Mapping of New Quantitative Trait Loci for Sudden Death Syndrome and Soybean Cyst Nematode Resistance in Two Soybean Populations

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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 F_7 derived recombinant inbred lines from each population AX19286 (A95-684043 x LS94-3207) and AX19287 (A95-684043 x 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.

Keywords: pathogen resistance, soybean, *Fusarium virguliforme*, soybean sudden death syndrome, *Heterodera glycines,* soybean cyst nematode, quantitative trait loci mapping, recombinant inbred lines

Author contribution statement

S. R. C. and M. K. B. devised the strategy and planned the experiments; S. R. C. generated the recombinant inbred lines and critically reviewed the results and manuscript; S. S. devised and conducted the SDS screening experiments, interpreted results and wrote the manuscript; S. S. and M. L. conducted the SCN screening experiments; N. S. A. conducted quantitative trait loci

mapping and analyzed the results; J. M. K. conducted the statistical analysis and interpreted the analysis results; J. D. and M. E. H. conducted copy number analysis.

Key message

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

Conflict of interests

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

Compliance of ethical standards

There are no conflicts of interest.

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

All authors have communicated their consent.

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 6,000 soybean plant introduction (PI) lines and 2,000 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 (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; Liu et al. 2017; Yu et al. 2016). The *rhg1* locus was found to be complex with a 31.2 kb interval repeated from one to ten

116 times and the number of repeats shown to be related to host resistance (Cook et al. 2012; Yu et
117 al. 2016). The *Rhg4* gene was cloned from the cultivar 'Forrest' and found to be a serine
118 hydroxymethyltransferase (SHMT) protein (Liu et al. 2012).

119 Approximately 95% of the soybean cultivars in the U.S. trace SCN resistance to *rhg1*
120 donated by PI 88788 (Mitchum 2016). It is a matter of concern that the resistant monoculture of
121 the *rhg1* locus has exposed the nematode populations to high selection pressure which could
122 overcome the *rhg1* encoded resistance (Faghihi et al. 2010; Mitchum et al. 2007; Niblack et al.
123 2008). It might be necessary to incorporate multiple diverse SCN resistance mechanisms into
124 single cultivars and/or rotate different sources of resistance with the *rhg1* locus to improve SCN
125 management (Mitchum 2016; Rincker et al. 2017).

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

133 In the present investigation we used two populations (AX19286 and AX19287) of F_7-
134 derived lines created by crossing one SCN resistant parent to each of two SCN and SDS resistant
135 parents. Phenotyping with each pathogen was done in the greenhouse, using either the fungus or
136 the nematode for artificial inoculations. In previous research, Swaminathan et al. (2016)
137 evaluated fungal toxin resistance using the same two populations. In this study, we report QTL,
138 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.

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Materials and Methods

Plant material

Two hundred RILs were developed from each of the two soybean filial populations, AX19286 (A95-684043 x LS94-3207), and AX19287 (A95-684043 x 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 x [‘Archer’ x (‘Cordell’ x 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’ x 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) x V71-480) was released as a high yielding cultivar with resistance to SCN HG type 0 (Schmidt et al. 1993). Hartwig (derived from Forrest x 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; Hartwig and Epps 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 x 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 x Asgrow A4595. Northrup King S46-44 was developed from the cross of another two Asgrow lines, Asgrow A5474 x 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 F_1 plants was confirmed with the morphological marker of flower color. For each cross, six F_1 seeds were obtained in January 2002. Each F_1 plant was identified and harvested individually in May 2002. The F_1 and F_2 plants were grown in Puerto Rico during the summer 2002. The identity of individual F_1 plants was maintained throughout the RIL development. The F_2 plants were also identified, maintaining the ID of the F_1 from which the seed had been harvested.

A total of 200 F_2 plants (seed at the F_3 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 F_7 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 control checks 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

184 communication, Iowa State University, IA, 2007), later patented by D. Lightfoot (Patent #
185 7,288,386; Lightfoot et al. 2007) and used with permission.

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

199 Clean Styrofoam cups (240 mL) were filled with 150 mL of a pasteurized 1:2 soil : sand
200 mixture, followed by 30 mL of the inoculum: soil-sand ::1:10 mixture added at the top of the
201 cup. Five seeds of each RIL were planted on the surface and covered with 30 mL of a
202 pasteurized 1:2 soil-sand mixture. The cups were placed in a growth chamber and watered once
203 daily. The seedlings were grown at 23°C for 16 h under light (200 µmol photons $m^{-2}s^{-1}$) and
204 16°C for 8 h under dark conditions.

205 The foliar disease score (FDS) of each plant was recorded five weeks after planting
206 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 in order 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 ^{0}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,

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

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 evaluated.

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 1X TE buffer pH 8.0 and stored at – 20ºC 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 260nm 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 1,536 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 1,536 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 1,000 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, 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 transferease (*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'-gtcaacgtccagccctactc-3') + Rhg4-1R (5'-tagtcgatgtagccggtggtg-3') and Rhg4-2F (5'-gtgggatctgagacctcttgg-3') + Rhg4-2R (5'-gttaccaattcgcactccacca-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 X LS94-3207), and AX19287 (A95-684043 X 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 three weeks after planting, and were scored five 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.

323 The FDS means of the SDS resistant cultivars MN1606 and Ripley were 1.2 ±0.09 and
324 1.4 ±0.11, respectively (data not shown). For LS94-3207 and LS98-0582, FDS means were 1.5
325 ±0.10 (Fig. 1), and 1.8 ±0.15, respectively (Fig 2). Each of the means for the LS parents were
326 significantly (p<0.05) different from Forrest that had a score of 2.0 (data not shown). The
327 susceptible control, Spencer has the highest FDS (4.0 ±0.19) among the parents and other
328 controls (Fig. 1 and 2). The second highest FDS, 3.8 ±0.18, was observed for Williams 82 (data
329 not shown). The FDS of the SCN resistant parent A95-684043 was 3.5 ±0.22 (Figs. 1 and 2),
330 which was significantly different (p<0.05) from Williams 82 and Spencer (Figs. 1 and 2).
331 Shapiro-Wilk (w) test for normality of FDS distribution of RILs indicated that both
332 AX19286 (p = 0.64; w = 0.99) and AX19287 (p = 0.32; w = 0.98) populations followed a normal
333 distribution (Figs. 1 and 2). For the AX19286 population, 7% of the RILs were highly resistant
334 (HR) (Table 1). Results were similar in the AX19287 population, with 7.5% classified as HR.
335 In general, the majority of the lines in both populations were either MR or had higher levels of
336 resistance. Several RILs had significantly greater FDS scores than the FDS of the SDS
337 susceptible parent A95-684043 (p <0.05) (Figs. 1 and 2). These lines are transgressive
338 segregants for susceptibility to the SDS pathogen.
339
340 **RILs response to SCN infection**
341 Shapiro-Wilk (w) test for normality of FI distribution indicated that both AX19286 (p = 0.00046;
342 w = 0.99) and AX19287 (p = 0.0270; w = 0.97) were not normally distributed (Figs. 3 and 4).
343 However the skewness and kurtosis values of both populations showed that they were having
344 only slight to moderate skewness of 0.33 and - 0.08 and kurtosis of 2.50 and 4.54, respectively
345 (Figs. 3 and 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 1,050 (data not shown). The average FI of A95-684043, LS94-3207, LS98-0582, and PI 88788 were 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 and 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 and 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 1,536 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 2,608 cM for AX19286 and 2,415 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 and 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. SDS-3 QTL accounts for 4.6 % of the total variation for FDS, with the resistance allele 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 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.

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

415 and AX19287 (Table 3). In each population several RILs carrying SCN and SDS resistance
416 QTL were also identified. In addition to the novel QTL, our study also detected SDS and SCN
417 QTL previously reported in similar regions of the chromosomes.

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

426 Chromosome 20 appears to have several QTL for resistance to *F. virguliforme* (Fig. 5). In
427 the AX19287 population, SDS-4 QTL was located in the region between 35.3 - 55.1 cM of
428 chromosome 20, where Swaminathan et al. (2016), in the same AX19287 population previously
429 identified QTL SDS 16-4 (SoyBase), in an interval of 22.8 - 35.3 cM, that confers tolerance to *F.*
430 *virguliforme* toxin(s). Similarly, just 3 cM downstream of SDS 16-4 QTL in the same
431 chromosome, Iqbal et al. (2001) identified SDS 7-6 QTL (SoyBase) in the interval of 38.9 - 50.1
432 cM, in a different population. The QTL SDS-1 that we identified, mapped to chromosome 20
433 within the interval 35.0 - 36.4 cM which overlaps with the SDS-4 QTL. SDS-4 QTL also
434 overlaps with a previously reported QTL, SDS 15-9 (50.1 – 63.3 cM) from AX19286
435 (Swaminathan et al. 2016). Additional research will be required to determine if the four SDS
436 resistance QTL, SDS-1, SDS-4, SDS 7-6 and SDS 15-9 are the same or are tightly linked QTL
437 (Fig. 5). It is also important to mention that in the same general region on chromosome 20 there

438 is also a SCN resistance QTL (SCN 12-1; 37.1 – 39.1 cM; SoyBase) (Qiu et al. 1999), a
439 *Phytophthora sojae* resistance QTL (Phytoph 8-2; 34.9 – 53.1 cM; SoyBase) (Tucker et al.
440 2010), and another QTL associated with resistance to *Sclerotinia sclerotiorum* (Sclero 7-3; 25.5
441 – 40.5 cM; SoyBase) (Huynh et al. 2010).

442 In chromosome 19, we identified a QTL, SDS-2 in the interval of 70.2 - 92.7 cM. In a
443 similar region of chromosome 9, Kassem et al. (2012) and Nitji and Lightfoot (2006) identified
444 SDS 9-2 QTL (61 – 93 cM; SoyBase) (Fig. 5), and Guo et al. (2005) mapped a SCN resistance
445 QTL inherited from PI 90763 (SCN 29-7; 87.4 – 93.9 cM; SoyBase).

446 Chromosome 9 also contained a previously reported SDS QTL. In AX19286, we
447 identified a minor SDS resistance QTL SDS-3 in the interval 46.4 – 51.5 cM. In a close position
448 and in the population AX19287, Swaminathan et al. (2016) identified a major QTL, SDS 16-1
449 (45.8 - 50.9 cM; SoyBase), that confers tolerance to *F. virguliforme* toxin(s). Yamanaka et al.
450 (2006), identified a SDS resistance QTL in a different population in a similar interval of 44.9 -
451 52.9 cM. For this work, the authors used *F. tucumaniae*, one of the causal fungi of SDS in South
452 America, not identified in the U.S. The two *Fusarium* species, *F. virguliforme* and *F.*
453 *tucumaniae*, are phylogenetically and morphologically different (Aoki et al. 2003; Huang et al.
454 2016), however, the close position of the two detected QTL suggest similar pathogenicity
455 mechanisms in both fungi species.

456 Chromosome 13 also contained regions in which SDS QTL were previously identified.
457 In population AX19287, we identified the SDS resistance QTL, SDS-5 mapped to the 20.6 - 32.3
458 cM interval, a similar region in which Kassem et al. (2007) and Njiti and Lightfoot (2006) also
459 identified a QTL (27.9 – 33.2 cM). Wen et al. (2014) in a genome-wide association study also
460 identified SDS QTL in a similar interval (18.1 – 33.2 cM). Swaminathan et al. (2016), identified

461 a SDS resistance QTL, SDS 15-1 from AX19287 population, in a different interval on
462 chromosome 13 (74.1 – 78.1 cM; SoyBase), downstream from previous reports.
463 Only three of the SDS resistance QTL, SDS-1, SDS-3 and SDS-4 that we identified in
464 our study matched to the same chromosomal locus of three of the 17 QTL identified associated
465 with tolerance to toxins in *F. virguliforme* culture filtrates (*Fv* toxins) (Swaminathan et al. 2016),
466 in spite of the fact that both studies used the same two sets of RIL populations. This may not be
467 surprising. One interpretation of the results is that the soybean hosts express different gene(s) in
468 response to each of the two modes of action by the fungus, either toxin exposition or fungus
469 invasion to roots. It is also possible that differences in the screening protocols (toxin filtrates *vs*
470 soil inoculation) as well as plant tissue used to assess disease symptoms (detached stem cut/root
471 *vs* seeds planted in soil) might have contributed to the differential QTL expression.
472 Similar to SDS several chromosomes were previously shown to possess SCN resistance
473 QTL in the same general regions in which we mapped QTL. As mentioned, further research is
474 necessary to determine if SCN QTL located in similar regions are the same QTL or not, or they
475 are tightly linked. For chromosome 8 in the population AX19286, in addition to the novel QTL,
476 SCN-3 (116.7 – 154.1 cM), two other SCN resistance QTL (SCN-1 and SCN-2), previously
477 reported were also mapped (Table 3; Fig. 6). We identified SCN-1 in the interval of 45.3 - 56.3
478 cM and the SCN resistance allele for SCN-1, being contributed by the parent LS94-3207. The
479 location of this QTL coincides with the previous reports in which the *Rhg4* locus (SoyBase) was
480 identified. *Rhg4*, a major SCN resistance locus (Chang et al. 1997; Concibido et al. 1994;
481 Concibido et al. 2004; Guo et al. 2006; Heer et al. 1998; Kadam et al. 2016; Meksem et al. 2001;
482 Webb et al. 1995; Weismann et al. 1992), has been identified in several accessions, of which
483 Peking and PI 437654, are in the pedigree of the SCN resistance parent, LS94-3207 (Schmidt

484 and Klein 2004). Our molecular analysis support the above finding (Table 3) that LS94-3207
485 inherited SCN resistance possibly from Peking by providing evidence that LS94-3207 has the
486 Peking-type resistance *SHMT* genotype and three copies of *rhg1* similar to that of Peking
487 (Supplementary Table 3 and Supplementary Fig. 3).

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

496 The new SCN QTL (SCN-3) located in chromosome 8 (116.7 – 154.1 cM) explained
497 15% of the FI variation and the resistance allele was contributed by the A95-684043 parent. The
498 presence of this QTL had not been reported earlier from either PI 88788, Peking, or PI 90763
499 which are the known sources of SCN resistance for A95-684043 (Cianzio et al. 2002). A
500 possible explanation might be that there was low coverage of genetic markers in the segregating
501 populations used in the earlier mapping studies.

502 The QTL SCN-4 we identified in AX19286 on chromosome 18 was mapped to a similar
503 interval, in which the *rhg1* locus was previously mapped (Concibido et al. 2004; Guo et al.
504 2006; Kadam et al. 2016; Vuong et al. 2010). The *rhg1* is one of the major SCN resistance loci
505 impacting SCN resistance (Chang et al. 2011; Concibido et al. 2004; Concibido et al. 1997; Guo
506 et al. 2005; Guo et al. 2006; Kadam et al. 2016; Kim et al. 2016; Yue et al. 2001). The region

507 containing this locus on chromosome 18 has also been reported to possess SDS resistance QTL
508 mapped in several other populations (Chang et al. 1996; Iqbal et al. 2001; Kazi et al. 2008; Njiti
509 et al. 2002; Prabhu et al. 1999; Wen et al. 2014). In our study, SCN-4 explained 30% of the total
510 FI variation, and the SCN resistance allele for SCN-4, being contributed by the A95-684043
511 parent (Table 3). The SNP haplotype analysis of *SHMT* gene and copy number analysis support
512 the above findings that A95-684043 inherited PI 88788-type susceptible genotype at *Rhg4* locus
513 and nine copies of *rhg1* as that of PI 88788, respectively (Supplementary Table 3 and
514 Supplementary Fig. 3). It is evident that the *rhg1* locus present in the AX19286 population,
515 might be donated by PI 88788, which is in the parentage of A95-684043 (Cianzio et al. 2002). In
516 the AX19287 population, a novel QTL SCN-5 (37.8 – 46.4 cM) was identified on chromosome
517 11 and three other SCN resistance QTL previously mapped are reported in different regions on
518 the same chromosome (58 - 63 cM, 84.2 - 98.9 cM, and 105.5 - 122.5 cM) (Guo et al. 2005; Wu
519 et al. 2009; Yue et al. 2001) (Fig. 6).

520 In our study three SCN resistant parents were used to generate the two RIL populations,
521 and two distinct patterns of segregation were observed in each population. In the population
522 AX19286, we identified four SCN resistance QTL (Table 3). In the population AX19287, we
523 identified one SCN resistance QTL that explained 12 % of the total variation. In the AX19286
524 population, both parents, A95-684043 and LS94-3207 are resistant to SCN. The SCN resistance
525 for A95-684043 is derived from three donors, Peking, PI 88788, and PI 90763 (Cianzio et al.
526 2002), also including SCN-4 QTL identified in this study. The major SCN resistance PI 88788-
527 type *rhg1* locus (SCN-4 QTL) was possibly inherited from PI 88788 (Table 3, Supplementary
528 Table 3 and Supplementary Fig. 3). For LS94-3207, SCN resistance is derived from PI 437654
529 and Peking (Schmidt and Klein 2004), including SCN-1 QTL identified in this study. 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 comes from LS94-3207, with A95-684043 having the allele for susceptibility (Table 3). For SCN-3 QTL and SCN-4 QTL (*rhg1* locus), however, the resistance allele comes 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 generation, 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 progeny. 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.

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

563 The complex nature of the SCN and SDS resistance mechanisms in the soybean
564 pathosystem may benefit from the identification and use of new resistance loci in addition to loci
565 previously identified for controlling both pathogens. Up to date, the progress in development of
566 mapping populations to identify QTLs for simultaneous resistance to both SDS and SCN has
567 been limited (Iqbal et al. 2009; Prabhu et al. 1999; Srour et al. 2012). In our study we could not
568 map a single QTL resistance to both pathogens because resistance QTL for SCN and SDS were
569 identified by inoculating each pathogen separately. Therefore, we are unable to hypothesize the
570 nature of the relationship between QTL for each pathogen. This opposes to the field situation,
571 in which SDS and SCN pathogens co-exist and simultaneously might attack the same soybean
572 root. The *Rhg1/Rfs2* locus on chromosome 18 has been identified to confer nearly complete
573 resistance to both SDS root rot and leaf symptoms caused by *F. virguliforme* and to also provide
574 partial resistance to three different populations of nematodes (Srour et al. 2012). The fact that so
575 far only one QTL has been detected to confer resistance to both pathogens suggests that in

576 general there might be different QTL along with other resistance mechanisms that might be
577 needed by the soybean host to fight thetwo soybean pathogens. A possible interpretation might
578 be that the biology/ infection mode /pathogenesis between *F. virguliforme* and SCN conditions
579 varying resistance mechanisms in soybean. Research is in progress at our lab that may contribute
580 to a better understanding of resistance expression and the inter-relation among QTL.

581 The research we report here will also result in the public future release of germplasm
582 lines possessing several QTL associated with resistance to SCN and to SDS (Cianzio et al.
583 unpublished). In brief, we identified three new QTL, one associated with SDS resistance and
584 two with SCN resistance. The QTL we identified, and those from previous studies using different
585 populations, placed in similar chromosomal regions contribute to validate the usefulness of some
586 of the QTL to improve resistance to SDS and to SCN.

587

588 **Figure Legends**

589 **Fig. 1** Frequency distribution of foliar disease scores among the AX19286 (A95-684043 x LS94-
590 3207) recombinant inbred lines (RILs). The foliar disease symptoms were scored 35 days
591 following infection with *F. virguliforme*. Arrows indicate the disease scores of parents and a
592 susceptible variety, Spencer. The values are means of three biological replications

593 **Fig. 2** Frequency distribution of foliar disease scores among the AX19287 (A95-684043 x LS98-
594 0582) recombinant inbred lines (RILs). The foliar disease symptoms were scored 35 days
595 following infection with *F. virguliforme*. Arrows indicate the disease scores of parents and a
596 susceptible variety, Spencer. The values are means of three biological replications

597 **Fig. 3** Segregation of soybean cyst nematode (SCN) resistance among the AX19286 (A95-
598 684043 x LS94-3207) recombinant inbred lines (RILs). Arrows indicate the phenotypes of

599 parents, the most resistant line, PI88788 and the most susceptible variety, Lee74. The female
600 indices are means of three biological replications calculated using the cysts numbers of Lee74 as
601 the denominator

602 **Fig. 4** Segregation of soybean cyst nematode (SCN) resistance among the AX19287 (A95-
603 684043 x LS98-0582) recombinant inbred lines (RILs). Arrows indicate the phenotypes of
604 parents, the most resistant line, PI88788 and the most susceptible variety, Lee74. The female
605 indices are means of three biological replications calculated using the cysts numbers of Lee74 as
606 the denominator

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

612 **Fig. 6** The composite genetic map of the soybean cyst nematode (SCN) resistance quantitative
613 trait loci (QTL) including the ones identified in this study. Stripped rectangles are QTL identified
614 in this study (Table 3); black rectangles are SCN resistance QTL identified previously. SoyBase
615 names were given for the previously identified QTL. (*) previously identified QTL not yet
616 named

617

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References:

Abeysekara NS, Bhattacharyya MK (2014) Analyses of the xylem sap proteomes identified candidate *Fusarium virguliforme* proteinacious toxins. PLoS One 9: e93667

Abney SA, Crochet WD (2004) The uniform soybean tests, northern region 2004. USDA-ARS, Department of Agronomy, Purdue University, West Lafayette, IN

Aoki T, O'Donnell K, Homma Y, Lattanzi A (2003) Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex-*F. virguliforme* in North America and *F. tucumaniae* in South America. Mycologia 95:660–684

Bernard RL, Noel GR, Anand SC, Shannon JG (1988) Registration of 'Fayette' soybean. Crop Sci 28:1028–1029

Bradley C, Allen T (2014) Estimates of soybean yield reductions caused by diseases in the United States. University of Illinois Urbana-Champaign, Department of Crop Science extension and outreach. (Accessed on 30 November 2016) http://extension.cropsciences.illinois.edu/fieldcrops/diseases/yield_reductions.php.

642 Brar HK, Swaminathan S, Bhattacharyya MK (2011) The *Fusarium virguliforme* toxin FvTox1
643 causes foliar sudden death syndrome-like symptoms in soybean. Mol Plant-Microbe
644 Interact 24:1179–1188
645 Brzostowski LF, Schapaugh WT, Rzodkiewicz PA, Todd TC, Little CR (2014) Effect of host
646 resistance to *Fusarium virguliforme* and *Heterodera glycines* on sudden death syndrome
647 disease severity and soybean yield. Plant Health Prog DOI:10.1094/PHP-RS-13-0100
648 Caviness CE, Riggs RD, Walters HJ (1975) Registration of Lee 74 soybean (Reg. No. 106).
649 Crop Sci 15:100
650 Chang SJC, Doubler TW, Kilo V, Suttner R, Klein J (1996) Two additional loci underlying
651 durable field resistance to soybean sudden death syndrome (SDS). Crop Sci 36:1684–
652 1688
653 Chang SJC, Doubler TW, Kilo VY, AbuThredeih J, Prabhu R (1997) Association of loci
654 underlying field resistance to soybean sudden death syndrome (SDS) and cyst nematode
655 (SCN) race 3. Crop Sci 37:965–971
656 Chang W, Dong L, Wang Z, Hu H, Han Y, Teng W, Zhang H, Guo M, Li W (2011) QTL
657 underlying resistance to two HG types of *Heterodera glycines* found in soybean cultivar
658 'L-10'. BMC Genomics 12:233
659 CIMMYT (2005) Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory.
660 Third Edition. Mexico, D.F.: CIMMYT.: p 2–4
661 Cianzio SR, Arelli P, Uphoff M, Mansur L, Schultz S, Ruff R (2002) Soybean germplasm line
662 A95-684043. ISURF Docket # 02975. Iowa State University, Ames, IA 50011-1010,
663 U.S.A.

664 Cianzio SR, Bhattacharyya MK, Swaminathan S, Westgate M, Gebhart G, Rivera-Velez N,
665 Lundeen P, Van Der Molen K, Pruski TI (2014) Registration of AR10SDS soybean
666 germplasm partially resistant to sudden death syndrome and resistant to soybean cyst
667 nematode. J Plant Regist 8: 200–210
668 Cianzio SR, Lundeen P, Gebhart G, Rivera-Velez N, Bhattacharyya MK, Swaminathan S (2016)
669 Registration of AR11SDS soybean germplasm resistant to sudden death syndrome,
670 soybean cyst nematode and with moderate iron deficiency chlorosis scores. J Plant Regist
671 10:177–188
672 Concibido VC, Denny RL, Boutin SR, Hautea R, Orf JH (1994) DNA marker analysis of loci
673 underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). Crop Sci
674 34:240–246
675 Concibido VC, Diers BW, Arelli PR (2004) A decade of QTL mapping for cyst nematode
676 resistance in soybean. Crop Sci 44:1121–1131
677 Concibido VC, Lange DA, Denny RL, Orf JH, Young ND (1997) Genome mapping of soybean
678 cyst nematode resistance genes in 'Peking', PI 90763, and PI 88788 using DNA markers.
679 Crop Sci 37:258–264
680 Cook DE, Lee TG, Guo X, Melito S, Wang K, Bayless AM, Wang J, Hughes TJ, Willis DK,
681 Clemente TE, Diers BW, Jiang J, Hudson ME, Bent AF (2012) Copy number variation of
682 multiple genes at *Rhg1* mediates nematode resistance in soybean. Science 338:1206–
683 1209
684 Cregan PB, Mudge J, Fickus EW, Danesh D, Denny R, Young ND (1999) Two simple sequence
685 repeat markers to select for soybean cyst nematode resistance conditioned by the *rhg1*
686 locus. Theor Appl Genet 99:811–818

687 Davis EL, Hussey R, Baum T (2004) Getting to the roots of parasitism by nematodes. Trends
688 Parasitol 20:134–141
689 Davis EL, Tylka GL (2000) Soybean cyst nematode disease. The Plant Health Instructor. DOI:
690 10.1094/PHI-I-2000-0725-01
691 de Farias-Neto AL, Hashmi R, Schmidt M, Carlson S, Hartman GL, Li S, Nelson RL, Diers BW
692 (2007) Mapping and confirmation of a new sudden death syndrome resistance QTL on
693 linkage group D2 from the soybean genotypes PI 567374 and 'Ripley'. Mol Breed 20:53–
694 62
695 Faghihi J, Donald PA, Noel G, Welacky TW, Ferris VR (2010) Soybean resistance to field
696 populations of *Heterodera glycines* in selected geographic areas. Plant Health Prog.
697 DOI:10.1094/PHP-2010-0426-01-RS
698 Fan JB, Oliphant A, Shen R, Kermani BG, Garcia F, Gunderson KL, Hansen M, Steemers F,
699 Butler SL, Deloukas P, Galver L, Hunt S, McBride C, Bibikova M, Rubano T, Chen J,
700 Wickham E, Doucet D, Chang W, Campbell D, Zhang B, Kruglyak S, Bentley D, Haas J,
701 Rigault P, Zhou L, Stuelpnagel J, Chee MS (2003) Highly parallel SNP genotyping. Cold
702 Spring Harb Symp Quant Biol 68:69–78
703 Fox J, Weisberg S (2011) An R companion to applied egression, Second Edition. Thousand Oaks
704 CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.
705 Gelin JR, Arelli PR, Rojas-Cifuentes GA (2006) Using independent culling to screen plant
706 introductions for combined resistance to soybean cyst nematode and sudden death
707 syndrome. Crop Sci 46:2081–2083
708 Grant D, Nelson RT, Cannon SB, Shoemaker RC (2010) SoyBase, the USDA-ARS soybean
709 genetics and genomics database. Nucl Acids Res 38 (suppl 1):D843–D846

710 Grinnan R, Carter TE Jr., Johnson, MTJ (2013) Effects of drought, temperature, herbivory, and
711 genotype on plant-insect interactions in soybean (*Glycine max*). Arthropod-Plant
712 Interactions 7:201–205
713 Guo B, Sleper DA, Arelli PR, Shannon JG, Nguyen HT (2005) Identification of QTLs associated
714 with resistance to soybean cyst nematode races 2, 3 and 5 in soybean PI 90763. Theor
715 Appl Genet 111:965–971
716 Guo B, Sun J, Sleper DA, Nguyen HT, Arelli PR, Shannon JG (2006) Pooled analysis of data
717 from multiple quantitative trait locus mapping populations. Theor Appl Genet 113:39–48
718 Guo X, Wang D, Gordon S, Helliwell E, Smith T, Berry S, St. Martin S, Dorrance A (2008)
719 Genetic mapping of QTLs underlying partial resistance to *Sclerotinia sclerotiorum* in
720 soybean PI 391589A and PI 391589B. Crop Sci 48:1129–1139
721 Hartman GL, Chang H-X, Leandro LF (2015) Research advances and management of soybean
722 sudden death syndrome. J Crop Prot 73:60–66
723 Hartman GL, Huang YH, Nelson RL, Noel GR (1997) Germplasm evaluation of *Glycine max* for
724 resistance to *Fusarium solani*, the causal organism of sudden death syndrome. Plant Dis
725 81:515–518
726 Hartman GL, Huang YH, Li S (2004) Phytotoxicity of *Fusarium solani* culture filtrates from
727 soybeans and other hosts assayed by stem cuttings. Australas Plant Pathol 33:9–15
728 Hartwig EE, Epps JM (1968) Registration of 'Dyer' soybean. Crop Sci 8:402
729 Hartwig EE, Epps JM (1973) Registration of 'Forrest' soybean. Crop Sci 13:287
730 Heatherly LG, Hodges HF (1998) Soybean production in the midsouth. CRC Press, 416 pages

731 Heer JA, Knap HT, Mahalingam R, Shipe ER, Arelli PR, Matthews BF (1998) Molecular
732 markers for resistance to *Heterodera glycines* in advanced soybean germplasm. Mol
733 Breed 4:359–367
734 Hnetkovsky N, Chang SJC, Doubler TW, Gibson PT, Lightfoot DA (1996) Genetic mapping of
735 loci underlying field resistance to soybean sudden death syndrome (SDS). Crop Sci
736 36:393–400
737 Huang X, Das A, Sahu BB, Srivastava SK, Leandro LF, O'Donnell K, Bhattacharyya M K
738 (2016) Identification of highly variable supernumerary chromosome segments in an
739 asexual pathogen. PLoS ONE 11(6):e0158183
740 Huynh T, Bastien M, Iquira E, Turcotte P, Belzile F (2010) Identification of QTLs associated
741 with partial resistance to white mold in soybean using field-based inoculation. Crop Sci
742 50:969–979
743 Hyten D, Choi I-Y, Song Q, Specht J, Carter T, Shoemaker R, Hwang EY, Matukumalli L,
744 Cregan P (2010) A high density integrated genetic linkage map of soybean and the
745 development of a 1536 universal soy linkage panel for quantitative trait locus mapping.
746 Crop Sci 50:960–968
747 Hyten D, Song Q, Choi I-Y, Yoon M-S, Specht J, Matukumalli L, Nelson R, Shoemaker R,
748 Young N, Cregan P (2008) High-throughput genotyping with the GoldenGate assay in
749 the complex genome of soybean. Theor Appl Genet 116:945–952
750 Iqbal MJ, Ahsan R, Afzal AJ, Jamai A, Meksem K, El Shemy H, Lightfoot DA (2009) Analysis
751 of the activity of the soybean laccase encoded within the Rhg1/Rfs2 locus. Curr Iss Mol
752 Biol 11:i11–19

753 Iqbal MJ, Meksem K, Njiti VN, Kassem M, Lightfoot DA (2001) Microsatellite markers identify
754 three additional quantitative trait loci for resistance to soybean sudden-death syndrome
755 (SDS) in Essex x Forrest RILs. Theor Appl Genet 102:187–192
756 Joehanes R, Nelson JC (2008) QGene 4.0, an extensible Java QTLanalysis platform.
757 Bioinformatics 24:2788–2789
758 Kadam S, Vuong TD, Qiu D, Meinhardt CG, Song L, Deshmukh R, Patil G, Wan J, Valliyodan
759 B, Scaboo AM, Shannon JG, Nguyen HT (2016) Genomic-assisted phylogenetic analysis
760 and marker development for next generation soybean cyst nematode resistance
761 breeding. Plant Sci 242:342–350
762 Kandel YR, Bradley CA, Wise KA, Chilvers MI, Tenuta AU, Davis VM, Esker PD, Smith
763 DL, Licht MA, Mueller DS (2015) Effect of glyphosate application on sudden death
764 syndrome of glyphosate-resistant soybean under field conditions. Plant Dis 99:347–354
765 Kassem MA, Meksem K, Wood AJ, Lightfoot DA (2007) Loci Underlying SDS and SCN
766 Resistance Mapped in the 'Essex' by 'Forrest' Soybean Recombinant Inbred Lines. Rev
767 Biol Biotech 6:2–10
768 Kassem MA, Ramos L, Leandro L, Mbofung G, Hyten DL, Kantartzi SK, Grier IV RL, Njiti
769 VN, Cianzio S, Meksem K (2012) The ‘PI 438489B’ by ‘Hamilton’ SNP-based genetic
770 linkage map of soybean [*Glycine max* (L.) Merr.] identified quantitative trait loci that
771 underlie seedling SDS resistance. J Plant Genome Sci 1:18–30
772 Kassem MA, Shultz J, Meksem K, Cho Y, Wood AJ, Iqbal MJ, Lightfoot DA (2006) An updated
773 'Essex' by 'Forrest' linkage map and first composite interval map of QTL underlying six
774 soybean traits. Theor Appl Genet 113:1015–1026

775 Kazi S, Njiti VN, Doubler TW, Yuan J, Iqbal JM, Cianzio S, Lightfoot DA (2007) Registration
776 of the Flyer × Hartwig recombinant inbred line mapping population. J Plant Regist
777 1:175–178
778 Kazi S, Shultz J, Afzal J, Johnson J, Njiti VN, Lightfoot DA (2008) Separate loci underlie
779 resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor
780 Appl Genet 116:967–977
781 Kazi S, Shultz J, Afzal J, Hashmi R, Jasim M, Bond J, Arelli PR, Lightfoot DA (2010) Iso-lines
782 and inbred-lines confirmed loci that underlie resistance from cultivar 'Hartwig' to three
783 soybean cyst nematode populations. Theor Appl Genet 120:633–644
784 Kim K-S, Vuong TD, Qiu D, Robbins RT, Grover Shannon J, Li Z, Nguyen HT (2016)
785 Advancements in breeding, genetics, and genomics for resistance to three nematode
786 species in soybean. Theor Appl Genet 129:2295–2311
787 Koenning SR, Wrather JA (2010) Suppression of soybean yield potential in the continental
788 United States from plant disease estimated from 2006 to 2009. Plant Health Prog
789 DOI:101094/PHP-2010-1122-01-RS
790 Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen
791 12:172–175
792 Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg LA, Newburg L
793 (1987) MAPMAKER: an interactive computer package for constructing primary genetic
794 linkage maps of experimental and natural populations. Genomics 1:174–181
795 Leandro LF, Tatalovic N, Luckew A (2012). Soybean sudden death syndrome-advances in
796 knowledge and disease management. CAB Rev 7:1–14

797 Lee TG, Diers BW, Hudson ME (2016) An efficient method for measuring copy number
798 variation applied to improvement of nematode resistance in soybean. Plant J 88:143–153
799 Li S, Hartman GL, Widholm JM (1999) Viability staining of soybean suspension cultured cells
800 and a stem-cutting assay to evaluate phytotoxicity of *Fusarium solani* culture filtrates.
801 Plant Cell Rep 18:375–380
802 Li X, Han Y, Teng W, Zhang S, Yu K, Poysa V, Anderson T, Ding J, Li W (2010) Pyramided
803 QTL underlying tolerance to *Phytophthora* root rot in mega-environments from soybean
804 cultivars Conrad and Hefeng 25. Theor Appl Genet 121:651–658
805 Lightfoot DA (2015) Two decades of molecular marker- assisted breeding for resistance to
806 soybean sudden death syndrome. Crop Sci 55:1460–1484
807 Lightfoot DA, Gibson PT, Meksem K (2007) Method of determining soybean sudden death
808 syndrome resistance in a soybean plant. U.S. Patent 7,288,386. Date issued: 30 October.
809 Liu S, Kandoth PK, Lakhssassi N, Kang J, Colantonio V, Heinz R, Yeckel G, Zhou Z, Bekal S,
810 Dapprich J, Rotter B, Cianzio SR, Mitchum MG, Meksem K (2017) The soybean
811 GmSNAP18 gene underlies two types of resistance to soybean cyst nematode. Nat
812 Commun 8:14822
813 Liu S, Kandoth PK, Warren S, Yeckel G, Heinz R, Alden J, Yang C, Jamai A, El Mellouki T,
814 Juvale P, Hill J, Baum T, Cianzio SR, Whitham S, Korkin D, Mitchum M, Meksem K
815 (2012) A soybean cyst nematode resistance gene points to a new mechanism of plant
816 resistance to pathogens. Nature 492:256–260
817 Liu ZH, Hu J, Anderson JA, Friesen TL, Rasmussen JB, Faris JD (2005) A wheat intervarietal
818 genetic linkage map based on microsatellite and target region amplified polymorphism
819 markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782–794

820 Lu P, Shannon JG, Sleper DA, Nguyen HT, Cianzio SR, Arelli PR (2006) Genetics of cyst
821 nematode resistance in soybean PIs 467312 and 507354. Euphytica 149:259–265
822 Mansur LM, Carriquiry AL, Rao-Arelli AP (1993) Generation mean analysis of resistance to
823 race 3 of soybean cyst nematode. Crop Sci 33:1249–1253
824 Meksem K, Pantazopoulos P, Njiti VN, Hyten LD, Arelli PR, Lightfoot DA (2001) 'Forrest'
825 resistance to the soybean cyst nematode is bigenic: saturation mapping of the *rhg1* and
826 *Rhg4* loci. Theor Appl Genet 103:710–717
827 Mitchum MG (2016) Soybean resistance to the soybean cyst nematode *Heterodera glycines*: An
828 update. Phytopathology 106:1444–1450
829 Mitchum MG, Wrather JA, Heinz RD, Shannon JG, Danekas G (2007) Variability in distribution
830 and virulence phenotypes of *Heterodera glycines* in Missouri during 2005. Plant Dis
831 91:1473–1476
832 Mueller D, Hartman G, Nelson R, Pedersen W (2002) Evaluation of *Glycine max* germ plasm for
833 resistance to *Fusarium solani* f. sp. *glycines*. Plant Dis 86:741–746
834 Mueller DS, Nelson RL, Hartman GL, Pedersen WL (2003) Response of commercially
835 developed soybean cultivars and the ancestral soybean lines to *Fusarium solani* f. sp.
836 *glycines*. Plant Dis 87:827–831
837 Niblack TL, Arelli PR, Noel GR, Opperman CH, Orf JH, Schmitt, DP, Shannon JG, Tylka GL
838 (2002) A revised classification scheme for genetically diverse populations of *Heterodera*
839 *glycines*. J Nemat 34:279–288
840 Niblack TL, Colgrove AL, Colgrove K, Bond JP (2008) Shift in virulence of soybean cyst
841 nematode is associated with use of resistance from PI 88788. Plant Health Prog.
842 DOI:10.1094/PHP-2008-0118-01-RS

843 Niblack TL, Tylka GL, Arelli P, Bond J, Diers B, Donald P, Faghihi J, Ferris VR, Gallo K,
844 Heinz RD, Lopez-Nicora H, Qualen RV, Welacky T, Wilcox J (2009) A standard
845 greenhouse method for assessing soybean cyst nematode resistance in soybean: SCE08
846 (standardized cyst evaluation 2008). Plant Health Prog. DOI:10.1094/PHP-2009-0513-
847 01-RV
848 Njiti VN, Lightfoot DA (2006) Genetic analysis infers *Dt* loci underlie resistance to *Fusarium*
849 *solani* f. sp *glycines* in indeterminate soybeans. Can J Plant Sci 86:83–90
850 Njiti VN, Meksem K, Iqbal MJ, Johnson JE, Kassem MA, Zobrist AF, Kilo VY, Lightfoot DA
851 (2002) Common loci underlie field resistance to soybean sudden death syndrome in
852 Forrest, Pyramid, Essex, and Douglas. Theor Appl Genet 104:294–300
853 Prabhu RR, Njiti VN, Johnson JE, Schmidt ME, Klein RJ, Lightfoot DA (1999) Selecting
854 soybean cultivars for dual resistance to cyst nematode sudden death syndrome with two
855 DNA markers. Crop Sci 39:982–987
856 Pudake R, Swaminathan S, Sahu B, Leandro L, Bhattacharyya MK (2013) Investigation of the
857 *Fusarium virguliforme fvtox1* mutants revealed that the FvTox1 toxin is involved in foliar
858 sudden death syndrome development in soybean. Curr Genet 59:107–117
859 Qiu BX, Arelli PR, Sleper DA (1999) RFLP markers associated with soybean cyst nematode
860 resistance and seed composition in a 'Peking' x 'Essex' population. Theor Appl Genet
861 98:356–364
862 R Core Team (2015) R: A language and environment for statistical computing. R Foundation for
863 Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
864 Rincker K, Cary T, Diers BW (2017) Impact of soybean cyst nematode resistance on soybean
865 yield. Crop Sci 57:1373–1382

866 Robertson A, Leandro L (2010) Answers to questions about soybean sudden death syndrome in
867 Iowa 2010. Integrated Crop Management News and Iowa State University Extension.
868 http://www.extension.iastate.edu/CropNews/2010/0907robertsonleandro.html
869 Roy KW, Hershman DE, Rupe JC, Abney TS (1997) Sudden death syndrome of soybean. Plant
870 Dis 81:1100–1111
871 Ruben E, Jamai A, Afzal J, Njiti VN, Triwitayakorn K, Iqbal MJ, Yaegashi S, Bashir R, Kazi
872 S, Arelli P, Town CD, Ishihara H, Meksem K, Lightfoot DA (2006) Genomic analysis
873 of the *rhg1* locus: candidate genes that underlie soybean resistance to the cyst
874 nematode. Mol Genet Genomics 276:503–516
875 Rupe JC (1989) Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with
876 sudden death syndrome. Plant Dis 73:581–584
877 Rupe J, Gbur E, Marx D (1991) Cultivar responses to sudden death syndrome of soybean. Plant
878 Dis 75:47–50
879 Sanogo S, Yang XB, Scherm H (2000) Effects of herbicides on *Fusarium solani* f. sp *glycines*
880 and development of sudden death syndrome in glyphosate-tolerant soybean.
881 Phytopathology 90:57–66
882 Scherm H, Yang XB (1996) Development of sudden death syndrome of soybean in relation to
883 soil temperature and soil water potential. Phytopathology 86:642–649
884 Schmidt ME, Klein JH (2004) Registration of 'LS94-3207' soybean. Crop Sci 44:1482–1483
885 Schmidt ME, Myers O Jr., Gibson PT (1993) Registration of 'Pharaoh' soybean. Crop Sci
886 33:210–211
887 Schmitt DP, Shannon JG (1992) Differentiating soybean reponses to *Heterodera glycines* races.
888 Crop Sci 32:275–277

889 Srour A, Afzal AJ, Saini N, Blahut-Beatty L, Hemmati N, Simmonds DH, El Shemy H, Town
890 CD, Sharma H, Liu X, Li W and Lightfoot DA (2012) The receptor like kinase at Rhg1-
891 a/Rfs2 caused pleiotropic resistance to sudden death syndrome and soybean cyst
892 nematode as a transgene by altering signaling responses. BMC Genomics 13:368
893 Stephens PA, Nickell CD, Kolb FL (1993) Genetic analysis of resistance to *Fusarium solani* in
894 soybean. Crop Sci 33:929–930
895 Swaminathan S, Abeysekara NS, Liu M, Cianzio SR, Bhattacharyya MK (2016) Quantitative
896 trait loci underlying host responses of soybean to *Fusarium virguliforme* toxins that cause
897 foliar sudden death syndrome. Theor Appl Genet 129:495–506
898 Tucker D, Maroof SM, Mideros S, Skoneczka J, Nabati D, Buss G, Hoeschele I, Tyler B, St.
899 Martin S, Dorrance A (2010) Mapping quantitative trait loci for partial resistance to
900 *Phytophthora sojae* in a soybean interspecific cross. Crop Sci 50:628–635Tylka GL,
901 Marett CC (2014) Distribution of the soybean cyst nematode, *Heterodera glycines*, in the
902 United States and Canada, 1954 to 2014. Plant Health Prog 15:85–87. DOI:10.1094/PHP-
903 BR-14-0006
904 Vuong T, Sleper D, Shannon J, Nguyen H (2010) Novel quantitative trait loci for broad-based
905 resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe) in soybean PI
906 567516C. Theor Appl Genet 121:1253–1266
907 Wang D, Diers BW, Arelli PR, Shoemaker RC (2001) Loci underlying resistance to Race 3 of
908 soybean cyst nematode in *Glycine soja* plant introduction 468916. Theor Appl Genet
909 103:561–566

910 Webb DM, Baltazar BM, Raoarelli AP, Schupp J, Clayton K (1995) Genetic mapping of soybean
911 cyst nematode race-3 resistance loci in the soybean PI 437654. Theor Appl Genet
912 91:574–581
913 Weismann JM, Matthews BF, Devine TE (1992) Molecular markers located proximal to the
914 soybean cyst nematode resistance gene, *Rhg4*. Theor Appl Genet 85:136–138
915 Wen Z, Tan R, Yuan J, Bales C, Du W, Zhang S, Chilvers MI, Schmidt C, Song Q, Cregan PB,
916 Wang D (2014) Genome-wide association mapping of quantitative resistance to sudden
917 death syndrome in soybean. BMC Genomics 15:809
918 Winstead NN, Skotian CB, Sasser JN (1955) Soybean cyst nematode in North Carolina. Plant
919 Dis Rep 39:9–11
920 Wrather JA, Ploper LD (1996) Soybean disease loss estimates for the top ten producing countries
921 during 1994. Phytopathology 86:S41
922 Wu X, Blake S, Sleper D, Shannon JG, Cregan P, Nguyen H (2009) QTL, additive and epistatic
923 effects for SCN resistance in PI 437654. Theor Appl Genet 118:1093–1105
924 Xing LJ, Westphal A (2013) Synergism in the interaction of *Fusarium virguliforme* with
925 *Heterodera glycines* in sudden death syndrome of soybean. J Plant Dis Prot 120:209–217
926 Yamanaka N, Fuentes F, Gilli J, Watanabe S, Harada K, Ban T, Abdelnoor R, Nepomuceno A,
927 Homma Y (2006) Identification of quantitative trait loci for resistance against soybean
928 sudden death syndrome caused by *Fusarium tucumaniae*. Pesquisa Agropecuária
929 Brasileira 41:1385–1391
930 Yuan J, Bashir R, Salas G, Sharma H, Srour A, Lightfoot DA (2012) New approaches to
931 selecting resistance or tolerance to SDS and *Fusarium* root rot. J Plant Genome Sci 1:10–
932 17

933 Yue P, Arelli PR, Sleper DA (2001) Molecular characterization of resistance to *Heterodera*
934 *glycines* in soybean PI 438489B. Theor Appl Genet 102:921–928
935 Yu N, Lee TG, Rosa DP, Hudson M, Diers BW (2016) Impact of *Rhg1* copy number, type, and
936 interaction with *Rhg4* on resistance to Heterodera glycines in soybean. Theor Appl Genet
937 129:2403–2412
938
939
940
941

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

	% of RILs [a]						
Population	(HR)[b]	(R)[b]	(MR)[b]	(S)[b]	(HS)[b]	Mean FDS ± Std. Error	Range
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

[a]200 Recombinant inbred lines (RILs) from each population were categorized according to the mean foliar disease score.

[b]Foliar disease score (FDS); 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)

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)

	% of RILs [a]					
Population	(R)[b]	(MR)[b]	(MS)[b]	(S)[b]	Mean FI ± Std. Error	Range
AX19286	61	33.5	5.5	0	10.68 ±0.36	1.54 - 46.93
AX19287	73	25.5	1.5	0	7.39 ±0.19	1.55 - 23.47

[a]200 Recombinant inbred lines (RILs) from each population were categorized according to the mean female index (FI).

[b]Resistant (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

964 **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[N]	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[N]	17/ D2	BARC-020357-04569-BARC-065705-19668	ss107913274-ss107930758	Sctt008 - Sct_192	3.2 - 11.8	3.2	7.5	LS98-0582	- 0.15
	SCN-5[N]	11/ B1	BARC-040851-07854 - BARC-016539-02087	ss107919849-ss107913087	Satt638 - Satt197	37.7 - 46.4	4.2	12	LS98-0582	- 16.0

965 [a]chromosome/linkage group

966 [b]position of QTL based on the soybean composite genetic map of soybean reference genome Glyma.Wm82.a2 (Gmax2.0) in SoyBase
967 (www.soybase.org)

968 [c]likelihood of odds (LOD) at the QTL peak

969 [d]per cent contribution of a QTL in the phenotypic variation

970 [e]additive effect of an allele substitution for the QTL based on foliar disease score (FDS) or female index (FI). Negative value means allele from
971 either LS94-3207 or LS98-0582 provides greater resistance (in lowering FDS or FI) than A95-684043. Estimated threshold LOD cut off value for
972 SDS loci was 4.3 and 3.4, for SCN loci was 4.5 and 4.0, respectively for AX19286 and AX19287 populations ($p = 0.05$). [N]novel QTL

973 **Supplementary Table 1** Statistics of the SNP-based linkage groups based on segregation in the AX19286 (A95-684043 X LS94-
974 3207) population

Chr. no. / LG	Coverage (cM)	No. of markers	Average cM/ marker
1/D1a	129.4	27	4.79
2/D1b	146.5	47	3.12
3/N	171	30	5.70
4/C1	121.9	16	7.62
5/A1	110.2	37	2.98
6/C2	112.8	33	3.42
7/M	75.9	31	2.45
8/A2	164.2	35	4.69
9/K	79.3	33	2.40
10/O	126.1	24	5.25
11/B1	150	20	7.50
12/H	56.2	12	4.68
13/F	296.3	40	7.41
14/B2	80.6	18	4.48
15/E	162.3	38	4.27
16/J	118.7	27	4.40
17/D2	143.3	31	4.62
18/G	130	48	2.71
19/L	116.5	19	6.13
20/I	117.4	14	8.39
Total	2608.6	580	4.50

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979 **Supplementary Table 2** Statistics of the SNP-based linkage groups based on segregation in the AX19287 (A95-684043 x LS98-
980 0582) population

Chr. no. / LG	Coverage (cM)	No. of markers	Average cM/ marker
1/D1a	136.6	14	9.76
2/D1b	154	39	3.95
3/N	149.4	16	9.34
4/C1	103.7	19	5.46
5/A1	171.7	26	6.60
6/C2	121.7	14	8.69
7/M	138	7	19.71
8/A2	177.9	27	6.59
9/K	93.5	24	3.90
10/O	125.3	21	5.97
11/B1	146.2	16	9.14
12/H	101.2	11	9.20
13/F	116.3	16	7.27
14/B2	90.2	14	6.44
15/E	143.2	30	4.77
16/J	58.3	17	3.43
17/D2	172.6	15	11.51
18/G	87.8	18	4.88
19/L	101.2	15	6.75
20/I	26.5	12	2.21
Total	2415.3	371	6.51

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Supplementary Table 3 SNP haplotype of *Rhg4* locus

Soybean line	*SHMT* 389 G/C [a]	*SHMT* 1165 T/A [a]	*Rhg4* haplotype	Phenotype
A95-684043	C	A	PI 88788-type	SCN resistance
LS94-3207	G	T	Peking-type	SCN resistance
LS98-0582	C	A	PI 88788-type	SCN resistance
PI 88788	C	A	-	SCN resistance
Peking	G	T	-	SCN resistance

[a]PCR was carried out to amplify the polymorphic region of serine hydroxylmethyl transferase (SHMT) and Sanger sequencing of the PCR products was carried out to find out the polymorphism of the soybean lines.

Fig. 1

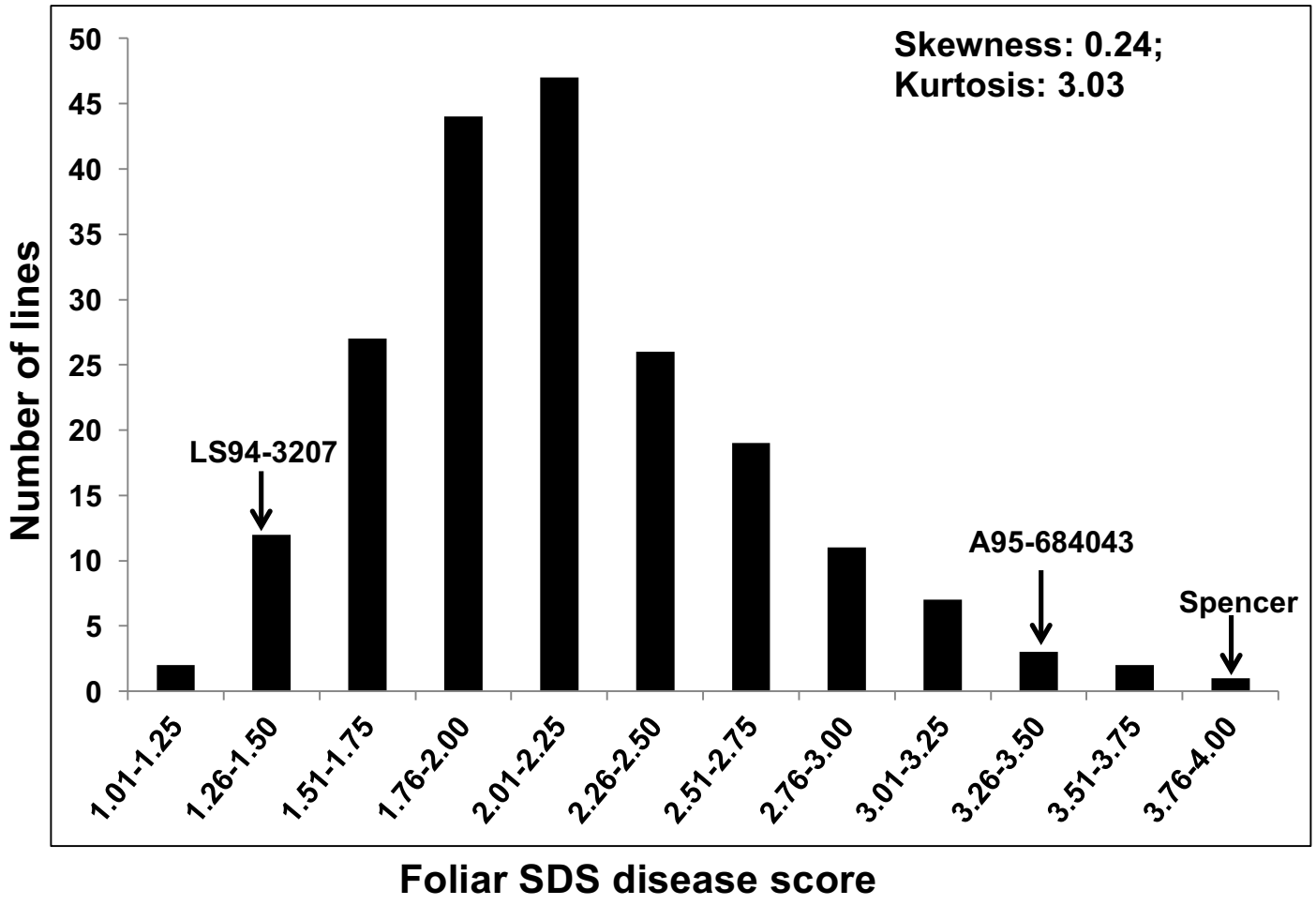

Skewness: 0.24;
Kurtosis: 3.03
Number of lines
50
45
40
35
30
25
20
15
10
5
0
LS94-3207
A95-684043
Spencer
1.01-1.25
1.26-1.50
1.51-1.75
1.76-2.00
2.01-2.25
2.26-2.50
2.51-2.75
2.76-3.00
3.01-3.25
3.26-3.50
3.51-3.75
3.76-4.00
Foliar SDS disease score

Fig. 2

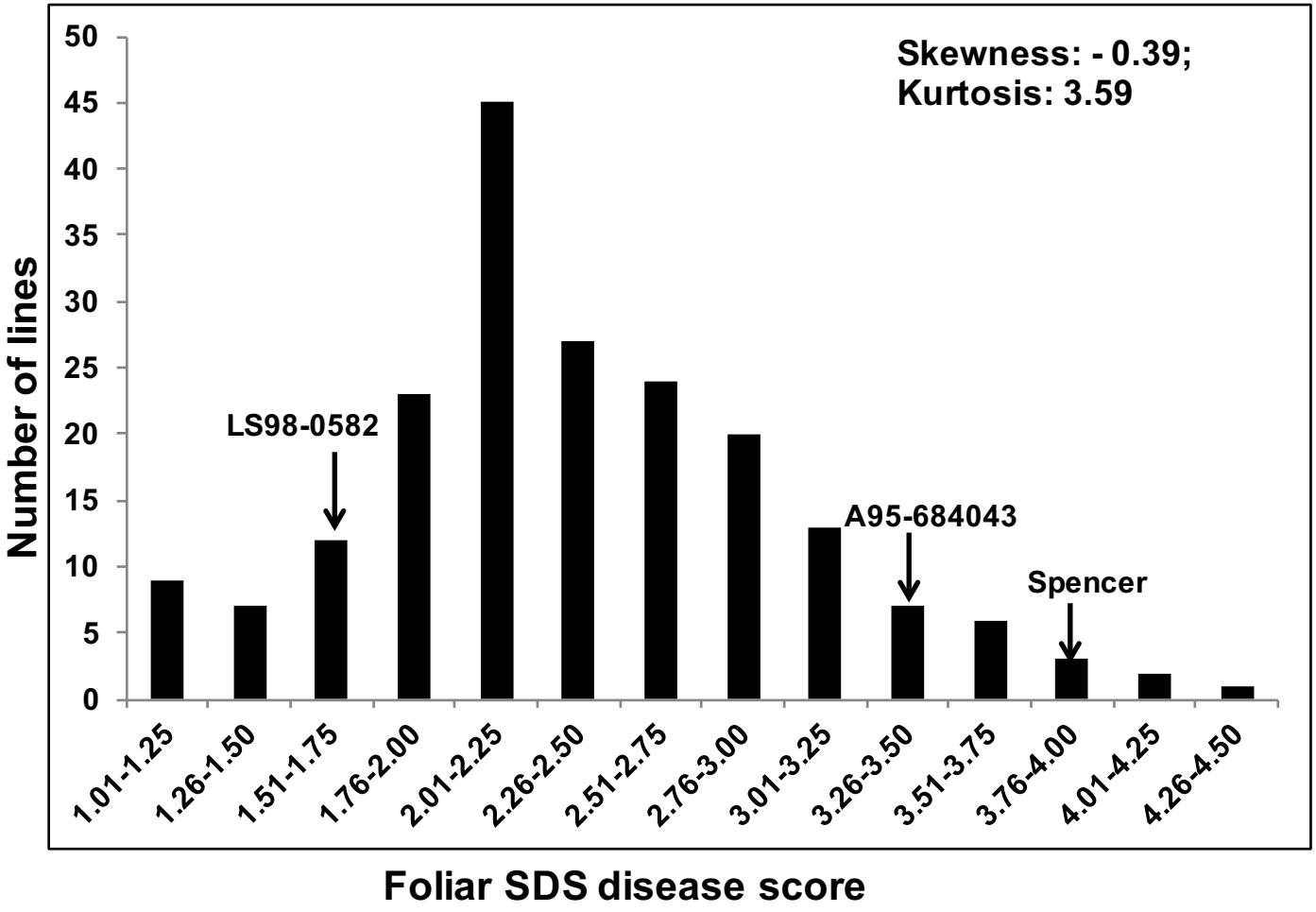

Skewness: - 0.39;
Kurtosis: 3.59
Number of lines
50
45
40
35
30
25
20
15
10
5
0
LS98-0582
A95-684043
Spencer
1.01-1.25
1.26-1.50
1.51-1.75
1.76-2.00
2.01-2.25
2.26-2.50
2.51-2.75
2.76-3.00
3.01-3.25
3.26-3.50
3.51-3.75
3.76-4.00
4.01-4.25
4.26-4.50
Foliar SDS disease score

Fig. 3

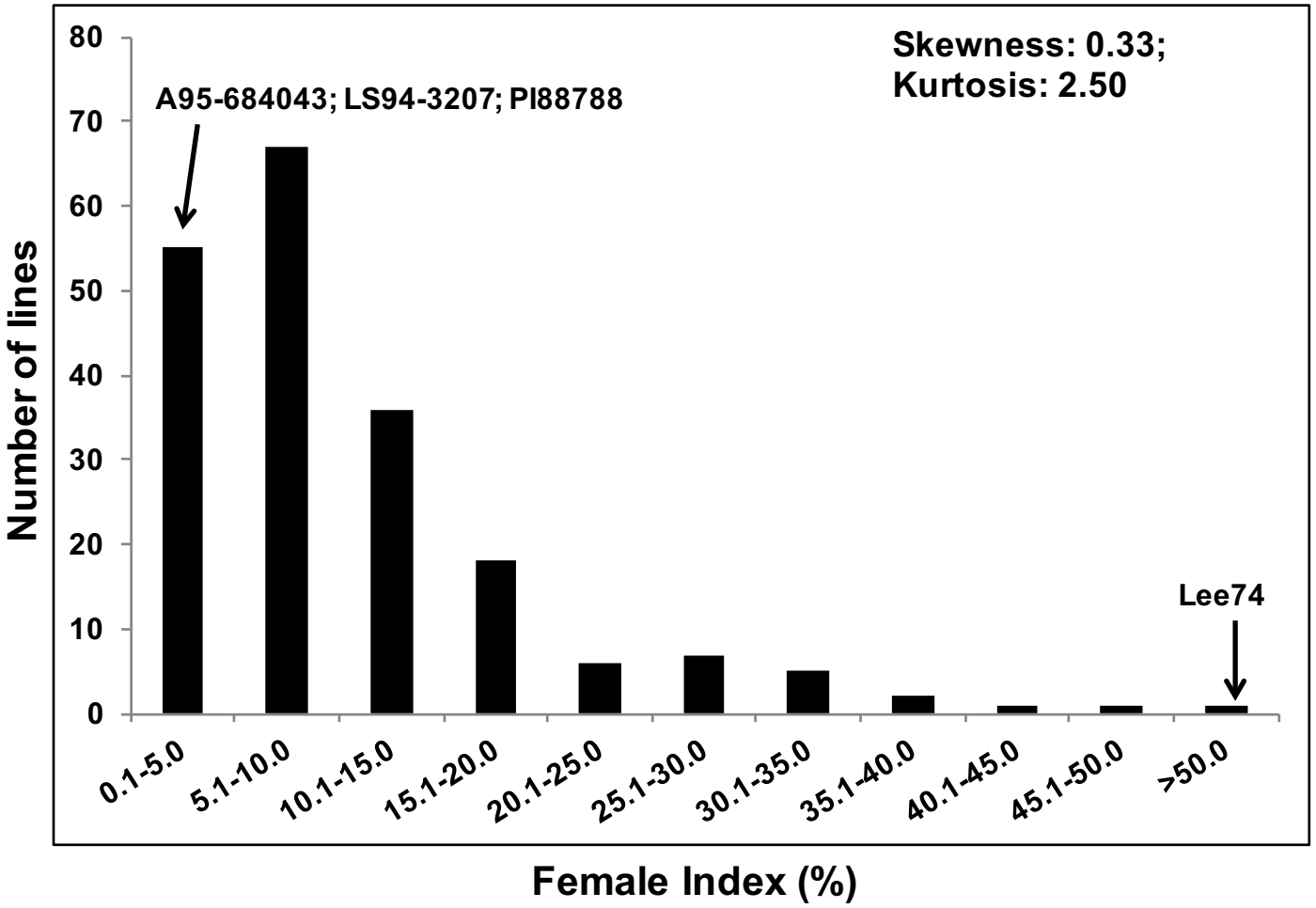

Skewness: 0.33;
Kurtosis: 2.50
A95-684043; LS94-3207; PI88788
Lee74
Number of lines
80
70
60
50
40
30
20
10
0
0.1-5.0
5.1-10.0
10.1-15.0
15.1-20.0
20.1-25.0
25.1-30.0
30.1-35.0
35.1-40.0
40.1-45.0
45.1-50.0
>50.0
Female Index (%)

Fig. 4

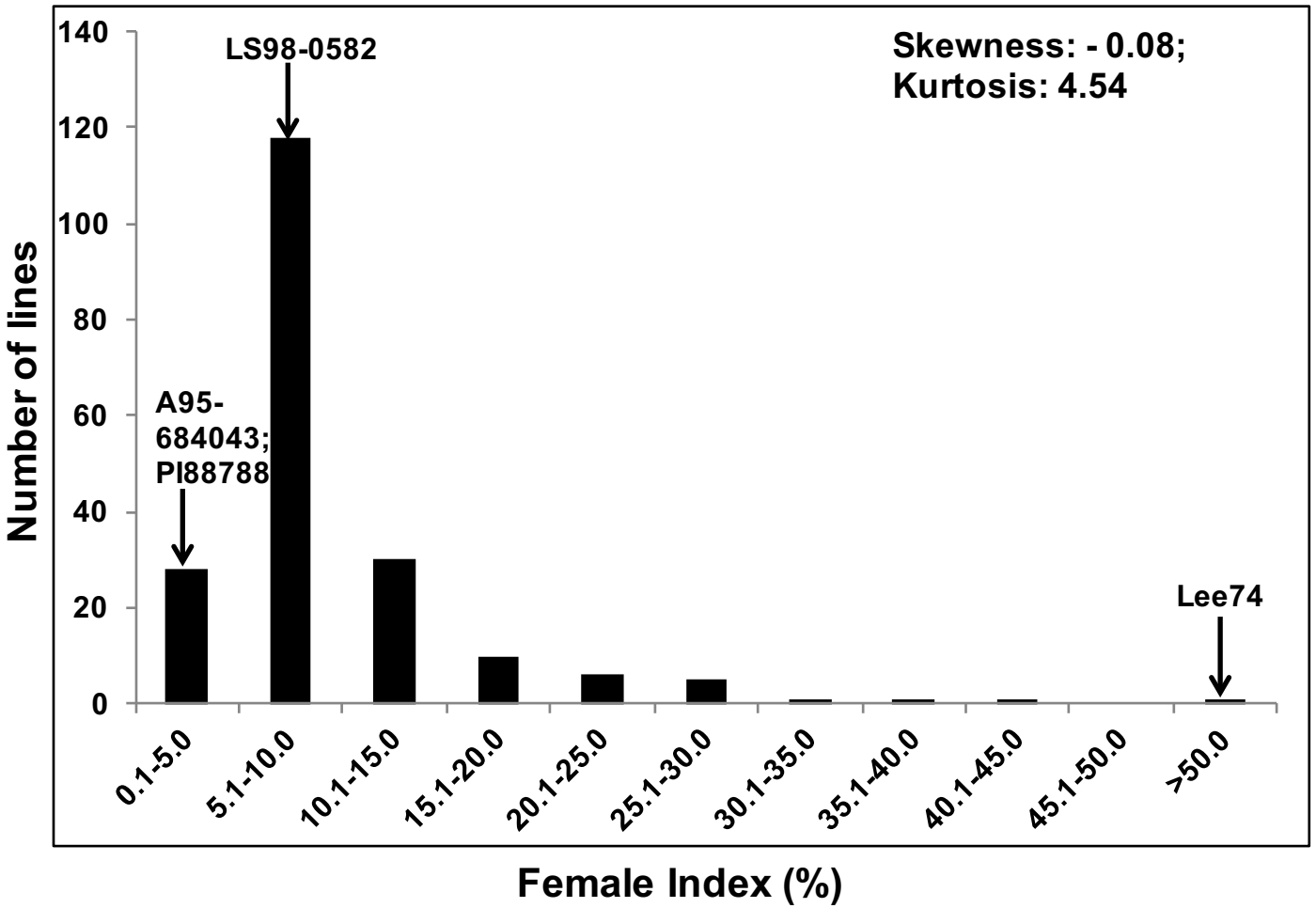

Skewness: - 0.08;
Kurtosis: 4.54
LS98-0582
A95-
684043;
PI88788
Lee74
Number of lines
140
120
100
80
60
40
20
0
0.1-5.0
5.1-10.0
10.1-15.0
15.1-20.0
20.1-25.0
25.1-30.0
30.1-35.0
35.1-40.0
40.1-45.0
45.1-50.0
>50.0
Female Index (%)

Fig. 5

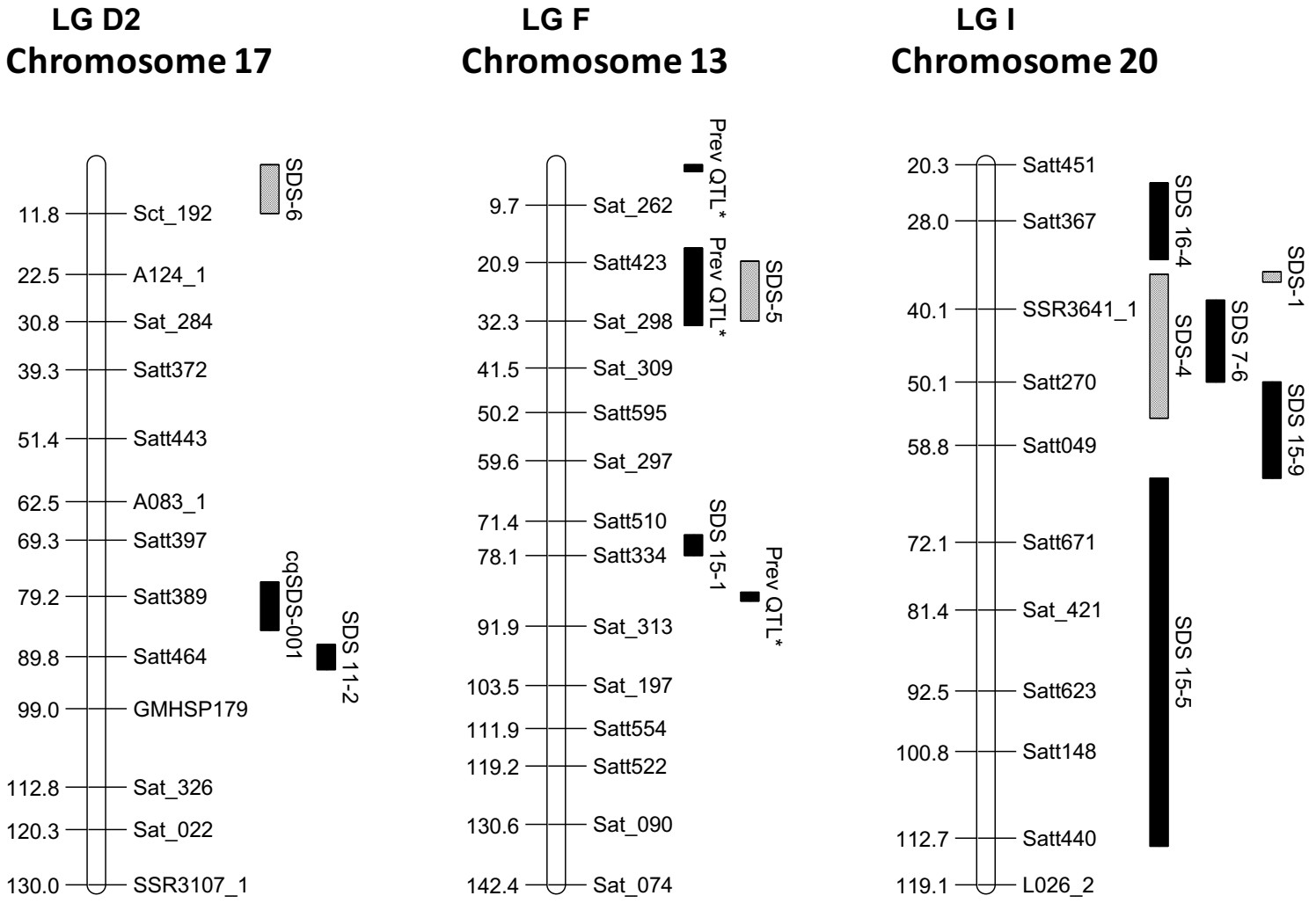

LG D2
Chromosome 17
11.8 Sct_192
22.5 A124_1
30.8 Sat_284
39.3 Satt372
51.4 Satt443
62.5 A083_1
69.3 Satt397
79.2 Satt389
89.8 Satt464
99.0 GMHSP179
112.8 Sat_326
120.3 Sat_022
130.0 SSR3107_1
SDS-6
cqSDS-001
SDS 11-2
LG F
Chromosome 13
9.7 Sat_262
20.9 Satt423
32.3 Sat_298
41.5 Sat_309
50.2 Satt595
59.6 Sat_297
71.4 Satt510
78.1 Satt334
91.9 Sat_313
103.5 Sat_197
111.9 Satt554
119.2 Satt522
130.6 Sat_090
142.4 Sat_074
Prev QTL*
Prev QTL*
SDS-5
SDS 15-1
Prev QTL*
LG I
Chromosome 20
20.3 Satt451
28.0 Satt367
40.1 SSR3641_1
50.1 Satt270
58.8 Satt049
72.1 Satt671
81.4 Sat_421
92.5 Satt623
100.8 Satt148
112.7 Satt440
119.1 L026_2
SDS 16-4
SDS-4
SDS 7-6
SDS-1
SDS 15-9
SDS 15-5

Fig. 5 continued

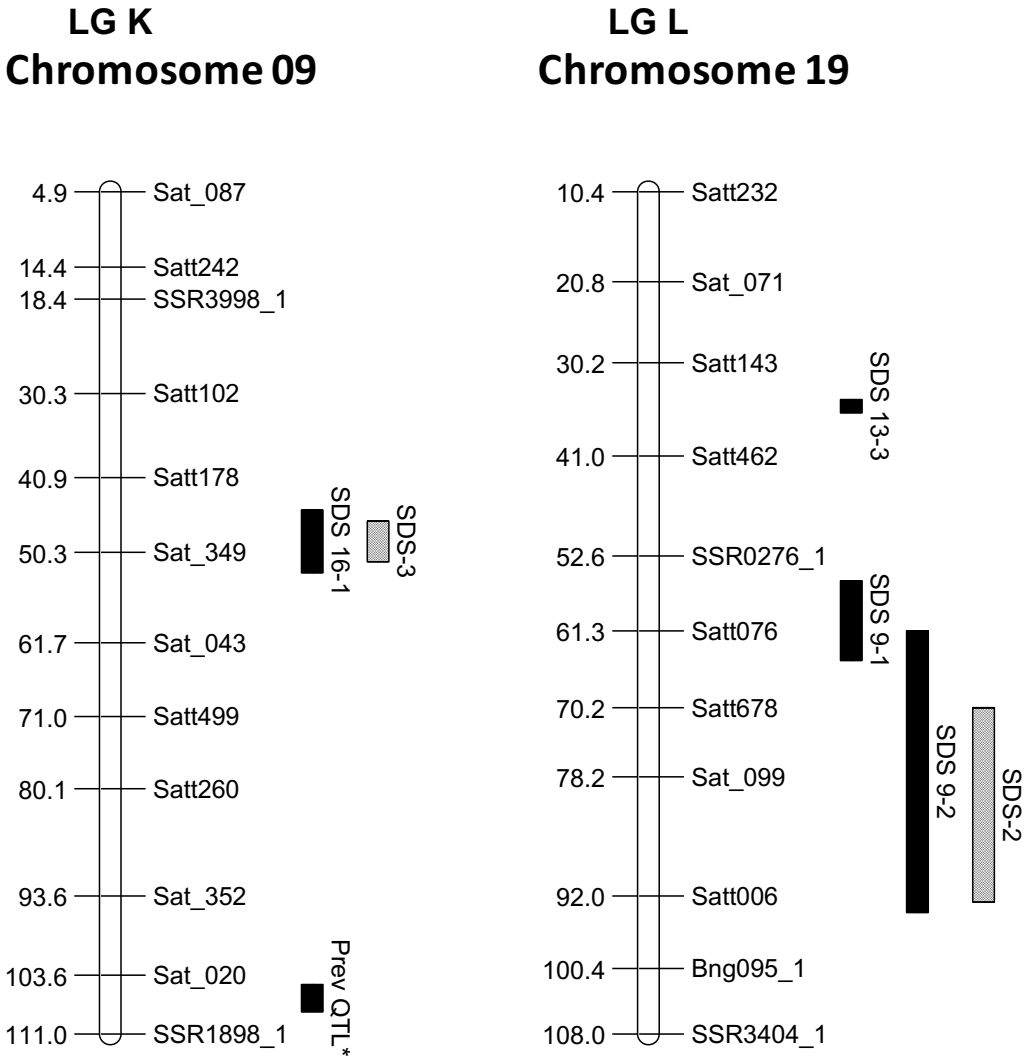
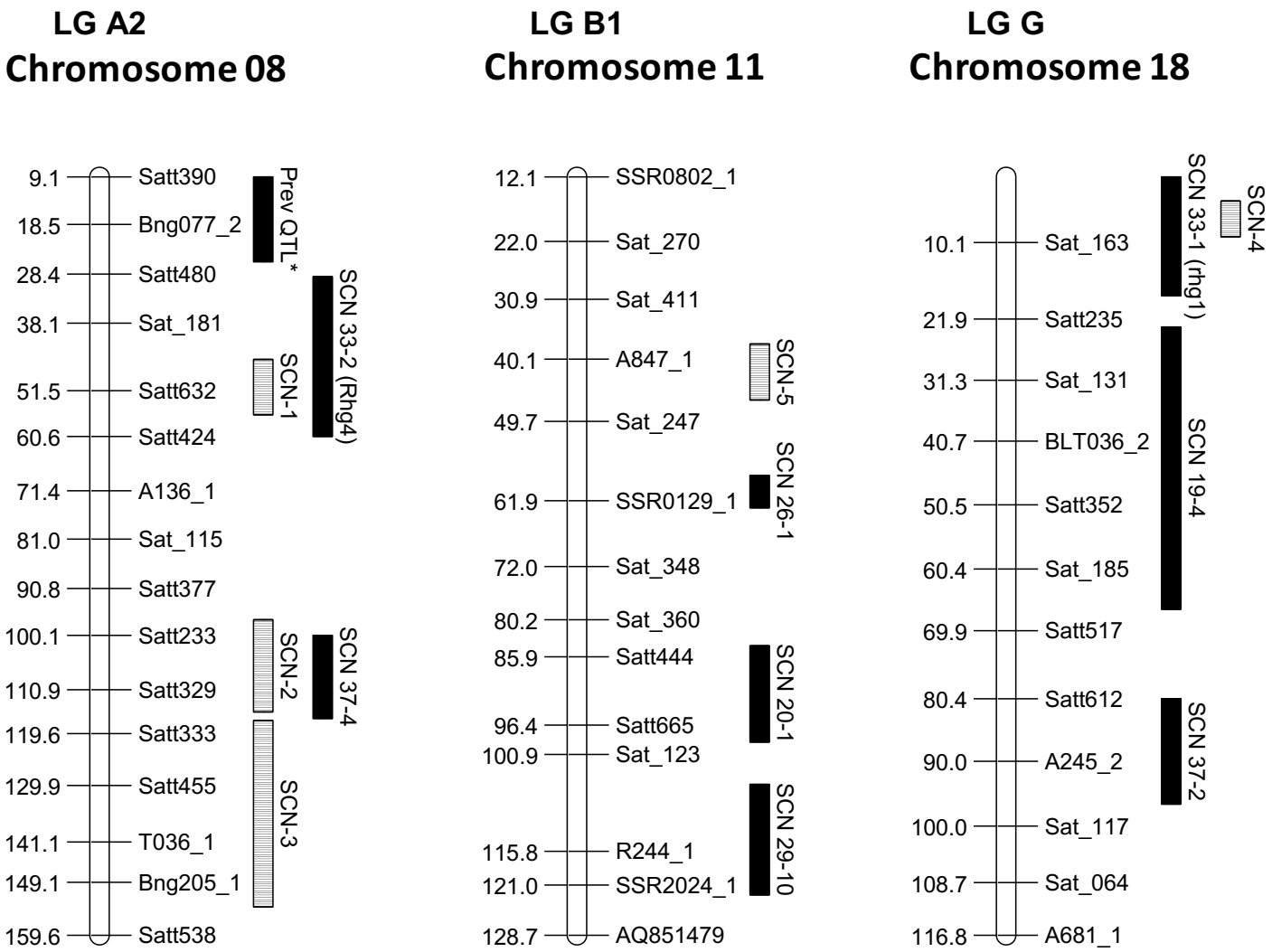
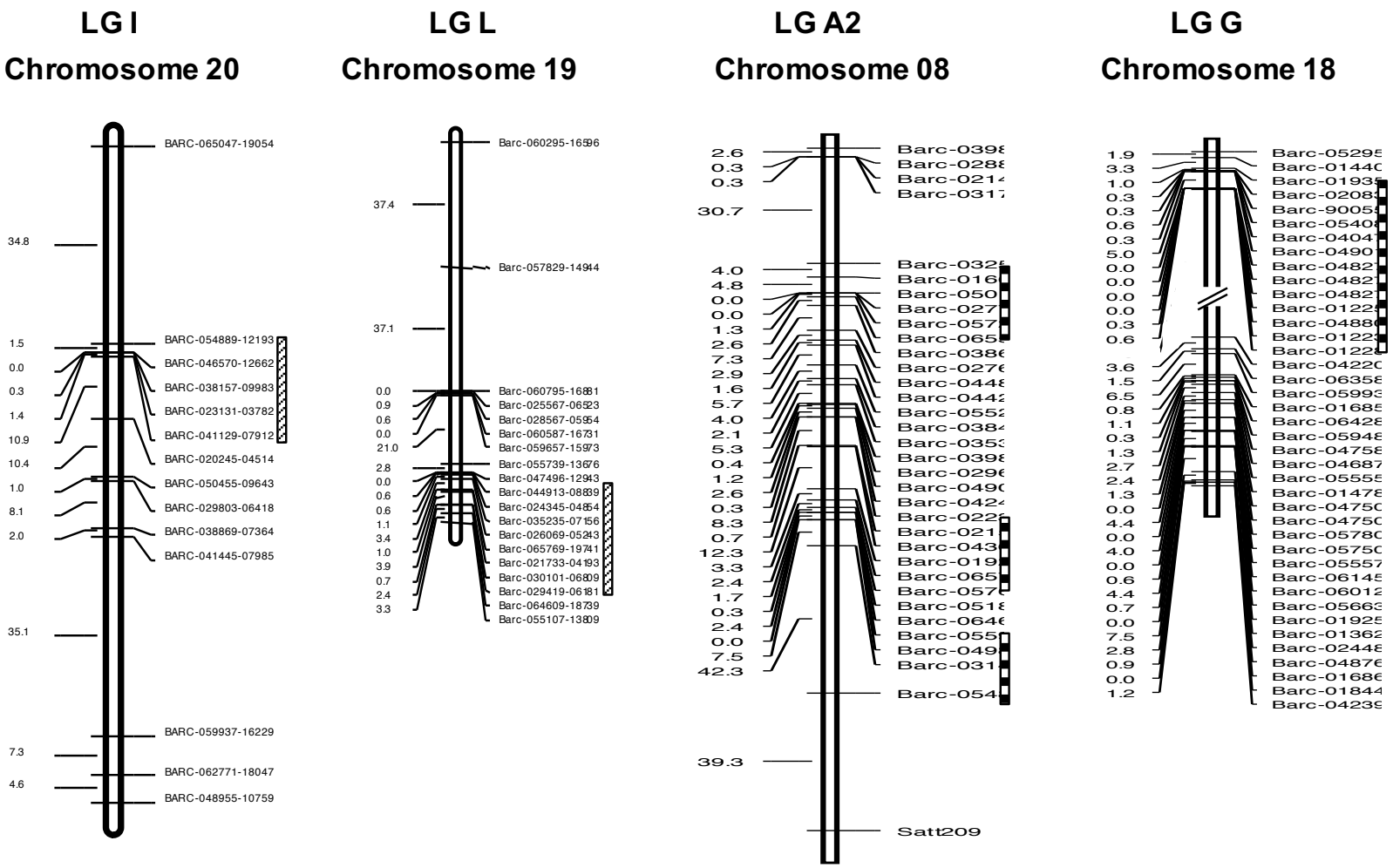
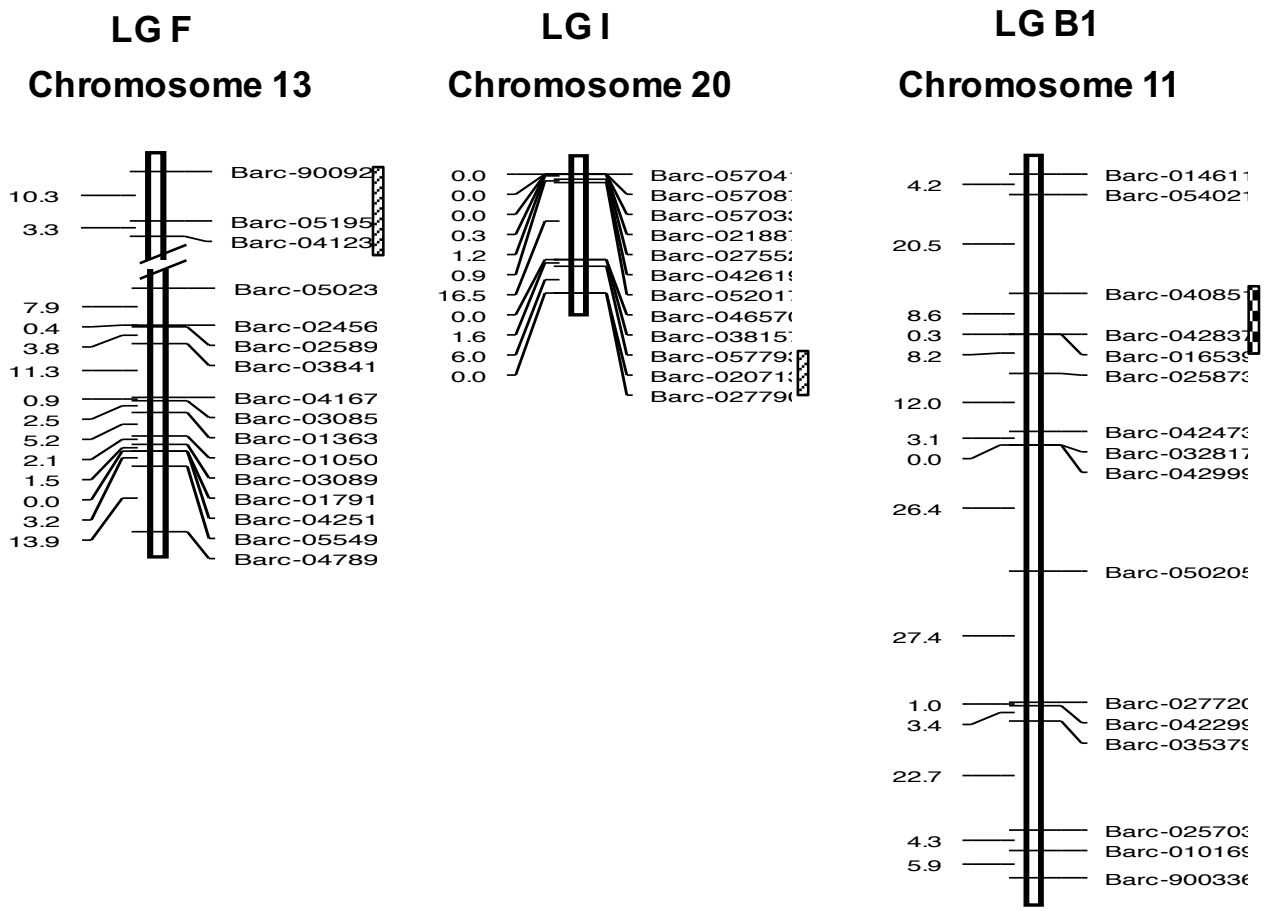


Fig. 6

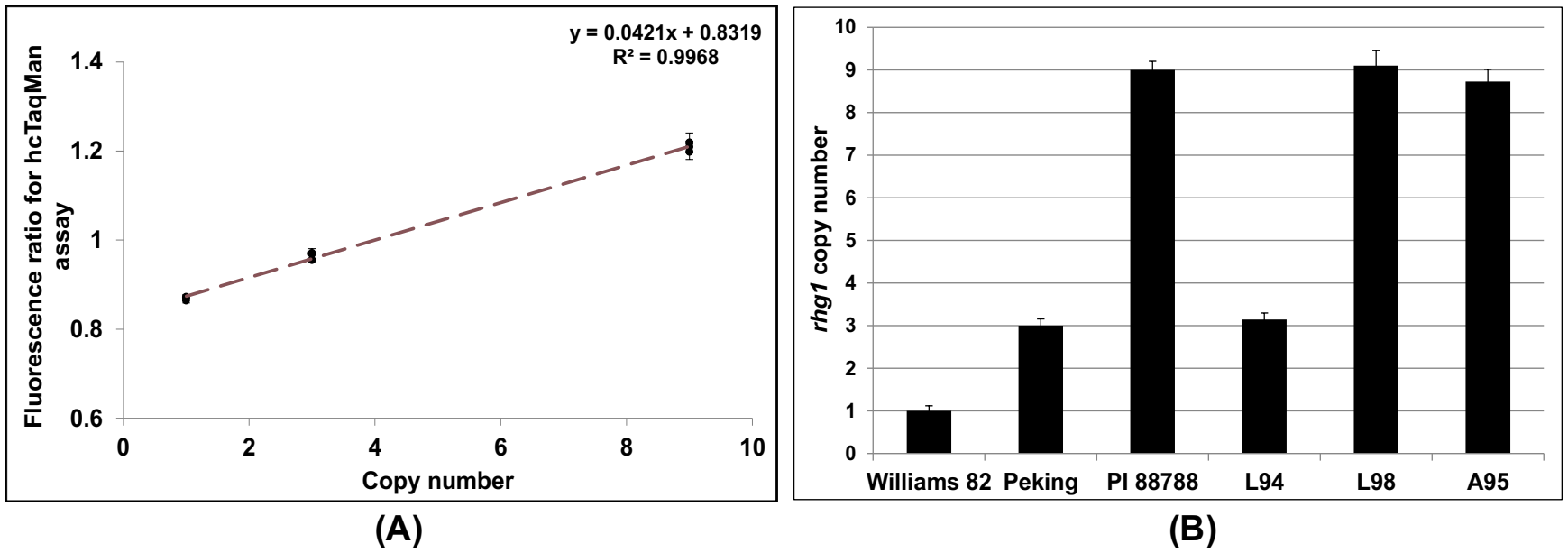

LG A2
Chromosome 08
9.1 Satt390
18.5 Bng077_2
28.4 Satt480
38.1 Sat_181
51.5 Satt632
60.6 Satt424
71.4 A136_1
81.0 Sat_115
90.8 Satt377
100.1 Satt233
110.9 Satt329
119.6 Satt333
129.9 Satt455
141.1 T036_1
149.1 Bng205_1
159.6 Satt538
Prev QTL*
SCN-1
SCN 33-2 (Rhg4)
SCN-2
SCN-3
SCN 37-4
LG B1
Chromosome 11
12.1 SSR0802_1
22.0 Sat_270
30.9 Sat_411
40.1 A847_1
49.7 Sat_247
61.9 SSR0129_1
72.0 Sat_348
80.2 Sat_360
85.9 Satt444
96.4 Satt665
100.9 Sat_123
115.8 R244_1
121.0 SSR2024_1
128.7 AQ851479
SCN-5
SCN 26-1
SCN 20-1
SCN 29-10
LG G
Chromosome 18
10.1 Sat_163
21.9 Satt235
31.3 Sat_131
40.7 BLT036_2
50.5 Satt352
60.4 Sat_185
69.9 Satt517
80.4 Satt612
90.0 A245_2
100.0 Sat_117
108.7 Sat_064
116.8 A681_1
SCN-4
SCN 33-1 (rhg1)
SCN 19-4
SCN 37-2



Supplementary Fig. 1 Composite interval mapping of major quantitative trait loci (QTL) associated with soybean sudden death syndrome (SDS) and soybean cyst nematode (SCN) for the AX19286 (A95-684043 X LS94-3207) recombinant inbred population. Genetic maps of the chromosomes with the markers indicated to the right and the centimorgan (cM) distances between loci shown on the left. (QTL associated with SDS resistance; QTL associated with SCN resistance)



Supplementary Fig. 2 Composite interval mapping of major quantitative trait loci (QTL) associated with soybean sudden death syndrome (SDS) and soybean cyst nematode (SCN) for the AX19287 (A95-684043 X LS98-0582) recombinant inbred population. Genetic maps of the chromosomes with the markers indicated to the right and the centimorgan (cM) distances between loci shown on the left. (QTL associated with SDS resistance; QTL associated with SCN resistance)



Supplementary Fig. 3 TaqMan assay of copy number analysis of *rhg1* locus of different genotypes. (**A**) A standard curve was established by using known single copy (Williams 82), three copy (Peking) and nine copy (PI 88788) genotypes. (B) The copy number estimation of *rhg1* locus different genotypes, Williams 82 (one copy), Peking (three copy) and PI 88788 (nine copy), LS94-3207 (L94), LS98-0582 (L98) and A95-684043 (A95). The bar represents three biological replicates and five technical replicates with their standard error. The results clearly shows that LS94-3207 has three copies and LS98-0582 and A95-684043 has nine copies of *rhg1* locus.