Theor Appl Genet (2010) 120:633–644
DOI 10.1007/s00122-009-1181-4

ORIGINAL PAPER

Iso-lines and inbred-lines confirmed loci that underlie resistance from cultivar 'Hartwig' to three soybean cyst nematode populations

Samreen Kazi · J. Shultz · J. Afzal · Rizwan Hashmi · Mohammed Jasim · Jason Bond · Prakash R. Arelli · David A. Lightfoot

Received: 7 December 2008 / Accepted: 6 October 2009 / Published online: 25 October 2009

Abstract Soybean [*Glycine max* (L.) Merr.] cultivars varied in their resistance to different populations of the soybean cyst nematode (SCN), *Heterodera glycines,* called HG Types. The *rhg1* locus on linkage group G was necessary for resistance to all HG types. However, the loci for resistance to *H. glycines* HG Type 1.3- (race 14) and HG Type 1.2.5- (race 2) of the soybean cyst nematode have varied in their reported locations. The aims were to compare the inheritance of resistance to three nematode HG Types in a population segregating for resistance to SCN and to identify the underlying quantitative trait loci (QTL). 'Hartwig', a soybean cultivar resistant to most SCN HG Types, was crossed with the susceptible cultivar 'Flyer'. A total of 92 F5-derived recombinant inbred lines (RILs; or inbred lines) and 144 molecular markers were used for map development. The *rhg1* associated QTL found in earlier studies were confirmed and shown to underlie resistance to all three HG Types in RILs (Satt309; HG Type 0, $P = 0.0001$ $R^2 = 22\%$; Satt275; HG Type 1.3, $P = 0.001$, $R^2 = 14\%$) and near isogeneic lines (NILs; or iso-lines; Satt309; HG Type 1.2.5-, $P = 0.001$ $R^2 = 24\%$). A new QTL underlying resistance to HG Type 1.2.5- was detected on LG D2 (Satt574; $P = 0.001$, $R^2 = 11\%$) among 14 RILs resistant to the other HG types. The locus was confirmed in a small NIL population consisting of 60 plants of ten genotypes ($P = 0.04$). This QTL (cqSCN-005) is located in an interval previously associated with resistance to both SDS leaf scorch from 'Pyramid' and 'Ripley' (cqSDS-001) and SCN HG Type 1.3- from Hartwig and Pyramid. The QTL detected will allow marker assisted selection for multigenic resistance to complex nematode populations in combination with sudden death syndrome resistance (SDS) and other agronomic traits.

Communicated by I. Rajcan.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1181-4) contains supplementary material, which is available to authorized users.

S. Kazi · J. Shultz · J. Afzal · R. Hashmi · M. Jasim · J. Bond · D. A. Lightfoot (✉)
Plant Biotechnology and Genomics Core-Facility,
Department of Plant, Soil, and Agricultural Systems,
Southern Illinois University, Carbondale, IL 62901, USA
e-mail: ga4082@siu.edu

S. Kazi · J. Shultz · J. Afzal · D. A. Lightfoot
Center for Excellence in Soybean Research,
Teaching and Outreach, Southern Illinois University,
Carbondale, IL 62901, USA

Present Address:
J. Shultz
School of Biological Sciences,
Louisiana Tech University, Ruston, LA, USA

Present Address:
P. R. Arelli
Soybean Genetics Laboratory,
USDA, ARS-MSA, Jackson, TN 38301, USA

R. Hashmi
Department of Plant Biology,
Southern Illinois University, Carbondale, IL 62901, USA

M. Jasim
Department of Biotechnology, Al-Anbar Uni, Al-Anbar, Iraq

Introduction

Heterodera glycines Ichinohe infestation of soybean fields leads to "Yellow dwarf" disease (Hussey and Grundler 1998; Davis et al. 2004) caused by the formation of cysts on the roots. The soybean cyst nematode (SCN) infections

of roots cause various other symptoms including; chlorotic (yellow) patches within leaflets; root necrosis; suppression of root and shoot growth; and reduced seed yield. Worldwide, SCN has become the most serious pest of soybean (Wrather et al. 1996, 2003). Once established in a field, the infestations have been difficult to eradicate due to the longevity of the eggs and the ability of the nematode populations to overcome soybean resistance genes.

The most economic and environmentally sound solution for managing areas infested with SCN has been the development of resistant germplasm (Concibido et al. 2004). However, the resistance has been biotype- or race-cultivar specific (*H. glycines* biotype are called HG Types; Niblack et al. 2003). However, HG Types and or plant resistance was shown to be temperature dependent (Palmateer et al. 2000). Further, pathogen populations have had the ability to mutate, recombine and/or drift quickly to new HG Types that overcome plant resistances (Niblack et al. 2003).

In most germplasm, SCN resistance was shown to be controlled by few major QTL (Concibido et al. 2004). For example, in the cultivar 'Forrest', resistance to SCN HG Type 0 required two loci, *rhg1* and *Rhg4*. (Meksem et al. 2001). The *rhg1* locus was on linkage group (LG) G (chromosome 18) and provided the major portion of resistance to SCN across many genotypes (Webb et al. 1995, 1996; Concibido et al. 1997, 2004). Recombination events suggest the *rhg1* locus lies between TMD1 and Satt309, a 42 kb region encompassing genes encoding the kinase of the RLK, a laccase like protein and a transporter like protein (Ruben et al. 2006; Afzal et al. 2008, 2009; Iqbal et al. 2009).

The *Rhg4* locus was consistently reported to be on LG A2 (chromosome 8; Weisemann et al. 1992; Webb et al. 1995, 1996; Meksem et al. 2001; Concibido et al. 2004). *Rhg4* provided an equal portion of resistance to SCN HG Type 0, and in some crosses HG Type 1.3- (Webb et al. 1995, 1996; Concibido et al. 1997, 2004). *Rhg4* did not contribute to resistance to HG Type 1.2.5-; or HG Type 2. Markers closest to the locus include the RFLP derived SCAR marker BLT65 (Weisemann et al. 1992), AFLP derived SCAR marker A2D8 (Meksem et al. 2001) and one (SIUC-SagH100B10b) of the two BES-SSRs from the BAC H100B10 anchored by A2D8 (Shultz et al. 2007). The other BES-SSR (SIUC-SagH100B10a) was derived from the opposite BAC end DNA sequence of the resistant cultivar Forrest. It was been located in the soybean genome (Lightfoot, unpublished) in 35 kbp region associated with the deletion underlying the *I* gene for seed coat color in a susceptible cultivar (Senda et al. 2002). The amplicons it generates are from LG A2 (resistant genotypes) and A1 (susceptible genotypes) and so it appears to map to linkage group A1 (Shultz et al. 2007). The *I* gene and the linked markers A2D8, SagH100B10b, and BLT65 are very strongly associated with *Rhg4*. Partly due to the deletions and insertions within the *Rhg4* to *I* region, that interfere with chromosome alignments, recombination events are very rare (Weisemann et al. 1992; Meksem et al. 2001). However, a few recombination events identified to date suggest the *Rhg4* locus lies between A2D8 and BLT65, a 142 kbp region encompassing genes encoding a RLK; a subtilisin-like protein and a transporter like protein (Meksem and Lightfoot 2000; Campbell et al. 2009).

Three additional QTL identified in 'PI 437654', 'Hartwig' and PI88788 for resistance to HG Types 1.3- and 1.2.5- were located on LGs J (*Rhg5*), D2 (*Rhg2*), and C1 (*Rhg3*; Webb et al. 1995, 1996; Schuster et al. 2001; Glover et al. 2004). Four different loci (on LGs A1, B1, F and G) underlying resistance to SCN were detected in PI437654 using inbred highly nematodes (Vierling et al. 1996). A tenth locus (LG I) from PI437654 was added recently (Wu et al. 2009). When epistasis among loci was measured, eighteen loci (on all LGs except B1 and D1b) were found to be involved in PI437654 derived resistance to SCN. Possibly the PI437654 genome encoded many redundant loci for resistance to SCN that were activated by crosses to different susceptible cultivars or by challenge with different HG Types.

The research described here sought to confirm previously reported QTL (Webb et al. 1995, 1996; Vierling et al. 1996; Schuster et al. 2001; Wu et al. 2009) or identify new QTL that underlie resistance to SCN. A recombinant inbred line (RIL) population was developed the cross of Flyer by Hartwig (Prabhu et al. 1999; Yuan et al. 2002; Kazi 2005; Kazi et al. 2007, 2008). The RIL population was challenged with three different HG Types (0, 1.3-, and 1.2.5-). QTL identified in RILs were tested using near isogeneic line (NIL) populations. One of the confirmed QTL was assigned the designation cqSCN-005.

Materials and methods

Plant material

Seeds were developed by the Genetics and Biotechnology Program at Southern Illinois University at Carbondale at the Agronomy Research Center (ARC) from 1993 to 2006. For this research, seeds were obtained from the collections of Dr. D.A. Lightfoot in the ARC seed store at SIUC in 2002. The RILs derived from a cross between two soybean cultivars, susceptible Flyer and resistant Hartwig ($F \times H$, $n = 92$; FH92) and the population was released in 2006 (Kazi et al. 2007). Flyer was originally released for high seed yield combined with a wide range of fungal resistance loci (McBlain et al. 1990).

All RILs in FH92 were selected for agronomic type. Fifty of the RILs were selected for the presence of approximately equal numbers of alleles at Satt038 (LG G) and BLT65 (LG A2; Prabhu et al. 1999). The selection for agronomic type tended to select against SCN resistant lines (Yuan et al. 2002; Brucker et al. 2005; Kopisch-Obuch et al. 2005). Also, the SCN resistance alleles at *rhg1* must be in phase with an unlinked modifier locus for gametes or zygotic seed to be viable (Webb et al. 1995, 1996; Afzal et al. 2008, 2009). Therefore, marker selection for approximately equal numbers of RILs with resistance alleles at *rhg1* and *Rhg4* was necessary to avoid the skewed and biased population otherwise formed by these unintentional selections.

SCN female index (FI) determination

The SCN assays here used previously described methods (Prabhu et al. 1999; Arelli et al. 2000; Yuan et al. 2002) for HG Types 0 and 1.3-, but with minor modifications for HG Type 1.2.5-. For the $F_{5:11}$ F × H RIL ($n = 94$) population SCN HG Types 0 and 1.3- used five and three single-plant replications, respectively. For HG Type 0 tests, the cultivar 'Hutcheson' was used as the susceptible control while 'Peking' (female index; FI 2%), 'PI 88788' (FI 3%), 'PI 90763' (FI 1%) and 'Pickett' (FI 3%) were used as the standard differentials to determine HG Type classification. For HG Type 1.3- tests, the cultivar 'Hutcheson' was used as the susceptible control while Peking (FI 98%), PI 88788 (FI 3%) and PI 90763 (FI 101%) and Pickett (FI 68%) were used as the standard differentials to determine the race classification (HG Type). Assays were carried out at the University of Missouri by Dr. P. Arelli's staff using near homogeneous nematode cultures. The development of the near homogenous nematode cultures was described in Qui et al. (1999).

The HG Type 1.2.5-(race 2) assays were replicated at SIUC by Dr. Bond's staff and at the University of Missouri by Dr. Arelli's staff. The RIL experiments each used two single-plant replications (4 plants in total), while the NIL experiments used six single-plant replications (12 plants in total). The cultivars 'Lee 74', 'Essex' and 'Hutcheson' were used as susceptible controls (Niblack et al. 2003). The differentials or indicator lines were 'PI54840' (FI 101%), PI 88788 (FI 24%), PI90763 (FI 1%), PI437654 (FI 0%), 'PI 209332' (FI 61%), 'PI89772' (FI 2%), 'PI548316' (FI 38%) and 'PI548402' (FI 35%) in the first experimental repeat. In the second repeat, the FIs were 19, 48, 0, 0, 63, 0, and 39%. Therefore, the standard differentials showed this HG Type to be 1.2.5- (Niblack et al. 2003) corresponding to race 2 (Riggs and Schmitt 1988).

For inoculation, seeds were surface sterilized with 5% (*v*/*v*) bleach for 2°min, rinsed three times with distilled water and placed on autoclaved filter paper in Petri dishes (Arelli et al. 2000). These dishes were incubated in the dark at 20°C for 48 h. Seeds were planted separately in plastic Micropots™ (Hummert International, Earth City, MO), then immersed in a 27°C water bath. Seven days after seedling emergence, each pot was inoculated with 1-ml of crushed cysts from a homogenous isolate of *H. glycines* in distilled water (2,000 ± 25 eggs per ml).

Thirty-eight days after inoculation, individual plants were uprooted, and the cysts were collected by washing the roots with pressurized water over wire mesh sieves. The leaves from the top of these plants were stored at −70°C. The total numbers of cysts were counted using a Nikon (Melville, NY, USA) dissecting scope (Model SMZ645) at 10× magnification. The mean number of cysts from two to six single-plant replications for each line and the susceptible checks were determined. The FI, previously called Index of Parasitism (IP), Schmitt and Shannon (1992) was calculated as the number of cysts or females on the evaluated genotype divided by the mean number of cysts or females present on the susceptible cultivar roots × 100.

DNA marker analysis

DNA was extracted and used for microsatellite amplifications as in Yuan et al. (2002) with modifications described in Kazi et al. (2008). Briefly, BARC-Satt markers were chosen at ~10 cM intervals from the soybean genetic map (Song et al. 2004). In addition, SIUC-BES-SSR markers from build 2 MTP BES clones (Shultz et al. 2006a, b, 2007) were chosen at ~10,000 kbp intervals within the soybean physical map (Shultz et al. 2006a, 2006b, 2007). Six additional markers in the regions of *rhg1* and *Rhg4* were added compared to Kazi et al. (2008). They were Satt038_1, Satt038_2 on LG G near *rhg1* and BLT65, SagH100B10b, SagH100B10a and A2D8 on LG A2 encompassing *Rhg4* (Supplemental Table 1).

Trait heritability and correlation estimations

Heritability (h^2) estimates, the ratio of genotypic variation over phenotypic variation of SCN FI%, 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 interactions. Therefore, heritability was considered narrow.

All correlations were calculated using the PROC CORR function of SAS (SAS Institute, Cary, NC, USA) using line mean data. The SDS data were described in detail in Kazi et al. (2007) as mean disease index (leaf scorch; two locations, 1 year) and mean infection severity (root infection; four locations 2 years). The mean seed yield data were

described in detail in Yuan et al. (2002) and was from four environments and 2 years. The SCN FI data were the mean number of cysts from two to six single-plant replications (for each line) and two experiments described above.

Construction of the genetic linkage map

The linkage map was created using MAPMAKER/EXP 3.0 (Lander et al. 1987) as described by Kazi et al. (2008). Briefly, map distances were calculated in Haldane units; heterogeneous lines were excluded; the RI-self genetic model was used. The log of the odds (LOD) score for grouping markers was set at 3.0 with a maximum distance of 50 cM and computed with error detection. Most markers were anchored on the LGs on the basis of the locations expected from the composite map (Song et al. 2004). Conflicts among the positions of linked markers in F × H were resolved in favor of experimental evidence if there was DNA sequence evidence for the existence of paralogs of marker amplicons and priming sites. Paralogs were inferred when the maps generated by Mapmaker had linkage probabilities above LOD 3.0 that disagreed with the composite map. Most markers have homeologous loci in soybean (Shultz et al. 2006a, b, 2007; Saini et al. 2008).

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). Mean separations were by LSD as described by Kazi et al. (2008) for RILs and Njiti et al. (1998) for NILs. Markers were compared with SCN FI measures by the *F*-test of ANOVA. Heterogeneous lines were excluded from analysis (numbers of lines ranged from 3 to 15 with a mean of 8.3 per marker).

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. The value of $P \leq 0.0005$ was suggested by an approximate Bonferroni correction ($P < 0.05/100$) for the set of about 100 DNA markers about 10 cM apart. However, at genomic regions where markers were sparse and gaps between adjacent markers were 20–40 cM, associations in the range $0.005 > P > 0.0005$ were considered significant. If the interval was even larger or was flanking, a single marker the uncorrected *P* value of <0.05 was accepted. Precedents with first-pass mapping of other quantitative traits (Hnetkovsky et al. 1996; Chang et al. 1997; Njiti et al. 2002) have shown these criteria to be valid. During subsequent saturation mapping of intervals inferred at marginal *P* values most have been confirmed (Njiti et al. 1998; Meksem et al. 2001; Yuan et al. 2002; Triwitayakorn et al. 2005; Ruben et al. 2006).

Interval maps of QTL

Maps of all 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. 1997; Njiti et al. 2002; Kazi et al. 2008). Putative QTL were inferred when LOD scores exceeded 2.0 at some point in an 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 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 Kazi et al. (2008), 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. To confirm linkages, an experiment-wise threshold was calculated from 1,000 permutations of each marker genotype compared with the phenotype in the population.

Results

Polymorphism and linkage

The linkage map used was described in Kazi et al. (2008) with six additional markers. Briefly, 150 markers were found to be polymorphic within this Flyer × Hartwig (FxH) RIL population (104 of 250 BARC-SSRs, 40 of 143 BES-SSRs and 3 of 3 SCARs tested). There were 3–10 markers per linkage group and distance between markers was 10–25 cM. The exceptions were linkage groups A2, G and K that were tested with additional markers due QTL detections reported previously (Prabhu et al. 1999; Yuan et al. 2002, Kazi et al. 2008). Sixty-one markers formed 17 linkage groups encompassing 534 cM. The map had large gaps, probably because the RIL population was derived from a cross between cultivars with a high coefficient of common ancestry (~0.25) from a very small germplasm base, the southern gene pool of north American adapted soybean cultivars (Kazi et al. 2007, 2008). However, assuming 10 cM as a distance for QTL detection, the 17 groups formed plus the 81 unlinked markers would allow

the detection of QTL over 2,494 cM (Supplementary Fig. 1). Therefore, the recombination distances, the orders of markers in linkage groups (with 2–3 exceptions) and the genome size (2,512 cM) agreed with those reported (Song et al. 2004).

Variation of resistance within F × H RILs to three races of SCN

The mean FI and standard errors within all three HG Types were relatively constant with minor variations among the two parents and RILs (Table 1). Transgressive segregation was observed in HG Type 0 (race 3) FI scores but not in HG Type 1.2.5- (race 2) and HG Type 1.3- (race 14; Fig. 1a, b, c). The RIL population mean was intermediate to the two parents for each HG Type (Table 1). Narrow sense heritability estimates for SCN FI had relatively high values (0.95 for HG Type 0, 0.96 for HG Type 1.2.5-, and 0.86 for HG Type 1.3-; Table 1).

Table 1 Mean female index plus or minus standard errors of parents, narrow sense heritabilities and probabilities of normality among the F × H ($F_{5:11}$) RILs for SCN HG Type 1.2.5-, HG Type 0 and HG Type 1.3- (races 2, 3 and 14)

HG type	1.2.5- (n = 14)	0 (n = 92)	1.3- (n = 92)
Hartwig[a]	3 ± 0.4	2 ± 0.4	2 ± 0.4
Flyer[a]	109 ± 23	103 ± 25	123 ± 33
F × H (F5:11)[a]	50.4 ± 8.4	55.1 ± 4.0	76.2 ± 2.4
Heritability (%)	95	96	86
Probability[b]	<0.0001	<0.0001	<0.0001

[a] Allelic mean ± SEMs

[b] Probability that the trait distribution was normal

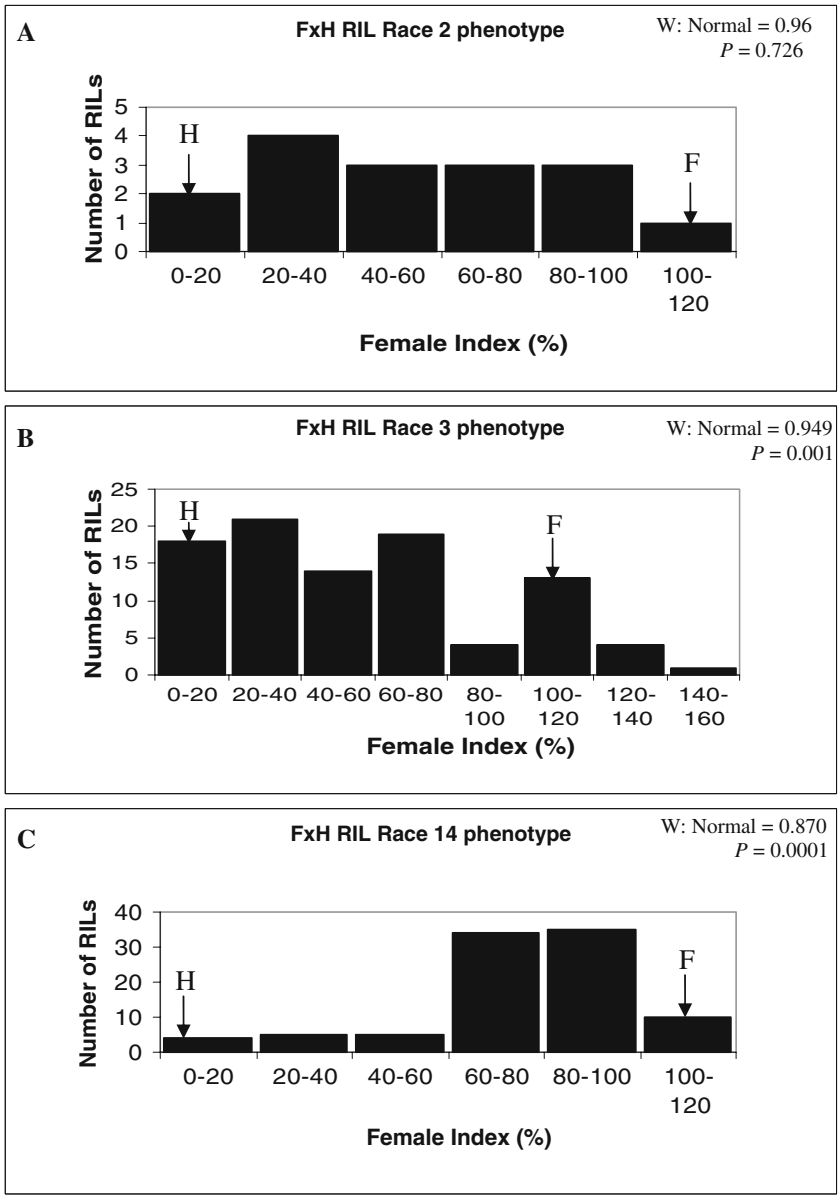

Fig. 1 Frequency distributions of the F × H (F5:11) RIL response to SCN HG Type 1.2.5- (race 2) HG Type 1.3-(race 14) and HG Type 0 (race 3). Letters *H* and *F* directed by *arrows* indicate where the mean FI of the Hartwig and Flyer, respectively, fall within the distribution

HG Type 1.2.5- FI distribution showed a normal distribution ($W = 0.95$; $P = 0.73$; Fig. 1a). The distribution was skewed (−0.58) towards the low female index (Hartwig). A negative kurtosis (−0.59) reflected a platykurtic distribution caused by a few extreme scores (two resistant RILs FI < 10; F × H35 and F × H93). There were no significant transgressive segregants.

For resistance to HG Type 0, often a bigeneic trait (Meksem et al. 2001), the distribution was not normal ($W = 0.95$; $P = 0.001$; Fig. 1b). The distribution was skewed (−0.44) towards the low female index (Hartwig). The distribution was negatively kurtotic (−0.72). There were about 18 lines with a mean FI higher than Flyer that were transgressive segregants (Fig. 1b). There were 14 resistant lines (FI < 10) and another 14 moderately resistant lines (10 > FI > 30). Only five lines were equal in resistance to Hartwig (FI < 1.0) but none were significant transgressive segregants.

HG Type 1.3- FI distribution was not normal as $W = 0.87$ ($P = 0.0001$; Fig 1c) and was skewed (1.5) towards the high female index (Flyer). There was a significant positive kurtosis (2.75) that reflected a leptokurtic distribution. No significant transgressive segregants were observed. There were only 4 resistant lines (FI < 10), no moderately resistant lines (10 > FI > 30) and only 11 moderately susceptible lines (30 < FI < 60). None of the four best lines equaled the resistance found in Hartwig (FI < 1.0).

Correlations among SCN, SDS and seed yield traits

Correlations among SDS (DX, ISR6, ISR8), SCN (FI 0, FI 1.3-) and seed yield (Mg/Ha) were made (Supplemental Table 2) to expand on the reports of Yuan et al. (2002) and Kazi et al. (2008). Resistance to SCN HG Type 0 was negatively correlated with seed yield ($R^2 = 0.69$). Resistance to HG Type 1.3- was also negatively correlated with seed yield but more weakly ($R^2 = 0.26$). Positive correlations were observed when comparing resistance to SCN HG Type 0 with the resistance to infection (IS) of roots by *Fusarium virguliforme* (the pathogen that causes SDS). FI was correlated with IS at both growth stages R6 ($R^2 = 0.71$) and R8 ($R^2 = 0.75$). However, FI and SDS DX were weakly correlated ($R = 0.31$). In contrast, the correlation between IS and HG Type 1.3- FI showed negative correlations at both R6 ($R^2 = 0.27$) and R8 ($R^2 = 0.63$). The relationships between loci underlying responses to SCN, SDS and seed yield appear complex and would be predicted to include linkages in attraction and repulsion phase at separate loci.

Identification of QTL underlying resistance to SCN FI HG Types 0 (race 3) and 1.3- (race 14) in the RIL population

The major genomic region associated with variation in resistance to HG Type 0 and HG Type 1.3- was found on linkage group G, the expected position for *rhg1* (Table 2). The marker Satt309 was the marker most strongly associated with resistance to HG Type 0 ($P = 0.0001$, $R^2 = 22.3\%$). The beneficial allele was derived from Hartwig (H) not Flyer ($F = 64.3 \pm 4.1$, $H = 20.8 \pm 6.8$). The TMD1 marker from the intron in the RLK gene at *rhg1* was also strongly associated ($P = 0.008$, $R^2 = 11\%$) and closely linked to Satt309 (3.3 cM). The beneficial allele at TMD1 was derived from Hartwig ($F = 60.8 \pm 4.2$, $H = 32.6 \pm 9.1$). The Satt309 to TMD1 interval had a peak-LOD score of 3.9 and explained 21% of the total variation in the HG Type 0 FI. Linked markers Satt275 (2.2 cM; $P = 0.002$, $R^2 = 13.7\%$) and Satt163 (3.2 cM; $P = 0.005$, $R^2 = 14\%$) located to the telomeric side of TMD1 were less strongly associated with resistance to HG Type 0.

Markers to the centromeric side of Satt309 were also strongly associated with resistance to HG Type 0. Satt610 ($P = 0.001$, $R^2 = 17\%$), 15.5 cM from Satt309, had the beneficial allele from Hartwig ($F = 66.5 \pm 5.6$, $H = 34.2 \pm 6.5$; Table 2). A high peak- LOD score of 4.85 underlay 27% of the trait variation. However, selection against Hartwig alleles at *rhg1* was observed for Satt 309, TMD1, Satt163 and Satt275. In contrast, Satt038_2 and Satt610 showed segregation ratios that were not significantly skewed away from 1:1 ratios among the RILs.

Table 2 Markers and intervals those were associated with resistance to HG Type 0 (race 3) in the F × H RIL population on linkage group G

Marker	*P* value	R^2 (%)	FI mean ± SEM		Intervals			
			Flyer	(*n*)[a]	Hartwig	(*n*)	LOD	QTL var[b]
Satt163	0.002	14	64.0 ± 5.2	(57)	37.6 ± 5.9	(20)		
Satt275	0.005	14	62.5 ± 4.6	(47)	34.8 ± 8.4	(33)	3.6	18
TMD1	0.008	11	60.8 ± 4.2	(59)	32.6 ± 9.1	(19)	3.9	18
Satt309	0.0001	22	64.3 ± 4.1	(69)	20.8 ± 6.8	(18)	3.9	21
Satt610	0.00117		66.5 ± 5.6	(35)	34.2 ± 6.5	(23)	4.9	27

[a] Number of lines after heterogenous lines were excluded

[b] % of trait variation explained by each marker-trait association

Consequently, Satt038_2 was placed between Satt309 and Satt610 (Fig. 3; Kazi et al. 2008), a different position from Satt038_1 on the composite map (Song et al. 2004). Therefore, interacting loci may be involved and caution should be exercised in changing the position of the QTL at *rhg1* from the TMD1 to Satt309 interval expected (Ruben et al. 2006).

Markers linked to *Rhg4* on LG A2 (BARC-BLT65, SIUC-SagH100B10b and SIUC-A2D8) were significantly associated with resistance to HG Type 0. The marker most strongly associated was A2D8 ($P < 0.001$, $R^2 = 19\%$) and the beneficial allele was again from Hartwig ($F = 51.2 \pm 4.5$, $H = 25.0 \pm 2.5$). The association was not detected by Prabhu et al. (1999), since only 50 lines were phenotyped at that time and only BLT65 was used. Here, 92 lines and the parents were tested with HG Type 0. Further SagH100B10b, A2D8 and SagH100B10a markers were more closely linked to *Rhg4* (0.5, 0.25, and 0.1 cM) than BLT65 (about 1.25 cM; Meksem et al. 2001). As in the Essex by Forrest population, the SagH100B10a marker mapped into the deletion in the *Rhg4* to *I* region (Shultz et al. 2007) in the susceptible lines.

For HG Type 1.3-, the Satt309 region of LG G was significantly associated with resistance ($P = 0.001$, $R^2 = 16.6\%$; Table 3), with the beneficial allele from Hartwig ($F = 81.2 \pm 2.30$, $H = 69.1 \pm 5.76$). The linked (3.2 cM) marker Satt163 ($P = 0.001$, $R^2 = 6\%$) was also associated. The interval had a peak-LOD score of 2.87 and explained about 14.6% of trait variation in the F × H RIL population. Markers linked to *Rhg4* on LG A2 (BLT65, SagH100B10b and A2D8) were weakly associated with resistance to HG Type 1.3- ($0.05 < P < 0.01$).

For HG Type 1.2.5-, as sub-set of RILs ($n = 14$) was used for ANOVA, the lines that were HG Type 0 resistant. Analysis indicated that the region of LG G from Hartwig identified by Satt309 was significantly associated with resistance ($P = 0.001$, $R^2 = 24\%$) in the RIL sub-population. Markers linked to *Rhg4* on LG A2 (BLT65, SagH100B10b and A2D8) were not significantly associated with resistance to HG Type 1.2.5- in this small sub-set (data not shown). However, another marker Satt543 on LG D2 was significantly associated with resistance to HG Type 1.2.5- ($P = 0.005$, $R^2 = 12\%$). The possible associations with resistance to HG Type 1.2.5- were tested in NIL populations to attempt QTL confirmation.

Confirmation of loci associated with resistance to SCN FI HG Type 1.2.5- (race 2) in a NIL population

Because there were only two RILs resistant to HG Type 1.2.5-, QTL analysis in the 14 lines most resistant to HG Type 0 was followed by analysis in NILs derived from these RILs. Judged by single-plant responses, two RILs were segregating for resistance to SCN and were selected for further NIL analysis (Kazi 2005).

A near-isogenic line (NIL) population, derived from RIL F × H19 was used to confirm the location of the locus underlying the SCN HG Type 1.2.5-resistance. Ten NILs were extracted and six plants per NIL were used for FI determination. The frequency distribution of the FxH19-derived NIL population appeared continuous and positively kurtotic (data not shown; see Kazi 2005). The female index ranged from 12 to >114 and was skewed toward a high female index. Heritability was calculated at 91%. Line 19–8 was the only fully resistant iso-line, two iso-lines were moderately susceptible (FI < 60), and seven iso-lines were susceptible. Within intermediate iso-lines (12 > FI < 100), there were some individual plants with FI < 10, suggesting genetic segregation within the NILs; or line contamination; or some escapes from SCN infection.

The RIL F × H19 and derived F5:7 derived NILs FH19-1 to 20 all carried the Hartwig allele at *rhg1* (TMD1) and *Rhg4* (BLT65) as judged by DNA markers. The NILs FH19-1 to 10 were expected to have about 6.25% of the genome segregating. However, the NILs did not segregate for 140 of the 144 markers tested suggesting only 3% of loci were heterozygous. The four markers that were heterozygous in F × H RIL 19 all segregated in the derived near-isogenic line population. Table 4 shows that mean FI was significantly lower ($P < 0.046$, $R^2 = 19\%$) for genotypes carrying the Hartwig allele for Satt543 (LG D2; 88.02 cM). However, Hartwig alleles at Satt594 (LG G; 52.93 cM), Satt237 (LG N; 74.98 cM) and Satt530 (LG N; 32.84) were not associated with different SCN FI means. The most resistant NIL 19-8 showed a single plant that was susceptible (FI 60%) and five completely resistant plants (Fig. 2b).

Table 3 Markers and intervals that were associated with resistance to HG Type 1.3- (race 14) in the F × H RIL population on linkage group G

Marker	*P* value	R^2 (%)	FI mean ± SEM	Intervals		
			Flyer	Hartwig	LOD	QTL var[a]
Satt163	0.04	6	81.2 ± 2.3	69.1 ± 5.8		
Satt309	0.001	17	81.6 ± 2.1	61.5 ± 6.9	2.87	14.6

[a] Percentage of trait variation explained by each marker-trait association

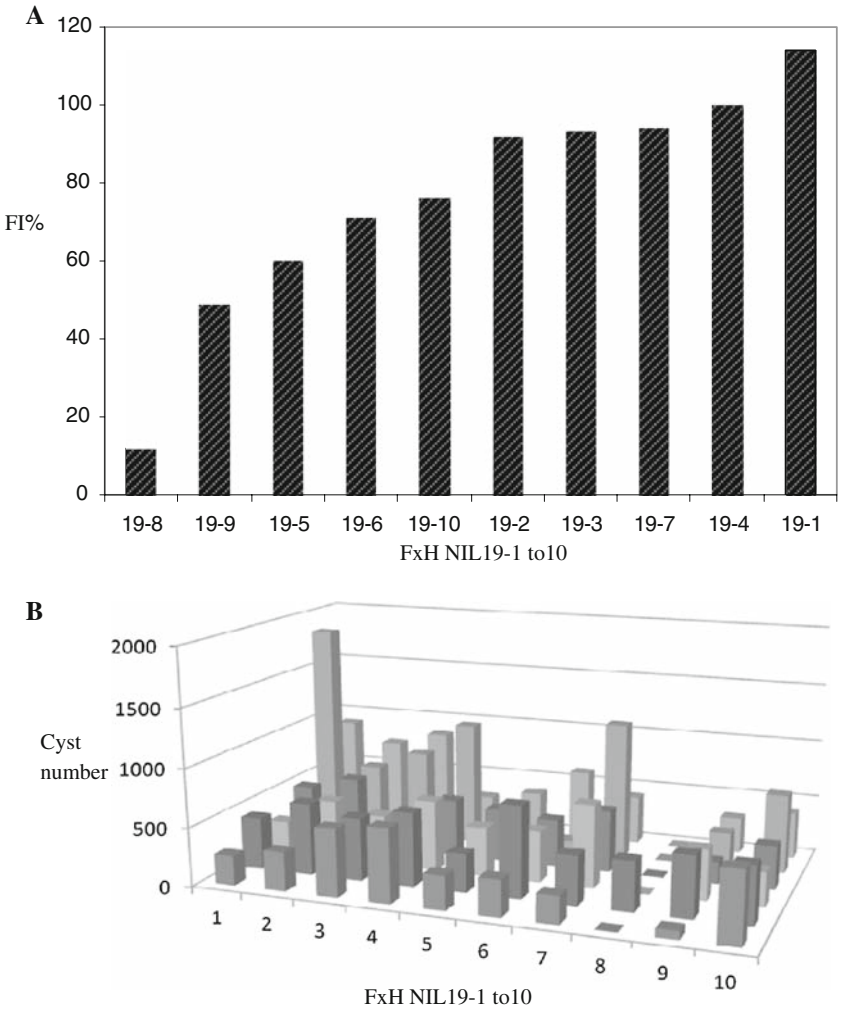

Fig. 2 Frequency distribution of F × H19-derived NIL population analyzed for SCN HG Type 1.2.5-female index (FI). **a** NIL 19-1 to 10 ranked by mean FI%. FIs below 30% were moderately resistant; FIs from 30 to 60% were moderately susceptible; FIs from 60 to 100% were susceptible and FIs greater than 100% were highly susceptible. **b** The cyst count for each of six plants per NIL 1-10 in numeric order

The susceptible plant had the Flyer allele at Satt543 suggesting the NIL 19-8 was still segregating at this locus.

A second small NIL population ($n = 10$) derived from F × H33 showed segregation at markers linked to *rhg1* (cqSCN-001) on LG G. There was association between the HG Type 0, 1.3- and 1.2.5- traits and those markers (data not shown; Kazi 2005). This was an expected result that validated the usefulness of the small NIL populations for QTL confirmation.

The seed yield QTL on LG D2

The region on LG D2 (Fig. 3) identified by the microsatellite marker Satt514 was significantly ($P = 0.0006$, $R^2 = 7.4\%$) associated with seed yield in the RILs of FH92. The QTL was detected in the mean seed yield in the complete population of 92 RILs grown at four locations (two row plots, three replications, 2 years). The beneficial allele was from Hartwig (3.00 ± 0.04 Mg ha^{-1}) not Flyer (2.77 ± 0.05 Mg ha^{-1}). The adjacent marker was Satt488. The interval between these two markers spanned a genetic distance of about 3.5 cM. The yield QTL had peak LOD of 2.57 and explained about 13.3% of total variation in seed yield (0.42 ± 0.04 Mg ha^{-1}).

Discussion

The correlations among SCN SDS and seed yield traits were significant (Supplemental Table 2). Different linkage phases were inferred among the separate loci underlying seed yield and resistances to SCN and SDS on LGs G and D2 (Tables 1, 2, 3; Fig. 3). On LG G, the alleles underlying resistance to SCN and SDS were linked to a seed yield depression allele. On linkage group D2, the SCN resistance allele was linked to a seed yield increase allele. Both were linked to alleles underlying SDS susceptibility. Therefore, progress can be made in soybean breeding by identifying recombination events in both LGs D2 and G that create new linkage relationships.

Trait heritabilities were high (86–96%) reflecting repeatable assays under controlled conditions (Table 1).

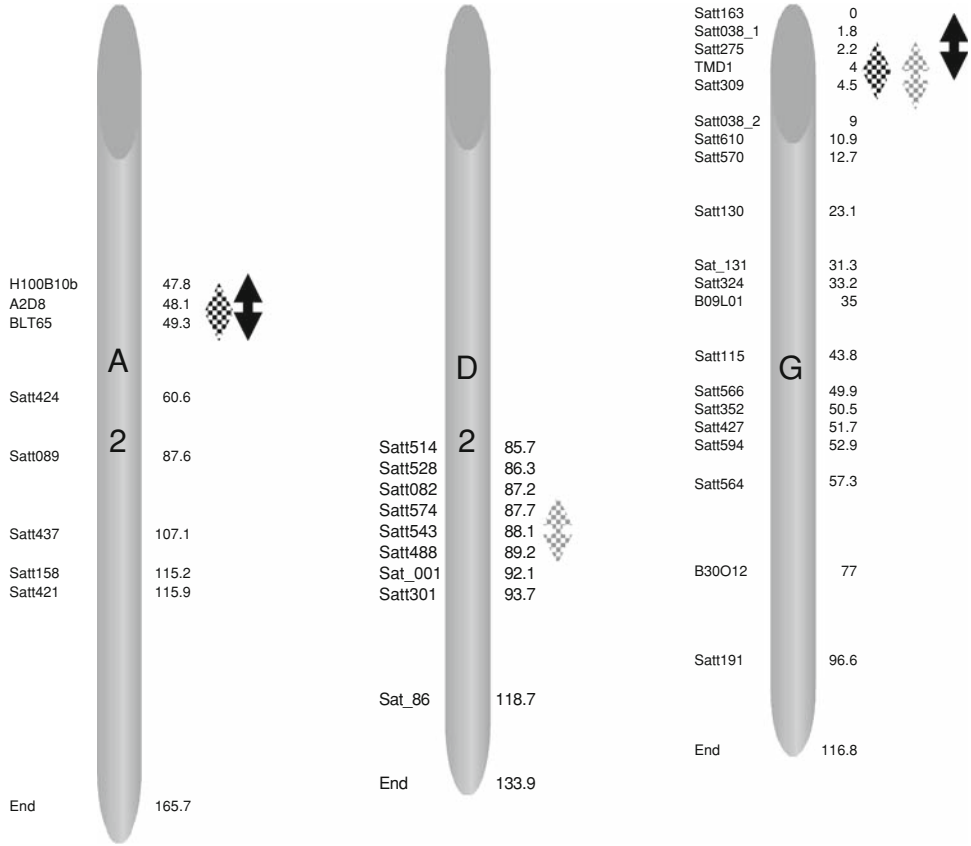

Fig. 3 Locations of the QTL found in the Flyer by Hartwig population on linkage groups *A2*, *D2* and *G* for resistance to SCN HG Type 1.2.5- (*grey stippled arrows*) HG Type 0 (*black stippled arrows*) and HG Type 1.3 (*black solid arrow*). The size of the arrow reflects the interval significantly associated by QTL Cartographer or Mapmaker at LOD >2.5 or ANOVA at $P < 0.0005$. H100B10a lies physically between A2D8 and BLT65 in resistant lines but within the deletion at *I* and *Rhg4* found in susceptible lines

Table 4 Four markers that were associated with the QTL underlying resistance to HG Type 1.2.5- segregated in the F × H19-derived near-isogenic line (NIL) population

Marker	LG	Allele[a]	N^b	Pr > *F*	R^{2c}	Allelic mean ± SEM
Satt543	D2	*H*	18	0.046*	0.19	30.8 ± 7.8
		F	28			86.2 ± 9.4
Satt594	G	*H*	23	0.31	0.006	58.7 ± 12.4
		F	21			82.4 ± 8.9
Satt237	*N*	*H*	22	0.076	0.9	63.1 ± 9.8
		F	31			84.6 ± 8.8
Satt530	*N*	*H*	25	0.12	0.7	70.6 ± 9.1
		F	25			79.7 ± 6.2

Six plants from each of ten lines within the F × H19-derived NIL population were tested for resistance to HG Type 1.2.5-. There were six plants per genotype. The sixty plants were scored for each of the four markers. Heterozygous lines were excluded

* Significance at 0.05 level

[a] *H* was the Hartwig allele, *F* was the Flyer allele

[b] *N* was the number of individuals scored. Heterozygous lines and absent marker scores not included

[c] R^2 was the sum of squares for the source of the variance divided by the total sum of squares

However, the loci identified each explained just 17–24% of the heritability (Tables 2, 3, 4). Joint heritabilities were less than 50% for the 2–3 loci underlying each trait. Hg Type 0 resistance should be explained fully by the 2 loci detected (Webb et al. 1995; Meksem et al. 2001) but skewed segregation ratios were detected for the most closely linked markers. For the other two Hg Types at least one additional locus was inferred to underlie the phenotype. Additional markers from SNP collections or BAC ends may help identify the location of these loci (Shultz et al. 2007).

Resistance to SCN Hg Type 1.3- was shown to require resistance alleles at both HG Type 0 loci (*rhg1, Rhg4*) and D2 (cqSCN005, *Rhg3*). An additional locus for resistance to Hg Type 1.3- was expected on LgC1 (*Rhg2,* Webb et al. 1995). The locus could be inferred from the fact that only 4 lines resistant to Hg Type 1.3- were identified from the 14 resistant to HgType 0. The linkages observed among QTL on D2 could also explain how resistance to SCN Hg Type 0 was positively correlated with resistance to SDS whereas resistance to SCN Hg Type 1.3.5 was negative correlated with SDS. That phenomenon was found both in this population (Kazi et al. 2008) and across diverse germplasm (Gibson et al. 1994).

Only two lines were resistant to HgType 1.2.5-. Therefore, another locus on Lg J in addition to LG G and D2 may underlie resistance (Table 5; *Rhg5*, Webb et al. 1995). The major effect of *rhg1* on resistance to all HG Types (Webb et al. 1995; Concibido et al. 1997, 2004) was confirmed (Table 5). The effect of *Rhg4* on resistance to Hg type 0 and Hg Type 1.3- was also confirmed.

Table 5 Comparison of the loci underlying resistance to SCN detected in inheritance studies using PI437654 and Hartwig

LG	A2	C1	G	M	J	D2
Gene	*Rhg4*	*Rhg2*	*Rhg1*	*Rzd*	*Rhg5*	*Rhg3*
HG Type (race)						
2.5–(1)	x	x	x	x	x	
1.2.5–(2)			**x**	x	x	**x**
0–(3)	**x**		**x**	x		
2–(5)		x	x	x		
1.3–(14)	**x**	x	**x**	x		

Data from were assembled from Patent # 6,162,967 (Webb et al. 1995); Prabhu et al. (1999); Yuan et al. (2002) and this work. In bold are the loci confirmed by the analysis in F × H. The *Rhg* gene annotations are from Webb et al. (1995). *Rzd* was a gene whose allele must match *Rhg1* to prevent zygote death

Interactions among *rhg*-loci are common phenomena and no single gene is sufficient for resistance (Webb et al. 1995; Meksem et al. 2001; Concibido et al. 2004; Wu et al. 2009). The set of genes identified within the *rhg1* locus (Ruben et al. 2006) have a syntenic set of paralogs at another genomic location on LG B1 (Afzal and Lightfoot 2007; http://www.phytozome.net/cgi-bin/gbrowse/soybean/; Afzal et al. 2008, 2009). However, the interacting locus on LG M inherited in phase with *rhg1* (Webb et al. 1995, 1996) did not appear to segregate in FH92 since no marker on LG M was polymorphic among the 20 tested. The equivalent locus discovered in E × F in the middle of LG G was not co-inherited with *rhg1* in FH92 either. Therefore, the known modifiers of *rhg1* action could not be implicated in the shift in apparent location of the loci.

On LG D2, the locus underlying resistance to HG Type 1.2.5- might have been the same allele as that underlying race 1.3- in the population derived from Hartwig by BR-92-31983 (Schuster et al. 2001) or simply linked to it. However, it was not associated with resistance to Hg Type 1.3- in the experiments reported here. The locus detected here was confirmed to underlie resistance to Hg Type 1.2.5- in a small NIL population and therefore represented a confirmed QTL. The designation cqSCN-005 was assigned by the Soybean Genetics Committee in 2008 (http://www.soygenetics.org/committee.php). The Hartwig allele at the region seemed to increase seed yield by 0.3 Mg ha^{-1} (either by resistance to low SCN infestations or linkage to a second locus) but caused greater susceptibility to root infection (IS by 15%) and leaf scorch (by 7%; Kazi 2005; Kazi et al. 2008). Therefore, the D2 locus should be targeted for fine mapping, genomic analyses, and marker selection of new recombination events that improve all three agronomic traits.

The molecular basis of the interactions among loci; within resistance to SCN (Webb et al. 1995, 1996; Wu et al. 2009); among diseases (Kazi et al. 2007); and with agronomic traits (Yuan et al. 2002) is not clear. A comparison of Williams, Forrest, Hartwig and PI437654 DNA sequences in both the G and D2 regions associated with resistance to SCN (Lightfoot 2008; Afzal et al. 2009; Campbell et al. 2009) might show the molecular mechanisms underlying these interactions, correlations and phenomena.

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 or United States Department of Agriculture. 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 Drs. P. Gibson, O. Myers Jr. and M. Schmidt for assistance with germplasm development and maintenance from 1991 to 2000. We thank the "Soybean Genome Project", at DoE Joint Genome Institute, for the timely release of the WGS reads, scaffolds and genome sequence.

References

Afzal AJ, Lightfoot DA (2007) Soybean disease resistance protein RHG1-LRR domain expressed, purified and refolded from *Escherichia coli* inclusion bodies: preparation for a functional analysis. Protein Expr Purif 53:346–355

Afzal AJ, Saini N, Srour A, Lightfoot DA (2008) The multigeneic *rhg1* locus: a model for the effects on root development, nematode resistance and recombination suppression. Nature Precedings hdl:10101/npre.2008.2726.1 (online)

Afzal AJ, Saini N, Srour A, Lightfoot DA (2009) The multigeneic *rhg1* locus complete sequence identifies alleles of three genes underlying root development, nematode resistance and recombination suppression. Nat Genet (in review)

Arelli PR, Sleper DA, Yue P, Wilcox JA (2000) Soybean reaction to races1 and 2 of *Heterodera glycines*. Crop Sci 40:824–826

Basten CJ, Weir BS, Zeng Z (2001) QTL Cartographer Version 2.0. Department of Statistics, North Carolina State University, Raleigh, NC, USA

Brucker E, Niblack T, Kopisch-Obuch FJ, Diers BW (2005) The effect of *rhg1* on reproduction of *Heterodera glycines* in the field and greenhouse and associated effects on agronomic traits. Crop Sci 45:1721–1727

Campbell N, Warner AL, Lightfoot DA, Matthews BF, Knap HT (2009) Duplication of a chromosomal region from linkage group A2 involved in cyst nematode resistance in soybean. Mol Gen Genet (in press)

Chang SJC, Doubler TW, Kilo V, Suttner RJ, Klein JH III, Schmidt ME, Gibson PT, Lightfoot DA (1997) Association of field resistance to soybean sudden death syndrome (SDS) and cyst nematode (SCN). Crop Sci 37:965–971

Concibido VC, Lange DA, Denny RL, Orf JH, Young ND (1997) Genome mapping of soybean cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers. Crop Sci 37:258–264

Concibido VC, Diers BW, Arelli PR (2004) A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci 44:1121–1131

Davis EL, Hussey RS, Baum TJ (2004) Getting to the roots of parasitism by nematodes. Trends Parasitol 20:134–141

Fehr W (1987) Principals of cultivar development, vol 1: theory and techniques. McMillan, New York

Gibson PT, Shenaut MA, Njiti VN, Suttner RJ, Myers Jr O (1994) Soybean varietal response to sudden death syndrome. In: Wilkinson D (ed) Proceedings of Twenty-fourth Soybean Seed Research Conference, Chicago, IL. 6–7 Dec 1994. American Seed Trade Association, Washington DC, pp 436–446

Glover KD, Wang D, Arelli PR, Carlson SR, Cianzio SR, Diers BW (2004) Near isogenic lines confirm a soybean cyst nematode resistance gene from PI88788 on linkage group. J Crop Sci 44:936–941

Hnetkovsky N, Chang SJC, Doubler TW, Gibson PT, Lightfoot DA (1996) Genetic mapping of loci underlying field resistance to soybean sudden death syndrome (SDS). Crop Sci 36:393–400

Hussey RS, Grundler FMW (1998) Nematode parasitism of plants. In: Perry RN, Wright DJ (eds) The physiology and biochemistry of free-living and plant parasitic nematodes. CAB International, Wallingford, pp 213–243

Iqbal MJ, Ahsan R, Afzal AJ, Jamai A, Meksem K, El-Shemy HA, Lightfoot DA (2009) Multigeneic QTL: the laccase encoded within the soybean *Rfs2/rhg1* locus inferred to underlie part of the dual resistance to cyst nematode and sudden death syndrome. Curr Issues Mol Biol i11:11–19

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Kazi S (2005) Minimum tile derive microsatellite markers improve the physical map of the soybean genome and the Flyer by Hartwig genetic map at *Rhg*, *Rfs* and yield loci. MS Thesis SIUC Carbondale IL, USA, p 212

Kazi S, Njiti VN, Doubler TW, Yuan J, Iqbal MJ, Cianzio S, Lightfoot DA (2007) Registration of the Flyer by Hartwig recombinant inbred line mapping population. J Plant Regis 1:175–178

Kazi S, Shultz J, Bashir R, Afzal J, Njiti V, Lightfoot DA (2008) Separate loci underlie resistance to soybean sudden death syndrome in 'Hartwig' by 'Flyer'. Theor Appl Genet 116:967–977

Kopisch-Obuch FJ, McBroom KL, Diers BW (2005) Association between SCN resistance loci and yield in soybean. Crop Sci 45:956–965

Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174

Lightfoot DA (2008) Soybean genomics: developments through the use of cultivar Forrest. Int J Plant Genome 2008:1–22. doi:10.1155/2008/793158

McBlain BA, Fioritto RJ, St Martin SK, Calip-DuBois A, Schmitthenner AF, Cooper RL, Martin RJ (1990) Registration of 'Flyer' soybean. Crop Sci 30:425

Meksem K, Lightfoot DA (2000) Novel polynucleotides and polypeptides relating to loci underlying resistance to soybean cyst nematode and methods of use thereof. Patent pending. # 2002 0144310). Filing Date 01-29-2001

Meksem K, Pantazopoulos P, Njiti VN, Hyten DL, Arelli PR, Lightfoot DA (2001) 'Forrest' resistance to the soybean cyst nematode is bigenic: saturation mapping of the *rhg1* and *Rhg4* loci. Theor Appl Genet 103:710–717

Niblack TL, Noel GR, Lambert KL (2003) The Illinois SCN type test: practical application of the HG Type classification system. J Nematol 35:345–355

Njiti VN, Doubler TW, Suttner RJ, Gray LE, Gibson PT, Lightfoot DA (1998) Resistance to soybean sudden death syndrome and root colonization by *Fusarium solani* f. sp. glycines in near-isogeneic lines. Crop Sci 38:472–477

Njiti VN, Meksem K, Iqbal MJ, Johnson JE, Kassem MA, Zobrist KF, Kilo VY, Lightfoot DA (2002) Common loci underlie field resistance to soybean sudden death syndrome in Forrest, Pyramid, Essex, and Douglas. Theor Appl Genet 104:294–300

Palmateer AJ, Schmidt ME, Stetina SR, Russin JS (2000) Temperature effects on race determination in *Heterodera glycines*. J Nematol 32:349–355

Prabhu RR, Njiti VN, Johnson JE, Schmidt ME, Klein RJ, Lightfoot DA (1999) Selecting soybean cultivars for dual resistance to cyst nematode sudden death syndrome with two DNA markers. Crop Sci 39:982–987

Qui BX, Arelli PR, Sleper DA (1999) RFLP markers associated with soybean cyst nematode resistance and seed composition in a 'Peking' × 'Essex' population. Theor Appl Genet 98:356–364

Riggs RD, Schmitt DP (1988) Complete characterization of the race scheme for *Heterodera glycines*. J Nematol 20:392–395

Ruben E, Aziz J, Afzal J, Njiti VN, Triwitayakorn K, Iqbal MJ, Yaegashi S, Arelli PR, Town CD, Ishihara H, Meksem K, Lightfoot DA (2006) Genomic analysis of the 'Peking' *rhg1* locus: candidate genes that underlie soybean resistance to the cyst nematode. Mol Genet Genome 276:320–330

Saini N, Shultz JL, Lightfoot DA (2008) Re-annotation of the physical map of *Glycine max* for ploidy by BAC end sequence driven whole genome shotgun read assembly. BMC Genomics 9:323–338

Schmitt DP, Shannon G (1992) Differentiating soybean responses to *Heterodera glycines*. Crop Sci 32:275–277

Schuster I, Abdelnoor RV, Marin SRR, Carvalho VP, Kiihl AS, Silva JFV, Sedyama CS, Barros EG, Moreira MA (2001) Identification of a new major QTL associated with resistance to the soybean cyst nematode (*Heterodera glycines*). Theor Appl Genet 102:91–96

Senda M, Kasai A, Yumoto S, Akada S, Ishikawa R, Harada T, Niizeki M (2002) Sequence divergence at chalcone synthase gene in pigmented seed coat soybean mutants of the inhibitor locus. Genes Genet Syst 77:341–350

Shultz JL, Kurunam D, Shopinski K, Iqbal MJ, Kazi S, Zobrist K, Bashir R, Yaegashi S, Lavu N, Afzal AJ, Yesudas CR, Kassem MA, Wu C, Zhang HB, Town CD, Meksem K, Lightfoot DA (2006a) The Soybean Genome Database (SoyGD): a browser for display of duplicated, polyploid, regions and sequence tagged sites on the integrated physical and genetic maps of *Glycine max*. Nucleic Acids Res 34:D758–D765

Shultz JL, Yesudas CR, Yaegashi S, Afzal J, Kazi S, Lightfoot DA (2006b) Three minimum tile paths from bacterial artificial chromosome libraries of the soybean (*Glycine max* cv. 'Forrest'): tools for structural and functional genomics. Plant Methods 2:9–18

Shultz JL, Kazi S, Afzal JA, Bashir R, Lightfoot DA (2007) The development of BAC-end sequence-based microsatellite markers and placement in the physical and genetic maps of soybean. Theor Appl Genet 114:1081–1090

Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theor Appl Genet 109:122–128

Triwitayakorn K, Njiti VN, Iqbal MJ, Yaegashi S, Town CD, Lightfoot DA (2005) Genomic analysis of a region encompassing *QRfs1* and *QRfs2*: genes that underlie soybean resistance to sudden death syndrome. Genome/Génome 48:125–138

Vierling RA, Faghihi J, Ferris VR, Ferris JM (1996) Association of RFLP markers conferring broad-based resistance to the soybean cyst nematode (*Heterodera glycines*). Theor Appl Genet 92:83–86

Webb DM, Baltazar BM, Arelli PR, Schupp J, Keim P, Clayton K, Ferreira AR, Owens T, Beavis WD (1995) QTL affecting soybean cyst-nematode resistance. Theor Appl Genet 91:574–581

Webb, DM, Baltazar BM, Arelli PR, Schupp J, Keim P, Clayton K, Ferreira AR, Owens T, Beavis WD (1996) QTL affecting soybean cyst-nematode resistance. United States Patent 5,491,081, Feb 16, 1996

Weisemann JM, Matthews BF, Devine TE (1992) Molecular markers located proximal to the soybean cyst nematode resistance gene *Rhg4*. Theor Appl Genet 85:136–138

Wrather JA, Anderson TR, Arsyad DM, Gai J, Ploper DL, Portapuglia A, Ram HH, Yorinori JT (1996) Soybean disease loss estimates for the top ten producing countries during. Plant Dis 79:107–110

Wrather JA, Koenning SR, Anderson TR (2003) Effect of diseases on soybean yields in the United States and Ontario (1999 to 2002). Plant Health Progr. doi:10.1049/PHP-2003-0325-01-RV

Wu X, Blake S, Sleper D, Shannon G, Cregan P, Nguyen H (2009) QTL, additive and epistatic effects for SCN resistance in PI 437654. Theor Appl Genet 118:1093–1105

Yuan J, Njiti VN, Meksem K, Iqbal MJ, Triwitayakorn K, Kassem MA, Davis GT, Schmidt ME, Lightfoot DA (2002) Quantitative trait loci in two soybean recombinant inbred line populations segregating for yield and disease resistance. Crop Sci 42:271–277