**) were analyzed for SDS disease index (**A, E**), *F. solani* infection severity (**B, F**), SCN IP1 (**C, G**) and SCN IP2 (**D, H**)


A
ExF34 DX
DX (%)
B
ExF34 IS
IS (%)
C
ExF34 IP1
IP (%)
D
ExF34 IP2
IP (%)
NILs
E
ExF11 DX
DX (%)
F
ExF11 IS
IS (%)
G
ExF11 IP1
IP (%)
H
ExF11 IP2
IP (%)
NILs

the QTLs that were not separated in the RIL population analysis (Chang et al. 1997) can be distinguished by analysis of NIL populations due to the high numbers of recombination events.

Mapping the QTLs for disease resistance as qualitative markers

The discontinous distribution of the SCN IP, and to a lesser extent SDS DX and SDS IS, suggested that separation of genotypes into resistance classes was valid for many genotypes for each trait (Fig. 3, Tables 2 and 3). We selected mean separations based on the LSD 0.01 (appropriate for these small populations) and evidence of discontinuity in the populations (Fig. 3).

The marker order shown minimizes double-recombination events within the ExF34 population and is the most likely order. Flanking and intervening marker analysis has confirmed that the order is correct (Meksem et al. 1999). As expected from the marker-trait associations (Table 5), IS and DX do no agree in six of seven of the NILs representing recombination events. Thee recombination events infer a cluster of different genes within the locus for reistance to SDS. The phenotypes of the NILs with recombination events are unlikely to represent scoring errors for DX or IS becaue they are in good agreement among 10 of the 15 NILs that have not had a recombination event and for which IS scores are above 54% or below 34% (Fig. 3). Further, the DX data is a mean of three environments over 2 years providing a total of nine replicates. Within the data-set the variability among NILs representing recombination events was normal. Nine replications have been sufficient to distinguish resistant from susceptible RILs and cultivars without error compared to data from 18 replicates over 5 years (Gibson et al. 1994). The IS data is from a single year and location, providing a total of three replicates within which variability was normal. Three replications have been sufficient to distinguish resistant from susceptible NILs and cultivars without error when compared to data for the same genotypes from 18 replicates over 3 years (Njiti et al. 1997, 1998).

There were four ExF34-derived lines that are heterogeneous at the microsatellite marker SATT309 (Fig. 2). Three of these lines (# 13, # 19, # 26) are heterogeneous for SCN resistance (Table 2) and contain plants that contrast for their SCN resistance (data not shown). SCN resistance was expected to behave as a recessive gene, *rhg1* (Rao-Arelli et al. 1988, 1992; Webb et al. 1995, Concibido et al. 1996). Two of the three heterogeneous lines are susceptible by mean SDS DX; however, DX is biased by calculation from DS scores based only on symptomatic plants within a row. Therefore, lines heterogeneous for SCN resistance would have higher DX scores than the intermediate score if pleiotropy or close clustering of loci occurs.

Within the ExF11-derived population 17 of the 40 lines appear to contain recombination events in some interval. This includes all six of the lines that are heterogeneous for Bng122D and one of the five lines heterogeneous for the microsatellite marker SATT309 (Table 3; Fig. 2). Three of the lines (# 4, # 14 and # 33) are heterogeneous for SCN resistance, which is expected to behave as a recessive gene, *rhg1* (Rao-Arrelli et al. 1988, 1992; Webb et al. 1995; Concibido et al. 1996), or be codominant (unpublished). Three of the six lines (# 14, # 28, and # 33) appear to contain double recombination events becaue they are susceptible by SDS DX. However, mean DX is biased by calculation from DS scores based only on symptomatic plants within a row. Therefore, heterogeneous-line DX scores would be higher than the intermediate score.

There are three genotypic classes within the six lines heterogeneous for Bng122D based on DNA markers and SCN IP, suggesting that these lines are not produced by sampling error. Three lines (# 1, # 5, # 35), that may represent double recombination events based on the RIL and ExF34-derived NIL population gene order, were identified that could not be explained by a bias in DX caused by heterogeneous lines. Overall, recombination events in the three intervals were about equal with 4–6 each, depending of whether ambiguous SDS scores were heterogeneous (H) or not. The marker and gene order shown in Table 3 generates the fewest double-recombination events and is the most-likely order. As in the ExF34-derived population, IS and DX do not agree in six of the non-heterogeneous recombination event near-isogenic lines (Table 3) but show strong association in the population as a whole (Table 6; Fig. 3). These six EXF11-derived genotypes and the six ExF34-derived genotypes infer a cluster of different genes within the locus for resistance to SDS. Alternately, they might represent scoring errors for DX or IS. DX might be mis-scored more often in ExF11-derived populations due to the higher degree of resistance in this population derived from SDS QTL-2G (Fig. 3; Njiti et al. 1998). However, as discussed above, agreement among the NILs that have no recombination events between the markers, and the extensive replication of trait data, make wholesale error unlikely.

Error in trait data among 1–2 lines per population would not alter the major conclusion that QTLs for resistance to SDS can be separated by both DX scores and IS scores, two contrasting methods. The errors become more critical as marker saturation and the numbers of recombination events studied increase. We are currently engaged in AFLP and recombination-event saturation of this interval to generate a 0.1-cM map for *rhg1* isolation. However, the error in scoring phenotype that cannot be avoided with field trait QTLs such as resistance to SDS is a problem. We are attempting to bypass the problem by large-scale physical mapping of contiguous BAC clones across a large (2-20 cM) interval (Zhang and Wing 1997; Meksem et al. 1999) followed by the transgenic complementation of phenotype in a T-DNA BAC vector (Hamilton et al. 1996; Meksem et al. 1998). Saturation and physical mapping will allow unequivocal sep-

aration of *rhg1* and *rfs1*, dissection of each locus structure, and, ultimately lead to the isolation and DNA sequence determination of genes providing resistance to agriculturally important plant root pathogens.

Implications for breeding for resistance

We have shown that the cluster of QTLs underlying resistance to SDS and resistance to SCN race 3 can be isolated in near-isogenic-line populations (Tables 5 and 6). Within these populations the QTLs behave as qualitative genes and accordingly can be assigned gene symbols (Cregan et al. 1995). Recombination events between DNA markers and qualitative resistance to SDS (IS) and SCN show that the traits are encoded by separate loci that may correspond to *rhg1* (Webb et al. 1995) and *rfs1* (Torto et al. 1996). The DX data infer that *rhg1* or a very closely linked locus (*rfl* resistance to fusarial leaf scorch?) has a significant effect in reducing leaf necrosis by SDS. This may explain why SCN race-3 resistance can explain 50% of the variability in SDS response when *rfs1* alone can explain just 25% of that variation in RIL populations (Chang et al. 1997).

Elevated recovery of heterozygous lines was noted in the ExF11 population, accompanied by elevated recombination frequencies. The genetic element responsible probably lies within the resistance gene cluster since there were more heterogeneous lines with the SATT309 and Bng122D markers than with the others tested (Meksem et al. 1999). The genetic element might be a single gene or a complex locus. Fine mapping with codominant markers may distinguish these possibilities.

Genetic elements causing an increase in recovery of heterozygotes have been noted in several interspecific crosses among inbred crops including tomato (*Lycopersicon esculentum* × *L. chimlewski*; Paterson et al. 1990) and bean (*Phaseolus vulgaris* × *P. coccineus*; Guo et al. 1991). In soybean, within an intraspecific cross (PI437.654 × BSR101; Webb et al. 1995), the PI437.654 allele of *rhg1*, the region we studied, was rarely recovered without the PI437.654 allele at a locus on linkage group M. In our laboratory we have noted elevated recombination in the same region in Pyramid × Douglas RILs (Abu-Thredeih 1998) and Manokin × Flyer RILs (unpublished). However, recombination was not elevated in Hartwig × Flyer RILs (Prabhu et al. 1999), PI437654 × BSR 101 RILs, or in RILs from susceptible by susceptible crosses (Mansur et al. 1996). Therefore, the genetic element detected in ExF11- and ExF34-derived NIL populations may be rare or be repressed by outcrossing. Such a locus close to a major-fitness determinant may be adaptive during inbreeding, generating increased opportunity for reassortment of the resistance gene cluster. The locus may promote meiotic drive, gametic selection or both.

Ackowledgements Particular thanks to Dr. M.E. Schmidt, R.T. Suttner and J.H. Klein III for excellent management of the field program in southern Illinois. Thanks also to all the workers on the SDS field team at Southern Illinois University at Carbondale from 1994–1997. Finally, we thank Dr. O. Myers Jr. for critical reading of the manuscript. This work was supported in part by grants from the Illinois Soybean Program Operating Board Nos. 93-19-132-3 and 94-20-143-3, and North Central Soybean Program Operating Board Nos. 95-20-431 and 95-20-432.

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