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ORIGINAL ARTICLE

Quantitative trait loci underlying host responses of soybean to *Fusarium virguliforme* toxins that cause foliar sudden death syndrome

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Abstract

Key message **Soybean deploys multiple genetic mechanisms to confer tolerance to *Fusarium virguliforme* toxins. This study revealed that *F. virguliforme* culture filtrates could be used in mapping QTL underlying foliar SDS resistance.**

Abstract Sudden death syndrome (SDS) is a major soybean disease throughout most of the soybean growing regions in the world including the United States. The disease is caused by the fungal pathogen, *Fusarium virguliforme* (*Fv*). The fungus produces several toxins that are responsible for development of interveinal leaf chlorosis and necrosis, which are typical foliar SDS symptoms. Growing of resistant cultivars has been the most effective method in controlling the disease. The objective of the present study was to identify quantitative trait loci (QTL) underlying host responses of soybean to *Fv* toxins present in culture filtrates. To accomplish this objective, two recombinant inbred line (RIL) populations, AX19286 (A95-684043 × LS94-3207) and AX19287 (A95-684043 × LS98-0582), segregating for SDS resistance were evaluated for foliar symptom development by applying two screening protocols, the stem cutting and the root feeding assays. The AX19286 population revealed two major and seven minor QTL for SDS resistance. In the AX19287 population, we identified five major QTL and three minor QTL. The two QTL mapped to Chromosome 7 [molecular linkage group (MLG) M] and Chromosome 20 (MLG I) are most likely novel, and were detected through screening of the AX19287 population with stem cutting and root feeding assays, respectively. This study established that *Fv* culture filtrates could be employed in mapping QTL underlying foliar SDS resistance. The outcomes of the research also suggest that multiple genetic mechanisms might be used by soybean to overcome the toxic effects of the toxins secreted by the pathogen into culture filtrates.

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Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important crops with high economic value worldwide. Sudden death syndrome (SDS) is one of the most serious soybean diseases in both North and South America. In North America, it is caused by the soil-borne fungus, *Fusarium virguliforme* O'Donnell and T. Aoki (formerly *F. solani* (Mart.) Sacc. f. sp. *glycines*). In South America, the disease is caused by four *Fusarium* spp., *F. virguliforme*, *F. tucumaniae, F. brasiliense* and *F. cuneirostrum* (Aoki et al. 2003; 2005). The pathogen has never been isolated from diseased foliar tissues. It is becoming evident that one or more fungal toxins secreted by the pathogen in infected roots are

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responsible for the foliar SDS development (Pudake et al. 2013). The pathogen secretes a large number of peptides and proteins to the culture filtrates including five discovered in the xylem sap of the *F. virguliforme* (*Fv*)-infected soybean plants (Abeysekara and Bhattacharyya 2014). Toxins secreted by the pathogen to the culture filtrates cause foliar SDS-like symptoms in the susceptible soybean varieties (Brar et al. 2011; Ji et al. 2006; Jin et al. 1996; Li et al. 1999).

Fusaria have been reported to produce a variety of phytotoxins including the isomeric compounds, marticin, and isomarticin (Kern 1972), anhydrofusarubin, fusarubin and javanicin (Baker et al. 1981), fusaric acid (Kern 1972), lycomarasmin (Vesonder and Heeseltine 1983), enniatin (Burmeister and Plattner 1987), fumonisin and moniliformin (Abbas et al. 1995), and 2,5-anhydro-Dglucitol (Tanaka et al. 1996). Two phytotoxins, monorden (Baker and Nemec 1994) and a 17-kDa proteinaceous toxin (Jin et al. 1996), were isolated from *F. virguliforme* (formally known as *F. solani* f. sp. *glycines*) culture filtrates that cause necrosis in soybean tissues. Recently a 13.5 kDa proteinaceous toxin, FvTox1, was isolated from the cell-free *F. virguliforme* culture filtrates. It is a major causal agent of foliar SDS in soybean (Pudake et al. 2013).

First discovered in Arkansas in 1971, SDS has spread rapidly throughout most of the soybean growing areas of the North Central United States (Leandro et al. 2012; Roy et al. 1997; Rupe 1989) and now has become a major soybean disease. SDS management options are limited and the use of SDS resistant cultivars has been the most effective method of managing the disease. Unfortunately, SDS resistance is partial and several QTL must be incorporated into a single cultivar to provide soybean with sufficient resistance against the pathogen.

Stephens et al. (1993) reported a single dominant gene, *Rfs,* responsible for SDS resistance in the soybean cultivar 'Ripley.' However, investigation of segregating populations developed from the cross 'Essex' × 'Forrest' revealed nine QTL for SDS resistance (Kassem et al. 2006, 2007). These QTL were distributed among Chromosome 13 (MLG F), 16 (MLG J), 6 (MLG C2), 20 (MLG I), and 18 (MLG G). In the RIL from 'Pyramid' × 'Douglas,' three QTL for SDS resistance were found in Chromosome 3 (MLG N), 6 (MLG C2), and 18 (MLG G) (Kassem et al. 2007; Njiti et al. 2002). In the 'Hartwig' × 'Flyer RIL' population, a QTL mapped to Chromosome 18 (MLG G) was identified (Prabhu et al. 1999). Study of the RIL population from 'Ripley' × 'Spencer' revealed three QTL for SDS resistance mapped to Chromosome 4 (MLG C1), 17 (MLG D2), and 19 (MLG L) (de Farias-Neto et al. 2007). From study of an RIL population developed from PI 567374 × 'Omaha,' two QTL for SDS resistance, mapped to Chromosomes 17 (MLG D2) and 20 (MLG I), were discovered (de Farias-Neto et al. 2007).

An investigation of the RILs developed from the cross 'Hartwig' x 'Flyer' revealed three QTL mapped to Chromosome 6 (MLG C2), 17 (MLG D2) and 18 (MLG G) (Kazi et al. 2007, 2008). Study of a RIL population generated from PI 438489B × 'Hamilton' revealed 14 QTL for SDS resistance (Kassem et al. 2012). Seven of these QTL had been previously identified on Chromosome 4 (MLG C1), Chromosome 6 (MLG C2), Chromosome 2 (MLG D1b), Chromosome 18 (MLG G), Chromosome 19 (MLG L), Chromosome 3 (MLG N) and Chromosome 10 (MLG O); the other seven QTL were novel and mapped to Chromosome 8 (MLG A2), Chromosome 11 (MLG B1), Chromosome 6 (MLG C2), Chromosome 1 (MLG D1a), Chromosome 2 (MLG D1b) and Chromosome 10 (MLG O) (Kassem et al. 2012). A recent genome-wide association mapping using more than 300 elite soybean cultivars resulted in the identification of 20 loci underlying SDS resistance, of which 13 were novel loci (Wen et al. 2014). In summary, by 2014 more than 40 QTL for SDS resistance had been identified from nine different recombinant inbred line populations.

Soybean breeders are constantly looking for new genetic sources to improve SDS resistance in soybean cultivars. Although a large number of QTL for foliar SDS resistance have been identified in response to toxins produced by the pathogen in infected diseased roots, no mapping experiments have been conducted to identify genetic loci that underlie tolerance of soybean just to *F. virguliforme* toxins involved in foliar SDS development. Here we have mapped the QTL underlying tolerance of soybean to *F. virguliforme* toxins secreted to the culture filtrates. Two sets of RILs, AX19286 (A95-684043 × LS94-3207) and AX19287 (A95-684043 × LS98-0582) were considered for this study. Progenitors of one SDS resistant parent (LS94-3207) were previously used in mapping QTL for SDS resistance; whereas, the other one (LS98-0582) has never been used in SDS resistance QTL mapping studies. We identified two putative novel QTL for foliar SDS resistance from the AX19287 mapping population. In addition to identifying the QTL, this study established that cell-free *Fv* culture filtrates could be used to identify QTL for SDS resistance. It also laid a strong foundation for future studies to determine if any of the QTL identified in this study carry the toxin-recognizing receptors for triggering resistance or susceptibility to the pathogen.

Materials and methods

Plant materials

Two hundred F_7-derived RILs each from the AX19286 (A95-684043 × LS94-3207) and AX19287

(A95-684043 × LS98-0582) populations were used in this study. The parent A95-684043 (Cianzio et al. 2002) is an F_4 plant selection from the cross of Jacques J285 × [Archer × (Cordell × Asgrow A2234)] developed at the Iowa State University soybean research site located at the Puerto Rico Agricultural Experiment Station, University of Puerto Rico. A95-684043 is a maturity group (MG) III, high yielding line that carries soybean cyst nematode (SCN) resistance introgressed from 'Peking,' PI 88788 and PI 90763. LS94-3207 is a soybean maturity group IV (Reg. no. CV-467, PI 634335) line developed at Southern Illinois University, Carbondale, IL. It carries resistance to multiple races of SCN and the SDS pathogen (Schmidt and Klein 2004). LS94-3207 is a maturity group IV (Reg. no. CV-467, PI 634335) cultivar developed at Southern Illinois University, Carbondale, IL. It carries resistance to multiple races of SCN and the SDS pathogen (Schmidt and Klein 2004). LS94-3207 originated from an individual F_5 plant selection from the cross 'Pharaoh' × 'Hartwig'. Pharaoh has the pedigree 'Forrest' (3) × V71-480. Hartwig was derived from 'Forrest' × 'PI 437654'. LS98-0582 is a MG IV soybean cultivar (Northrup King S46-44 × Asgrow A4138) developed at Southern Illinois University (Bowers and Russin 1998). It has high levels of SCN and SDS resistance.

Stem cutting and root feeding assays

200 RILs from AX19286 and AX19287 populations were evaluated by feeding roots with cell-free *Fv* culture filtrates. Stem cutting and root feeding assays were carried out with cell-free *Fv* culture filtrates according to published protocols (Li et al. 1999; Mbofung et al. 2012). To prepare the cell-free *Fv* culture filtrates, three isolates of the pathogen, Clinton 1B, Scott F2II 1a and Scott B2 collected from Iowa (Cianzio et al. 2014), were grown separately on solid Bilay medium for 14 days. Mycelial plugs were then transferred to 100 ml liquid modified Septoria medium (MSM) and incubated at room temperature in dark for 14 days (Song et al. 1993). Fungal culture medium was then sequentially filtered through Whatman No. 1 paper, 0.45 and 0.22 µm Stericups (Millipore, Inc., Billerica, MA). The protein content of the fungal culture filtrates was determined using the Bradford colorimetric assay (Bio-Rad, Inc., Hercules, CA). The liquid cell-free *Fv* culture filtrates of Clinton 1B, Scott F2II 1a and Scott B2 isolates were mixed in equal proportions. The protein estimation of liquid culture filtrates was done just before the assay.

Seeds of each RIL were planted in growth chambers under light at 23 °C for 16 h and maintained in the dark at 16 °C for 8 h. The light intensity was 200 µmol photons m^{-2} s^{-1}. Single 21-day-old seedlings were cut below the cotyledons and placed in individual 50 mL plastic tubes each containing 25 mL diluted cell-free *Fv* culture filtrates in sterile double distilled water containing 225 µg of *F. virguliforme* excreted proteins (75 µg from each isolate) (Li et al. 1999). Ten seedlings per RIL were assayed in each experiment. The experiment was repeated three more times. The cut seedlings were kept in the growth chambers watered as needed.

The root feeding assay was adopted from the method developed in Leandro lab (Mbofung et al. 2012). For the root feeding assay, the 21-day-old seedlings were carefully pulled up from the soil mixture (SB 300 Universal Professional Growing Mix, Sunshine; Sun Gro Horticulture, Agawam, MA) without causing damage to the roots, which were then washed in tap water and placed into 50 mL tubes containing 25 mL diluted cell-free *Fv* culture filtrates as described for stem cutting assays.

In both stem and root assays, foliar SDS symptoms appeared 5–6 days following the feeding of seedlings with the *Fv* culture filtrates. Disease scoring was done 14 days after the start of the assays. The disease scoring scheme was: 0, no symptoms; 1, leaves showing slight yellowing and/or chlorotic flecks or blotches (1–10 % of the foliage affected); 2, leaves with obvious, interveinal chlorosis (11–20 % foliage affected) symptoms; 3, necrosis along the margin of leaves (>2 cm wide in size; 21–40 % foliage affected); 4, necrosis along the entire margin of the leaves and leaves curled with irregular shapes (41–75 % foliage affected); 5, interveinal necrosis and most of the leaf areas necrotic (75–100 % foliage affected); 6, leaves starting to defoliate. On the basis of foliar disease scores, the RILs were grouped as highly resistant with scores <1.5, resistant with scores 1.50–2.00, moderately resistant with scores 2.01–2.50, susceptible with scores 2.51–3.00, and highly susceptible with scores >3.00 (Brar et al. 2011; Brar and Bhattacharyya 2012; Hartman et al. 2004; Pudake et al. 2013).

Genotyping of RILs, genome map construction and QTL analysis

Genomic DNA was isolated by CTAB extraction (CIMMYT, 2005) and quantified by Nanodrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE, USA). DNA samples were diluted to a final concentration of 100 ng DNA/µL. The DNA was subjected to Illumina Golden Gate Genotyping assay at the Cregan lab (USDA ARS, Beltsville, MD). The 1536 Universal Soy Linkage Panel 1.0 was used for the Golden Gate assay (Fan et al. 2003; Hyten et al. 2008, 2010). Genetic linkage maps were constructed individually for the AX19286 and AX19287 populations using MAPMAKER V2.0. QTL for SDS resistance were mapped by conducting composite interval mapping using the computer program QGene. A permutation test with

1000 iterations was executed to calculate the critical LOD threshold values.

Results

Foliar SDS scores in the stem cutting assays of the RILs suggest distinct patterns of inheritance of SDS resistance from the two SDS resistant parents

The highest disease severity was recorded for the susceptible control line Spencer (mean foliar SDS score 4.4) followed by the susceptible parent of each of the two crosses, A95-684043 (mean foliar SDS score, 3.7) (Figs. 1, 2). The foliar SDS mean scores of the resistant parents LS98-0582 and LS94-3207 were 2 and 1.6, respectively. We observed several transgressive segregants ($p < 0.05$) with foliar SDS scores exceeding either the susceptible or the resistant parent (Figs. 1 and 2).

We observed that 12.5 % of the RILs showed foliar SDS scores that were either similar to or lower than the resistant parent in the AX19286 population; whereas in the AX19287 population, only 5 % of the RILs had foliar SDS scores either similar to or lower than the resistant parent (Table 1). We also observed twice the number of susceptible RILs with foliar SDS scores >3.00 in the AX19287 population as compared to that in the AX19286 population (Table 1).

The foliar SDS scores in root feeding assays of the RILs suggest distinct patterns of inheritance of SDS resistance from the two SDS resistant parents

The overall segregation patterns of the RILs of the two populations were comparable for scores obtained through stem cutting assays of the lines (Figs. 3 and 4; Table 2). In both methods, host responses to cell-free *Fv* culture filtrate preparations were recorded as foliar SDS scores. The foliar SDS scores in the root feeding assays were however higher than the scores observed in the stem cutting assays (Figs. 3 and 4). In the root feeding assays, the foliar SDS scores were 4.13, 2.13, and 2.10 for the soybean lines A95-684043, LS94-3207, and LS98-0582, respectively. The foliar SDS score was also higher for the susceptible check cultivar Spencer, 5.2. Again, more foliar SDS resistant transgressive segregants ($p < 0.05$) were observed for the AX19286 population compared to the AX19287 population.

Identification of QTL underlying the foliar SDS resistance

Composite interval mapping was conducted to identify QTL for SDS resistance based on the foliar SDS scores

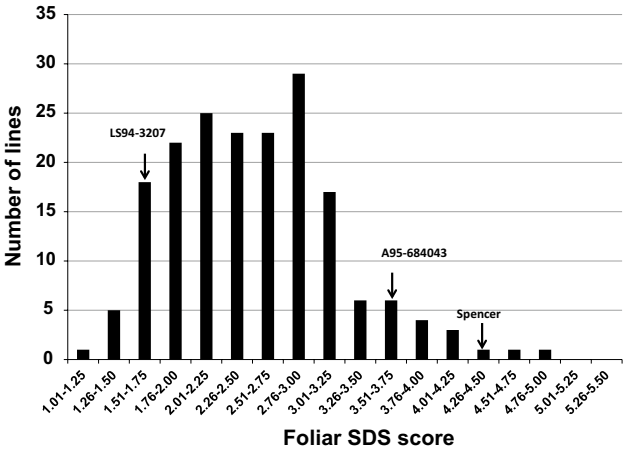


Fig. 1 Frequency distribution of host responses to cell-free *Fv* culture filtrates evaluated by stem cutting assay. Frequency distribution of foliar SDS scores of the F_7 RILs of the AX19286 population developed by crossing A95-684043 with LS94-3207 is presented. *Arrows* indicate the parents and the most susceptible variety, 'Spencer'. The *values* are means of three biological replications. The disease symptoms were determined according to Pudake et al. (2013)

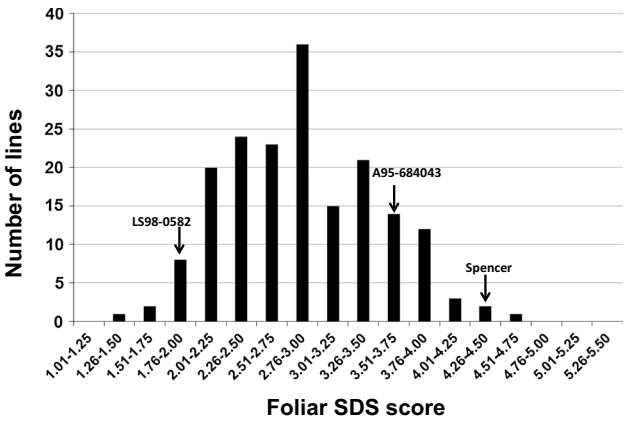


Fig. 2 Frequency distribution of host responses to cell-free *Fv* culture filtrates evaluated by stem cutting assay. Frequency distribution of foliar SDS scores of the F_7 RILs of the AX19287 population developed by crossing A95-684043 with LS98-0582 is presented. *Arrows* indicate the parents and the most susceptible variety, 'Spencer'. The *values* are means of three biological replications. The disease symptoms were determined according to Pudake et al. (2013)

obtained by stem cutting and root feeding assays and the results are presented in Tables 3 and 4, and Fig. 5. In the stem cutting assays, two major QTL for foliar SDS resistance were identified from the study of RILs of the AX19286 population. The two QTL were mapped to Chromosome 2 (MLG D1b) and 13 (MLG F). The LOD score for the QTL mapped to Chromosome 2 (MLG D1b) was 3.6. This QTL contributes 8.4 % of the total variation and was mapped to the BARC-041581-08046–BARC-046084-10230 interval (Supplemental Fig. 1; Table 3). The QTL on

Table 1 Distribution of foliar SDS scores of two segregating populations gathered by stem-cut assays

Population	Percentage of RILs[a]					Mean (±St. Dev)	Range
	<1.50[b] (HR)	1.51–2.00[b] (R)	2.01–2.50[b] (MR)	2.51–3.00[b] (S)	>3.00[b] (HS)		
AX19286	3	20	24	33	20	2.50 (±0.71)	1.21–4.75
AX19287	1	5	22	30	42	2.89 (±0.45)	1.44–4.67

HR highly resistant, *R* resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

[a] 200 RILs from each population were categorized according to the mean disease score

[b] Foliar SDS score

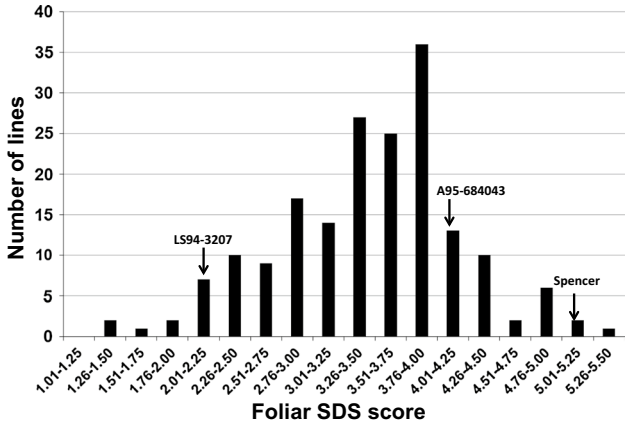


Fig. 3 Frequency distribution of host responses to cell-free *Fv* culture filtrates evaluated by root feeding assay. Frequency distribution of foliar SDS scores of the F_7 RILs of the AX19286 population developed by crossing A95-684043 with LS94-3207 is presented. *Arrows* indicate the parents and the most susceptible variety, 'Spencer'. The *values* are means of three biological replications. The disease symptoms were determined according to Pudake et al. (2013)

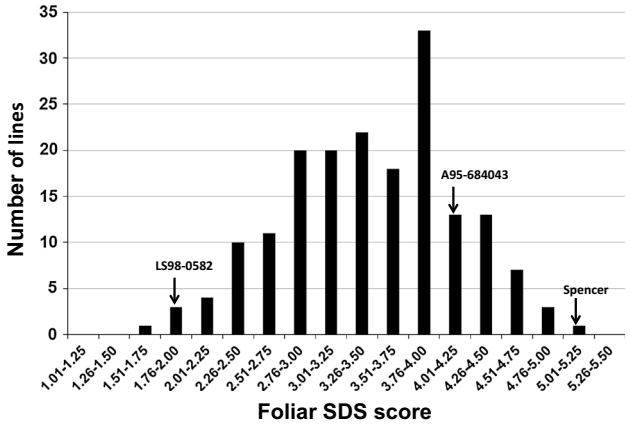


Fig. 4 Frequency distribution of host responses to cell-free *Fv* culture filtrates evaluated by root feeding assay. Frequency distribution of foliar SDS scores of the F_7 RILs of the AX19287 population developed by crossing A95-684043 with LS98-0582 is presented. *Arrows* indicate the parents and the most susceptible variety, 'Spencer'. The *values* are means of three biological replications. The disease symptoms were determined according to Pudake et al. (2013)

Chromosome 13 (MLG F) is located in the BARC-065495-19507–BARC-030899-06963 interval (Supplemental Fig. 1). The QTL interval was 5 cM with a LOD score value of 4.4. The QTL explained 16 % of the total variation (R^2 = 16 %; Table 3). Five minor QTL with LOD scores of ≤3.6 were mapped to the Chromosomes 8 (MLG A2), 16 (MLG J) and 20 (MLG I) in this population (Table 3). These five QTL had been previously identified in studies, in which different segregating populations were evaluated through root-infection with the pathogen (Table 4).

In the root feeding assays of the AX19286 population, two QTL were mapped to Chromosome 5 (MLG A1) and 20 (MLG I) with LOD score values of 3.0 and 2.5, respectively. The two QTL however explained only 7.0 and 6.3 % of the total variation, respectively (Table 3). Both of the QTL identified in root feeding assays were minor and had been previously identified (de Farias-Neto et al. 2007; Kassem et al. 2007; Meksem et al. 1999; Yamanaka et al. 2006) (Table 4). Interestingly on Chromosome 20 (MLG I), the QTL obtained in stem cutting and root feeding assays both mapped to the same region of 50.0–65.0 cM marker interval (Tables 3 and 4; Fig. 5).

In the AX19287 population, two major foliar SDS QTL were identified by the stem cutting assays and mapped to Chromosome 7 (MLG M) and 9 (MLG K). The QTL on Chromosome 7 (MLG M) was located in the 30 cM marker interval between BARC-028517-05936 and BARC-065255-19294. The LOD score for this QTL was 6.6. This QTL explains 12.2 % of the total variation. The QTL on Chromosome 9 (MLG K) was mapped to an approximately 6 cM marker interval between BARC-056323-14257 and BARC-010353-00615 with a LOD score value of 6.2. It explains 13 % of the total variation (Table 3; Supplemental Fig. 2). From analysis of the foliar SDS scores of this population gathered through root feeding assays, a major QTL was mapped to a 23 cM marker interval between BARC-052017-11314 and BARC-020713-04700 on Chromosome 20 (LG I) with a LOD score value of 5.4. It explains 15 % of the total phenotypic variation (Table 3). Two additional QTL were mapped to Chromosomes 6 (MLG C2) and 13 (MLG F) from analyses of the foliar SDS scores obtained

from the root feeding assays of the RILs of this population. Each QTL contributes 12 % of the phenotypic variation (Table 3). We identified three minor QTL mapped to Chromosomes 6 (MLG C2), 8 (MLG A2) and 10 (MLG O) from analyses of this population through root feeding assays (Table 3).

In between the AX19286 and AX19287 populations, we did not identify major common QTL. However, two minor common QTL with low LOD scores were mapped to the 14.99–67.86 cM interval of Chromosome 8 (MLG A2) and to the 74.12–78.05 cM interval of the Chromosome 13 (MLG F) (Tables 3 and 4; Fig. 5).

Discussion

Considering the fact that foliar SDS is easily scorable, most of the QTL for SDS resistance were discovered based on foliar SDS. We therefore investigated if (1) stem cutting and (2) root feeding assays can be applied to identify QTL for SDS resistance. Our study revealed several new findings: (1) Although the same culture filtrates was used in phenotyping the RILs of two independent segregating populations, the two assays resulted in discovering different QTL for foliar SDS resistance. This observation suggests a possible differential uptake of toxins through cut stems and roots from the culture filtrates. This observation warrants further study to determine if such differences do exist. (2) Some of the QTL identified in this study had been previously identified by scoring for SDS resistance following root inoculation with the pathogen. Our results thus establish that cell-free *Fv* culture filtrates can be employed to screen soybean germplasm to map QTL for foliar SDS resistance. (3) We identified two novel QTL, which could be due to either novel SDS resistance mechanisms in the new parents included in this mapping study or identified due to the novel methodologies used in this study.

Fifteen of the 17 QTL identified in this research from the two populations with stem cutting and root feeding assays had been reported earlier from investigation of other segregating materials through inoculation of roots with *F. virguliforme* (Tables 3 and 4; Fig. 5). From the study of the AX19286 population, seven and two QTL were identified through stem cutting and root feeding assays, respectively (Tables 3 and 4; Fig. 5). The study of the AX19287 population led to identification of two and six QTL through stem cutting and root feeding assays, respectively. The two QTL mapped to Chromosome 20 (MLG I) and Chromosome 7 (MLG M) using the AX19287 population are most likely novel (Tables 3 and 4; Fig. 5).

Fig. 5 The composite genetic map of the previously identified SDS resistance QTL (*black rectangles*) and the QTL for SDS resistance identified in this study (*gray rectangles*). The maps were constructed using Mapchart 2.2, marker information from SoyBase (http://www.soybase.org), and data from published papers (see Table 4 for details)

In our study we have identified, nine QTL from the AX19286 (A95-684043 × LS94-3207) population. The SDS resistant parent of this population, LS94-3207 was developed from the cross between 'Forrest' with 'Hartwig'. Both 'Hartwig' and 'Forrest' have been previously used to map SDS resistance QTL (Chang et al. 1996; Hnetkovsky et al. 1996; Kassem et al. 2006, 2007; Kazi et al. 2008; Meksem et al. 1999; Njiti et al. 2002; Yuan et al. 2012). Six of the nine QTL identified from the AX19286 population of this study, mapped to Chromosomes 13 (MLG F), 8 (MLG A2), 20 (MLG I starting at 63.33 cM), 20 (MLG I starting at 55.09 cM), 16 (MLG J), and 20 (MLG I starting at 50.11), were previously identified either from 'Forrest' (Njiti et al. 2002) or 'Hartwig' (Kazi et al. 2008) (Tables 3 and 4; Fig. 5). The rest of the nine QTL identified from the AX19286 population, mapped to Chromosomes 5 (MLG A1; Yamanaka et al. 2006), 8 (MLG A2; Kassem et al. 2012) and 2 (MLG D1b; Kassem et al. 2012), were previously identified from SDS resistant parents, other than 'Forrest' and 'Hartwig' (Tables 3 and 4; Fig. 5). From the population AX19287 (A95-684043 × LS98-0582), eight QTL were identified in our mapping study. LS98-0582 was developed from two lines that had never been used in SDS resistance QTL mapping studies. However, except for the two putative novel QTL mapped to Chromosomes 7 (MLG M) and 20 (MLG I) the rest of the six QTL were identified in previous studies (Tables 3 and 4; Fig. 5).

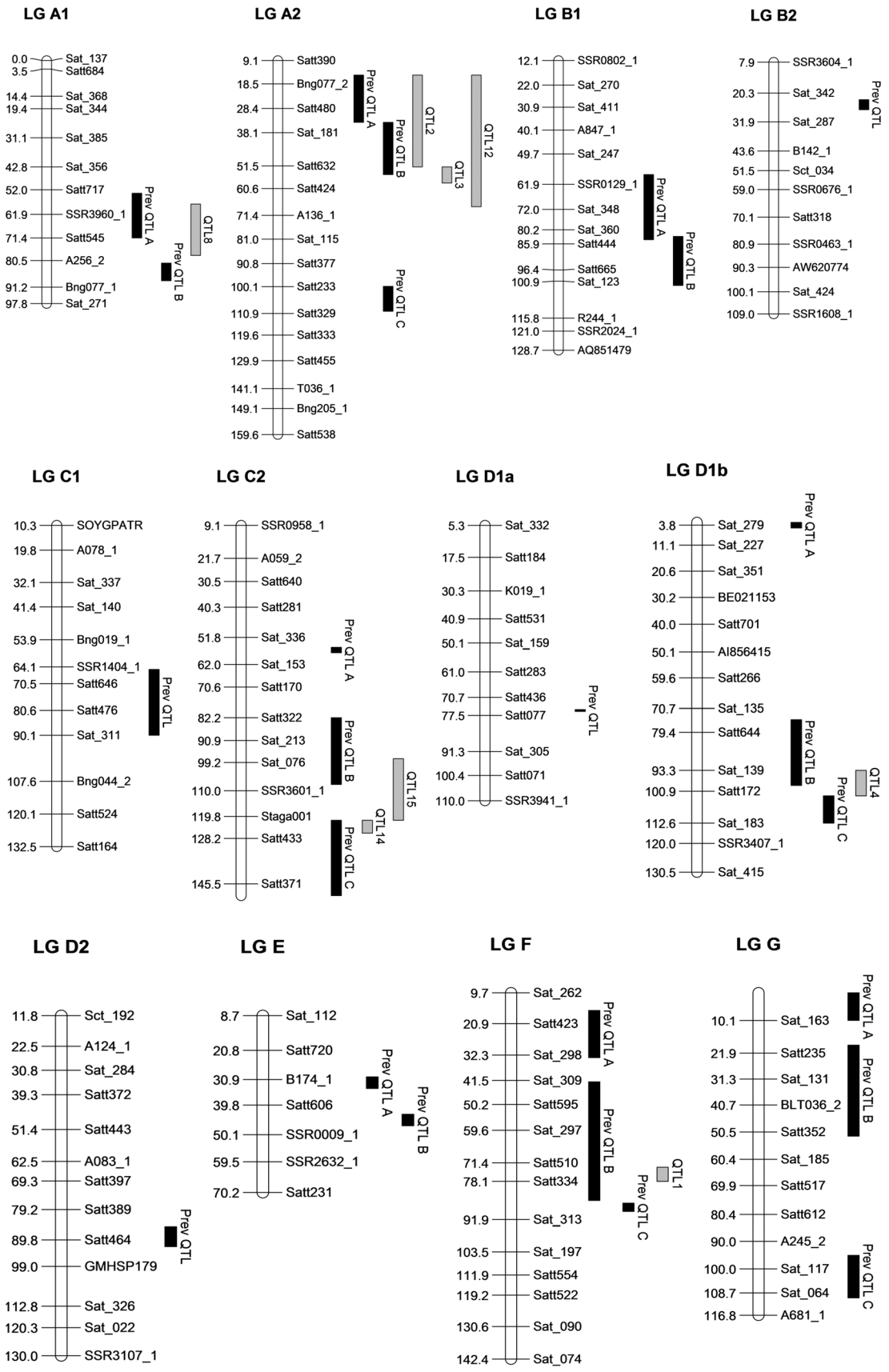
Table 2 Distribution of foliar SDS scores of two segregating populations gathered by root-feeding assays

Population	Percentage of RILs[a]					Mean (±St. Dev)	Range
	<1.50[b] (HR)	1.51–2.00[b] (R)	2.01–2.50[b] (MR)	2.51–3.00[b] (S)	>3.00[b] (HS)		
AX19286	1	2	9	13	75	3.49 (±0.66)	1.33–5.42
AX19287	0	2	7	16	75	3.46 (±0.54)	1.56–5.06

HR highly resistant, *R* resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

[a] 200 RILs from each population were categorized according to the mean disease score

[b] Foliar SDS score


LG A1
0.0 Sat_137
3.5 Satt684
14.4 Sat_368
19.4 Sat_344
31.1 Sat_385
42.8 Sat_356
52.0 Satt717
61.9 SSR3960_1
71.4 Satt545
80.5 A256_2
91.2 Bng077_1
97.8 Sat_271
Prev QTL A
Prev QTL B
QTL8
LG A2
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 A
Prev QTL B
Prev QTL C
QTL2
QTL3
QTL12
LG B1
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
Prev QTL A
Prev QTL B
LG B2
7.9 SSR3604_1
20.3 Sat_342
31.9 Sat_287
43.6 B142_1
51.5 Sct_034
59.0 SSR0676_1
70.1 Satt318
80.9 SSR0463_1
90.3 AW620774
100.1 Sat_424
109.0 SSR1608_1
Prev QTL
LG C1
10.3 SOYGPATR
19.8 A078_1
32.1 Sat_337
41.4 Sat_140
53.9 Bng019_1
64.1 SSR1404_1
70.5 Satt646
80.6 Satt476
90.1 Sat_311
107.6 Bng044_2
120.1 Satt524
132.5 Satt164
Prev QTL
LG C2
9.1 SSR0958_1
21.7 A059_2
30.5 Satt640
40.3 Satt281
51.8 Sat_336
62.0 Sat_153
70.6 Satt170
82.2 Satt322
90.9 Sat_213
99.2 Sat_076
110.0 SSR3601_1
119.8 Staga001
128.2 Satt433
145.5 Satt371
Prev QTL A
Prev QTL B
Prev QTL C
QTL14
QTL15
LG D1a
5.3 Sat_332
17.5 Satt184
30.3 K019_1
40.9 Satt531
50.1 Sat_159
61.0 Satt283
70.7 Satt436
77.5 Satt077
91.3 Sat_305
100.4 Satt071
110.0 SSR3941_1
Prev QTL
LG D1b
3.8 Sat_279
11.1 Sat_227
20.6 Sat_351
30.2 BE021153
40.0 Satt701
50.1 AI856415
59.6 Satt266
70.7 Sat_135
79.4 Satt644
93.3 Sat_139
100.9 Satt172
112.6 Sat_183
120.0 SSR3407_1
130.5 Sat_415
Prev QTL A
Prev QTL B
Prev QTL C
QTL4
LG D2
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
Prev QTL
LG E
8.7 Sat_112
20.8 Satt720
30.9 B174_1
39.8 Satt606
50.1 SSR0009_1
59.5 SSR2632_1
70.2 Satt231
Prev QTL A
Prev QTL B
LG F
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 A
Prev QTL B
Prev QTL C
QTL1
LG G
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
Prev QTL A
Prev QTL B
Prev QTL C

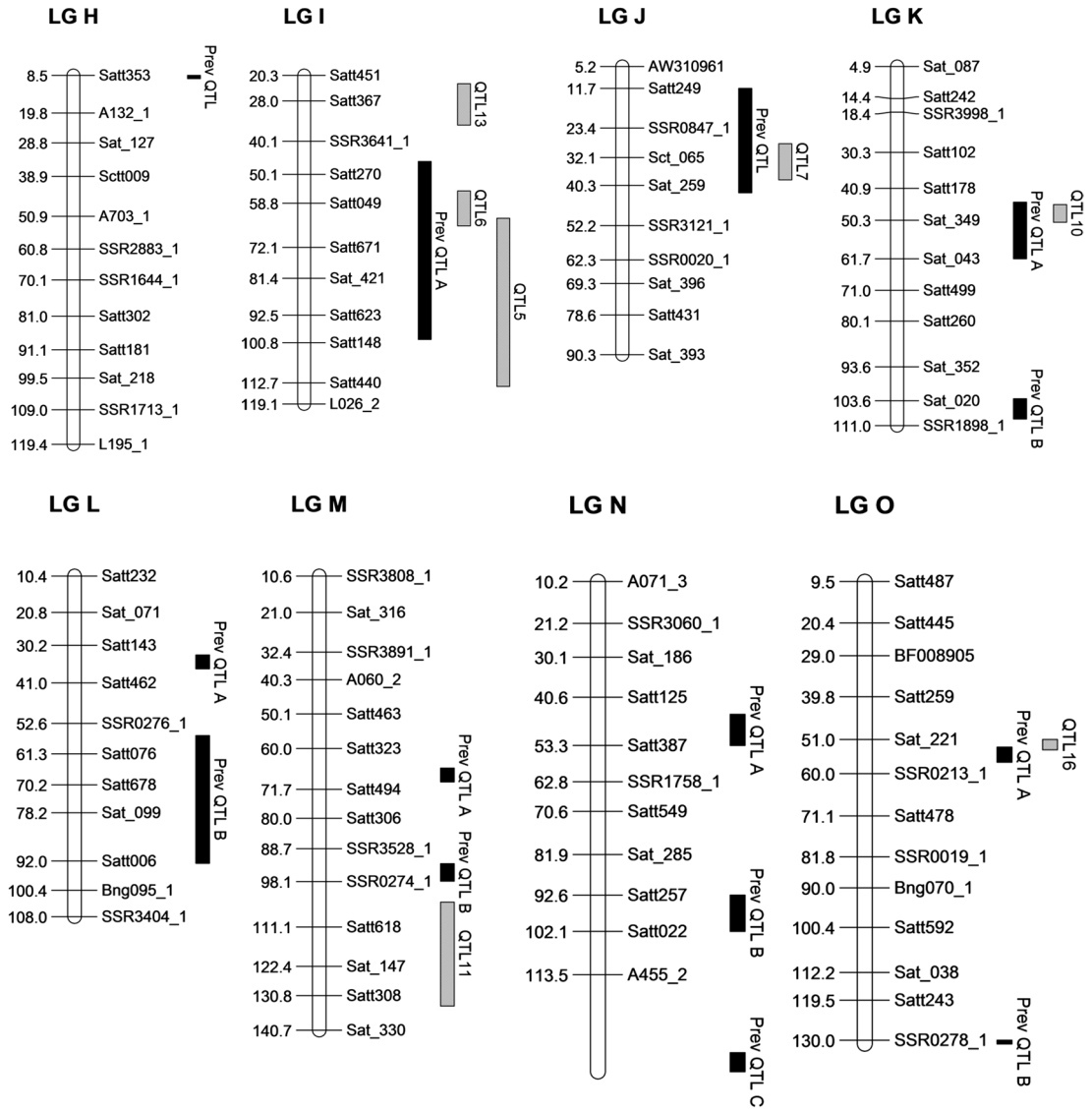


Fig. 5 continued

In this investigation we have shown for the first time, that screening of mapping populations can be conducted using cell-free *Fv* culture filtrates containing toxins to map QTL for SDS resistance. The fact that 15 of the 17 QTL identified in this study mapped to genomic regions that have previously been reported to contain QTL for SDS resistance, as stated above, establishes that the use of cell-free *Fv* culture filtrates can be used for mapping QTL for SDS resistance and identifying novel SDS resistant germplasm. Application of cell-free *Fv* culture filtrates in the evaluation of soybean genotypes for foliar SDS resistance is more reproducible than the traditional assays based on root inoculation with the pathogen. In this new approach, we can control the amounts of culture filtrates with a greater precision and more uniformly across the genotypes. In the root inoculation method, much of the variations can be attributed to variation in the amount and distribution of inocula in soil and other environmental conditions generally encountered during the assays. The inconsistency of inoculum levels can greatly influence the outcomes of the root infection assay as high inoculum levels can lead to breakdown of SDS resistance (Gray and Achenbach 1996; Yamanaka et al. 2006).

The positions of the two putative QTL identified in this study, one on Chromosome 5 (MLG A1) from analysis of the AX19286 population via root feeding assay, and the other one on Chromosome 9 (MLG K) from the AX19287 population through stem cutting assay match with the map locations of two previously reported QTL for SDS resistance (Yamanaka et al. 2006). It is worth noting that the

Table 3 Locations of QTL associated with SDS resistance determined by stem-cut and root-feeding assays

Population	Assay	QTL	Ch/MLG[a]	Marker/interval	Flanking SSR markers	Position (cM)[b]	LOD[c]	R^2 (%)[d]	Parent contribution
AX19286	Stem cut	1**	13/F	BARC-065495-19507 to BARC-030899-06963	Satt334-Sat_317	72.97–78.05	4.4	16	A95-684043
		2	08/A2	BARC-031701-07215 to BARC-016685-03321	Sct_067-Sat_162	14.99–51.86	3.4	8.4	A95-684043
		3	08/A2	BARC-016685-03321 to BARC-038631-07266	Sat_162-GMENOD2B	51.86–58.43	2.3	5.8	A95-684043
		4**	02/D1b	BARC-041581-08046 to BARC-046084-10230	Sat_139-Sat_069	93.34–102.59	3.6	8.4	LS94-3207
		5	20/I	BARC-038869-07364 to BARC-059937-16229	Satt650-Sct_189	63.33–113.76	2.4	5.9	LS94-3207
		6	20/I	BARC-029803-06418 to BARC-041445-07985	Sat_268-Sat_104	55.09–65.62	2.5	6.2	LS94-3207
		7	16/J	BARC-016775-02320 to BARC-014745-01638	Sat_339-Satt280	27.99–38.70	2.1	5.2	LS94-3207
	Root feeding	8	05/A1	BARC-059081-15595 to BARC-065229-19273	Sat_171-Sat_267	57.79–78.44	3.0	7.0	LS94-3207
		9	20/I	BARC-020245-04514 to BARC-038869-07364	Satt270-Satt650	50.11–63.33	2.5	6.3	LS94-3207
AX19287	Stem cut	10**	09/K	BARC-056323-14257 to BARC-010353-00615	Satt167-Satt617	45.74–50.93	6.2	13	LS98-0582
		11**	07/M	BARC-028517-05936 to BARC-065255-19294	Satt336-Sat_121	103.98–133.83	6.6	12.2	LS98-0582
	Root feeding	12	08/A2	BARC-042199-08209-BARC-044217-08646	Sct_067-AW132402	14.99–67.86	3.6	10.2	LS98-0582
		13**	20/I	BARC-052017-11314 to BARC-057793-14926	Satt562-Satt127	22.84–35.34	5.4	15	LS98-0582
		14	06/C2	BARC-010457-00640 to BARC-025767-05060	Satt307-Satt202	121.26–126.23	3.2	8.6	A95-684043
		15**	06/C2	BARC-021735-04194 to BARC-062515-17881	Satt376-Satt307	97.83–121.26	6.3	12	A95-684043
		16	10/O	BARC-017045-02182 to BARC-060901-16948	Sat_221-Satt466	51.00–53.66	2.0	5	A95-684043
		17**	13/F	BARC-010501-00676 to BARC-042515-08280	Sct_033-Satt334	74.12–78.05	6.0	12	LS98-0582

[a] Ch, Chromosome/MLG, molecular linkage group

[b] Position of QTL based on the composite interval mapping (www.soybase.org)

[c] Logarithm of odd ratios (LOD) at the QTL peak. Threshold LOD cut off value in AX19286 was 3.6 and 3.7, respectively for stem cutting and root feeding assays. Threshold LOD cut off value in AX19287 was 3.8 and 3.7, respectively for stem cutting and root feeding assays ($p = 0.05$)

[d] Per cent contribution of particular QTL for the phenotypic variation in a particular population

**, Major QTL found in this study, which was selected based on the threshold LOD cut off value ($p = 0.05$). Other QTL were treated as minor

segregating population used in the previous study was screened in the greenhouse through root inoculation with *F. tucumaniae,* the common SDS pathogen in the South America.

In the present investigation, alleles for SDS resistance among some of the segregating RILs were most likely contributed by both susceptible and resistant parents. Similar observations were also made during mapping QTL for SDS resistance using segregating populations generated from crosses between 'Pyramid' and 'Douglas', 'Essex' and 'Forrest', and 'Ripley' and 'Spencer' (de Farias-Neto et al. 2007; Iqbal et al. 2001; Njiti et al. 2002). In these studies, the SDS susceptible parents were shown to carry QTL for SDS resistance; for examples, 'Douglas' was shown to carry QTL on Chromosome 6 (MLG C2), 'Essex' to carry QTL on Chromosome 6 (MLG C2) and Chromosome 20 (MLG I), and 'Spencer' to carry QTL on Chromosome 4 (MLG C1). In the population developed from the cross between 'Misuzudaizu' and 'Moshidou Gong 503', the susceptible parent 'Misuzudaizu' contributed beneficial

Table 4 QTL for SDS resistance reported prior to 2015 and identified in this investigation

LG	Essex x Forrest	Pyramid x Douglas	Hartwig x Flyer	Ripley x Spencer	PI 567354 x Omaha	PI 438489B x Hamilton	Minsoy X Noir1	Asgrow X Cordell	Misuzudaizu x Moshidou Gong 503	Elite soybean cultivars	*A95-684043 X LS94-3207*	*A95-684043 x LS98-0582*	[a]Map position (cM)
A1									■		▒		53.36-71.38
A1									■				81.51-88.58
A2						■					▒		14.99 – 33.95
A2			■	■		■					▒	▒	34.00 – 55.00
A2										■			105.18
B1	■					■							58.00 – 84.19
B1										■			82.88- 102.55
B2										■			25.9
C1				■		■	■						65.08 – 90.11
C2										■			56.50
C2	■					■						▒	82.23 – 107.58
C2	■	■	■			■				■		▒	121.26 – 150.0
D1a													75.25
D1b										■			3.79
D1b						■					▒		74.79- 98.75
D1b										■			102.59- 112.62
D2		■	■	■	■			■					85.15- 92.12
E										■			32.09
E													45.04
F	■						■						16.08 – 33.18
F											▒	▒	42.00 – 85.00
F										■			87.01
G	■	■	■			■							0.00 – 10.00
G	■		■										19.00 – 52.00
G	■					■							95.10 – 110.50
H	■												8.50
I												▒	22.84- 36.40
I	■		■		■			■			▒		46.22 – 99.83
J	■										▒		11.74 - 42.50
K									■			▒	44.99 – 61.66
K										■			104.79
L													36.04
L				■		■	■						56.13 – 92.66
M										■			67.6
M													95.44
M												▒	103.98-133.83
N	■	■	■	■									45.13-53.25
N						■							92.55 - 102.05
N	■												136
O				■								▒	55
O						■							130
References	1-7	3, 8	3, 9	3, 10 ,11	10	12	13	3	14	15			

References: 1 Kassem et al. (2006); 2 Kassem et al. (2007); 3 Meksem et al. (1999); 4 Yuan et al. (2012); 5 Hnetkovsky et al. (1996); 6 Njiti et al. (2002); 7 Chang et al. (1996); 8 Njiti et al. (2002); 9 Kazi et al. (2008); 10 de Farias-Neto et al. (2007); 11 Hashmi, (2004); 12 Kassem et al. (2012); 13 Njiti and Lightfoot (2006); 14 Yamanaka et al. (2006); 15 Wen et al. (2014)

[a] The approximate map position is calculated based on the marker information available for the QTL/QTL peak from the literature and it is based on the composite genetic map at http://www.soybase.org. Black boxes indicate the confirmed QTL from published literature

■, (Dark horizontal) stem cutting assay; ▒, (Small checker board) root feeding assay

genes for SDS resistance mapped to Chromosome 5 (MLG A1) (Yamanaka et al. 2006). Presumably, the contribution of beneficial alleles for SDS resistance from both parents could explain the generation of transgressive segregants. We speculate that similar mechanism might be involved in generating the transgressive segregants in this investigation (Figs. 1, 2, 3 and 4). Transgressive RILs with increased SDS resistance as compared to their respective resistant parents are ideal for realizing genetic gain for SDS resistance in soybean. Several RILs with enhanced tolerance to toxins of the cell-free *Fv* culture filtrates identified in this study could be used as new germplasm sources for genetic improvement of soybean for foliar SDS resistance.

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Author contribution statement M. K. B. and S. R. C. devised the strategy and planned the experiments; S. R. C. generated the recombinant inbred lines; S. S. lead the screening experiments, interpreted results and wrote the manuscript; M. L. conducted screening experiments; N. S. A. conducted quantitative trait loci mapping and analyzed the results; M. K. B. critically reviewed the results and edited the manuscript.

Conflict of interest The authors declare that they do not have any conflict of interest.

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