



Mapping of new quantitative trait loci for sudden death syndrome and soybean cyst nematode resistance in two soybean populations

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Abstract

Key message Novel QTL conferring resistance to both the SDS and SCN was detected in two RIL populations. Dual resistant RILs could be used in breeding programs for developing resistant soybean cultivars.

Abstract Soybean cultivars, susceptible to the fungus *Fusarium virguliforme*, which causes sudden death syndrome (SDS), and to the soybean cyst nematode (SCN) (*Heterodera glycines*), suffer yield losses valued over a billion dollars annually. Both pathogens may occur in the same production fields. Planting of cultivars genetically resistant to both pathogens is considered one of the most effective means to control the two pathogens. The objective of the study was to map quantitative trait loci (QTL) underlying SDS and SCN resistances. Two recombinant inbred line (RIL) populations were developed by crossing ‘A95-684043’, a high-yielding maturity group (MG) II line resistant to SCN, with ‘LS94-3207’ and ‘LS98-0582’ of MG IV, resistant to both *F. virguliforme* and SCN. Two hundred F₇ derived recombinant inbred lines from each population AX19286 (A95-684043 × LS94-3207) and AX19287 (A95-684043 × LS98-0582) were screened for resistance to each pathogen under greenhouse conditions. Five hundred and eighty and 371 SNP markers were used for mapping resistance QTL in each population. In AX19286, one novel SCN resistance QTL was mapped to chromosome 8. In AX19287, one novel SDS resistance QTL was mapped to chromosome 17 and one novel SCN resistance QTL was mapped to chromosome 11. Previously identified additional SDS and SCN resistance QTL were also detected in the study. Lines possessing superior resistance to both pathogens were also identified and could be used as germplasm sources for breeding SDS- and SCN-resistant soybean cultivars.

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Introduction

Worldwide, soybean [*Glycine max* (L.) Merrill] is one of the most economically and nutritionally valuable legumes for oil and protein production. However, every year a number of abiotic and biotic factors threaten soybean production and greatly decrease yield (Grinnan et al. 2013). As per 2014 estimates in the USA, soybean cyst nematode (SCN) (*Heterodera glycines*, Ichinohe) and sudden death syndrome (SDS), caused by the soil-borne fungus *Fusarium virguliforme* O’Donnell and T. Aoki (formerly *F. solani* (Mart.) Sacc. f. sp. *glycines*), are ranked first and second, respectively, as yield-reducing pathogens of soybean (Bradley and Allen 2014). The estimated losses are 3.4 million metric tons (125 million bushels) due to SCN and 1.7 million metric tons (62 million bushels) due to SDS, together representing a loss of US 1.9 billion dollars (Bradley and Allen 2014). Both pathogens, first identified in the southern regions of the U.S., have spread to the northern soybean production areas

(Koenning and Wrather 2010; Roy et al. 1997; Rupe 1989; Scherm and Yang 1996; Tylka and Marett 2014; Winstead et al. 1955).

Fusarium virguliforme infects and colonizes soybean roots, causing necrosis and root rot, later causing foliar symptoms, although the pathogen has never been isolated from leaves (Li et al. 1999). It has been reported that one or more toxins move from the infected roots through the xylem finally reaching the leaves to cause foliar SDS symptoms (Abeysekara and Bhattacharyya 2014; Brar et al. 2011; Li et al. 1999; Pudake et al. 2013). The name ‘sudden death syndrome’ is descriptive of the disease, since normal-appearing plants in fields suddenly turn yellow and quickly die (Hartman et al. 2015; Leandro et al. 2012). The management options for controlling the disease are limited (Robertson and Leandro 2010), with some agronomic practices reducing disease incidence (Mueller et al. 2003). The planting of resistant varieties is the most effective and feasible method to reduce SDS yield losses (Kandel et al. 2015).

The inheritance of SDS resistance is complex and quantitative (Chang et al. 1996; de Farias Neto et al. 2007; Hnetkovsky et al. 1996; Kassem et al. 2006, 2007, 2012; Kazi et al. 2007, 2008; Njiti et al. 2002; Njiti and Lightfoot 2006; Prabhu et al. 1999; Stephens et al. 1993; Swaminathan et al. 2016; Yuan et al. 2012). A recent publication reported 40 plus SDS resistance QTL mapped to 18 of the 20 soybean chromosomes from studies on 15 different segregating populations (Swaminathan et al. 2016).

The complex nature of SDS resistance makes breeding of high-yielding SDS-resistant cultivars difficult, with significant efforts devoted to identify new SDS resistance sources. More than 6000 soybean plant introduction (PI) lines and 2000 public/private developed soybean cultivars have been evaluated for SDS resistance with only a fraction being partially resistant (Hartman et al. 1997; Mueller et al. 2002, 2003; Rupe et al. 1991). No major resistance genes have yet been identified, suggesting that for breeding purposes, it might be useful to pyramid some of the important SDS resistance QTL from different sources into a single genotype (Lightfoot 2015).

SCN is the other even more destructive pathogen to soybean production (Brzostowski et al. 2014). The nematode infests the roots of the soybean and leads to what at times is called “Yellow dwarf” symptom in soybean (Davis et al. 2004). The nematode causes root necrosis, suppression of root and shoot growth, chlorotic patches within leaflets, reducing seed yield. Once established in a field, the nematode is difficult to eradicate due to high longevity of the eggs and the ability of the nematode populations to overcome soybean resistance genes (Wrather and Ploper 1996). This pathogen is best controlled by planting SCN resistant cultivars (Davis and Tylka 2000).

The inheritance of resistance to SCN has also been reported as multigenic (Kazi et al. 2010; Lu et al. 2006; Mansur et al. 1993). Many SCN resistance QTL have been identified in more than 18 PIs using molecular techniques (Concibido et al. 2004; Guo et al. 2006; Lu et al. 2006). More than 60 SCN resistance QTL have been reported and mapped to almost all soybean chromosomes, except chromosome 2, 9 and 10 (<http://www.soybase.org>). Five major resistance genes have also been mapped, i.e., *rhg1*, *rhg2*, *rhg3*, *Rhg4* and *Rhg5* (Chang et al. 2011; Concibido et al. 2004; Meksem et al. 2001; Ruben et al. 2006). The major resistance loci *rhg1* (chromosome 18) and *Rhg4* (chromosome 8) have been consistently mapped in multiple populations and both were cloned (Concibido et al. 2004; Cook et al. 2012; Liu et al. 2012, 2017; Yu et al. 2016). The *rhg1* locus was found to be complex with a 31.2 kb interval repeated from one to ten times and the number of repeats shown to be related to host resistance (Cook et al. 2012; Yu et al. 2016). The *Rhg4* gene was cloned from the cultivar ‘Forrest’ and found to be a serine hydroxymethyltransferase (SHMT) protein (Liu et al. 2012).

Approximately 95% of the soybean cultivars in the U.S. trace SCN resistance to *rhg1* donated by PI 88788 (Mitchum 2016). It is a matter of concern that the resistant monoculture of the *rhg1* locus has exposed the nematode populations to high selection pressure which could overcome the *rhg1* encoded resistance (Faghihi et al. 2010; Mitchum et al. 2007; Niblack et al. 2008). It might be necessary to incorporate multiple diverse SCN resistance mechanisms into single cultivars and/or rotate different sources of resistance with the *rhg1* locus to improve SCN management (Mitchum 2016; Rincker et al. 2017).

The soil-borne pathogens *F. virguliforme* and *H. glycines* have been detected in soil samples collected in many commercial fields (A. Robertson, personal communication, Iowa State University, IA, 2010). In these soils, synergistic effects have been observed resulting in greater plant damage and yield losses than when only one of the pathogen is present (Brzostowski et al. 2014; Gelin et al. 2006; Xing and Westphal 2013). Improved germplasm lines carrying both SDS and SCN resistance are considered important as a means to control the pathogens (Cianzio et al. 2014, 2016).

In the present investigation, we used two populations (AX19286 and AX19287) of F₇-derived lines created by crossing one SCN-resistant parent to each of two SCN- and SDS-resistant parents. Phenotyping with each pathogen was done in the greenhouse, using either the fungus or the nematode for artificial inoculations. In previous research, Swaminathan et al. (2016) evaluated fungal toxin resistance using the same two populations. In this study, we report QTL, some new and some likely previously identified associated with resistance to *F. virguliforme* and SCN. We also

identified RILs that simultaneously possess resistance SDS QTL and SCN QTL.

Materials and methods

Plant material

Two hundred RILs were developed from each of the two soybean filial populations, AX19286 (A95-684043 × LS94-3207), and AX19287 (A95-684043 × LS98-0582) for this study. A95-684043 is susceptible to SDS but resistant to SCN HG types 0, 2 and 2.5.7 (Cianzio et al. 2002). The line A95-684043 (Cianzio et al. 2002; ISURF Docket # 02975), is of maturity group (MG) II, derived from the cross of Jacques J285 × [‘Archer’ × (‘Cordell’ × Asgrow A2234)]. Cordell is a SCN-resistant cultivar with resistance to SCN HG types 0, 2.5.7 and 1.2.3.5.7, developed from the cross of ‘Bedford’ × D72-8927. Bedford has the SCN resistance sources ‘Peking’ and PI 88788 in its pedigree. D72-8927 derived its SCN resistance from PI 90763.

The parent, LS94-3207, was developed at Southern Illinois University, Carbondale, IL (Schmidt and Klein 2004). It is of MG IV, resistant to SCN HG types 0, 2, 2.5.7, 1.2.5.7 and 1.3.6.7 and to SDS. It is a selection from the cross ‘Pharaoh’ × ‘Hartwig’. Pharaoh [derived from ‘Forrest’ (3) × V71-480] was released as a high yielding cultivar with resistance to SCN HG type 0 (Schmidt et al. 1993). Hartwig (derived from Forrest × PI 437654) is a cultivar resistant to SDS leaf scorch caused by *F. virguliforme* and resistant to SCN HG Type 1.3.6.7. Forrest derives SCN resistance from Peking through ‘Dyer’ (Hartwig and Epps 1968, 1973). Both Peking and PI 437654 are in the pedigree of the SCN resistance of LS94-3207.

LS98-0582 derived from the cross of Northrup King S46-44 × Asgrow A4138, is also of MG IV, and highly resistant to SCN HG types 0 and 1.3.6.7 (Heatherly and Hodges 1998). Asgrow A4138 was developed from the cross of Asgrow A4009 × Asgrow A4595. Northrup King S46-44 was developed from the cross of another two Asgrow lines, Asgrow A5474 × Asgrow A3127. LS98-0582 derives its SCN resistance from the source ‘Fayette’, which in turn traces SCN resistance to PI 88788 (Bernard et al. 1988).

The two crosses, AX19286 and AX19287 and the RILs were generated at the ISU soybean research site located at the Isabela Substation, University of Puerto Rico, Isabela, Puerto Rico between 2002 and 2006. The hybrid nature of the F₁ plants was confirmed with the morphological marker of flower color. For each cross, six F₁ seeds were obtained in January 2002. Each F₁ plant was identified and harvested individually in May 2002. The F₁ and F₂ plants were grown in Puerto Rico during the summer 2002. The identity of individual F₁ plants was maintained throughout the RIL

development. The F₂ plants were also identified, maintaining the ID of the F₁ from which the seed had been harvested.

A total of 200 F₂ plants (seed at the F₃ generation) were harvested for each of the two crosses. The subsequent generations were advanced by single seed descent. Generation advances were conducted for each line from December 2002 until February 2006, when the F₇ individual plants were harvested. F_{7,8} plant rows were grown for a seed increase and harvested in bulk.

SDS resistance screening

The 200 RILs from each of the two populations, the parents and controls were screened for SDS resistance/susceptibility using the protocol described by Cianzio et al. (2014). The screening method was originally developed by X. B. Yang (personal communication, Iowa State University, IA, 2000) and Hartman et al. (1997), modified by P. Lundeen (personal communication, Iowa State University, IA, 2007), later patented by D. Lightfoot (Patent # 7288386; Lightfoot et al. 2007) and used with permission.

Two *F. virguliforme* isolates were used in the screening study namely, Clinton-1b and Scott-F2I11a. Clinton-1b isolate is from Clinton County, IA, and Scott-F2I11a isolate from Scott County, IA. Isolates were obtained from roots of SDS symptomatic plants from commercial soybean fields (Sanogo et al. 2000). The isolates are stored and maintained in the Leandro lab culture collections at Iowa State University with the unique ID numbers viz., Clinton-1b (LL0059) and Scott-F2I11a (LL0063). Five weeks before planting soybean seeds, a mixture of *F. virguliforme* Clinton-1b and Scott-F2I11a isolates was grown on sorghum (*Sorghum bicolor*) seed under sterile conditions in 2-quart Mason jars. Four hundred grams of the sorghum seed was weighed, soaked overnight in distilled water, and autoclaved twice before spore inoculation. Ten plugs containing spores of *F. virguliforme* each of Clinton1b and Scott isolates grown on 1/3rd strength potato dextrose agar (PDA) plates were added to the autoclaved sorghum seed. *F. virguliforme* isolates were grown on the sorghum seed for 5 weeks, harvested, dried and ground in a blender.

Clean styrofoam cups (240 mL) were filled with 150 mL of a pasteurized 1:2 soil:sand mixture, followed by 30 mL of the inoculum: soil–sand: 1:10 mixture added at the top of the cup. Five seeds of each RIL were planted on the surface and covered with 30 mL of a pasteurized 1:2 soil–sand mixture. The cups were placed in a growth chamber and watered once daily. The seedlings were grown at 23 °C for 16 h under light (200 μmol photons/m²/s) and 16 °C for 8 h under dark conditions.

The foliar disease score (FDS) of each plant was recorded 5 weeks after planting using the scale of 1 = no foliar symptoms; 2 = slight yellowing and/or chlorotic flecks or blotches

(1–10% foliage affected); 3 = interveinal chlorosis (11–20% foliage affected); 4 = necrosis along a portion > 2 cm of its leaf margin (21–40% foliage affected); 5 = necrosis along the entire margin of leaves and leaves showing cupped and/or irregular shapes (41–75% foliage affected); 6 = interveinal necrosis and most of leaf area necrotic (75–100% foliage affected) and/or leaf drops including defoliation of the entire plants. On the basis of FDS, the RILs were classified as highly resistant (HR; FDS < 1.50), resistant (R; FDS 1.51–2.00), moderately resistant (MR; FDS 2.01–2.50), susceptible (S; FDS 2.51–3.00) and highly susceptible (HS; FDS > 3.00) (Hartman et al. 2004; Pudake et al. 2013).

Each experiment was repeated three different times (one experiment = one run) with three replications in each experiment. The cups of each genotype were placed in the chamber following a completely randomized design. Each cup represented an experimental unit. The 200 RILs from each of the two populations were evaluated separately for SDS disease resistance, along with the parental lines and other SDS resistant ('MN1606', 'Ripley', Forrest) and susceptible ('Essex', 'Williams 82', 'Spencer') control lines. In all experiments, the same controls were used to compare outcomes among runs. The mean FDS of each genotype from individual replications was subjected to statistical analysis.

SCN screening

SCN screening was carried out by following the protocol of Niblack et al. (2009) as modified in the Tylka laboratory (Iowa State University). Two seeds from each RIL were planted in individual cone-tainer filled with SCN HG type 0 infested soil (collected from Muscatine, Iowa) amounting to 50 cysts per cone-tainer. The HG type of Muscatine soil was classified at the SCN Diagnostics Center (University of Missouri-Columbia) as described by Niblack et al. (2002). After germination, only one plant was allowed to grow in the cone-tainer. Each cone-tainer represents one experimental unit and the experiment was replicated three times. The cone-tainers were randomly placed in a bucket with sand; 18 cone-tainers were accommodated in each bucket.

The buckets containing the cone-tainers were placed in a completely randomized arrangement in the water bath in a greenhouse room. Temperature of the water bath and greenhouse room was maintained at 27 ± 1 °C and under natural lighting conditions. Plants in the cone-tainers were watered once a day. Thirty days after planting, individual plants were gently pulled from the cone-tainer, and the female nematode cysts attached to the roots of each plant were gently removed from roots by washing with high-pressure tap water. The washing was done on nested sieves of 20 mesh (850 µm pore) placed over 60 mesh (250 µm pore) so that the washed cysts were collected over the 60 mesh sieve. The cysts were

collected in a small beaker and the number of cysts was counted under a microscope.

The female index (FI) based on the standard classification system (Schmitt and Shannon 1992) was used to evaluate the SCN reaction of individual genotypes. The female index as a percentage was,

$$FI(\%) = \frac{\text{Mean number of cysts on roots of a genotype}}{\text{Mean number of cysts on roots of Lee74}} \times 100$$

The standard classification system on the basis of the FI was as follows, RIL were rated as resistant (R; FI equal or < 10), moderately resistant (MR; FI range from 11 to 29), moderately susceptible (MS; FI range from 30 to 60), and susceptible (S; FI > 60) (Schmitt and Shannon 1992). The experiment was repeated three times. The parents of the populations, the highly SCN-susceptible cultivar 'Lee 74' (Caviness et al. 1975), and the highly SCN-resistant genotype PI 88788 were also included in the evaluation.

Genotyping the RILs

Genomic DNA was isolated from leaf samples following a CTAB extraction method (CIMMYT 2005). The DNA pellet was resuspended in 300 µL of 1× TE buffer pH 8.0 and stored at – 20 °C until further use. Two µL of the DNA was run on a 1% agarose gel to check the DNA quality. DNA concentration was quantified by absorbance at 260 nm using a Thermo Fisher Scientific (Waltham, MA) NanoDrop spectrophotometer. DNA samples were diluted to a final concentration of 100 ng/µL.

Plants were genotyped using the 1536 Universal Soy Linkage Panel 1.0 (Hyten et al. 2010) and the Illumina GoldenGate Genotyping assay. The genotyping was carried out at the Soybean Genomics and Improvement Lab, Beltsville Agricultural Research Center-West, USDA ARS, Beltsville, MD. The GoldenGate assay was performed according to Fan et al. (2003) and Hyten et al. (2008). Automatic allele calling for each locus was accomplished using BeadStudio version 3.2 software (Illumina Inc., San Diego, CA). All BeadStudio data for the 1536 SNPs were visually inspected and re-scored if any errors in calling the homozygous or heterozygous clusters were detected.

Whole-genome map construction and QTL analysis

Genetic linkage maps were constructed using MAPMAKER V2.0 for Macintosh (Lander et al. 1987), a logarithm of odds (LOD) value of 3.0 as described by Liu et al. (2005), and the Kosambi mapping function (Kosambi 1944). Marker order was validated using the "RIPPLE" (LOD > 3.0) command. QTL analysis was performed using composite interval-regression mapping (CIM) with QGene (Joehanes

and Nelson 2008). A permutation test with 1000 iterations was executed to determine the critical LOD threshold. The threshold LOD cut off value in AX19286 was 4.3 and 4.5, respectively, for SDS and SCN resistance loci ($p = 0.05$). The threshold LOD cut off value in AX19287 was 3.4 and 4.0, respectively, for SDS and SCN resistance loci ($p = 0.05$). The QTL map was generated using Mapchart 2.3.

Statistical analysis

All experimental data were analyzed using R 3.2.3 Software (R Core Team 2015). Normality of each experiment was analyzed by the Shapiro–Wilk, skewness, and kurtosis tests. A population with a skewness of 0 and a kurtosis of 3 was considered ideal for a normal distribution. The data were subjected to analysis of variance and tested for homogeneity of variances across lines in a population using the Levene test in the R package car (Fox and Weisberg 2011).

SNP haplotype analysis of *Rhg4* locus

PCR was carried out with two sets of primers flanking the two polymorphic SNPs of the serine hydroxymethyl transferase (*SHMT*) gene at the *Rhg4* locus that governs the SCN resistance (Liu et al. 2012). The two SNPs, 389 G/C and 1165 T/A were PCR amplified by the primers, Rhg4-1F (5'-gtcaacgtccagcctactc-3') + Rhg4-1R (5'-tagtcgatgtagccgggtgtg-3') and Rhg4-2F (5'-gtgggatctgagacctcttg-3') + Rhg4-2R (5'-gttaccattcgcactccacca-3'), respectively. The amplified PCR products were run on 1.2% agarose gel, the correct size bands were excised out, gel eluted by columns (Qiagen Inc, Germantown, MD) to get the purified DNA. The DNA was submitted for Sanger sequencing by using the forward primers at Iowa State University DNA facility.

Copy number estimation of *rhg1* locus

The copy number of the *rhg1* locus was estimated at the Hudson's lab (University of Illinois-Urbana Champaign) as described by Lee et al. (2016). The genomic DNA extracted

from the three parental lines, the SCN-resistant accessions PI 88788 and Peking, and the reference *rhg1* single-copy accession Williams 82 were characterized using a homeolog-controlled TaqMan (hcTaqMan) assay and primers described by Lee et al. (2016).

Results

Two hundred RILs from each of the AX19286 (A95-684043 × LS94-3207) and AX19287 (A95-684043 × LS98-0582) populations were screened for their SDS and SCN resistances in individual experiments. They were also genotyped with SNPs to identify QTL associated with resistance to the pathogens.

RILs response to *F. virguliforme* infection

The foliar disease symptoms began to appear 3 weeks after planting, and were scored 5 weeks after planting. The AX19286 population had a foliar SDS (FDS) mean of 2.24 and the AX19287 population had a mean of 2.16 (Table 1). There were significant differences for FDS means among RILs within each segregating populations ($p < 0.05$). The Levene test ($p > 0.05$) revealed that the variances for FDS were similar between the two populations.

The FDS means of the SDS resistant cultivars MN1606 and Ripley were 1.2 ± 0.09 and 1.4 ± 0.11 , respectively (data not shown). For LS94-3207 and LS98-0582, FDS means were 1.5 ± 0.10 (Fig. 1), and 1.8 ± 0.15 , respectively (Fig. 2). Each of the means for the LS parents were significantly ($p < 0.05$) different from Forrest that had a score of 2.0 (data not shown). The susceptible control, Spencer had the highest FDS (4.0 ± 0.19) among parents and other controls (Figs. 1, 2). The second highest FDS, 3.8 ± 0.18 , was observed for Williams 82 (data not shown). The FDS of the SCN resistant parent A95-684043 was 3.5 ± 0.22 (Figs. 1, 2), which was significantly different ($p < 0.05$) from Williams 82 and Spencer (Figs. 1, 2).

Shapiro–Wilk (w) test for normality of FDS distribution of RILs indicated that both AX19286 ($p = 0.64$; $w = 0.99$)

Table 1 Phenotypic frequency distribution of foliar disease scores among 200 recombinant inbred lines in each of two soybean segregating populations, AX19286 (A95-684043 × LS94-3207) and AX19287 (A95-684043 × LS98-0582)

Population	% of RILs ^a					Mean FDS ± std error	Range
	HR	R	MR	S	HS		
AX19286	7	35	37	15	6	2.24 ± 0.03	1.10–4.20
AX19287	7.5	17.5	36.5	22	16.5	2.16 ± 0.02	1.06–5.25

FDS foliar disease score, HR highly resistant (FDS < 1.50), R resistant (FDS 1.51–2.00), MR moderately resistant (FDS 2.01–2.50), S susceptible (FDS 2.51–3.00), HS highly susceptible (FDS > 3.00)

^a200 Recombinant inbred lines (RILs) from each population were categorized according to the mean foliar disease score

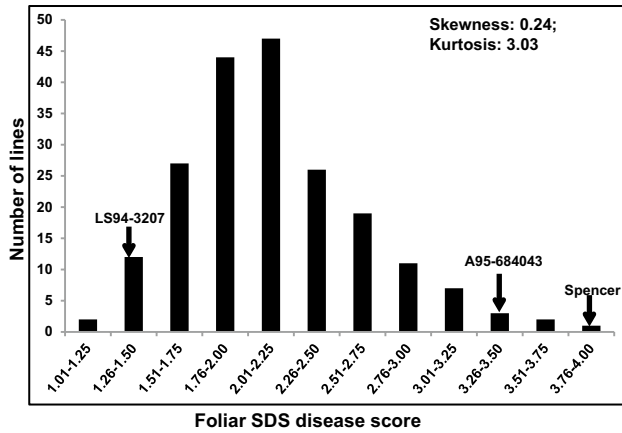


Fig. 1 Frequency distribution of foliar disease scores among the AX19286 (A95-684043 × LS94-3207) recombinant inbred lines (RILs). The foliar disease symptoms were scored 35 days following infection with *F. virguliforme*. Arrows indicate the disease scores of parents and a susceptible variety, Spencer. The values are means of three biological replications

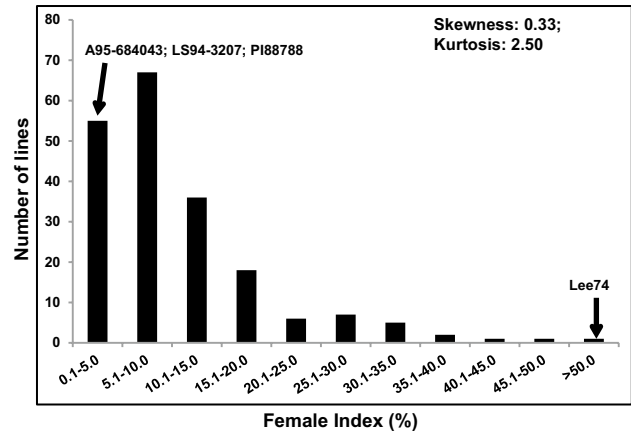


Fig. 3 Segregation of soybean cyst nematode (SCN) resistance among the AX19286 (A95-684043 × LS94-3207) recombinant inbred lines (RILs). Arrows indicate the phenotypes of parents, the most resistant line, PI88788 and the most susceptible variety, Lee74. The female indices are means of three biological replications calculated using the cysts numbers of Lee74 as the denominator

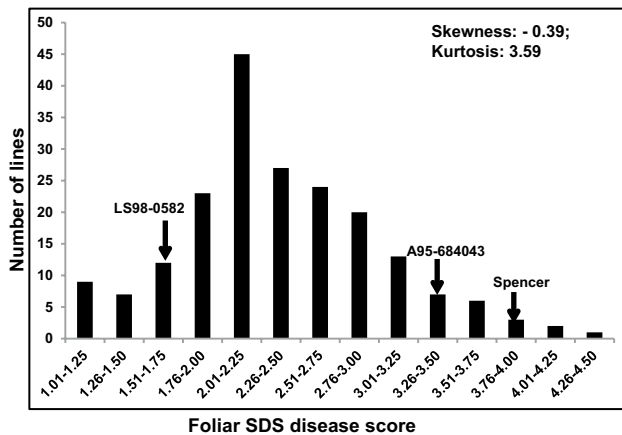


Fig. 2 Frequency distribution of foliar disease scores among the AX19287 (A95-684043 × LS98-0582) recombinant inbred lines (RILs). The foliar disease symptoms were scored 35 days following infection with *F. virguliforme*. Arrows indicate the disease scores of parents and a susceptible variety, Spencer. The values are means of three biological replications

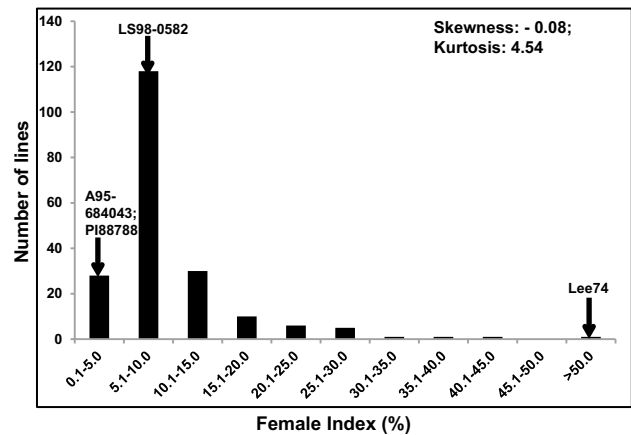


Fig. 4 Segregation of soybean cyst nematode (SCN) resistance among the AX19287 (A95-684043 × LS98-0582) recombinant inbred lines (RILs). Arrows indicate the phenotypes of parents, the most resistant line, PI88788 and the most susceptible variety, Lee74. The female indices are means of three biological replications calculated using the cysts numbers of Lee74 as the denominator

and AX19287 ($p = 0.32$; $w = 0.98$) populations followed a normal distribution (Figs. 1, 2). For the AX19286 population, 7% of the RILs were highly resistant (HR) (Table 1). Results were similar in the AX19287 population, with 7.5% classified as HR. In general, the majority of the lines in both populations was either MR or had higher levels of resistance. Several RILs had significantly greater FDS scores than the FDS of the SDS susceptible parent A95-684043 ($p < 0.05$) (Figs. 1, 2). These lines are transgressive segregants for susceptibility to the SDS pathogen.

RILs response to SCN infection

Shapiro–Wilk (w) test for normality of FI distribution indicated that both AX19286 ($p = 0.00046$; $w = 0.99$) and AX19287 ($p = 0.0270$; $w = 0.97$) were not normally distributed (Figs. 3, 4). However, the skewness and kurtosis values of both populations showed that they were having only slight to moderate skewness of 0.33 and -0.08 and kurtosis of 2.50 and 4.54, respectively (Figs. 3, 4) after log transformation to normalize the data. The mean number of cysts observed in the SCN resistant PI 88788 was 45, while

the mean cyst number for the SCN susceptible Lee 74 check was 1050 (data not shown). The average FI of A95-684043, LS94-3207, LS98-0582, and PI 88788 was 4.0, 4.5, 7.0, and 4.1, respectively, indicating that all four are SCN resistant (R) with a FI of < 10.0 (Figs. 3, 4). Most of the RILs in both populations were either resistant or moderately resistant to SCN, with few lines being moderately susceptible and none being susceptible (Figs. 3, 4; Table 2).

The ANOVA results indicated that the AX19286 population had a SCN mean of 10.7, while the AX19287 population had a mean of 7.4 (Table 2). Significant variation ($p < 0.05$) among lines was observed in each population. The Levene test of homogeneity of variance was done for FI across populations revealing that both populations had similar variance ($p > 0.05$).

SNP mapping of the soybean genome

Of the 1536 SNPs, 580 SNPs were polymorphic between the two parents in the AX19286 population and 371 SNPs were polymorphic in the AX19287 population (Supplementary Tables 1 and 2). The two sets of polymorphic SNPs were used to construct the genetic linkage map for each population and were used for QTL analysis. The Map coverage was 2608 cM for AX19286 and 2415 cM for AX19287 populations. The average distance between markers was 4.9 cM in the AX19286 population, and 7.3 cM in the AX19287 population. SDS resistance QTL map positions based on the composite interval map (Glyma.Wm82.a2 (Gmax2.0); Grant et al. 2010; <http://soybase.org>) are presented in Table 3. QTL identified in this study and those previously reported are shown in the Mapchart (version 2.3) generated linkage maps (Figs. 5, 6).

Identification of SDS resistance QTL

In the AX19286 population, two QTL for SDS resistance mapped to chromosomes 19 and 20, designated as SDS-1 and SDS-2, respectively (Table 3; Fig. 5; Supplementary Fig. 1). The SDS-1 QTL on chromosome 20 had a R^2 value of 11%, which explains the percentage of the

total variation for FDS. The resistance allele was inherited from the parent A95-684043. The SDS-2 QTL on chromosome 19 accounts for 16% of the total variation for FDS (Table 3), and the resistance allele was contributed by the parent LS94-3207. A minor QTL, SDS-3 was mapped to chromosome 9 accounting for 4.6% of the total variation for FDS. This resistance allele was also inherited from the parent LS94-3207.

In AX19287, three QTL associated with SDS resistance were identified on chromosomes 20, 13 and 17, which were designated as SDS-4, SDS-5 and SDS-6, respectively (Table 3, Fig. 5; Supplementary Fig. 2). SDS-4 QTL on chromosome 20 explained 7.6% of the total variation for FDS, and SDS-5 QTL on chromosome 13 explained 9.0% of the total variation for FDS. In both cases, the resistance allele was contributed by the parent LS98-0582. SDS-6 QTL on chromosome 17 explained 7.5% of the total variation on FDS with the resistance allele inherited from the parent LS98-0582.

Identification of SCN resistance QTL

In the AX19286 population four SCN resistance QTL were identified, three on chromosome 8 and one on chromosome 18, named as SCN-1, SCN-2, SCN-3 and SCN-4, respectively (Table 3, Fig. 6). The QTL SCN-1 on chromosome 8 (Supplementary Fig. 1) explained 34% of the total variation for FI, SCN-2 explained 10% of the total FI variation. The resistance alleles for SCN-1 and SCN-2 were inherited from LS94-3207. SCN-3 QTL explained 15% of the FI variation and the resistance allele was contributed by A95-684043. SCN-4 QTL identified on chromosome 18 explained 30% of the FI variation, and the resistance allele was contributed by A95-684043.

In the AX19287 population, only one SCN resistance QTL, SCN-5 mapped to chromosome 11, was identified (Table 3; Supplementary Fig. 2). It explains 12% of the total FI variation and the resistance allele was contributed by the parent LS98-0582.

Table 2 Phenotypic frequency distribution of female indices among 200 recombinant inbred lines of two soybean populations, AX19286 (A95-684043 X LS94-3207) and AX19287 (A95-684043 X LS98-0582)

Population	% of RILs ^a				Mean FI \pm std error	Range
	(R) ^b	(MR) ^b	(MS) ^b	(S) ^b		
AX19286	61	33.5	5.5	0	10.68 \pm 0.36	1.54–46.93
AX19287	73	25.5	1.5	0	7.39 \pm 0.19	1.55–23.47

^a200 recombinant inbred lines (RILs) from each population were categorized according to the mean female index (FI)

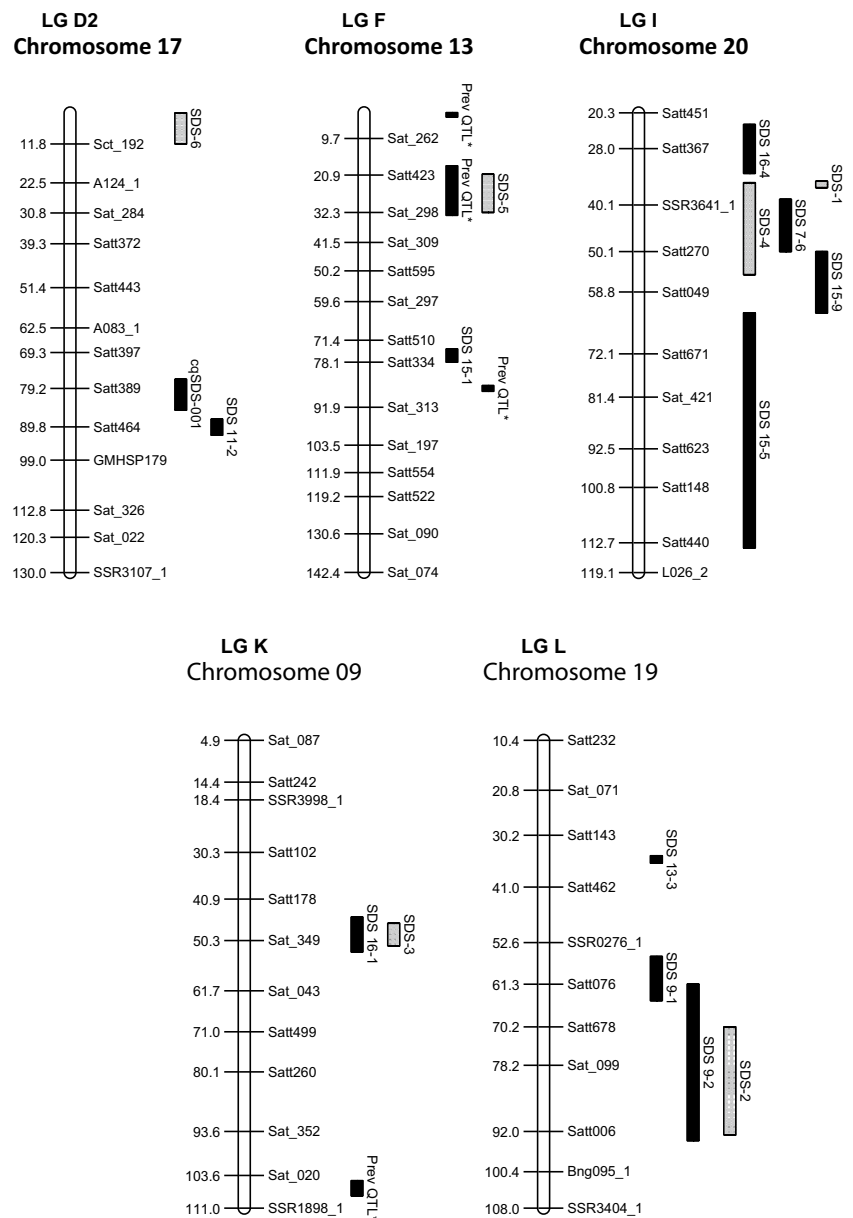
^bResistant (R; FI is < 10), moderately resistant (MR; FI of 11–29), moderately susceptible (MS; FI of 30–60) or susceptible (S; FI of > 60) based on the female index (FI) number

Table 3 Locations of the quantitative trait loci each underlying either SDS or SCN resistance

Population	QTL	Chr./LG ^a	Marker/interval	dbSNP ID	Flanking SSR markers	Position (cM) ^b	LOD ^c	R ² (%) ^d	Parent contribution	Additive effect ^e
AX19286	SDS-1	20/I	BARC-054889-12193-BARC-041129-07912	ss107924192-ss107913844	Satt700-Satt496	35.0–36.4	4.7	11	A95-684043	0.5
	SDS-2	19/L	BARC-047496-12943-BARC-029419-06181	ss107921208-ss107918449	Satt678-Satt664	70.2–92.7	7.0	16	LS94-3207	-0.28
	SDS-3	9/K	BARC-058901-15494-BARC-050815-09887	ss107926758-ss107912828	Satt552-Satg002	46.4–51.5	2.3	4.6	LS94-3207	-0.11
	SCN-1	8/A2	BARC-032503-08989-BARC-065571-19573	ss107912616-ss107930663	Satt315-Sat_212	45.3–56.3	15.0	34	LS94-3207	-57.0
	SCN-2	8/A2	BARC-022387-04319-BARC-057653-14889	ss107913364-ss107926089	Satt525-Satt158	97.0–115.3	6.0	10	LS94-3207	-30.0
	SCN-3 ^f	8/A2	BARC-055945-13878-BARC-054887-12192	ss107925080-ss107924191	Satt470-Satt228	116.7–154.1	7.5	15	A95-684043	48.2
	SCN-4	18/G	BARC-019351-03885-BARC-012289-01799	ss107912541-ss107914461	Sat_210-Sat_141	3.7–9.2	14	30	A95-684043	48.1
AX19287	SDS-4	20/I	BARC-057793-14926-BARC-025913-05152	ss107926125-ss107912572	Satt127-Sat_268	35.3–55.1	3.5	7.6	LS98-0582	-0.21
	SDS-5	13/F	BARC-900926-00961-BARC-041237-07944	ss107931019-ss107912652	Sat_298-Satt423	20.6–32.3	3.9	9	LS98-0582	-0.19
	SDS-6 ^f	17/D2	BARC-020357-04569-BARC-065705-19668	ss107913274-ss107930758	Sett008-Set_192	3.2–11.8	3.2	7.5	LS98-0582	-0.15
	SCN-5 ^f	11/B1	BARC-040851-07854-BARC-016539-02087	ss107919849-ss107913087	Satt638-Satt197	37.7–46.4	4.2	12	LS98-0582	-16.0

^aChromosome/linkage group^bPosition of QTL based on the soybean composite genetic map of soybean reference genome Glyma.Wm82.a2 (Gmax2.0) in SoyBase (<http://www.soybase.org>)^cLikelihood of odds (LOD) at the QTL peak^dPercent contribution of a QTL in the phenotypic variation^eAdditive effect of an allele substitution for the QTL based on foliar disease score (FDS) or female index (FI). Negative value means allele from either LS94-3207 or LS98-0582 provides greater resistance (in lowering FDS or FI) than A95-684043. Estimated threshold LOD cut off value for SDS loci was 4.3 and 3.4, for SCN loci was 4.5 and 4.0, respectively, for AX19286 and AX19287 populations ($p = 0.05$)^fNovel QTL

Fig. 5 The composite genetic map of the sudden death syndrome (SDS) resistance quantitative trait loci (QTL) including the ones identified in this study. Striped rectangles are QTL identified in this study (Table 3); black rectangles are SDS resistance QTL identified previously. SoyBase names were given for the previously identified QTL. Asterisk previously identified QTL not yet named



Molecular analysis of *Rhg4* locus and *rhg1* locus

The haplotype characterization to determine two key *SHMT* nucleotide sequence polymorphism at *Rhg4* locus showed that A95-684043 and LS98-0582 inherited the PI 88788-type susceptible *SHMT* genotype, whereas LS94-3207 inherited the Peking-type resistant *SHMT* genotype (Supplementary Table 3). Copy number estimates using hcTaqMan assay showed that A95-684043, LS98-0582 and PI 88788 contained nine copies of *rhg1* (Supplementary Fig. 3). The analysis also confirmed that LS94-3207 and Peking have three copies each of *rhg1*, whereas the SCN-susceptible Williams 82 has one copy of *rhg1* (Supplementary Fig. 3).

Discussion

The combined presence of SDS and SCN pathogens in commercial fields results in economically important soybean yield losses (Bradley and Allen 2014; Brzostowski et al. 2014; Gelin et al. 2006; Xing and Westphal 2013). The present study was undertaken to search for QTL associated with resistance to these two major soybean pathogens, and separate screenings for each disease were conducted. The research identified one novel SDS resistance QTL, on chromosome 17 and two novel SCN resistance QTL, one each on chromosomes 8 and 11 in populations AX19286 and AX19287 (Table 3). In each population, several RILs carrying SCN and SDS resistance QTL were also identified.

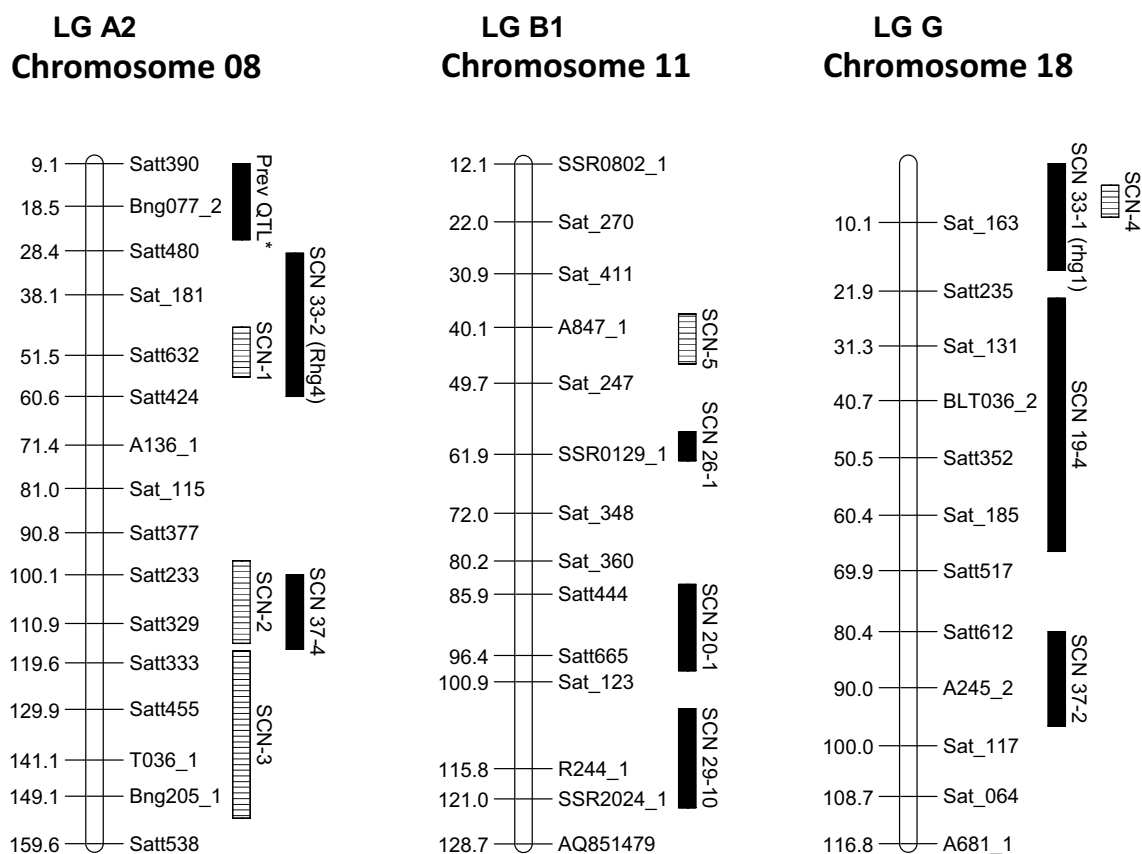


Fig. 6 The composite genetic map of the soybean cyst nematode (SCN) resistance quantitative trait loci (QTL) including the ones identified in this study. Stripped rectangles are QTL identified in this

study (Table 3); black rectangles are SCN resistance QTL identified previously. SoyBase names were given for the previously identified QTL. Asterisk previously identified QTL not yet named

In addition to the novel QTL, our study also detected SDS and SCN QTL previously reported in similar regions of the chromosomes.

To decide if the QTL identified in this research were novel, genetic distances of QTL for SDS and SCN resistances were compared with genetic distances of previously identified QTL compiled in SoyBase (<http://www.soybase.org>). Genetic distances were also compared to those reported in published research that were not included in SoyBase. In our study, a QTL was considered novel if the genetic distance was more than 10 cM of the previously reported QTL. It is important to indicate that final validation of a novel QTL will require a separate study independent from the research we report. QTL identified in this study and QTL previously reported are shown in the Mapchart (version 2.3)-generated linkage maps (Figs. 5, 6).

Chromosome 20 appears to have several QTL for resistance to *F. virguliforme* (Fig. 5). In the AX19287 population, SDS-4 QTL was located in the region between 35.3 and 55.1 cM of chromosome 20, where Swaminathan et al. (2016), in the same AX19287 population previously identified QTL SDS 16-4 (SoyBase), in an interval

of 22.8–35.3 cM, that confers tolerance to *F. virguliforme* toxin(s). Similarly, just 3 cM downstream of SDS 16-4 QTL in the same chromosome, Iqbal et al. (2001) identified SDS 7-6 QTL (SoyBase) in the interval of 38.9–50.1 cM, in a different population. The QTL SDS-1 that we identified mapped to chromosome 20 within the interval 35.0–36.4 cM which overlaps with the SDS-4 QTL. SDS-4 QTL also overlaps with a previously reported QTL, SDS 15-9 (50.1–63.3 cM) from AX19286 (Swaminathan et al. 2016). Additional research will be required to determine if the four SDS resistance QTL, SDS-1, SDS-4, SDS 7-6 and SDS 15-9 are the same or are tightly linked QTL (Fig. 5). It is also important to mention that in the same general region on chromosome 20 there is also a SCN resistance QTL (SCN 12-1; 37.1–39.1 cM; SoyBase) (Qiu et al. 1999), a *Phytophthora sojae* resistance QTL (Phytoph 8–2; 34.9–53.1 cM; SoyBase) (Tucker et al. 2010), and another QTL associated with resistance to *Sclerotinia sclerotiorum* (Sclero 7-3; 25.5–40.5 cM; SoyBase) (Huynh et al. 2010).

In chromosome 19, we identified a QTL, SDS-2 in the interval of 70.2–92.7 cM. In a similar region of chromosome 9, Kassem et al. (2012) and Nitji and Lightfoot (2006)

identified SDS 9-2 QTL (61–93 cM; SoyBase) (Fig. 5), and Guo et al. (2005) mapped a SCN resistance QTL inherited from PI 90763 (SCN 29-7; 87.4–93.9 cM; SoyBase).

Chromosome 9 also contained a previously reported SDS QTL. In AX19286, we identified a minor SDS resistance QTL SDS-3 in the interval 46.4–51.5 cM. In a close position and in the population AX19287, Swaminathan et al. (2016) identified a major QTL, SDS 16-1 (45.8–50.9 cM; SoyBase), that confers tolerance to *F. virguliforme* toxin(s). Yamanaka et al. (2006), identified a SDS resistance QTL in a different population in a similar interval of 44.9–52.9 cM. For this work, the authors used *F. tucumaniae*, one of the causal fungi of SDS in South America, not identified in the U.S. The two *Fusarium* species, *F. virguliforme* and *F. tucumaniae*, are phylogenetically and morphologically different (Aoki et al. 2003; Huang et al. 2016); however, the close position of the two detected QTL suggests similar pathogenicity mechanisms in both fungi species.

Chromosome 13 also contained regions in which SDS QTL were previously identified. In population AX19287, we identified the SDS resistance QTL, SDS-5 mapped to the 20.6–32.3 cM interval, a similar region in which Kassem et al. (2007) and Njiti and Lightfoot (2006) also identified a QTL (27.9–33.2 cM). Wen et al. (2014) in a genome-wide association study also identified SDS QTL in a similar interval (18.1–33.2 cM). Swaminathan et al. (2016), identified a SDS resistance QTL, SDS 15-1 from AX19287 population, in a different interval on chromosome 13 (74.1–78.1 cM; SoyBase), downstream from previous reports.

Only three of the SDS resistance QTL, SDS-1, SDS-3 and SDS-4 that we identified in our study matched to the same chromosomal locus of three of the 17 QTL identified associated with tolerance to toxins in *F. virguliforme* culture filtrates (*Fv* toxins) (Swaminathan et al. 2016), in spite of the fact that both studies used the same two sets of RIL populations. This may not be surprising. One interpretation of the results is that the soybean hosts express different gene(s) in response to each of the two modes of action by the fungus, either toxin exposition or fungus invasion to roots. It is also possible that differences in the screening protocols itself (toxin filtrates vs soil inoculation) as well as plant tissue used to assess disease symptoms (detached stem cut/root vs seeds planted in soil) might have contributed to the differential QTL expression.

Similar to SDS, several chromosomes were previously shown to possess SCN resistance QTL in the same general regions in which we mapped QTL. As mentioned, further research is necessary to determine if SCN QTL located in similar regions are the same QTL or not, or they are tightly linked. For chromosome 8 in the population AX19286, in addition to the novel QTL, SCN-3 (116.7–154.1 cM), two other SCN resistance QTL (SCN-1 and SCN-2), previously reported were also mapped (Table 3; Fig. 6). We identified

SCN-1 in the interval of 45.3–56.3 cM contributed by the parent LS94-3207. The location of this QTL coincides with the previous reports in which the *Rhg4* locus (SoyBase) was identified. *Rhg4*, a major SCN resistance locus (Chang et al. 1997; Concibido et al. 1994, 2004; Guo et al. 2006; Heer et al. 1998; Kadam et al. 2016; Meksem et al. 2001; Webb et al. 1995; Weismann et al. 1992), has been identified in several accessions including Peking and PI 437654, which are in the pedigree of the SCN resistance parent, LS94-3207 (Schmidt and Klein 2004). Our molecular analysis supports the above finding (Table 3), LS94-3207 inherited the SCN resistance possibly from Peking. Further evidence of this is provided by the observation that LS94-3207 has the Peking-type resistance *SHMT* genotype and three copies of *rhg1* similar to that of Peking (Supplementary Table 3 and Supplementary Fig. 3).

The region on chromosome 8 (96.9–115.2 cM) that the SCN-2 QTL was mapped in our study overlaps with an earlier SCN resistance QTL (SCN 37-4; 100.1–118.6 cM; SoyBase) (Satt233–Sat_040), reported by Vuong et al. (2010). Further research will be necessary to determine if the QTL in our study and that of Vuong et al. (2010) are the same. Also mapped to this region and in addition to SCN-2, there is a QTL for *Sclerotinia sclerotiorum* stem rot resistance (Sclero 9-1; 104.8–114.8 cM; SoyBase) (Guo et al. 2008), and another QTL for *Phytophthora sojae* resistance (Phytoph 6-4; 100.8–107.5 cM; SoyBase) (Li et al. 2010), that were reported previously (data not shown).

The new SCN QTL (SCN-3) located in chromosome 8 (116.7–154.1 cM) explained 15% of the FI variation and the resistance allele was contributed by the A95-684043 parent. The presence of this QTL had not been reported earlier from either PI 88788, Peking, or PI 90763 which are the known sources of SCN resistance for A95-684043 (Cianzio et al. 2002). A possible explanation might be that there was low coverage of genetic markers in the segregating populations used in the earlier mapping studies.

The QTL SCN-4 we identified in AX19286 on chromosome 18 was mapped to a similar interval, in which the *rhg1* locus was previously mapped (Concibido et al. 2004; Guo et al. 2006; Kadam et al. 2016; Vuong et al. 2010). The *rhg1* is one of the major SCN resistance loci impacting SCN resistance (Chang et al. 2011; Concibido et al. 1997, 2004; Guo et al. 2005, 2006; Kadam et al. 2016; Kim et al. 2016; Yue et al. 2001). The region containing this locus on chromosome 18 has also been reported to possess SDS resistance QTL mapped in several other populations (Chang et al. 1996; Iqbal et al. 2001; Kazi et al. 2008; Njiti et al. 2002; Prabhu et al. 1999; Wen et al. 2014). In our study, SCN-4 explained 30% of the total FI variation. This SCN resistance allele, SCN-4, was contributed by the A95-684043 parent (Table 3). The SNP haplotype analysis of *SHMT* gene and copy number analysis support the above findings that

A95-684043 inherited PI 88788-type susceptible genotype at the *Rhg4* locus along with nine copies of *rhg1* as those of PI 88788, respectively (Supplementary Table 3 and Supplementary Fig. 3). It is evident that the *rhg1* locus present in the AX19286 population might be donated by PI 88788, which is in the parentage of A95-684043 (Cianzio et al. 2002). In the AX19287 population, a novel QTL SCN-5 (37.8–46.4 cM), was identified on chromosome 11 and three other SCN resistance QTL previously mapped are reported in different regions on the same chromosome (58–63, 84.2–98.9, and 105.5–122.5 cM) (Guo et al. 2005; Wu et al. 2009; Yue et al. 2001) (Fig. 6).

In our study, three SCN-resistant parents were used to generate the two RIL populations, and two distinct patterns of segregation were observed in each population. In the AX19286 population, we identified four SCN resistance QTL (Table 3). In AX19287, we identified only one SCN resistance QTL explaining 12% of the total variation. In the AX19286 population, both parents, A95-684043 and LS94-3207 are resistant to SCN. The SCN resistance for A95-684043 is derived from three donors, Peking, PI 88788, and PI 90763 (Cianzio et al. 2002), also including SCN-4 QTL we identified. The major SCN resistance PI 88788-type *rhg1* locus (SCN-4 QTL) was possibly inherited from PI 88788 (Table 3, Supplementary Table 3 and Supplementary Fig. 3). For LS94-3207, SCN resistance is derived from PI 437654 and Peking (Schmidt and Klein 2004), including the SCN-1 QTL we identified. The major SCN resistance Peking-type *Rhg4* locus (SCN-1 QTL) was inherited possibly from Peking (Table 3, Supplementary Table 3 and Supplementary Fig. 3). For SCN-1 QTL (*Rhg4* locus) and SCN-2 QTL, the resistance allele was derived from LS94-3207, with A95-684043 having the allele for susceptibility (Table 3). For SCN-3 and SCN-4 QTL (*rhg1* locus), however, the resistance allele came from A95-684043, with LS94-3207 having the allele for susceptibility (Table 3). The observations on QTL mapping results (Table 3) and the molecular analysis results (Supplementary Table 3 and Supplementary Fig. 3) combined with the pedigree information suggest that the SCN resistance mechanisms of A95-684043 and LS94-3207, parents of the AX19286 population, might be different and complementary, thus releasing additional genetic variation in the segregating generations, which resulted in the mapping of four SCN resistance QTL.

In the AX19287 population, both parents, A95-684043 and LS98-0582 are resistant to SCN. The cultivar Fayette is a distant donor in the pedigree of LS98-0582 that traces SCN resistance to PI 88788 (Abney and Crochet 2004). The molecular analysis showed that both the A95-684043 and LS98-0582 genotypes have similar genetic background for the major SCN resistance locus, *rhg1* and both demonstrated to inherit the PI 88788-type susceptible *SHMT* genotype at the *Rhg4* locus and nine copies of *rhg1* locus possibly

from PI 88788 (Supplementary Table 3 and Supplementary Fig. 3). Our data and pedigree information suggest that alleles at major SCN resistance loci in the two parents are likely similar, which resulted in diminished genetic variation in the progeny of this cross compared to the AX19286 population. This may also explain the fact that only one SCN resistance QTL was detected in this population. This observation is also supported by the skewed distribution of the resistant lines observed for the AX19287 population, in which, 145 RILs of the 200 studied, showed FI equal to or < 10.

Ours and previous results in which chromosomes and regions in chromosomes identified several SDS and SCN QTL may contribute to a better understanding of the host resistance inheritance to each of the two pathogens. It will be important to determine if the different QTL on the same chromosomes associated with each pathogen are the same or tightly linked. This information will contribute to decide the QTL that might be used for introgression to improve resistance, particularly to the SDS disease. It is also important to note that some of the QTL identified are located in proximity of QTL associated with resistance to other important pathogens of soybeans, i.e., *P. sojae* and *S. sclerotiorum*. These findings suggest the importance of some genomic regions in soybean to breeding programs considering resistance improvement against multiple pathogens.

The complex nature of the SCN and SDS resistance mechanisms in the soybean pathosystem may benefit from the identification and use of new resistance loci in addition to loci previously identified for controlling both pathogens. Up to date, the progress in development of mapping populations to identify QTLs for simultaneous resistance to both SDS and SCN has been limited (Iqbal et al. 2009; Prabhu et al. 1999; Srour et al. 2012). In our study, we could not map a single QTL resistance to both pathogens because resistance QTL for SCN and SDS were identified by inoculating with each pathogen separately. Therefore, we are unable to hypothesize the nature of the relationship between QTL for each pathogen. This opposes to the field situation, in which SDS and SCN pathogens co-exist and simultaneously might attack the same soybean root. The *Rhg1/Rfs2* locus on chromosome 18 has been identified to confer nearly complete resistance to both SDS root rot and leaf symptoms caused by *F. virguliforme* and to also provide partial resistance to three different populations of nematodes (Srour et al. 2012). The fact that so far only one QTL has been detected to confer resistance to both pathogens suggests that in general there might be different QTL along with other resistance mechanisms that might be needed by the soybean host to fight the two soybean pathogens. A possible interpretation might be that the biology/infection mode/pathogenesis between *F. virguliforme* and SCN conditions varying resistance mechanisms in soybean. Research is in progress at our lab that may

contribute to a better understanding of resistance expression and the inter-relation among QTL.

In brief, we identified three new QTL, one associated with SDS resistance and two with SCN resistance. This research will also result in the public future release of germplasm lines possessing several QTL associated with resistance to SCN and to SDS (Cianzio et al. unpublished), which may benefit breeding programs. Additionally, the QTL we identified, and those from previous studies resulting from different populations and placed in similar chromosomal regions contribute to validate the usefulness of some of the QTL to improve resistance to both SDS and SCN pathogens.

Author contribution statement SRC and MKB devised the strategy and planned the experiments; SRC generated the recombinant inbred lines and critically reviewed the results and manuscript; SS devised and conducted the SDS screening experiments, interpreted results and wrote the manuscript; SS and ML conducted the SCN screening experiments; NSA conducted quantitative trait loci mapping and analyzed the results; JMK conducted the statistical analysis and interpreted the analysis results; JD and MEH conducted copy number analysis.

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Compliance of ethical standards

Conflict of interest To the best knowledge of each and all authors, there are no conflicts of interests.

Human and/or animal participants The research does not involve human and/or animal participants.

Informed consent All authors have communicated their consent.

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