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ORIGINAL PAPER

Usefulness of 10 genomic regions in soybean associated with sudden death syndrome resistance

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Abstract Sudden death syndrome (SDS) is an important soybean [*Glycine max* (L) Merrill] disease caused by the soilborne fungus *Fusarium virguliforme.* Currently, 14 quantitative trait loci (QTL) had been confirmed associated with resistance or tolerance to SDS. The objective of the study was to evaluate usefulness of 10 of these QTL in controlling disease expression. Six populations were developed providing a total of 321 F2-derived lines for the study. Recombinant inbred lines (RIL) used as parents were obtained from populations of 'Essex' × 'Forrest' (EF), 'Flyer' × 'Hartwig' (FH), and 'Pyramid' × 'Douglas' (PD). Disease resistance was evaluated in the greenhouse at three different planting times, each with four replications, using sorghum infested with *F. virguliforme* homogeneously mixed in the soil (Luckew et al., Crop Sci 52:2215–2223, 2012). Four disease assessment criteria—foliar disease incidence (DI), foliar leaf scorch disease severity (DS), area under the disease progress curve (AUDPC), and root rot severity—were used. QTL were identified in more than one of the disease assessment criteria, mainly associated with lines in the most resistant categories. Five QTL (*qRfs4, qRfs5, qRfs7, qRfs12,* and *Rfs16*) were associated with at least one of the disease assessments across multiple populations. Of the five, *qRfs4* was associated with DI, AUDPC, and root rot severity, and *Rfs16* with AUDPC and root rot severity. The findings suggest it may be possible for plant breeders to focus on stacking a subset of the previously identified QTL to improve resistance to SDS in soybean.

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Introduction

Sudden death syndrome (SDS) of soybean [*Glycine max* (L.) Merrill) is caused by the soilborne fungus *Fusarium virguliforme* (formerly *Fusarium solani* f. sp. *glycines*) (Aoki et al. 2003; Roy 1997). The pathogen infects soybean roots causing a light-brown to black discoloration, leading to reductions in root mass and root nodules (Rupe 1989; Stephens et al. 1993). Above-ground symptoms of foliar chlorosis and necrosis result from the translocation of toxins released by the pathogen, and can cause premature defoliation and pod abortion (Jin et al. 1996; Rupe 1989).

SDS reduces yield in most of the major soybean producing countries (Wrather et al. 2010). In the United States it ranked second to fifth compared to all other soybean diseases regarding estimated yield impact in the Midwestern soybean producing region from 1996 to 2007, leading to estimated average losses of $190 million a year (Roy et al. 1997; Wrather and Koenning 2009; Wrather et al. 2010). In 2010, SDS was severe in Iowa and yield losses were estimated to exceed 20 % in some production fields (Robertson and Leandro 2010), with a yield loss estimate of 70 million

bushels for the US (Wrather and Koenning 2011). Disease management focuses on planting resistant cultivars and using cultural practices that may improve soil drainage and reduce soil compaction (Hershman et al. 1990; Roy et al. 1997; Wrather et al. 1995).

Resistant cultivars are primarily identified through field screens planted on SDS-infested soils (Gibson et al. 1994). However, environmental factors such as temperature and moisture, play important roles in disease expression (de Farias Neto et al. 2006; Roy et al. 1997; Rupe et al. 1994), creating challenges in the field evaluation of cultivars (Njiti et al. 2001). As a means to overcome this limitation, and to increase breeding efficiency by allowing phenotypic selection of resistant plants during the winter, greenhouse screening methods have also been developed (de Farias Neto et al. 2008; Hashmi et al. 2005; Hartman et al. 1997; Njiti et al. 2001; Lightfoot et al. 2007). A modified greenhouse protocol subjecting plants to higher disease pressure than that in original methods has been developed by Luckew et al. (2012).

Molecular analysis in conjunction with greenhouse and field assays have been used as a means to determine quantitative trait loci (QTL) associated with resistance to SDS (Kazi et al. 2008). Currently, 14 QTL associated with SDS resistance have been confirmed in several recombinant inbred line (RIL) populations (Chang et al. 1996; de Farias Neto et al. 2007; Hnetkovsky et al. 1996; Iqbal et al. 2001; Kassem et al. 2007, 2012; Kazi et al. 2008; Lightfoot et al. 2005; Meksem et al. 1999; Njiti et al. 1997, 2002; Prabhu et al. 1999; Sanitchon et al. 2004; Yamanaka et al. 2006). Research has shown these QTL may confer resistance to foliar disease severity, root rot severity, or both (Kassem et al. 2012; Kazi et al. 2008; Njiti et al. 1998; Triwitayakorn et al. 2005). Two of the QTL are common across four populations, 'Essex' × 'Forrest' (EF), 'Pyramid' × 'Douglas' (PD), 'Flyer' × 'Hartwig' (FH) and 'PI438489B' × 'Hamilton'; one on linkage group (LG) C2 and the other on LG G (Table 1). Two other QTL are common to RIL populations EF and FH, one on LG G and one on LG I (Table 1). The RIL populations PD, FH, and 'Ripley' × 'Spencer' have one QTL in common on LG D2, and PD, FH, and 'PI438489B' × 'Hamilton' populations have a QTL in common on LG N (Table 1). The other QTL are unique to populations in which they were detected, and could be considered population-specific. Up to date, there is no published information on relative importance of the QTL compared to one another in conferring resistance to SDS, even if they are introgressed in similar genetic backgrounds.

Since resistance is quantitative, breeding SDS-resistant cultivars requires accumulation of multiple genes. With the present state of technology, it would be impractical for plant breeders to try to accumulate numerous QTL from different populations into a single genetic background. This stacking particularly would require unusually large population sizes that may not always be feasible to obtain and to manage in breeding (Prabhu et al. 1999). A relevant question is how many QTL and which ones may be needed to confer high levels of SDS resistance that may be necessary to protect yield. The objective of this study was to determine, for the control of SDS disease expression, the usefulness of the known QTL previously associated with SDS resistance in the field and detected in the three RIL populations of EF, FH, and PD.

Materials and methods

Plant material

Populations were developed with different combinations of RIL parents possessing 12 of the QTL previously identified (Chang et al. 1996; Hnetkovsky et al. 1996; Iqbal et al. 2001; Kassem et al. 2007; Kazi et al. 2007, 2008; Lightfoot et al. 2005; Meksem et al. 1999; Njiti et al. 2002; Prabhu et al. 1999). Parents for crossing were identified from the RIL mapping populations of 'Essex' × 'Forrest' (EF-23), 'Pyramid' × 'Douglas' (PD-98), and 'Flyer' × 'Hartwig' (FH-13, FH-33, FH-35). Plant genotypes used included the five parental lines (EF-23, PD-98, FH-13, FH-33, and FH-35), 'Ripley' (Cooper et al. 1990) as resistant control, 'Spencer' (Wilcox et al. 1989) as susceptible control, and 321 $F_{2:3}$-derived lines ($F_{2:3}$) developed from nine different crosses among RIL. Population identification, parentage and number of lines per population are described in Table 2. Some of the populations were obtained by reciprocal crosses between the RIL parents. The parental RIL had been genotyped at the Lightfoot lab and QTL molecular information was published and it is summarized in Table 3 (Njiti et al. 2002; Kassem et al. 2006; Kazi et al. 2008).

Populations were developed at the Iowa State University (ISU) research site located, at Isabela, Puerto Rico, University of Puerto Rico. F_1 seed was obtained in November 2006. The F_1 plants were harvested individually, and identified even if morphological markers were available to confirm the hybrid nature of the crosses. The F_1 plant identity was maintained throughout generation advances. $F_{2:3}$ plants were individually harvested during April 2007. The $F_{2:3}$ lines were randomly selected among all lines available in each population, with seed numbers on an individual plant basis adequate to conducting several runs of the experiment with replications.

Table 1 Quantitative trait loci (QTL) for resistance to *Fusarium virguliforme* described by name of the QTL, simple sequence repeat (SSR) marker, linkage group (LG) in which QTL was assigned, primer sequences, and the articles that identified and characterized each QTL

QTL	SSR	LG	Upper primer sequence (5′ → 3′)	Lower primer sequence (5′ → 3′)	Allele size (bp)	References
Rfs	Sat_403	G	GCGGCGTCATGT TAGTTGGAACC	GCGAGCCATTTTTCTCT TTTAGACAAT	127	Njiti et al. (1998); Meksem et al. (1999); Prabhu et al. (1999); Iqbal et al. (2001); Njiti et al. (2002); Triwitayakorn et al. (2005)
Rfs2	Satt309	G	GCGCCTTCAAATTG-GCGTCTT	GCGCCTTAAATAAAAC CCGAAACT	142	Meksem et al. (1999); Iqbal et al. (2001); Njiti et al. (2002); Triwitayakorn et al. (2005)
qRfs3	Satt163	G	GCGGCACGAGA AAAGGAGAGAGAG	GCGGGGGAAAAACTAT-GTTCT	231	Chang et al. (1996); Iqbal et al. (2001); Kazi et al. (2008)
qRfs4	Satt371	C2	TGCAAACTAACTG GATTCACTCA	GAGATCCCGAAATTT TAGTGTAACA	245	Hnetkovsky et al. (1996); Iqbal et al. (2001); Njiti et al. (2002); Kazi et al. (2008); Kassem et al. (2012)
qRfs5	Satt354	I	GCGAAAATGGACA CCAAAAGTAGTTA	GCGATGCACATCAATTA GAATATACAA	248	Iqbal et al. (2001)
qRfs6	Satt80	N	CCATAAAATAATAAAG GTCAAT	TAATCAGTGGAAAAAAAGT TAT	177	Chang et al. (1996); Njiti et al. (2002)
qRfs7	Sat_001	D2	GCGGATACGAC-CAAAAATTGTT	GCGAACTGCGAAGATAC TACCC	214	de Farias Neto et al. (2007); Kazi et al. (2008)
qRfs11	Satt138	G	GACATTTTTCCACG GATATTGAAT	AACGGGCGATTTATG GCTAT	294	Lightfoot et al. (2007)
qRfs12	Satt160	F	TCCCACACAGTTTTCAT ATAATATA	CATCAAAAGTT TATAACGTGTAGAT	251	Kassem et al. (2006)
Rfs16	Satt353	H	CATACACGCATTGC CTTTCCTGAA	GCGAATGGGAATGC CTTCTTATTCTA	169	Dr. Lightfoot, personal communication

Inoculum preparation

Fusarium virguliforme isolates Clinton1b and Scott obtained in 1996 from roots of SDS symptomatic plants from production fields in Clinton and Scott counties in Iowa, were used in the screening experiments. Isolates were grown on antibiotic-amended potato dextrose agar (PDA) (0.150 g L^{-1} of streptomycin sulfate, and chlortetracycline hydrochloride, 39 g Difco PDA per liter) for 6 weeks under natural day length, and at room temperature (19–23 °C). Sterile white sorghum [*Sorghum bicolor* (L.) Moench] kernels (500 g) were placed in quart mason jars filled with water for 24 h. Water was drained and flasks were autoclaved for 1 h on two consecutive days. After cooling, sorghum kernels were inoculated with five mycelia plugs 7 mm in diameter of a single *F. virguliforme* culture. Jars were incubated at room temperature (21 ± 2 °C) for 2 weeks, shaken daily by hand for 1 to 2 min to ensure uniform fungal growth. Jars were visually inspected to verify uniform fungal growth and emptied on racks in a fume hood for 24 h to allow infested sorghum kernels to dry. After drying, infested kernels each with a *F. virguliforme* isolate, were combined in equal parts into a single plastic biohazard bag. Isolates were combined in equal proportions as described by Sanogo et al. (2000). Inoculum was prepared separately for each run.

Infested sorghum previously prepared in the lab following the same techniques, had been quantified using qPCR using specific primers identified by Li et al. (2008) in the Hartman lab at the University of Illinois (Bowen and Bond, 2012). The batches ranged from 21.4 ng *F. virguliforme* DNA/mg sorghum to 31.1 ng DNA/mg sorghum (dry weight). The specific batch of infested sorghum used in the study was not quantified.

Table 2 ANOVA table of six populations and four disease assessment criteria: disease incidence (DI), foliar disease severity (DS), area under the disease progress curve (AUDPC), and root rot severity

Main effect	Disease assessment criteria							
	DI		DS		AUDPC		Root rot	
	df[a]	MS	*df*	MS	*df*	MS	*df*[a]	MS
Population 1, FH13 × EF23, 52 lines								
Run	1	0.736	2	34,452***	2	2,863,402***	1	30,685***
Rep	3	0.158	3	5,179.8***	3	263,607.3**	3	7,415.0***
Run*Rep	3	0.934*	5	8,381.7***	5	466,360.3***	3	16,018***
Genotype	51	0.290	51	690.19	51	53,548.49	51	839.24
Error	305	0.259	423	634.95	424	49,916.93	304	655.00
Population 2, FH13 × PD98, 62 lines								
Run	1	8.794***	2	59,407***	2	6,029,312***	1	90,339***
Rep	3	0.297	3	2,759.9*	3	177,498.4*	3	5,140.1***
Run*Rep	3	1.727***	5	6,397.9***	5	454,922.5***	3	18,054***
Genotype	61	0.152	61	943.62*	61	86,399.91*	61	830.09
Error	388	0.157	538	588.30	537	61,282.19	388	618.09
Population 3, FH33 × EF23, 45 lines								
Run	1	6.923***	2	40767***	2	4,182,412***	1	53,358***
Rep	3	0.528*	3	738.98	3	144,273.7	3	3,986.9**
Run*Rep	3	0.396	5	673.88	5	93,220.52	3	8,245.9***
Genotype	43	0.152	44	465.69	44	40,226.83	43	662.92
Error	271	0.170	343	578.67	343	65,369.22	270	578.16
Population 4, FH33 × PD98, 59 lines								
Run	1	11.01***	2	45,258***	2	3,470,430***	1	56,847***
Rep	3	0.397*	3	4,216.7***	3	176,313.6*	3	7,805.2***
Run*Rep	3	1.805***	5	6,596.5***	5	265,332.0***	3	19,218***
Genotype	58	0.103	58	623.15*	58	62,299.13	58	510.24
Error	376	0.126	470	459.95	472	48,135.21	379	554.80
Population 5, PD98 × FH35, 38 lines								
Run	1	5.515***	2	33539***	2	1,985,523***	1	19,969***
Rep	3	0.067	3	2,472.4**	3	113,916.3	3	9,325.5***
Run*Rep	3	1.028**	5	1,919.1*	5	96,552.26	3	6,650.4***
Genotype	37	0.176	37	800.74	37	70,671.16	37	962.20
Error	215	0.148	287	610.07	289	60,693.95	215	671.99
Population 6, PD98 × EF23, 65 lines								
Run	1	3.567***	2	47,801***	2	3,272,560***	1	54,173***
Rep	3	0.427*	3	2,905.3**	3	117,508.9	3	7,913.9***
Run*Rep	3	3.027***	5	8,354.1***	5	417,585.5***	3	18,283***
Genotype	62	0.166	64	810.55	64	77,277.52	62	771.98
Error	331	0.146	431	627.52	432	69,453.76	331	702.21

[a] DI and Root rot severity data was only collect in two runs of the experiment

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Greenhouse experiment

The 321 $F_{2:3}$-derived lines, parents, the resistant control Ripley and the susceptible control Spencer were screened for foliar leaf scorch and root rot resistance to *F. virguliforme* at the ISU Plant Pathology Greenhouses, Ames, IA.

The infested sorghum inoculum was homogeneously mixed with pasteurized 2:1 sand: soil mixture at the ratio of 1 part inoculum to 20 parts sand/soil (v/v) following Luckew et al. (2012). This ratio uses more inoculum than what is used in other screening methods and what would be found naturally in the field (Luckew et al. 2012). The

Table 3 Presence (+) or absence (−) of the beneficial allele according to SSR band sizes for 10 quantitative trait loci (QTL) associated with resistance to soybean sudden death syndrome (SDS) in the five RIL parents used to develop the 321 $F_{2:3}$-derived lines screened for resistance to SDS in the greenhouse (16 h photoperiod at 23 ± 5 °C)

QTL	Parent				
	E × F23	F × H13	P × D98	F × H33	F × H35
Rfs	+	−	+	−	−
Rfs2	−	+	−	+	+
qRfs3	+	+	−	−	−
qRfs5	+	−	+	−	−
qRfs4	−	+	−	+	+
qRfs7	−	+	−	+	+
Rfs16	+	+	−	+	+
qRfs12	+	−	+	−	−
qRfs6	−	+	−	+	+
qRfs11	+	−	+	−	−

high level of inoculum was used to overcome the QTL stacks present in the 321 lines. Sterile non-infested sorghum mixed with pasteurized sand/soil at the same ratio as the infested sorghum, was used as negative control for the resistant and susceptible check cultivars.

A 240-ml Styrofoam cup with five seeds planted at a depth of 2 cm was considered a plot. There were four cups per soybean genotype, each considered a replication. The two checks were planted into sterile soil mixture and infested soil mixtures to verify contamination was not present. Cups were incubated in the greenhouse at 23 ± 5 °C with a 16 h photoperiod. Plants were watered once daily to maintain soil moisture. Experimental design was a randomized complete block design (RCBD) with genotypes as treatments and with four replications. The experiment was conducted three times (runs) planted on May 2010 (Run 1), December 2010 (Run 2), and April 2011 (Run 3).

Disease assessment

SDS foliar leaf scorch symptoms were evaluated 21, 24, 27, 30, 33, and 36 days after planting (dap). Foliar leaf scorch disease severity (DS) was scored as a percentage of leaf area showing typical SDS symptoms (Roy et al. 1997). Foliar disease incidence (DI) was calculated as number of plants per cup showing typical SDS foliar symptoms, divided by the total number of plants in each cup. Root rot was assessed at 36 dap and scored visually as the percentage of root area showing dark brown to black discoloration (Roy et al. 1997). Following the midpoint rule of Campbell and Madden (1990), the area under the disease progress curve (AUDPC) was calculated.

Molecular marker analysis

For all lines, and parents, DNA was isolated from leaf samples of 10 individual plants collected 36 dap, corresponding to soybean plant growth stage V3–V4 (Fehr and Caviness 1977). Leaves of each of five plants were collected from two of the three runs.

DNA was isolated following a modification of the method described in the CIMMYT laboratory protocols manual, section entitled 'Small scale extraction of high quality DNA' (CIMMYT 2005). Leaves of each line were combined over the four replications, keeping runs separate, and were finely ground with mortar and pestle. The procedure described by CIMMYT (2005) was followed.

The DNA pellet was resuspended in 100 µl of TE buffer. Simple sequence repeat (SSR) markers (Table 1) were used to test the isolated DNA for the known QTL. A step down polymerase chain reaction (PCR) was performed. An initial 2 min at 94 °C was followed by five cycles of 94 °C for 30 s, then 60 °C for 30 s with a step of −2 °C every cycle, and finished with 72 °C for 1 min. The last 35 cycles mimicked the first 5 cycles starting with 30 s at 94 °C followed with 50 °C for 30 s, then 72 °C for 45 s. After all cycles finished, the products were kept at 72 °C for 10 min followed by 15 °C for 10 min. Products from the PCR were electrophoresed on 8 % (w/v) agarose gels for 3 h, visualized by ethidium bromide fluorescence and scored for presence/absence of the corresponding marker allele associated with SDS resistance using the known base pair size of the marker.

Data analysis

For each population the three runs of the greenhouse experiment were combined and analysis of variance was performed using the PROC GLM statement in SAS version 9.2 (SAS Institute 2008) on foliar DS and DI at 36 dap, root rot severity, and AUDPC. Main effects tested were run, replication, and genotype, and interactions, all considered random effects. Control genotypes were considered fixed effects.

Allele frequencies of the 10 QTL for which parents were polymorphic, were calculated within two defined groups or categories, the 10 % most resistant, 10 % least resistant for each population and the five parents. Allele frequencies were compared between groups to identify differences in frequencies for disease assessment criteria (Appendix 1–4).

The PROC GLM statement was used to perform contrast analyses of the 10 % most and the 10 % least resistant categories for DS and root rot severity. A Spearman rank correlation was performed on all 321 lines for DI, DS, AUPDC and root rot using the PROC CORR statement. A Pearson correlation was also performed on DI,

DS, AUDPC, root rot, and the 10 QTL using the PROC CORR statement. PROC UNIVARIATE was run to obtain a Shapiro–Wilk test for normality of the curves for each of the disease assessment criteria, DI, DS, AUDPC, and root rot severity.

Results

The four disease assessment criteria, DI (Fig. 1a), DS (Fig. 1b), root rot severity (Fig. 1c), and the calculated AUDPC curve (Fig. 1d), had frequency distribution curves that approached normal uni-modal distributions. For each disease criteria there were four parental lines that had a calculated average over runs that approximated the general combined average calculated for the 321 $F_{2:3}$-derived lines (Fig. 1). Mean squares for run, replicate, and their interaction were significant ($P < 0.05$) in all populations for DS, AUDPC, and root rot severity, except for populations FH33 × EF23, PD98 × FH35 and PD98 × EF23 (Table 2). Mean squares of the interaction terms (genotype × run and genotype × rep) were non-significant in all populations.

Spearman rank correlations were calculated for all 321 lines and populations among disease assessment criteria (Table 4). These correlations were significant ($P < 0.0001$), with r values ranging from 0.48 to 0.89 (Table 4). The highest correlation was obtained between AUDPC and DS recorded at 36 dap, $r = 0.89$.

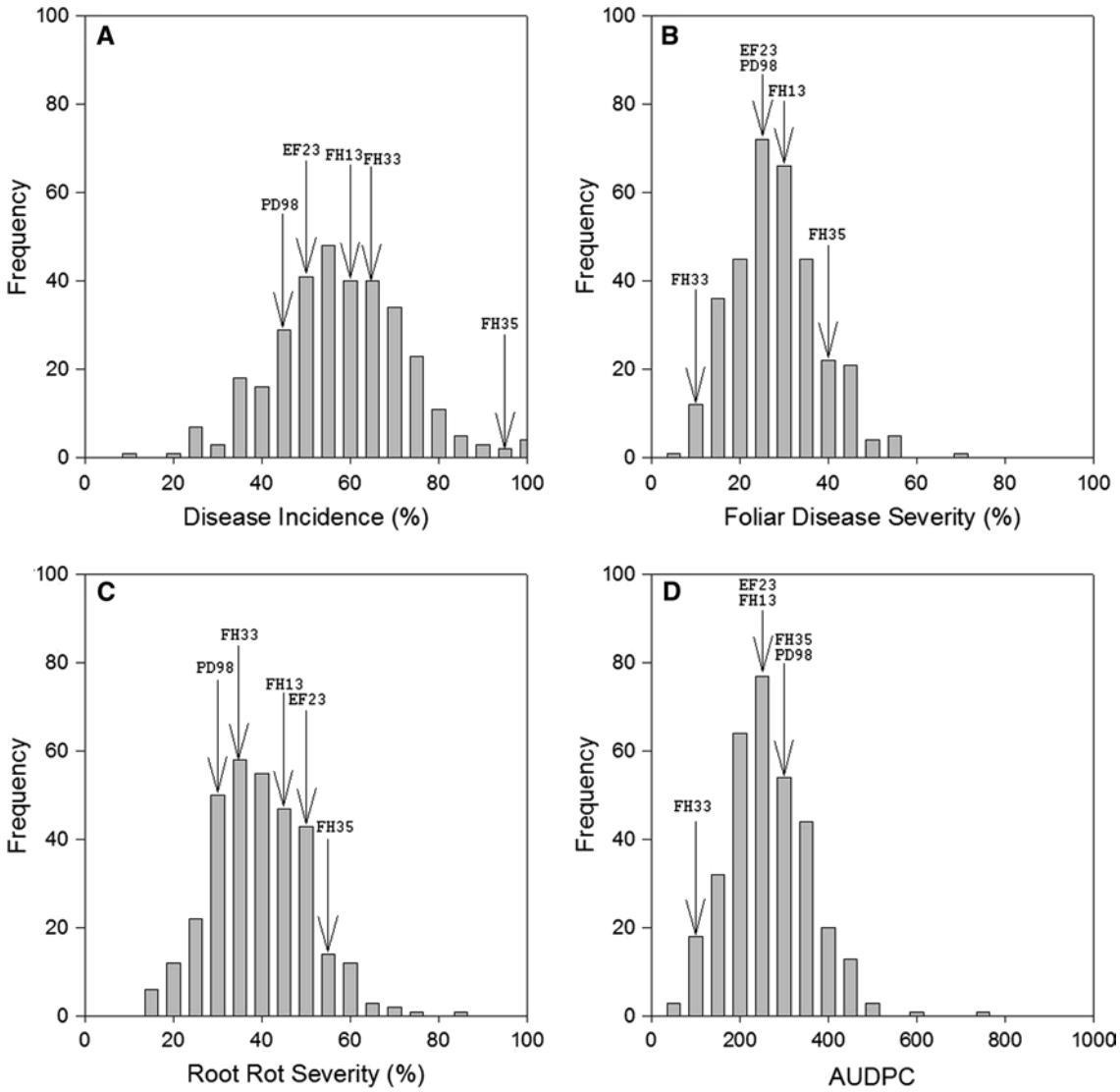

Fig. 1 Distribution of 321 $F_{2:3}$-derived soybean lines and the five parents averaged over three runs based on symptoms resulting from infection of *Fusarium virguliforme* **a** disease incidence (%), **b** foliar leaf scorch severity (%), **c** root rot severity (%), and **d** area under the disease progress curve. Plants were grown in the greenhouse at 23 ± 5 °C with a 16 h photoperiod. The *vertical arrows* represent the position of the parents in each of the three histograms

Table 4 Spearman rank correlation of foliar disease incidence (DI) at 36 dap, foliar leaf scorch disease severity (DS) at 36 dap, area under the disease progress curve (AUDPC), and root rot severity of soybean sudden death syndrome for 321 $F_{2:3}$-derived lines screened in the greenhouse

	DI	DS	AUDPC	Root Rot
DI		0.74	0.64	0.48
DS			0.89	0.59
AUDPC				0.54

The Pearson correlation between the QTL and disease assessment criteria using only the 10 % most and 10 % least resistant showed population specificity in their values (Tables 4, 5, 6). Half of the populations showed only one significant ($P < 0.05$) r value or none, while the other half of the populations had many significant relationships. One QTL, *qRfs12*, was associated ($P < 0.05$) with DS, root rot, and AUDPC in two populations, FH33 × PD98 and PD98 × FH35. Two QTL were associated ($P < 0.05$) with a disease assessment criterion in two populations, in one population having a positive relationship and in the other a negative relationship. For root rot *qRfs7* had a positive relationship in FH33 × EF23 and a negative relationship in FH13 × EF23. The second QTL to act similarly was *qRfs4* with a positive relationship in PD98 × FH35 and a negative relationship in PD98 × EF23 for AUDPC. Many QTL showed ($P < 0.05$) correlations to other QTL (Tables 5, 6, 7).

Considering QTL and disease assessment over populations, significant differences ($P < 0.0001$) between the 10 % most resistant and 10 % least resistant groups were observed. For DI, QTL *qRfs4* was present in the 10 % most resistant group in five of the six populations (Table 8). The DI assessment also showed QTL *Rfs* and *qRfs3* were present in four of the six populations in the 10 % least resistant group. Focusing on DS, a different set of QTL common across the six populations was observed. QTL *qRfs7* was identified in five of the populations in the 10 % most resistant group and *qRfs11* in four populations in the 10 % least resistant category. Root rot severity had three common QTL across four populations, *qRfs5, qRfs12*, and *qRf4*. The 10 % most resistant groups had *qRfs5* and *qRfs12* present in five of the six populations, while *qRfs4* was present in the 10 % least resistant group in four of the populations. Although AUDPC is related to DS, AUDPC showed a different set of QTL present across populations with *qRfs4* and *qRfs12* being common in the 10 % most resistant group in four populations as well as *Rfs16* in five of the populations. AUDPC also had two QTL common across populations in the 10 % least resistant group: *Rfs2* in four populations and *qRfs11* in five populations.

Common QTL were also observed among disease assessment criteria (Table 8). One QTL, *qRfs4*, was present across three disease assessments; DI, AUDPC, and root rot severity, although not always present in the same most resistant/least resistant category of each of the three populations. The QTL was present in the 10 % most resistant group for DI and AUDPC, while for root rot severity, the common QTL was in the 10 % least resistant group. QTL *qRfs11* was also common across two disease assessments, DS and AUDPC, present for both disease criteria in the 10 % least resistant group. QTL *qRfs11* had in general, a higher frequency in the 10 % least resistant group of root rot severity. The last QTL common across disease assessment criteria was *qRfs12* for AUDPC and root rot severity. For both criteria *qRfs12* was in the 10 % most resistant group. For DI and DS, *qRfs12* was present in the 10 % most resistant group at a frequency higher than in the 10 % least resistant group.

Discussion

Using RILs obtained from the populations of Essex x Forrest, Flyer x Hartwig, and Pyramid x Douglas, and making crosses among them, new soybean populations were developed to conduct this study. The results from this study identified certain QTL showing association with SDS resistance under high disease pressure, over different populations and disease assessment criteria. QTL detected in comparisons of the most and least resistant categories were not always significant in all populations, suggesting a population background effect. Screening under high disease pressure allowed for discrimination of resistant QTL, providing indications on which and how many QTL might be important to consider in breeding.

The results indicated that of the 10 QTL for which the parental lines were polymorphic, five—*qRfs4, qRfs5, qRfs7, qRfs12,* and *Rfs16*—may have potentially greater effects on SDS resistance, particularly in the four disease assessment criteria (DI, DS, root rot severity and AUDPC). The QTL effects on disease resistance expression were evident when within population comparisons were done between the 10 % most and least resistant lines.

Five other QTL *Rfs, Rfs2, qRfs3, qRfs4,* and *qRfs11,* were observed in the 10 % least resistant category for at least one disease assessment criteria. The QTL were previously reported as associated with SDS resistance. Our observations do not fully contradict the reports, since genetic backgrounds used in this and previous studies were different. Different genetic backgrounds and possibly QTL

Table 5 Pearson correlation coefficients and the probability they differ from zero for the 10 QTL

FH13 × PD98	FH13 × EF23													
	DI	DS	Rot	AUDPC	*Rfs*	*Rfs2*	*Rfs3*	*qRfs4*	*qRfs5*	*qRfs6*	*qRfs7*	*qRfs11*	*qRfs12*	*Rfs16*
DI		0.49***	0.42***	0.44***	0.14	0.06	0.10	0.06	−0.03	N/A[a]	−0.07	0.03	0.02	0.03
DS	0.57***		0.58***	0.87***	−0.08	0.06	0.06	−0.00	−0.03	N/A[a]	−0.03	0.02	−0.08	−0.01
Rot	0.40***	0.65***		0.52***	0.01	0.06	0.01	0.11	−0.11	N/A[a]	-0.16*	-0.13	0.01	0.08
AUDPC	0.49***	0.86***	0.65***		−0.07	0.10	0.06	−0.00	−0.01	N/A[a]	−0.01	0.03	−0.06	−0.00
Rfs	−0.02	0.02	0.01	0.03		−0.07	0.00	0.32***	0.23***	N/A[a]	−0.20***	0.03	0.05	0.22***
Rfs2	0.06	0.09	−0.03	0.12	0.13*		0.25***	−0.15**	0.11	N/A[a]	−0.05	0.22***	−0.10	0.33***
qRfs3	−0.01	−0.08	0.01	−0.02	−0.02	0.33***		−0.03	0.24***	N/A[a]	0.03	0.54***	0.17**	0.19**
qRfs4	−0.01	0.05	0.00	0.07	−0.13*	−0.20***	−0.19***		0.09	N/A[a]	−0.30***	−0.49***	0.05	0.21***
qRfs5	0.01	−0.05	−0.01	−0.07	0.36***	−0.23***	−0.16**	−0.10		N/A[a]	−0.18**	0.32***	0.29***	0.10
qRfs6	0.05	0.04	−0.00	0.05	0.13*	0.21***	0.18**	0.13*	0.12*		N/A[a]	N/A[a]	N/A[a]	N/A[a]
qRfs7	−0.08	−0.08	0.04	−0.04	0.04	0.13*	0.14*	0.15**	−0.03	0.44***		0.05	−0.23***	−0.25***
qRfs11	−0.03	0.05	0.06	0.04	0.42***	0.24***	−0.02	0.02	0.14*	0.04	0.17**		0.06	−0.06
qRfs12	0.04	0.04	−0.00	0.00	−0.42***	0.05	0.16**	−0.13*	0.01	0.14*	−0.31***	−0.16**		−0.37***
Rfs16	−0.04	−0.01	−0.03	−0.05	−0.08	−0.03	−0.06	−0.31***	0.15**	0.10	−0.12*	−0.14*	0.16**	

The four disease assessments disease incidence (DI), disease severity (DS), area under the disease progress curve (AUDPC), and root rot severity (rot) based on the data from the 10 % most and least resistant categories for the populations FH13 × EF23 in the top half and FH13 × PD98 in the bottom half

[a] *qRfs6* was not present in the 10 % most and least resistant categories for population 1, FH13 × EF23

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 6 Pearson correlation coefficients and the probability they differ from zero for the 10 QTL

FH33 × PD98	FH33 × EF23													
	DI	DS	Rot	AUDPC	*Rfs*	*Rfs2*	*Rfs3*	*qRfs4*	*qRfs5*	*qRfs6*	*qRfs7*	*qRfs11*	*qRfs12*	*Rfs16*
DI		0.67***	0.50***	0.59***	0.03	0.11	−0.07	−0.08	−0.06	−0.09	−0.00	0.12	−0.13	0.06
DS	0.61***		0.65***	0.89***	0.06	0.07	0.01	−0.08	−0.03	0.00	0.06	0.12	−0.02	0.03
Rot	0.55***	0.66***		0.65***	−0.00	0.10	−0.07	0.04	−0.09	0.06	0.16*	0.07	−0.04	0.06
AUDPC	0.58***	0.86***	0.52***		0.08	0.01	0.00	−0.04	−0.00	0.02	0.03	0.04	0.02	0.05
Rfs	0.10	−0.10	−0.05	−0.12		−0.23***	−0.23***	0.01	−0.44***	0.22***	−0.16**	0.14*	0.11	−0.02
Rfs2	−0.05	−0.16*	−0.04	−0.12	−0.04		−0.09	−0.29***	0.04	0.00	0.69***	0.31***	−0.31***	0.05
qRfs3	0.19*	0.27***	0.10	0.32***	−0.23***	−0.23***		−0.29***	0.21***	−0.30***	−0.06	−0.22***	0.00	0.05
qRfs4	−0.03	−0.13	0.04	−0.14	−0.04	0.12	−0.48***		0.16**	0.28***	−0.20***	−0.11	−0.06	−0.35***
qRfs5	−0.01	0.02	−0.11	0.05	−0.36***	−0.23***	0.08	0.00		−0.14*	−0.09	0.02	−0.14*	−0.05
qRfs6	0.07	−0.03	−0.02	−0.08	0.54***	0.04	−0.11	−0.20**	−0.48***		0.21***	0.15*	0.26***	−0.16**
qRfs7	0.01	−0.09	−0.10	−0.06	0.32***	0.55***	−0.13	−0.30***	−0.13	0.35***		0.09	−0.22***	0.30***
qRfs11	0.03	0.08	0.05	0.10	0.03	−0.24***	−0.05	0.00	−0.19**	0.15*	−0.13*		−0.25***	0.05
qRfs12	−0.10	−0.19*	−0.17*	−0.17*	0.03	0.10	−0.05	−0.07	0.23***	0.26***	0.21**	−0.31***		0.00
Rfs16	0.01	−0.12	0.01	−0.13	−0.01	−0.05	−0.25***	0.25***	−0.25***	0.32***	−0.21**	0.18**	0.27***	

The four disease assessments disease incidence (DI), disease severity (DS), area under the disease progress curve (AUDPC), and root rot severity (rot) based on the data from the 10 % most and least resistant categories for the populations FH33 × EF23 in the top half and FH33 × PD98 in the bottom half

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 7 Pearson correlation coefficients and the probability they differ from zero for the 10 QTL

PD98 × EF23	PD98 × FH35													
	DI	DS	Rot	AUDPC	*Rfs*	*Rfs2*	*Rfs3*	*qRfs4*	*qRfs5*	*qRfs6*	*qRfs7*	*qRfs11*	*qRfs12*	*Rfs16*
DI		0.56***	0.38***	0.43***	0.08	0.09	0.08	0.05	−0.14	0.22*	−0.03	−0.10	−0.12	0.07
DS	0.55***		0.42***	0.89***	0.13	0.01	−0.00	0.24**	−0.03	0.11	−0.14	−0.15	−0.22*	−0.02
Rot	0.45***	0.54***		0.37***	−0.21	0.13	0.17	0.13	−0.23*	0.27*	−0.16	−0.11	−0.31**	−0.17
AUDPC	0.51***	0.88***	0.52***		0.10	0.03	−0.11	0.25**	0.01	0.11	−0.14	−0.12	−0.19*	−0.05
Rfs	0.12	0.07	0.10	0.05		−0.09	−0.47***	0.03	0.41***	−0.12	0.07	−0.04	−0.08	0.52***
Rfs2	N/A[a]	N/A[a]	N/A[a]	N/A[a]	N/A[a]		0.12	0.22**	0.42***	−0.16	0.19*	0.11	−0.23**	−0.40***
qRfs3	−0.13	−0.03	−0.06	−0.00	−0.28***	N/A[a]		−0.07	−0.50***	0.11	0.07	0.12	−0.30***	−0.40***
qRfs4	0.01	−0.12	−0.09	−0.15*	0.02	N/A[a]	−0.11		0.31***	−0.26***	−0.48***	−0.03	−0.43***	−0.34***
qRfs5	0.21*	0.10	−0.01	0.05	0.03	N/A[a]	0.12*	−0.33***		−0.63***	0.19*	0.10	0.05	−0.09
qRfs6	N/A[a]	N/A[a]	N/A[a]	N/A[a]	N/A[a]	N/A[a]	N/A[a]	N/A[a]	N/A[a]		0.09	0.00	0.07	−0.10
QRfs7	−0.20*	−0.06	−0.05	−0.06	−0.18**	N/A[a]	−0.07	0.01	−0.45***	N/A[a]		0.57***	0.50***	−0.30***
qRfs11	−0.17*	0.11	0.18*	0.16*	−0.18**	N/A[a]	0.10	−0.30***	−0.06	N/A[a]	0.17**		0.07	−0.47***
qRfs12	0.04	0.04	−0.06	0.02	−0.06	N/A[a]	−0.11	0.36***	0.11	N/A[a]	0.14*	−0.20***		0.14
Rfs16	−0.09	−0.12	−0.04	−0.11	−0.28***	N/A[a]	−0.05	0.38***	−0.48***	N/A[a]	0.23***	0.13*	−0.15*	

The four disease assessments disease incidence (DI), disease severity (DS), area under the disease progress curve (AUDPC), and root rot severity (rot) based on the data from the 10 % most and least resistant categories for the populations PD98 × FH35 in the top half and PD98 × EF23 in the bottom half

[a] *Rfs2* and *qRfs6* were not present in the 10 % most and least resistant categories for population 6, PD98 × EF23

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

Table 8 Number of populations possessing each of the 10 quantitative trait loci (QTL) in either the 10 % most resistant or least resistant categories

Disease assessment criteria	Category	QTL									
		Rfs	*Rfs2*	*qRfs3*	*qRfs4*	*qRfs5*	*qRfs6*	*qRfs7*	*qRfs11*	*qRfs12*	*Rfs16*
DI	Most Res.	1/6	2/6	1/6	5/6	2/6	1/6	3/6	3/6	3/6	3/6
	Least Res.	4/6	2/6	4/6	1/6	1/6	3/6	0/6	3/6	3/6	1/6
DS	Most Res.	3/6	2/6	2/6	3/6	1/6	1/6	5/6	2/6	3/6	3/6
	Least Res.	2/6	2/6	2/6	3/6	1/6	1/6	0/6	4/6	1/6	2/6
AUDPC	Most Res.	2/6	1/6	1/6	4/6	1/6	1/6	2/6	1/6	5/6	4/6
	Least Res.	3/6	4/6	2/6	2/6	2/6	2/6	0/6	5/6	1/6	2/6
Root rot severity	Most Res.	3/6	1/6	2/6	1/6	5/6	1/6	3/6	2/6	1/6	5/6
	Least Res.	1/6	2/6	2/6	4/6	0/6	3/6	3/6	3/6	1/6	1/6

There were six populations, FH13 × EF23, FH13 × PD98, FH33 × EF23, FH33 × PD98, PD98 × FH35, and PD98 × EF23 assessed by four disease assessment criteria, disease incidence (DI), foliar disease severity (DS) at 36 days after planting (dap), area under the disease progress curve (AUDPC), and root rot severity

interacting with them may explain the differential contribution of the QTL to the final expression of SDS resistance. An example of interaction between QTL and genetic background was reported for QTL *Rfs2* (Afzal et al. 2012; Srour et al. 2012). The authors indicated that *Rfs2* has pleiotropic effect under the presence of *rhg1*, one of the alleles conferring resistance to soybean cyst nematode (SCN). One of the populations used in our study traces SCN resistance to PI 88788, while the other two trace to the Peking source of resistance for SCN.

Another significant aspect of the study was to identify SDS-resistant QTL common across populations. Three QTL, *qRfs4*, *qRfs11*, and *Rfs16*, which were common to more than one disease assessment criteria, were also common across populations. Additionally, two of the QTL were also observed in the 10 % most resistant category—*qRfs4* and *Rfs16*. These results suggest the two QTL could be used by plant breeders as initial genes to improve SDS resistance. The QTL *qRfs4* and *Rfs16* both confer resistance to foliar leaf scorch caused by the fungus and results also provided indications that *Rfs16* appears to favor root rot resistance.

Our observations for *Rfs16* indicate the QTL is associated with foliar and root resistance in the populations. This is the first published result in which the association of *Rfs16* with both DS at the leaves and root rot severity is reported. A QTL having SDS-resistant expression at the two plant levels has important implications for soybean breeding. On the basis of up to date observations it had been hypothesized that resistance to SDS in soybean may be governed by two different genetic systems, one acting at the foliar level, and the other at root level (Kazi et al. 2008; Njiti et al. 1997; Triwitayakorn et al. 2005). Recently however, and confirming our observation for *Rfs16,* work in the Meksem lab has reported other QTL with significant association for resistance simultaneously expressed both at the foliar and root levels (Kassem et al. 2012).

In contrast to *Rfs16,* QTL *qRfs4* was only associated with foliar resistance, which agrees with previous results of independent resistance mechanisms for foliar and root resistance (Kazi et al. 2008; Triwitayakorn et al. 2005). In our work, *qRfs4* was observed in the 10 % least resistant category for root rot severity, although for foliar resistance (DI and AUDPC), the QTL was present in the 10 % most resistant category. In previous work, Iqbal et al. (2001) identified the QTL associated with resistance for DI, later Kazi et al. (2008) reported an association with the calculated DX resistance. It is important to mention that both disease assessment criteria, AUDPC and DX, are mathematically related since DS is part of the equation to calculate AUDPC. This fact justifies the highly significant ($P < 0.0001$) correlation of $r = 0.89$ we observed in the study.

In this study, the association of *qRfs5* with root rot severity is a novel result. Previously, Iqbal et al. (2001) reported *qRfs5* was associated with resistance to SDS under the DI assessment criteria. The authors observed that in the population of Essex x Forrest, the QTL explained 11.5 % of the total variation for resistance. Our observations seem to indicate that the association reported by Iqbal et al. (2001) may be in reality a consequence of resistance QTL acting at root level.

The association between *qRfs7* with DS observed is not a new observation. Kazi et al. (2008) previously reported the QTL explained 25 % of the variation in root rot severity, and also a weak association with DS. A partial explanation for the differences between studies may be the higher disease pressure used in this study (Luckew et al. 2012). The disease protocol by Luckew et al. (2012) causes rotted roots even when foliar disease symptoms are not visible.

It is important to mention that results of our study be interpreted with some measure of caution. Several limitations may be identified, one is that results were based solely on greenhouse screening tests, even though the screening protocol used (Luckew et al. 2012), was purposely designed to create increased disease pressure as compared to classical greenhouse screening methods (Hartman et al. 1997; X.B. Yang, Plant Pathology Dept., ISU, personal communication; Patent #7,288,386 issued to Lightfoot et al. 2007). In the study, we did not conduct field screening tests and this is an important limitation to confirm and validate mode of action of QTL, particularly when the QTL tested were identified in previous research through field screens. Counteracting this limitation, however, Luckew et al. (2012) reported in previous work that genotypic rankings between greenhouse and field screenings can also be highly correlated. Another limitation refers to the lack of genetic variance component estimates, since the study was not planned with that objective in mind. This precludes consideration of QTL effects associated with components of the genetic variance, and interactions among them, such as additive x additive, and other higher interactions. It is important to note, however, that this may not be relevantly important, since contradictory results on higher order genetic interactions have been reported (Iqbal et al. 2001; Njiti et al. 2002).

Our results suggest it may be possible for plant breeders to focus on stacking fewer QTL in breeding lines to improve resistance to SDS in soybean, than those identified associated with resistance. Research is in progress to conduct line evaluations in field test conditions, both on SDS-infested and non-infested soil conditions to obtain SDS disease symptoms and yield estimates. Reports have indicated that foliar symptoms are strongly and clearly associated with seed yield reduction (Gibson et al. 1994; Yuan et al. 2002; Kazi et al. 2008), similarly as root rot symptoms (Njiti et al. 1997; Kazi et al. 2007, 2008). The work in progress may contribute to establish potential associations between important resistance QTL and yield expression, increasing efficiency of breeding programs to improve resistance to SDS.

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