Theor Appl Genet (2008) 116:967–977
DOI 10.1007/s00122-008-0728-0

ORIGINAL PAPER

Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome

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Received: 27 February 2007 / Accepted: 1 February 2008 / Published online: 7 March 2008

Abstract Soybean [*Glycine max* (L.) Merr.] cultivars show differences in their resistance to both the leaf scorch and root rot of sudden death syndrome (SDS). The syndrome is caused by root colonization by *Fusarium virguliforme* (ex. *F. solani* f. sp. *glycines*). Root susceptibility combined with reduced leaf scorch resistance has been associated with resistance to *Heterodera glycines* HG Type 1.3.6.7 (race 14) of the soybean cyst nematode (SCN). In contrast, the *rhg1* locus underlying resistance to Hg Type 0 was found clustered with three loci for resistance to SDS leaf scorch and one for root infection. The aims of this study were to compare the inheritance of resistance to leaf scorch and root infection in a population that segregated for resistance to SCN and to identify the underlying quantitative trait loci (QTL). "Hartwig", a cultivar partially resistant to SDS leaf scorch, *F. virguliforme* root infection and SCN HG Type 1.3.6.7 was crossed with the partially susceptible cultivar "Flyer". Ninety-two F5-derived recombinant inbred lines and 144 markers were used for map development. Four QTL found in earlier studies were confirmed. One contributed resistance to leaf scorch on linkage group (LG) C2 (Satt277; $P = 0.004$, $R^2 = 15\%$). Two on LG G underlay root infection at R8 (Satt038; $P = 0.0001$ $R^2 = 28.1\%$; Satt115; $P = 0.003$, $R^2 = 12.9\%$). The marker Satt038 was linked to *rhg1* underlying resistance to SCN Hg Type 0. The fourth QTL was on LG D2 underlying resistance to root infection at R6 (Satt574; $P = 0.001$, $R^2 = 10\%$). That QTL was in an interval previously associated with resistance to both SDS leaf scorch and SCN Hg Type 1.3.6.7. The QTL showed repulsion linkage with resistance to SCN that may explain the relative susceptibility to SDS of some SCN resistant cultivars. One additional QTL was discovered on LG G underlying resistance to SDS leaf scorch measured by disease index (Satt130; $P = 0.003$, $R^2 = 13\%$). The loci and markers will provide tagged alleles with which to improve the breeding of cultivars combining resistances to SDS leaf scorch, root infection and SCN HG Type 1.3.6.7.

Introduction

Among the top four loss causing diseases of soybean [*Glycine max* (L.) Merrill.], worldwide were the root rot and leaf scorch called Sudden Death Syndrome (SDS; Wrather et al. 1996, 2003). Over a 5-year period, 1999–2004, average losses around 1%, or 0.9 million Mg per harvest, worth \$190 million a year, were reported. The syndrome was

Communicated by I. Rajcan.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0728-0) contains supplementary material, which is available to authorized users.

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accurately predicted to intensify and spread over the next 20 years (Scherm and Yang 1996). Improved genetic resistance in germplasm releases will be key to containing soybean losses to SDS (Gibson et al. 1994; Kazi et al. 2007).

SDS was shown to be caused by the blue-pigmented soil borne fungus *Fusarium virguliforme* (Aoki et al. 2003; ex. *Fusarium solani* (Mart.) Sacc. f. sp. *glycines*; Fsg; Roy 1997). *F. virguliforme* is a member of an evolutionary group known as the "*F. solani* complex" that colonize a wide variety of habitats and hosts (Gray et al. 1999; O'Donnell 2000). They are serious pathogens of many crops. Analysis in North America showed that only *F. virguliforme* prompted the symptoms of SDS on soybean but in South America two separate species, *F. tucumaniae* and *F. virguliforme*, were both responsible for SDS (Aoki et al. 2003; Covert et al. 2007).

The genetics of resistance to SDS is complex. Stephens et al. (1993) reported that a single dominant gene, *Rfs* controls SDS resistance in "Ripley" soybean in greenhouse conditions. In contrast, the "Essex" × "Forrest" (E × F) population (Hnetkovsky et al. 1996; Chang et al. 1996; Kassem et al. 2006) showed that the SDS resistance was conditioned by several quantitative trait loci (QTL). By 2007, more than 20 detections of QTL for resistance to SDS have been reported among eight different recombinant inbred line (RIL) populations (Supplementary Table 1). By assigning QTL detected in overlapping intervals to the effect of a single locus, the QTL may be assigned to as few as 12 *qRfs* loci on nine linkage groups (LGs) including A2, C2, D2, F, G, I, J, L and N. The map of E × F showed three (Kassem et al. 2006) or four QTL (Iqbal et al. 2001) that mapped to LG G and one on each of LGs C2, F, J, I, L and N (*qRfs*1 to *qRfs* 9).

Some QTL for resistance to SDS have been confirmed and suffixed *cqRfs*- (Triwitayakorn et al. 2005; Lightfoot 2008). The confirmed QTL either mapped to a similar location in separate populations or were mapped for a second time in near isogeneic lines (NILs) derived from RILs segregating across regions that encompassed the QTL. The confirmed QTL include C2 (Hnetkovsky et al. 1996; Njiti et al. 1998; 2002), one on D2 (Lightfoot et al. 2001; Farias-Neto et al. 2007), three all on G (Prabhu et al. 1999; Iqbal et al. 2001; Njiti et al. 2002), J (Sanitchon et al. 2004; Kassem et al. 2006) and N (Njiti et al. 2002; Hashmi 2004). The E × F QTL on F and I (Iqbal et al. 2001) were not yet confirmed by association in a second population by late 2007. Similarly not confirmed to date were the QTL found on A2 in Ripley by "Spencer" (Hashmi 2004; Farias-Neto et al. 2007); L in "Minsoy" × "Noir 1" (Njiti and Lightfoot 2006) and H in E × F grown in Argentina (Bashir 2007).

Some cultivars of soybean have a dual resistance to SDS leaf scorch and root infection by the causal organism, *F. virguliforme* that was consistent in both field and greenhouse (Njiti et al. 1997, 2001, 2003; Hartman et al. 1997). Among dually resistant lines are Forrest, "Hartwig", "Jack", Ripley and several commercial lines. Most of the dually resistant lines are also resistant to *Heterodera glycines* HG Type 0 (race 3) of the soybean cyst nematode (SCN). Subsequently, linkage and pleiotropy with loci underlying resistance to SDS have been detected at the SCN resistance locus *rhg1* but not *Rhg4* (Meksem et al. 1999; Triwitayakorn et al. 2005; Ruben et al. 2006).

In contrast, cultivars that show root susceptibility to *F. virguliforme* combined with SDS leaf scorch resistance (like "Pyramid", "Fayette" and "LS92-1920") have been associated with resistance to *H. glycines* HG Type 1.3.6.7 (race 14) of SCN (Gibson et al. 1994) across a wide collection of germplasm. Consequently, repulsion linkage and/or pleiotropy is expected with loci underlying resistance to SDS at loci that underlie resistance to Hg Type 1.3.6.7 (Webb et al. 1995; Lightfoot et al. 2001; Schuster et al. 2001; Concibido et al. 2004).

Preliminary separation of loci underlying root and leaf resistance used NILs to show a single root resistance locus in Forrest (*cqRfs1*, requested to be renamed *cqSDS-003*) was about 10 cM from cq*Rfs2/rhg1* gene cluster that separately conferred partial resistance to SCN and SDS leaf scorch (Njiti et al. 1998; Meksem et al. 1999; Triwitayakorn et al. 2005; Supplementary Table 1). The other loci on G (*cqRfs2*; or *cqSDS-002*) the locus *Rhg4* on LG A2 and the locus on C2 (*cqRfs4*; or *cqSDS-004*) were shown to have no effect on root infection (Njiti et al. 1998; Triwitayakorn et al. 2005).

The cultivar Hartwig was resistant to both leaf scorch and root rot (Wrather et al. 1995; Njiti et al. 1997, 2001; Mueller et al. 2003) and HG Type 1.3.6.7 (race 14) of SCN. Therefore, Hartwig might contain superior alleles underlying a combined SCN and SDS resistance. Cultivar Flyer was susceptible to SCN and both leaf scorch and root rot of SDS (Njiti et al. 1997, 2001). RILs were developed from the cross of Flyer × Hartwig (F × H), released (Kazi et al. 2007) and used for preliminary QTL detection (Prabhu et al. 1999). A locus for resistance to root infection (*Rfs1*) was detected on LG G in the same interval as *rhg1* but not *Rhg4* in E × F (Prabhu et al. 1999; Supplementary Table 1).

The mechanisms underlying resistance to root infection by *F. virguliforme* appear to include the increases in the abundance of transcripts encoded by stress- and defense-related genes (Iqbal et al. 2005). The response, over time, prevents the inhibition of cellular transcription found in susceptible roots. In turn, the *F. virguliforme* genome encodes several pathogenicity factors found in other plant pathogenic species within the section Martiella of the genus *Fusarium* (Dr. K. Meksem, SIUC, personal communication; and Dr. S. Covert, University of Georgia, personal communication 2007). These general plant pathogen responses might underlie the association between resistance to SCN and SDS. However, other mechanisms of resistance do operate. For example, since the pathogen is active in lignin degradation (Lozovaya et al. 2005), plant processes related to isoflavonoid production (Iqbal et al. 2002; Lozovaya et al. 2004), lignin deposition or modification (Triwitayakorn et al. 2005) might help prevent infection.

Mechanisms for leaf scorch development were expected to include infection rate and pathogen load (Njiti et al. 1997, 1998; Lightfoot et al. 2007). However, there is evidence that genotypes with root resistance in the absence of sufficient leaf scorch resistance alleles show unusually high leaf scorch indices (Triwitayakorn et al. 2005). Involved in the leaf scorch are at least four different toxins (Baker and Nemec 1994; Jin et al. 1996; Ji et al. 2006; Dr. M. Bhattacharryya, Iowa State University, personal communication 2007). Production, excretion, translocation, uptake and metabolism of the toxins are all stages at which plant genetic diversity might act. SCN infection might indirectly alter toxin responses by weakening the plants or altering translocation.

To explore the genetic relationship between root and leaf resistance to SDS and known loci for resistance to SCN this paper reports the identification of QTL underlying the inheritance of resistance to leaf scorch and root infection from a SCN Hg Type 0 and 1.3.6.7 resistant cultivar.

Materials and methods

Plant material

The genetic material used in this study consisted of 92 F × H F_5-derived RILs (RILs; Yuan et al. 2002). The population was advanced from the $F_{5:7}$ to the $F_{5:14}$ from 1997 to 2005. Seeds were released in 2006 (Kazi et al. 2007). Hartwig was resistant to the leaf scorch of SDS in nearly every replicate plot at all locations and the roots also appeared to be resistant to infection by *F. virguliforme* (Gibson et al. 1994; Wrather et al. 1995; Njiti et al. 1997, 2001; Mueller et al. 2003). However, the SDS resistance of Hartwig was partial and could be defeated by heavy fungal infestations (Njiti et al. 2001; Lightfoot et al. 2007). Hartwig was strongly resistant to most HG Types of SCN (Anand 1992; Niblack et al. 2003). Flyer was susceptible to most SCN HG Types and partially susceptible to SDS (McBlain et al. 1990; Gibson et al. 1994; Njiti et al. 1997, 2001; Yuan et al. 2002; Kazi 2005). Roots of Flyer did not appear to be resistant to infection by *F. virguliforme* (Njiti et al. 2001). However, Flyer was not completely susceptible to SDS (Gibson et al. 1994).

SDS disease evaluation

In 1997, 50 lines were selected in four groups based on the genotype at *rhg1* and *Rhg4* judged by DNA markers (Prabhu et al. 1999). Selected from the larger population were 12 lines with genotype H/H, 11 with H/F, 9 with F/H and 18 with F/F (at Satt038/BLT65). Selection was necessary because the root infection assay is labor intensive and because segregation distortion was observed at the *rhg1* locus in F × H. The lines were planted in SDS infested environments at Ullin (U) and Ridgway (R). The lines were chosen to reduce the cost of root infection assays. For disease rating RILs were planted in a randomized complete block design (RCBD), two-row plots and two replications. Disease incidence (DI), disease severity (DS) and root infection severity (IS) were measured. However, sufficient leaf scorch symptoms (DI and DS) to distinguish among genotypes did not develop due to insufficient rainfall during the growing seasons 1997–1999. In contrast, the IS was sufficient to separate genotypes at both locations (Prabhu et al. 1999) in 1997.

In 2000 the population was again planted at the ARC (Carbondale, IL, USA) and Ullin in SDS infested fields. Severe leaf symptoms developed that allowed DI, and DS to be measured and disease index (DX) to be calculated. Measurements of SDS DX and IS followed Njiti et al. (1998) as modified by Triwitayakorn et al. (2005). To provide accurate scores of SDS leaf scorch, adjustment to maturity dates of individual lines was critical (Hnetkovsky et al. 1996; Njiti et al. 1997). Therefore, the days after planting to maturity were measured for each line from growth stages R5 to R8 (Fehr and Caviness 1977). SDS leaf scorch DI was rated 0% (no disease) to 100% (death of all plants). Scores were taken within the R5 to R6 and R6 to R7 transitions and was interpolated to the estimated R6 by linear regression (Hnetkovsky et al. 1996; Njiti et al. 1996). SDS leaf scorch DS was rated between 1 and 9, where 1 = 0–10% chlorosis or 1–5% necrosis and 9 = premature death of plants and was adjusted to the R6. DX was calculated as DI*DS/9 after the maturity adjustments.

The IS was the mean percentage (0–100) of taproot slices with detectable *F. virguliforme* evident on restrictive media (Prabhu et al. 1999). The IS was measured in taproots recovered at both the R6 and R8 stages of growth (Njiti et al. 1997, 1998, 2003; Prabhu 1999; Triwitayakorn et al. 2005) and was determined from 100 slices per genotype per plot per location (36,800 slices were scored from RILs during the experiment). Several traits including seed yield in non-infested locations and resistance to SCN HG Type classifications for the RILs were as recorded as described in Yuan et al. (2002).

DNA marker analysis

DNA was extracted and used for microsatellite amplifications as in Yuan et al. (2002) with the following modifications. More than 350 BARC-Satt markers with either di- or tri-nucleotide repeat microsatellite markers from all 20 LGs were selected for polymorphism tests. Most (250) of the

BARC-Satt markers were chosen to be spaced at 10 cM intervals from the soybean genetic map (Song et al. 2004). In addition, 140 SIUC-BES-SSR primers from the build two MTP BES clones (Shultz et al. 2006a, b; 2007) were chosen to be spaced at 10,000 kbp intervals from the soybean physical map (Shultz et al. 2006a, b; 2007). Amplification reactions for RILs were performed after Shultz et al. (2007) with no modifications.

Heritability estimation

The heritability (h^2) estimates, a ratio of genotypic variation over phenotypic variation of SDS, were calculated using variance components obtained through ANOVA as described in Fehr (1987). Due to the low frequency of heterozygosity at the $F_{5:11}$, the genetic variance is almost entirely an additive and additive × additive interaction. Therefore the heritability estimate was considered narrow sense. All correlations were calculated using the PROC CORR function of SAS (SAS Institute, Cary, NC, USA).

Construction of the genetic linkage map

A linkage map was created using MAPMAKER/EXP 3.0 (Lander et al. 1987). Map distances between linked markers were calculated in centimorgans (cM, Haldane units) to construct a linkage map (heterogenous lines were excluded). The RIL (RI-selfing genetic model) was used. The $\log_{10}$ of the odds ratio (LOD) for grouping markers (threshold) was set at 3.0, maximum distance was 50 cM. A maximum likelihood map was computed with error detection. The microsatellite markers used in this study have been mapped (Song et al. 2004) in other soybean populations that form a composite map. Therefore, most markers were anchored on the LGs on the basis of the locations expected from the composite map. Conflicts among the positions of linked markers in F × H were resolved in favor of experimental evidence when the maps generated at LOD 3.0 disagreed with the composite map of Song et al. (2004) because most markers do have homeologous loci in soybean (Shultz et al. 2006a).

Construction of QTL maps

Single point analysis

For line mean comparisons, the data were subjected to analysis of variance (ANOVA; SAS Institute Inc., Cary, NY, USA), with mean separation by LSD as described by Njiti et al. (1998). Markers were compared with SDS response measures by the *F* test of ANOVA. The heterogeneous lines were excluded.

For SDS DX a significant difference ($P < 0.005$) was considered to be a preliminary indication of an association between a marker and a QTL for the trait in question. A $P \leq 0.0005$ was suggested by an approximate Bonferroni correction ($P < 0.05/100$) for the set of about 100 independent (unlinked or >10 cM apart) DNA markers (from the 144 mapped). However, at genomic regions where gaps between adjacent markers were greater than 10 cM in the map associations $0.005 > P > 0.0005$ were accepted as a potentially significant association. If the interval was large or was flanking a single marker the uncorrected $P < 0.05$ was accepted. Precedents with first-pass mapping of other quantitative traits (Hnetkovsky et al. 1996; Chang et al. 1996; Njiti et al. 2002) have shown these criteria to be valid during the later saturation mapping of the intervals that were inferred at marginal *P* values (Njiti et al. 1998; Meksem et al. 2001; Yuan et al. 2002; Triwitaykorn et al. 2005; Ruben et al. 2006).

Interval maps of QTL

The maps of all the linked markers and trait data were simultaneously analyzed with Mapmaker/QTL 1.1 using the F_2-backcross genetic model for trait segregation (Chang et al. 1996; Njiti et al. 2002). Putative QTL were inferred when LOD scores exceeded 2.0 at some point in each interval. LOD 2.0 was empirically determined to be equivalent (but not equal) to a single marker $P < 0.005$. The position of each QTL was inferred from the LOD peaks at individual loci detected by maximum likelihood tests at positions every 2 cM between adjacent linked markers.

Composite interval maps of QTL

For more accurate location of QTL among sets of linked markers, the composite interval map (CIM) function of WinQTL Cartographer (version 2.5) was used (Jansen and Stam 1994; Basten et al. 2001). Following Kassem et al. (2006) a walk speed of 2 cM and the forward regression method were selected. QTL were inferred when LOD score peaks exceeded 2.0 for the traits studied, considering a $P < 0.05$ corrected for the use of about 100 independent markers. To confirm linkage, experiment-wise threshold was calculated from 1,000 permutations of each genotype marker against the phenotype in the population.

Results

Polymorphism and linkage

One hundred and forty-four markers (Supplemental Data Table 2) were found to be polymorphic within the F × H

RIL population. Of those 104 were BARC-simple sequence repeats, 15 were BAC derived SSRs from different contigs and 23 were BAC derived SSRs from 11 contigs that contained loci syntenic with *rhg1* (3) or *Rhg*4 (8) and 2 SCARs were from genes in the loci (*rhg1* TMD1 and *Rhg4* BLT65). For IM just 61 loci mapped to 15 different LGs (Song et al. 2004) that encompassed just 534 cM (382 cM for CIM). Therefore, weakly linked markers (between LOD 1.5 and 2.9), unlinked markers and single marker ANOVAs were important for sampling genomic regions during QTL detection (see Supplemental Data Table 2). Assuming 10 cM as a distance for QTL detection by an unlinked or flanking marker, the 15 LGs and the 81 unlinked markers would allow the detection of QTL associated with SDS resistance over about 1,971 cM using single point analysis.

Frequency distributions of SDS mean DX

DX at two locations (R00 and ARC00) showed similar severity with uni-modal and relatively normal ($P < 0.01$) distribution so data were pooled and means used for further analyses. The distribution of mean DX was positively skewed (1.32) toward resistance. The distribution was continuous and had a significant kurtosis (0.88) that reflected a peaked distribution (Fig. 1). The mean, R6 adjusted, DX distribution ranged from negative 15.1 to positive 56.4%. The DX for Flyer was 31.5% and for Hartwig was 0%. The three most resistant and seven most susceptible lines were significant ($P < 0.05$) transgressive segregants. The lines with negative DX after adjustment to the R6 maturity date (less than Hartwig) were all lines that matured earlier than Hartwig.

Frequency distribution of IS

Mean IS at two locations across 2 years among the 50 RILs selected from F × H92 were used for QTL detection (Prabhu et al. 1999). The R6 and R8 data were not pooled for mapping because examination of both mean values and rank correlations across sampling dates, replicates and locations showed significant differences related to the temporal development of resistance (Table 1; Njiti et al. 1997; Iqbal et al. 2005). The frequency distribution of IS was continuous, not normal, kurtosis varied in direction and scale (Fig. 2). The IS ranged from 3.3 to 84.7%. IS for Flyer ranged from 24 to 70% and for Hartwig 16–42%. Six lines were significantly more resistant than Hartwig and eight were significantly more susceptible than Flyer.

Heritability estimates

The heritability estimates for mean SDS DX was 80%. This high value reflected the concordance between locations and severity of SDS. The heritability estimate for mean IS at R6 was 56% and mean IS at R8 was 49%. The lower values reflected the different severities at the locations and sampling dates, particularly the low severity at R6 at Ridgway (Supplementary Table 2). However, within R stage the genotype × environment (G × E) interaction was not significant and was used as the justification to use the mean data (Prabhu et al. 1999).

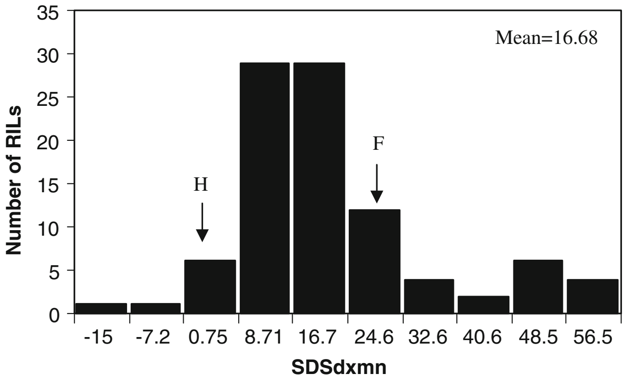

Fig. 1 Frequency distributions of mean DX among 92 RIL from the F × H cross. DX values were adjusted to the R6 by linear regression so some values are negative. Range mid-point values are given each range encompassed 7.85 DX units. The population mean DX was shown on the upper right. Flyer (*F*) and Hartwig (*H*) mean scores were *arrowed*. ARC'00 and U'00 were the environments used with sufficient leaf symptom development

Table 1 Mean square values from analysis of variance on IS and DX among 50 F × H recombinant inbred lines

	F test		Mean squares	
Source	*df*	Divisor	R6	R8
IS				
Location	1	4	1,809	10,933
Rep (loc.)	2	4	1,290**	455
RILs	49	5	286	617**
Loc. × RILs	49	4	275	236
Error	98		226	241
DX				
Replication	1	3	34	
RIL	49	3	451***	
Error	49		179	

IS and DX were measured in two locations with two replications per location

F test divisor = error term for *F* test

Rep (loc.) = replications within location

*** Significant at $P < 0.001$

** Significant at $P < 0.01$

Correlations among traits

The correlation method was used to measure the relationships between SDS and the SCN and seed yield of Yuan

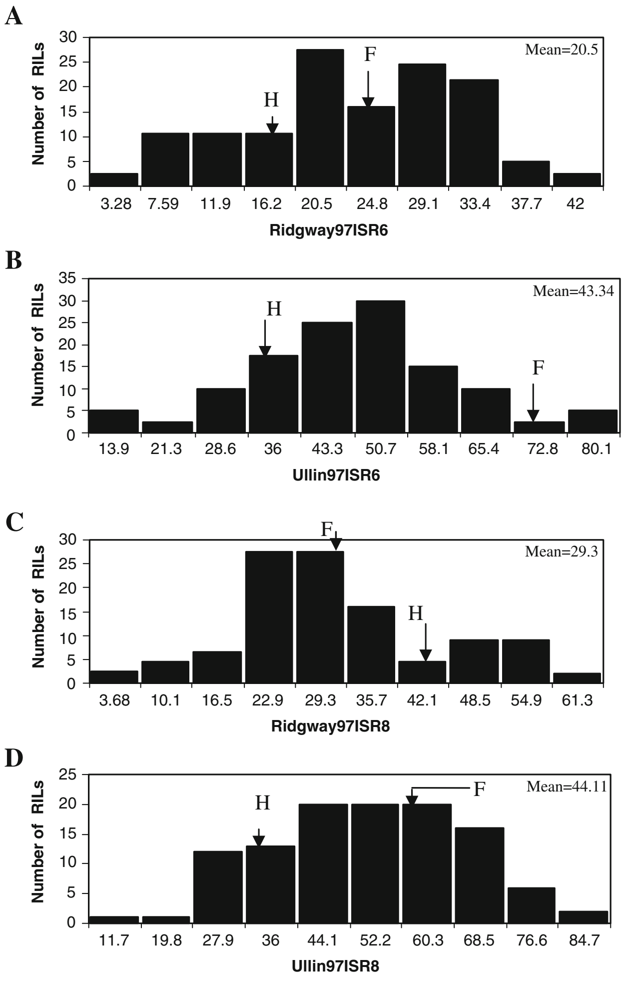

Fig. 2 Frequency distributions of IS in taproots of soybean during 1997 (97). IS was scored at R6 from Ridgway (**a**) and Ullin (**b**) and at R8 from Ridgway (**c**) and Ullin (**d**) among 92 RILs from the F × H cross. Range mid-point values are given. The population mean IS was shown on the *upper right*. The ranges into which "Flyer" (*F*) and Hartwig' (*H*) mean IS scores fall were *arrowed*. R6 was the full-pod reproductive development stage and R8 was the harvest maturity stage of soybean plant when samples were taken. At Ridgway one replicate of Hartwig was very susceptible

et al. (2002). The DX scores for each genotype at each location were highly correlated ($R = 0.99$). Also, SDS resistance as measured by mean IS at R8 and mean DX were correlated ($R = 0.37$) suggesting mean DX was a trait only partly dependent on mean IS (Supplementary Figure 1). Rank correlations between DX and IS also showed partial dependency of DX on IS. Among environments the correlations between IS and DX also varied. Consequently selection of the top ten lines for root resistance by mean IS recovered only five of the best ten lines for mean DX and vice versa. Therefore, separate selection for both traits will be necessary to improve germplasm for resistance to SDS (Lightfoot et al. 2007).

There was no correlation with mean seed yield in non-infested locations (Yuan et al. 2002) and any SDS trait. Therefore, in this population neither leaf scorch nor IS resistance genes caused significant reductions in seed yield in non-infested locations. This was an important result because it suggested that in most genotypes the presence of genes conferring resistance to leaf scorch, and to root IS were not associated with any deleterious effects on seed yield. In contrast, resistance to IS at R8 among the ten most resistant lines was significantly associated with more yield depression than at IS-R6 or DX.

SCN responses for lines in this population were measured previously (Yuan et al. 2002) with the AP3 isolate of HG Type 0 (race 3) and the AP 14 isolate of HG Type 1.3.6.7 (race 14). Root infection measured as IS at R6 and R8 were both strongly correlated with SCN HG Type 0 resistance among the F × H RIL population ($R = 0.71$ to 0.75) whereas SDS DX was weakly correlated ($R = 0.31$). The correlations of resistances to SDS and SCN in Hartwig may reflect both the close linkage (2–3 cM) of *cqRfs1* to *rhg1*and the clustering (less than 0.25 cM) between *cqRfs2* and *rhg1* found in resistant cultivars Forrest (Triwitayakorn et al. 2005; Ruben et al. 2006) and Pyramid (Njiti et al. 2002).

The correlations of resistance to mean IS metrics with responses to SCN HG Type 1.3.6.7 (race 14) was significant but less strong ($R = 0.27$ for ISR6; 0.43 for ISR8) but were significant. SDS DX was not significantly associated ($R = -0.06$ DX). The association between susceptibility to SDS and SCN race 14 resistant germplasm reported previously in cultivars derived from PI88788 (Gibson et al. 1994; Njiti et al. 2002) was evident in lines that derived from Hartwig. Therefore, the recombination events between loci conditioning SCN Hg Type 1.3.6.7 and resistance to SDS may be useful for breeding dually SDS and SCN resistant cultivars.

Consistent with the correlations between SDS and SCN scores the best line judged by DX (F × H13) ranked fourth by IS at R6, was HG Type 0 resistant and partially resistant to HG Type 1.3.6.7. However, the best ranked line with HG Type 1.3.6.7 resistance (F × H33) was also best ranked by IS at both R6 and R8 but ranked 15th by DX. In view of the correlations resistance to *F. virguliforme* infection may be co-inherited with both resistance to SCN Hg Type 1.3.6.7 reproduction and susceptibility to SDS leaf scorch.

Significant genomic regions for SDS mean DX

Two regions significantly associated with resistance to leaf scorch were detected based on the markers used. One region was detected on LG C2 that was associated with mean SDS DX across two environments (Table 2). The region on LG C2 (Fig. 3) of about 13 cM between the microsatellite markers BARC_Satt277 ($P = 0.004$, $R^2 = 14.8\%$) and BARC_Satt079 ($P = 0.003$, $R^2 = 9\%$)

Table 2 Intervals with the flanking markers by CIM (LOD; QTL variation) and single markers by ANOVA probability (P) and Variance (R^2) values associated with SDS mean DX (DXmn) and mean IS at the R6 and R8 stages in the Flyer by Hartwig (RIL) population

LG Locus name(s)[d]	Marker interval or CIM QTL	Trait	P	R^2 (%)	LOD[a]	QTL var.[b]	Mean ± SEM for RILs with allele from[c]	
							Flyer	Hartwig
C2	Satt277	DXmn[e]	0.004	14.8	2.1	19.0	10.9 ± 2 2	30.3 ± 5.6
cqRfs4	Satt079	DXmn	0.003	9.0	2.2	8.2	13.2 ± 2.7	24.5 ± 4.8
	QTL	DXmn	–	–	2.7	24.1	–	–
D2	Satt574	IS R6mn	0.001	10	2.2	10.2	34.3 ± 3. 0	47.3 ± 3.2
cqRfs11	Sat_001	IS R6mn	0.003	6.1	2.4	12	36.9 ± 3.0	52.1 ± 3.9
	QTL	IS R6mn	–	–	3.0	25.2	–	–
G								
cqRfs1	Satt038_2	IS R8mn	0.0001	28.1	–	–	41.6 ± 1.9	28.1 ± 1. 9
qRfs13	Satt130	DXmn	0.003	12.9	–	–	27.4 ± 5.1	12.0 ± 2.5
cqRfs3	Satt115	IS R6 R97	0.01	6.4	2.50	16	24.7 ± 1.4	17.6 ± 2.6
cqRfs3	Satt427	IS R6 R97	0.001	15	2.8	17	25.4 ± 1.8	15.5 ± 1.9
cqRfs3	QTL	IS R6 R97	–	–	3.6	38.5	–	–

[a] LOD: Log of the probability of a locus being present; LOD threshold was 2.0

[b] Amount of variability in the infection explained by the marker loci based on MapMarkerQTL1.1

[c] SEM: Mean ± SD/$\sqrt{N}$; where N was the number of each of allele

[d] QTL associated with resistance to root infection. QTL detected in common intervals in separate populations or derived NILs were considered confirmed and suffixed with c under Soybean Genetics Committee recommendations from 2000 to 2006 (http://soybase.agron.iastate.edu/nomenclature/QTL.html). QTL designations cqSDS00# were applied for

[e] DX was measured at Ullin (U) and at the Agronomy Research Center (ARC) in 2000. IS was measured at Ullin and Ridgway (R) in 1997. Allelic means were shown along with standard error of the mean (SEM)

encompassed the QTL detected by CIM. The interval had a peak-LOD score of 2.7 and explained about 24.1% of the total variation in SDS DX. The region derived the beneficial allele from Flyer that reduced DX by about 20%. The locus was significant for DX, DI and DS at both locations ($0.001 < P < 0.04$). The locus was located between 108 and 118 cM on the composite map and therefore may be *cqRfs4* (Supplementary Table 1) the same locus that was detected with a beneficial allele from susceptible parents Essex; crossed with Forrest (E × F94; Hnetkovsky et al. 1996); and “Douglas”; crossed with Pyramid (P × D90; Njiti et al. 2002).

The second locus underlying SDS DX variation was detected by BARC_Satt130 (Table 2). The marker did not have any significantly linked marker in the F × H RIL set. Satt130 was significantly associated with mean DX ($P = 0.003$, $R^2 = 12.9\%$) and DX, DI and DS ($0.003 < P < 0.04$) at each location. The locus identified derived the beneficial allele from Hartwig. The common allele of Satt130 was normally found on LG G at 20 cM on the composite map (Song et al. 2004; Fig. 3). However, in F × H the marker was not part of LG G and was not linked to Satt038, Satt324 or Satt275 of the composite map flanking markers mapped in F × H nor in any of the markers from other LGs. Therefore, in the F × H population, Satt130 may identify either *cqRfs1* (Supplementary Table 1), a new locus on LG G (*qRfs13*) or a paralog of the marker found on the composite map located on a yet unknown LG (Shultz et al. 2006a).

The amount of variation in SDS DX explained by the markers was significant. However, the two regions jointly contributed only about 31% of the total variation compared to trait heritability across locations of 80%. Therefore, both markers more closely linked to the QTL and additional loci for resistance to SDS leaf scorch remain to be discovered in this population.

Significant genomic regions for mean IS at R6 and R8

A QTL for resistance to root infection for the R6 sampling was identified by BARC_Satt574 ($P = 0.001$, $R^2 = 10\%$) that derived the beneficial allele from Flyer and reduced IS by about 13% (Table 2). The linked (15 cM) marker BARC_Sat_001 ($P = 0.005$, $R^2 = 6.1\%$) was also found associated with mean IS at R6 (Fig. 3). The markers were weakly associated with leaf scorch metrics DX, DI and DS and their means ($0.01 < P < 0.04$) in each location. The interval had a peak-LOD score of 3.0 and explained about 25% of the variation in SDS IS by CIM (Table 2). The locus was located on LG D2 of the composite map between 87 and 92 cM and so is likely to be *cqRfs11* (Supplementary Table 1) found in Pyramid by Lightfoot et al. (2001).

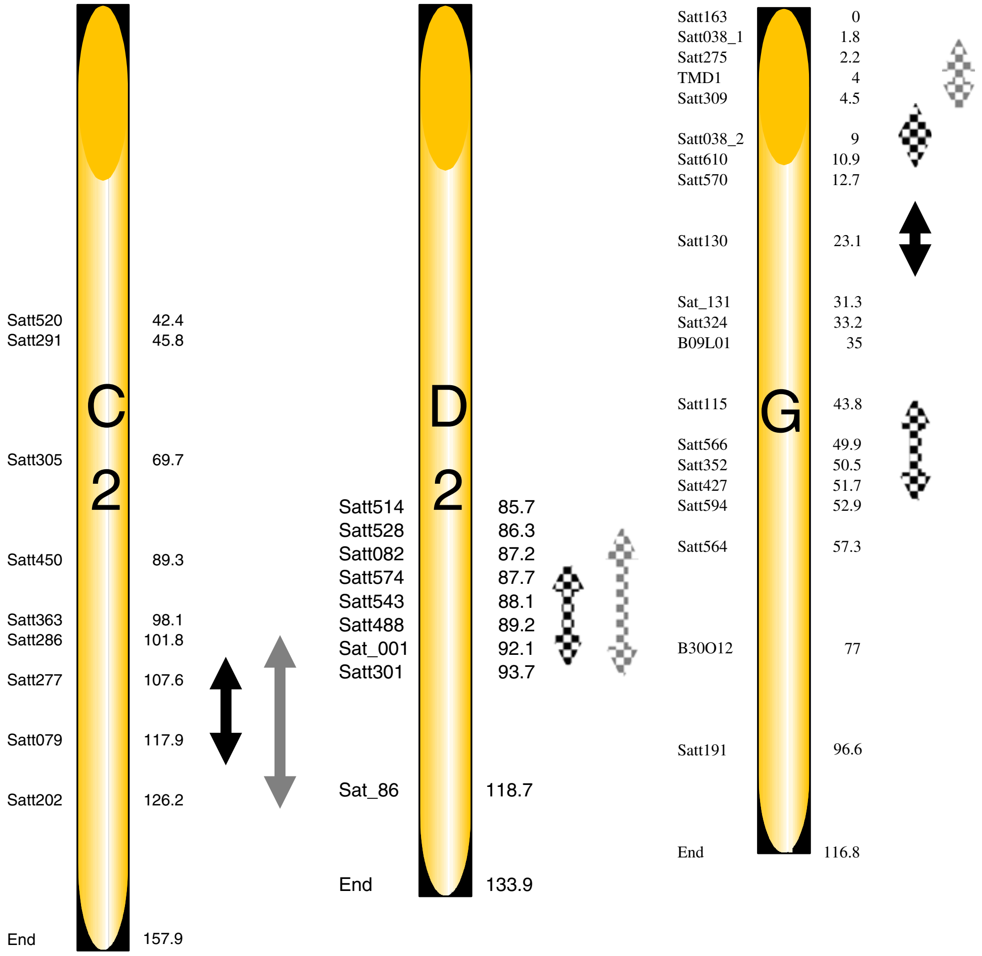

Fig. 3 Locations of the QTL found in the Flyer by Hartwig population on linkage groups C2, D2 and G for SDS mean DX (*black arrows*) and SDS IS (*black stippled arrows*). Also shown are QTL for resistance to SCN (*gray stippled arrows*) and date of maturity (*gray solid arrow*). The size of the arrow reflects the interval significantly associated by QTL Cartographer or Mapmaker at LOD > 2.0 or ANOVA at $P < 0.001$

The locus may also be the same as cqSDS001 reported by Farias-Neto et al. (2007).

Satt038_2, the single marker that identified the QTL on LG G reported by Prabhu et al. (1999; Table 2) was here placed between Satt309 and Satt610 by Mapmaker. That location was not the usual position for Satt038_1 on the composite map but was the location expected for *cqRfs1* (Supplementary Table 1). The marker was strongly linked (LOD > 3.0) to Satt309 (4.5 cM), TMD1 (5.0 cM), Satt 275 (8 cM) and Satt163 (9 cM). Those markers also showed significantly skewed segregation ratios away from the expected 1:1 ratio, with Hartwig alleles in the minority as had Satt038_2 in a larger population (Prabhu et al. 1999). However, Satt038_2 and Satt610 did not show skewed segregation ratios in the RILs selected for F × H92. None of the markers except Satt038_2 was significantly associated with IS or any measure of leaf scorch resistance. The skewed segregation ratios of all the markers (except Satt038_2, for which selection had been applied) may have caused real QTL to marker associations to be missed. Alternately, the association of Satt038_2 with IS at R8 may be an error caused be selection.

At Ridgway in 1997, a second region on LG G (Fig. 3) for resistance to root infection was identified. IS at the R6 sampling at Ridgway identified a QTL linked to BARC_Satt115 ($P = 0.01$, $R^2 = 6.4\%$). It derived the beneficial allele from Hartwig that reduced IS by about 7% (Table 2). The marker was not associated with leaf scorch metrics DX, DI and DS at any location or their means. The linked markers Satt427 Satt566 and Satt352 were weakly associated with the IS at R6 trait. The interval had a CIM peak-LOD score of 3.6 and explained about 38.5% of the total variation in SDS IS-R6 (Table 2). The locus was located on LG G of the composite map between 43 and 51 cM and probably was *qRfs3* (Supplementary Table 1) described previously (Chang et al. 1997; Iqbal et al. 2001, 2005).

The amount of variation in SDS IS explained by the two QTL underlying IS at R6 was significant; the two regions jointly contribute about 40% of the total variation compared to a trait heritability of 56%. In addition, only one QTL was identified with R8 IS data. Therefore, markers more closely linked to the QTL rather than additional loci for resistance to root infection by the SDS causal pathogen may remain to be discovered in this population.

Discussion

The F × H linkage map detected only two QTL for SDS DX, one with beneficial allele from Hartwig (on LG G) and one from Flyer (on LG C2). The number of QTL was less than the three found in P × D (Njiti et al. 2002) and the eight found in E × F (Kassem et al. 2006). It is possible that because Flyer is not as susceptible to SDS as Essex and Douglas some QTL are fixed in the F × H population that segregate in E × F and P × D. Considering the SDS root IS QTL, Hartwig contributed two QTL for resistance on G (Fig. 3) and Flyer contributed a cqSDS001-like locus on LG D2; even though Flyer was more susceptible to infection by *F. virguliforme* than Hartwig. In Ripley by Spencer and Pyramid by Douglas the SDS resistant parent provided the beneficial allele at the equivalent position (Lightfoot et al. 2001; Farias-Neto et al. 2007). The locus on D2 was in the same interval as a locus for resistance to Hg Type 1.3.6.7 from PI88788 (Schuster et al. 2001); Pyramid (Lightfoot et al. 2001); and PI437654 (Webb et al. 1995). Therefore, this genomic region may explain the negative association between resistance to SCN HG Type 1.3.6.7 and resistance to SDS in soybean germplasm (Gibson et al. 1994). The identification of recombination events in this region that separate the negative association will be important for germplasm improvement.

In earlier studies where R6 and R8 data were pooled, about half of the cultivars tested showed root resistance, suggesting single-gene inheritance (Njiti et al. 1997; Prabhu et al. 1999; Njiti et al. 2003). In this study either bi- or tri-geneic inheritance for root resistance was detected. That knowledge will significantly increase the ability to breed for increased root resistance. Molecular methods for detecting and quantifying the pathogen in the root will provide effective tools for germplasm testing at different developmental stages (Achenbach et al. 1996; Li and Hartman 2003) because the inheritance of resistance to infection was shown to be significantly affected by developmental stage in F × H. Further, the measurements of SDS by DX and IS were shown to be very different in heritability, trait distribution, trait correlation and selection based on rank. DX was a poor indicator of root resistance whether by value or rank (Njiti et al. 1997). Therefore, efficient breeding strategies should make selections by both DX and either IS or Hg type rating for the identification of the most resistant cultivars.

Perhaps the most surprising result was the absence of a set of QTL for resistance to SDS leaf scorch (*qRfs2*) clustered around or pleiotropic to *rhg1*. The region was well populated with markers (Supplementary Table 1; Triwitayakorn et al. 2005; Ruben et al. 2006). Therefore, Forrest and Hartwig differ significantly in this region, despite sharing the same allele of the receptor like kinase at *rhg1* (Ruben et al. 2006). This result also argues against pleiotropy between *rhg1* and *Rfs2* postulated by Triwitayakorn et al. (2005). Perhaps the location of the functional SCN and linked or pleiotropic SDS resistance QTL may have shifted to the loci paralagous to *rhg1* or even the non-paralagous D2 locus (Shultz et al. 2006a, b; Afzal and Lightfoot 2007; Lightfoot 2008). In this case NILs recombinant in the D2 region around Satt574 may help identify candidate genes using the genome framework at SoyGD (Supplementary Figure 2; Shultz et al. 2006a) and data from the DOE soybean genome sequencing project.

Acknowledgments This research was funded over the past 11 years in part by grants from the NSF 9872635, ISA 95-122-04; 98-122-02 and 02-127-03 and USB 2228-6228. The integrated genetic and physical map was based upon work supported by the National Science Foundation under Grant No. 9872635. Any opinions, findings, and conclusions or recommendations expressed in this material were those of the author(s) and do not necessarily reflect the views of the National Science Foundation. The continued support of SIUC, College of Agriculture and Office of the Vice Chancellor for Research to SK, JA and DAL was appreciated. The authors thank Dr. P. Gibson, O Myers Jr. and M. Schmidt for assistance with germplasm development and maintenance from 1991 to 2000 and Dr. Rizwan Hashmi for assistance with data analysis and interpretation.

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