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Confirmation of QTL that Underlie Resistance to Soybean Sudden Death Syndrome using NILs and SNPs

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Abstract

Soybean (*Glycine max* (L.) Merr.) cultivars differ in their resistance to sudden death syndrome (SDS), caused by *Fusarium virguliforme* (Aoki). Breeding for improving SDS response has been challenging, due to the large number of known resistance loci (more than 43) and interactions among them. The aims here were to compare the inheritance of resistance to SDS in a near isogenic line (NIL) population that was fixed for 91.5% of the genome but appeared to segregated at loci underlying partial resistance to SDS; to examine the interaction with the loci; and to identify regions containing candidate genes underlying QTL. Used were; a NIL population derived from residual heterozygosity in an $F_{5:9}$ recombinant inbred line EF60 (lines 1-40). The SDS disease index (DX) data were from two locations but two different years. There were 4 of 400 microsatellite and 456 of 5,361 SNP markers tested that were polymorphic (8-10%). The SNPs clustered into 23 genomic regions. Significantly associated with resistance to SDS (0.005 < P > 0.0001) were regions from 2,788 Kbp to 8,938 Kbp on chromosome (Chr.) 18 and 33,100 Kbp to 34,943 Kbp on Chr. 20. The marker to trait association values suggested that the two closely linked loci on Chr. 18 were really three loci (cq*Rfs1*, cq*Rfs*, and now *Rfs19*). They were clustered within 20 cM of the *rhg1* locus underlying resistance to soybean cyst nematode (SCN; HgType 7). An epistatic interaction between the Chr18 loci and the Chr 20 locus were inferred. Therefore, QTL for resistance to SDS were shown to be both internally complex and interacting.

Keywords: Fusarium; resistance; soybean; Glycine max; Forrest; SDS; near isogenic line; SNP.

Abbreviations: Receptor like kinase (RLK); soybean cyst nematode (SCN); sudden death syndrome (SDS); Chromosome (Chr.).

Introduction

Sudden death syndrome (SDS) of soybean (*Glycine max* [L.] Merr.) is one of the most devastating diseases in the Midwest (Wrather et al., 2009). SDS is caused by a soil-borne fungus *Fusarium virguliforme* (comma shaped spores; Aoki et al., 2003; Luckew et al., 2013; Hartman, 2015). SDS was first discovered in Arkansas in 1971, it later spread to neighboring states and was found throughout most soybean producing states by the

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late 1990's (Hartman et al., 2015). There are several hypotheses about how SDS spread to the US, it could be due to a species hop from common bean (*Phaseolus vulgaris* L.) production; natural selection from common soil organisms; or in soil or dust introduced from South America (Lightfoot, 2015). *F. virguliforme* infects the root causing a slightly tan to brown discoloration of the cortex, which leads to the loss of root mass and root nodules. The early above ground symptoms include; leaf mottling; yellowing between the major veins leading to necrosis; leaf abscission at the top of the petiole rather than the base; and finally early plant death. The diseases that comprise SDS are favored by; cool and wet environments; early planting; soil compaction; meristem determinacy; and genotypes with high seed yield potentials. Consequently, SDS can lead to severe yield losses with average losses estimated to increase from $95 million a year in 1996 to $190 million a year in 2014 in the US Midwest alone (Luckew et al., 2013; Lightfoot, 2015).

The most efficient approach to try to control SDS has been the use of resistant varieties (Yuan et al., 2012; Lightfoot, 2015; Swaminathan et al., 2016). Cultivars with higher genetic resistances may be the key for controlling soybean loss caused by SDS. Conventional breeding methods are time-consuming and labor-intensive; molecular methods can accelerate breeding programs and make selection for disease traits more effective. SDS Resistance is controlled by many (more than 43) quantitative trait loci (QTL; Lightfoot 2015; Swaminathan et al. 2016). Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) markers were the first markers used to detect QTL underlying SDS resistance in the 1990s (Hnetkovsky et al., 1996; Meksem et al., 1999; Njiti et al., 1998, 2002). Microsatellite markers (Simple Sequence Repeat; SSRs) were later developed to identify QTL associated with SDS (Meksem et al., 1999; Iqbal et al., 2001; Kassem et al., 2006). With the availability of integrated SSR and SNP maps of soybean, it has become possible to find many more QTL underlying SDS resistance (Wen et al., 2014; Bao et al., 2015), although care must be taken with the phenotypic data collected (Lightfoot, 2015). Estimates of 18 - 30 loci underlying resistance across 12 populations have been made based on field data (Lightfoot, 2015) or robust greenhouse data (Bao et al., 2015; Swaminathan et al., 2016). Resistance to SCN has been found to be linked or pleiotropic to resistance to SDS in two regions (Kazi et al., 2009; Srour et al., 2012). Up to 50% of resistance to SDS appeared to be co-inherited with resistance to SCN (Gibson et al., 1994).

Three populations of recombinant inbred lines (RILs) were used to map the loci affecting resistance to SDS and soybean cyst nematode (SCN) using phenotypic data in previous studies (Iqbal et al., 2001; Njiti et al., 2002; Kazi et al., 2008). The soybean cultivars 'Forrest', 'Hartwig' and 'Pyramid' showed a partial field resistance to SDS whereas the cultivars 'Essex', 'Flyer' and 'Douglas' were more susceptible to SDS (Lightfoot, 2015). The ten different SSRs that were polymorphic and linked to different SDS QTL were selected to determine whether the QTL associate with four different disease assessments (disease incidence, foliar scorch, disease severity, and area under the disease progression curve; Luckew et al., 2013). Among the ten tested, five QTL were strongly associated with at least one of the four disease metrics in multiple cross populations, providing more information on useful QTL for SDS resistance breeding (Luckew et al., 2013).

The Essex × Forrest (E × F) population was developed to study the inheritance of SDS resistance (Gibson et al., 1994). A major limitation in using the Essex × Forrest (E × F) population in genomics research is the small population size (n = 100) that could preclude fine mapping (Meksem et al., 1999). To overcome this problem, populations of near isogeneic lines (NILs; n < 40) were developed from each RIL (Lightfoot et al., 2005). The residual heterozygosis present in the F5 seed was subsequently fixed as heterogeneity (but not completely) and captured in these NILs. Heterogeneity within the RILs has been measured to be 8%, so each NIL population was expected segregate for about 8% of the genome on a continuing basis (herein the F5:9:13 generation). Residual heterozygosity was estimated at about 1% (Triwitayakorn et al., 2005) based on the ability to find heterozygous plants at Satt309. NIL populations derived from E × F RIL34 (EF34 lines 1-40) and E × F RIL11 (EF11 lines 1-40). Hundreds of their progeny, were used to fine map and isolate the receptor like kinase underlying cq*Rfs2* (Triwitayakorn et al., 2005; Srour et al., 2012). The forty NILS were estimated to be equivalent to a 480 line RIL population in the 8% of regions that were not fixed. However, to date many NIL populations derived from lines within the E × F RIL population have not been studied thoroughly. Further field trials, greenhouse assays, and molecular marker techniques, including SNPs, could help to evaluate additional lines for resistance to SDS and develop new cultivars highly resistant to SDS.

Nine QTL were identified using the E × F population (Lightfoot, 2015). Among those, 4 QTL were mapped on Linkage Group G (LG G); chromosome 18 (Chr,18), cqRfs, cqRfs1, cqRfs2, and cqRfs3, respectively. Those 4 QTL were reported to be a cluster of loci for resistance to SDS (Triwitayakorn et al., 2005). Meksem et al. (1999, 2001b) reported that each of the loci were located in 2- to 5- cM intervals and are mutually linked; the partial resistance beneficial alleles on LG G all derived from Forrest (Iqbal et al., 2001; Triwitayakorn et al., 2005; Lightfoot, 2008). Loci cq*Rfs2* (cqSDS002) and cq*Rfs3* were previously fine mapped in NIL populations (Meksem et al., 1999). Loci cqRfs and cq*Rfs1* were not previously fine mapped in NIL populations.

Another locus on Chr.20 (LG I; cq*Rfs5*) was reported (Iqbal et al., 2001; Kassem et al., 2006; de Farias-Neto et al., 2007; Swaminathan et al., 2016). However, the QTL had low value for breeding selections for leaf symptoms but high value for root rot when multiple crosses involving large populations were analyzed (Luckew et al., 2013). Interestingly, the locus was effective against leaf scorch caused by fungal exudates (Swaminathan et al., 2016). The locus cq*Rfs5* was not previously fine mapped in NIL populations.

Here were reported; field trials on a new NIL population EF60 lines 1-38; segregation data for SSR markers near cq*Rfs*, cqRfs1 (closely linked on Chr.18) and cq*Rfs5* (Chr.20) that were associated with resistance to SDS; and genome wide analysis of the NILs using the 5,361 SNP markers developed from Song et

al. (2013). Evidence of inter-locus interaction is presented. SNP based maps of both genomic regions identified small regions encoding putative candidate genes.

Materials and Methods

Plant Material

Essex was crossed with Forrest to generate 100 F5 derived RILs (Lightfoot et al., 2005). The RILs were evaluated for SDS resistance in many field trials from 1994 to 2015. The disease assessment methods used in the studies were described in Njiti et al. (1996). Briefly, disease incidence (DI) was the percentage of plants showing SDS leaf symptom (0-100%), disease severity (DS) was used to assess the severity of the disease (1-9 scale), the collected data were then converted into disease index (DX, DS × DI/9), the data were collected during the R6-R7 stage (full green seed at upper 4 nodes, pods > 1.5 cm) of soybean growth and adjusted to the R6.5. Several NIL populations were selected and generated based on the performance of several of the RILs (9, 11, 34, 60, 77) that appeared to segregate for DS based on a consistent DI score in the range of 10-60% (Matthews et al., 1991; Njiti et al., 1998; Meksem et al., 1999; Triwitayakorn et al., 2005). Some of those NILs segregated for SCN resistance (11 and 34) but some did not (9, 60 and 77). The RIL60 was selected at the F5:9 generation to isolate 40 NIL lines by collecting seed from 40 individual plants at random in disease free plots in 1994. The seed planted were extracted from RIL60 at the $F_{5:9}$ and advanced to the $F_{5:9:13}$ generation following the method of Njiti et al. (1998).

After seed increase the lines were planted in several locations (Carbondale, Carmi, Cora, Harrisburg, Ullin, and Villa Ridge) but significant disease was only found in the plots at the plantings at Harrisburg, Saline County, in 1998; and in Carmi, White County, in 2000. The locations were selected based on historical appearance of SDS symptom. The field trials used the Random Complete Block design (RCBD) with 2 replications at each location. The NILs were evaluated for disease performance using the disease assessment method previously described (Njiti et al., 1996). DX data for two locations are presented herein.

Molecular Marker Analysis

The NIL population EF 60 (lines 1-40) were planted in the greenhouse in 2000 and the leaf tissues were collected for DNA extraction at the F5:9:13 generation. The DNA of the NILs were extracted using the Qiagen (Hilden, Germany) DNAeasyTM kit from leaves of 10 plants per line. The DNA was stored at -20 C until 2014. In early 2014 the DNA samples were tested for polymorphisms with the 10 SSR markers described in Luckew et al. (2013) to determine the polymorphisms among the NIL EF 60 population. A step down Polymerase Chain Reaction (PCR) was modified and performed based on the method described in Luckew et al. 2013. The PCR products were electrophoresed on a 3% (w/v) agarose gel for 3 h and visualized using ethidium bromide. Aliquots of the DNA were shipped to the Soybean Breeding and Genetics lab at Michigan State University for the SoySNP6k Iselect BeadChipTM analysis (Illumina, San Diego, Calif. USA), which consists of 5,361 SNPs (Song et al., 2013; Akond et al., 2013).

Data Analysis

The SNPs significantly associated with the resistance to SDS as judged by the phenotypic data were selected using the R software, basic packages for T-tests. Data analysis was also performed by ANOVA using JMP 11 (SAS Institute, Cary NC, USA). The mean, standard error, and student t-test ($P < 0.05$) was recorded. Two-way ANOVA was used to look for interactions between the regions on Chr. 18 and 20. All the phenotypic traits were analyzed for heritability following the methods described in Kazi et al. (2008).

Table 1. SNP markers and intervals significantly associated with the Harrisburg SDS data. SNP intervals; allelic means and standard errors; probabilities; and variation explained are shown.

Markers and Intervals	LG	Disease index		P > F	R^2 (%)
		Essex Mean	Forrest Mean		
ss715630114 - ss715630131	G	17.2 ± 0.8	12.3 ± 1.1	0.0004	0.161
ss715630160 - ss715630479	G	17.2 ± 0.7	11.8 ± 1.3	0.0007	0.175
ss715630520 - ss715630660	G	17.3 ± 0.7	11.5 ± 1.2	0.0006	0.191
ss715630733	G	17.2 ± 0.7	11.9 ± 1.2	0.0006	0.164
ss715630903 - ss715631000	G	17.3 ± 0.7	11.9 ± 1.2	0.0005	0.173
ss715631531	G	17.3 ± 0.7	11.5 ± 1.2	0.0006	0.191
ss715631642	G	16.3 ± 0.7	12.2 ± 1.5	0.0281	0.054
ss715632529	G	16.5 ± 0.8	13.0 ± 1.1	0.015	0.055
ss715632537 - ss715632542	G	17.3 ± 0.7	12.5 ± 1.2	0.001	0.147
ss715632589 - ss715632835	G	17.1 ± 0.7	13.12 ± 1.2	0.0067	0.097
ss715637419 - ss715637459	I	-	-	0.16	-
ss715637485 - ss715637550	I	17.0 ± 0.7	13.6± 1.2	0.02	0.07
ss715637647 - ss715637657	I	16.7 ± 0.6	12.7 ± 1.7	0.0373	0.07

Table 2. SNP markers and intervals significantly associated with the White County SDS data. SNP intervals; allelic means and standard errors; probabilities and variation explained are shown.

Markers and Intervals		LG	Disease index		P > F	R^2
			Essex Mean	Forrest Mean		
ss715630114	- ss715630131	G	-	-	0.09	-
ss715630160	- ss715630479	G	28.9 ± 2.2	20.2 ± 3.4	0.0365	0.0563
ss715630520	- ss715630660	G	30.5 ± 2.1	17.2 ± 2.9	0.0007	0.136
ss715630733		G	29.9 ± 2.0	17.2 ± 3.2	0.001	0.131
ss715630903	- ss715631000	G	31.3 ± 2.3	16.4 ± 2.7	0.0001	0.187
ss715631531		G	29.9 ± 2.0	17.2 ± 3.2	0.001	0.131
ss715631642		G	-	-	0.0504	-
ss715632529		G	28.9 ± 2.1	20.1 ± 3.2	0.0292	0.047
ss715632537	- ss715632542	G	31.1 ± 2.2	18.8 ± 2.6	0.0007	0.135
ss715632589	- ss715632835	G	31.6 ± 2.2	18.9 ± 2.5	0.0009	0.149
ss715637419	- ss715637459	I	30.9 ± 2.3	21.1 ± 2.6	0.0052	0.08
ss715637485	- ss715637550	I	30.1 ± 2.4	21.1 ± 2.5	0.0091	0.074
ss715637647	- ss715637657	I	29.6 ± 2.1	21.3 ± 3.4	0.0409	0.046

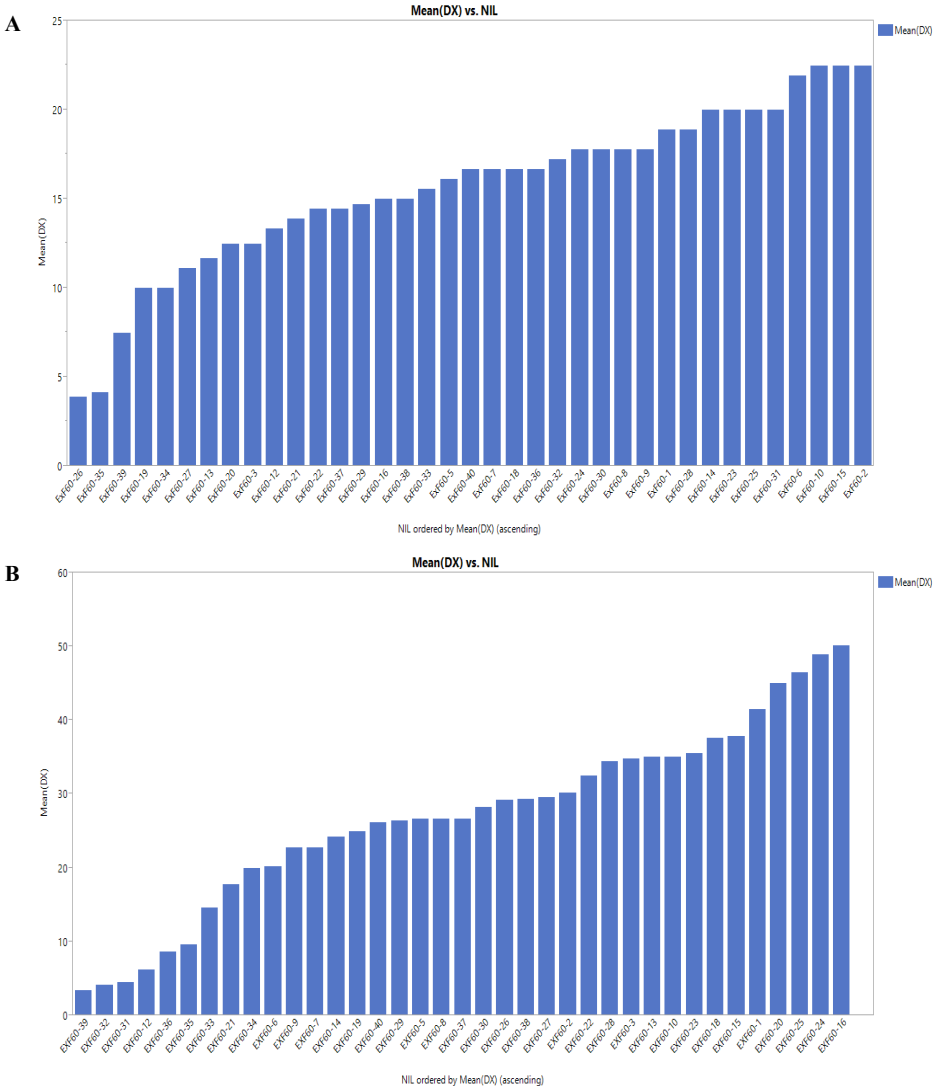

Figure 1. Ranked NILs showing a normal distribution of SDS scores in Harrisburg (A) and Carmi, White County (B).

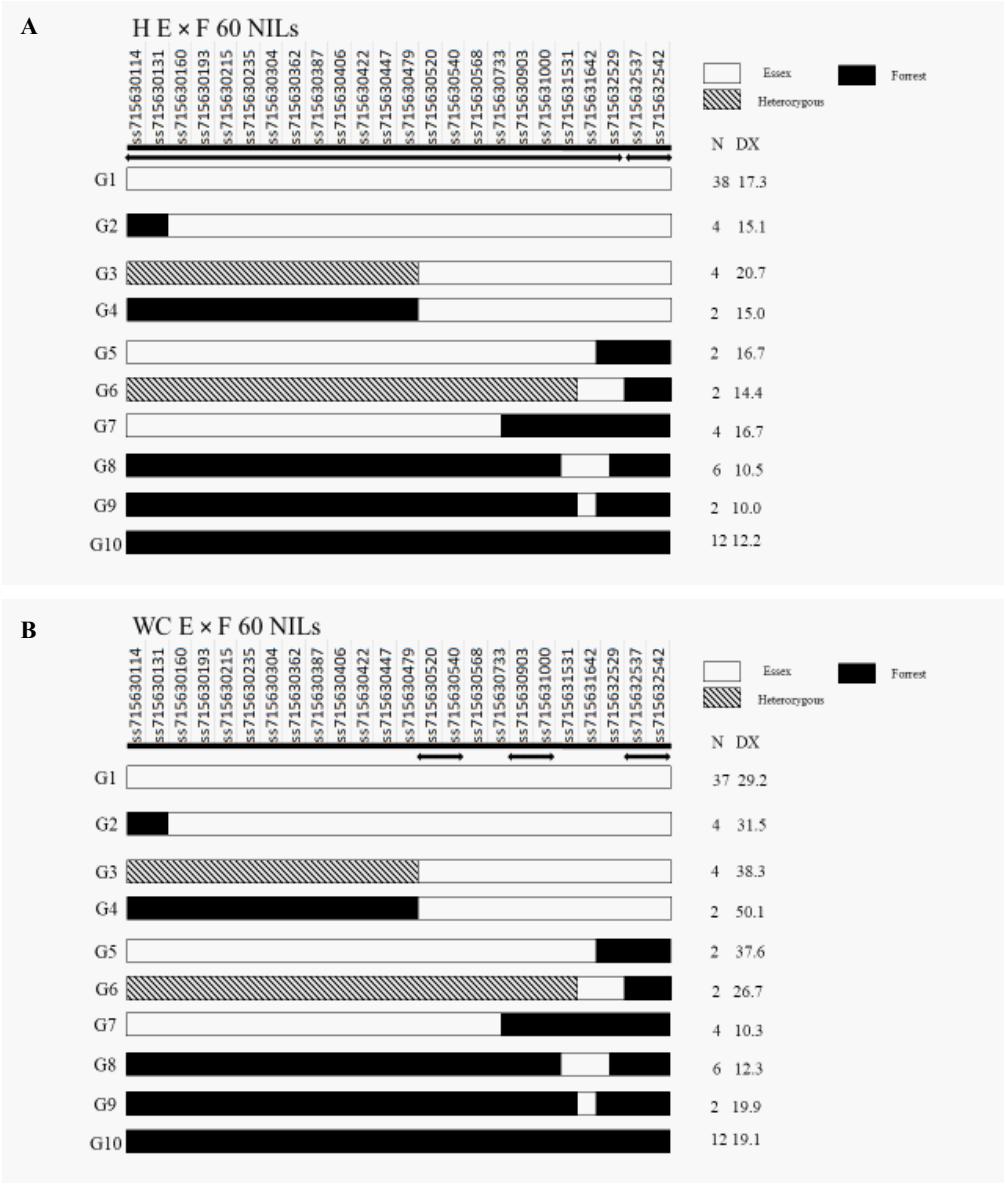

Figure 2. Ranked NILs showing a normal distribution of SDS scores in Harrisburg (A) and Carmi, White County (B).

SNP Physical Maps

The SNPs that associated with the phenotypic data were selected to identify genomic regions and several candidate genes based on their positions in the soybean genome. Those physical maps consisted of the SNPs that were segregating for the susceptible parent (Essex) and the resistant parent (Forrest), the monomorphic SNPs and the heterozygous or heterogenous SNPs.

Results

SDS DX trait data in NIL60 lines 1-40

SDS symptoms were less severe in the two locations in 1998, the highest DX value for Harrisburg was 25 and ARC was 15. The Carmi field trial in 2000 had a more significant SDS disease pressure, 58.1 being the highest DX value but Ridgway had no detectable disease. Therefore, this analysis only used the SDS data from Harrisburg and Carmi, White County. Trait data for SDS showed non-normal distributions for DX (Fig. 1). Frequency distributions of traits showed there was evidence for bi- or tri-phasic distribution. Traits representing field data, like DX, include some error variance that was reduced, but not removed, by replication. Heritability values were moderate for DX (49 and 58%) respectively at each location. Disease was more severe at Carmi (where individual lines DXs ranged from 3-58) than Harrisburg (where the individual lines DX ranged from 4-25). Further, the genotype by environment interactions were significant, so means that pooled both locations data were not used.

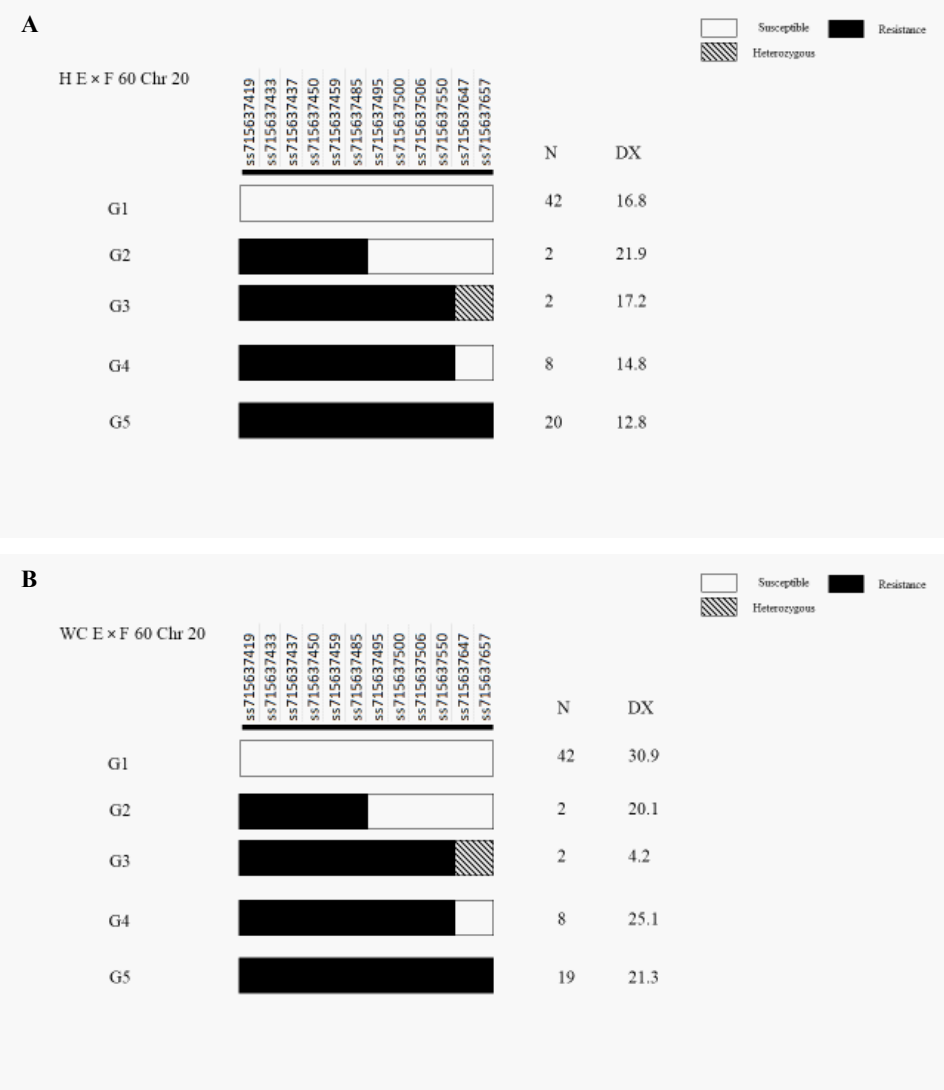

Figure 3. Fine map of the region of chromosome 20 polymorphic in the EF60 NIL populations in Harrisburg (A) and Carmi , White County (B).

DNA Marker Analyses of Polymorphic Regions

Among 5,361 high quality SNP scores made, just 49 of the Chr.18 markers were polymorphic (Supplemental Table 1). Most, 47-48, were significantly associated with the resistance to SDS trait (Fig. 2; Table 1 and 2). They were physically mapped to one region of 73 markers. Regions of monomorphism (15 encompassing the other 24 of the 73 markers within associated regions) were detected. These same regions were also monomorphic between the parents of the population, Essex and Forrest. Therefore, the polymorphic region was divided into 14 regions, separated by the 15 monomorphic regions. Eight of the polymorphic regions showed evidence of residual heterogeneity or heterozygosity suggesting heterozygosity was preserved allowing recombination to continue through many of the 13 generations. Genotype 9 (EF 60-9) appeared to have preserved the most heterogeneity and/or heterozygosity. Three of the regions were confirmed by microsatellite markers showing 2 bands on gels (not shown). Recombination events had generated 10 rare genotype classes (G2-G11 in Fig. 2 and 3) from the 2 common parental plant types (G1 and G12) developed from RIL60, and still found in RIL60. One of the common types (Essex, susceptible type) was more abundant than the other (Forrest, resistant type) suggesting non-intentional selection against resistance had occurred. Three lines (EF 60-4, EF 60-11, and EF 60-17) were determined to be contaminants from another NIL population based on the SNP polymorphism analysis and so were excluded from the analyses. The marker data analyses indicated that there was a region of about 6.2 Mbp where recombination events had occurred (G2-G11) on Chr.18 (Fig. 2; Tables 1 and 2). There were 3 previously reported SSR markers within the region (reviewed by Lightfoot 2015). They were Satt570 (start position 3,162,724 end position 3,162,756 bp); Satt130 (start position 4,639,943 end position 4,640,401 bp); and Sat_403 (start position 6,169,553 end position 6,169,618 bp). Satt570 was previously reported to be linked to cqRfs1. Satt130 and

Table 3. Possible epistatic interactions among the loci on chromosomes 18 and 20 from the means of the two replicates.

WC Epistasis		
	G^F	G^E
I^F	16.9 (N=18)	34.2 (N=7)
I^E	19.9 (N=2)	28.6 (N=28)

H Epistasis		
	G^F	G^E
I^F	11.6 (N=18)	17.1 (N=8)
I^E	10 (N=2)	17 (N=28)

Sat_403 were previously reported to be linked to cqRfs.

Among the 5,361 high quality SNP scores made just 12 of 21 were polymorphic on Chr.20 in the region encompassing cqRfs5 (Supplemental Table 1). Only 4-6 were associated with the SDS trait (Fig. 3; Tables 1 and 2) at one or both locations. Regions of monomorphism (4 encompassing 9 markers) were detected. Essex and Forrest parents were also monomorphic at these same SNPs. There were 5 polymorphic regions. Two of those regions showed evidence of residual heterogeneity. Recombination events had generated 3 rare genotype classes from the 2 common EF RIL60 parental types. The marker data analysis indicated that there was a region of about 1.8 Mbp where recombinant events had occurred on Chr.20 (Fig. 3). There was one SSR marker within the region, Satt354 (start position 33,013,951 end position 33,430,010 bp). Satt354 was previously reported to be linked to cqRfs5 (reviewed by Lightfoot 2015).

SNP based SDS Trait Association Mapping

On Chr. 18 the SNP to SDS score association data from Harrisburg appeared to show two regions associated with SDS DX. One was large (2.7 Mbp), from ss715630114 to ss715631531 (Table 1; $P < 0.007$; n was 37). The region corresponded to the position of cqRfs1. The second was small, from ss715632537 to ss715632542, a 0.5 Mbp region that corresponded to the position of cqRfs.

However, at Carmi there appeared to be three separated significant intervals, mapped between SNP markers ss715630520 to ss715630540; ss715630903 to ss715631000; and ss715632537 and ss715632542 (Table 2; $P < 0.0001$). The, second, middle putative QTL had not previously been detected. These finding suggested that there may be an additional QTL conferring resistance in this region. It might be named Rfs19. It was mapped to a 0.06 Mbp region. The first region was about 0.5 Mbp and corresponded to the position of cqRfs1. The third region was about 0.05 Mbp and corresponded to the position of cqRfs.

On Chr.20 the SNP to SDS score association data from Harrisburg showed weak significance (Table 1) across 2 of the 3 polymorphic regions. However, at Carmi, White County, 2 of 3 polymorphic regions were associated with resistance to SDS. One region was most strongly associated with SDS DX in each location, but they differed. That suggested a 0.4 Mbp region corresponded to the position of cqRfs5, but suggested that interactions were occurring.

QTL Interaction Analyses

The values of the 4 QTL segregating in the NIL population were compared to determine the breeding value of each of the QTL. The loci cqRfs1 and the closely linked (0.2 Mbp; 0.5 cM) Rfs19 explained 14-19% (6-13 DX units) and (6-15 DX) 17-19 % of variation in trait respectively (Table 1 and 2). cqRfs appeared to be a slightly weaker locus that explained just 14-15% of variation (5-12 DX) however it was only 2 Mp (5 cM) away from the other 2 loci on Chr.18. The cqRfs5 locus on Chr.20 was weakest explaining just 4-9% of variation (7-8 DX units). Beneficial alleles were all from Forrest.

Interaction analyses were made using the common parental genotypes and treating the cluster on Chr.18 as a single type (Table 3). Interactions appeared to be significant by two-way ANOVA ($P<0.05$). The most resistant genotype had the Forrest allele on both chromosomes (LGs G and I) when disease was severe. However, the Chr.20 (I) locus appeared to have no value when disease was mild. Notably, the genotype classes GF IE and GE IF were rare (n =2 and 8 respectively at both locations). Selection against those genotypes or drift in a small population may have occurred. Caution should be exercised in interpreting the significance of the QTL interaction data. Further experiments with larger populations are needed.

Discussion

An archived NIL population (EF60 lines 1-40) and its DNA samples were used to confirm the mapping of three loci (cqRfs, cqRfs1 and cqRfs5) within two QTL previously found to underlie resistance to SDS (Iqbal et al., 2001; Kassem et al., 2006; Kazi et al., 2008; Luckew et al., 2013) using newly available SNP markers. However, an apparently additional interval (Rfs19) with highly significant values was dissected within the Chr.18 region based on data from Carmi, White County in 2000. This may be due to the severe disease pressure in Carmi, White County.

NILs showed good variation for SDS DX because they were fixed to susceptible alleles at the 5 other known major loci for resistance to SDS and SCN in E × F (Lightfoot et al., 2005; Lightfoot 2008; 2015). SNP mapping allowed a clearer picture of the recombination events in a NIL population to emerge. The larger region of residual heterozygosity on Chr.18 had the greater number of recombination events in it than the smaller region on Chr.20. The recombination events allowed mapping of the QTL to small intervals (0.04-0.5 Mbp) and suggested the existence of a new QTL in the cluster on Chr.18.

A map of cqRfs (between ss715632537 and ss715632542) was used to infer the location of 7 candidate genes (Glyma.18.g070200 – Glyma.18.g07800) that encoded; three as not yet named proteins with no pFams; a DNA helicase TIP49, which is also a TBP-interacting protein; a non-named protein containing an armadillo/beta-catenin-like repeat; a chaperonin-like

RbcX protein; and a heterogeneous nuclear ribonucleoprotein.

A SNP map of Rfs19 (between ss715630903 and ss715631000) was used to infer the location of 5 candidate genes that encoded; a nodulin like major facilitator superfamily protein; a tetratricopeptide repeat (TPR)-like superfamily protein; an O-sialoglycoprotein endopeptidase in the serine threonine kinase family; an HXXXD-type acyl-transferase family protein; and a mitochondrial ribosomal death-associated protein 3 (Glyma.18g055900 - Glyma.18g056300).

None of these proteins were reported to be, or interact with, known partial resistance genes (Triwitayakorn et al., 2005; Srour et al., 2012). However, the genes that encode them may be good candidates for partial resistance genes and so will be tested in future experiments.

The value of the loci for breeding on the two chromosomes was shown to be unequal, as reported by Luckew et al. (2013). Chr.18 loci were equal in the amount of DX they controlled, but were closely linked so only a few recombinant lines contributed to differences. Chr.18 loci were not associated with resistance to fungal exudates (Swaminathan et al., 2016) so the loci may not underlie toxin resistance. Kazi et al. (2008) found loci in this region controlled root resistance (infection severity) but not leaf scorch in Flyer by Hartwig derived RILs. However, in that population the locus Rfs19 linked to Satt130 was associated with DX but not root resistance. They noted the Satt130 marker was not linked to any other Chr.18 marker. Genome sequence now shows the Satt130 amplicon (gi:14969847) has strong paralogs (5.8e-2 >E<3.9e-4) on 7 other chromosomes (3, 6, 7, 8, 10, 11 and 9) all of which contain at least one QTL. Therefore, it appears the loci detected by Satt130 in E × F and F × H are not on the same Chr. as was inferred previously by Lightfoot (2015).

The Chr.20 locus cqRfs5 controlled much less of the variation in DX than cqRfs, cqRfs1 and Rfs19. However, there were 18 loci associated with resistance to fungal exudates (Swaminathan et al. 2016) in their cross made with a Hartwig derived line (LS94-3207; Schmidt et al., 2004). Therefore, cqRfs5 may underlie toxin resistance. Some evidence for epistasis was found. The Chr.20 locus was only beneficial with the Forrest allele present on Chr.18. In fact the beneficial allele was change when the Essex allele was present on Chr.18. However, the low abundance of 2 of the 4 genotypes weakened those conclusions. The lower abundance of the GF IE and GE IF might have been caused by drift or unintentional selection. In future we will isolate more recombination events in these NIL populations from residual heterogeneity and heterozygosity found. The confirmed QTL have been sent to the Soybean Genetics Committee requesting the assignment of names cqSDS003-006.

Conflicts of Interest

None

Statement of Contributions

Yi Chen Lee carried out the marker work, analyzed the data and made the first draft of the paper. Victor Njiti carried out the field work and made the SDS scores. Javed Iqbal made the NIL DNAs. David. A. Lightfoot and Stella K. Kantartzi edited the paper and added analyses and insights. Stella K. Kantartzi provided the SNP data. Paul Gibson provided the E × F population to us in 1991 and developed the NIL population strategy with his team.

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References

Akond M, S Liu, L Schoener, JA Anderson, SK Kantartzi, K Meksem, Q Song, D Wang, Z Wen, DA Lightfoot DA, and MA Kassem (2013) SNP-based genetic linkage map of soybean using the SoySNP6K Illumina Infinium bead-chip genotyping array. J Plant Genome Sciences 1 (3): 80–89.

Aoki T, K O'Donnell, Y Homma, and AR Lattanzi (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.

Bao Y, JE Kurle, G Anderson, and ND Young (2015) Association mapping and genomic prediction for resistance to sudden death syndrome in early maturing soybean germplasm. Molec. Breed. 35: 128–130.

De Farias-Neto AF, R Hashmi, ME Schmidt, SR Carlson, GL Hartman, S Li, RL Nelson, and BW Diers (2007) Mapping and confirmation of a sudden death syndrome resistance QTL on linkage group D2 from the soybean genotypes 'PI 567374' and 'Ripley'. Mol. Breeding 20: 53–62.

Hartman GL, HX Chang, and LF Leandro (2015) Research advances and management of soybean sudden death syndrome. Crop Prot, http://dx.doi.org/10.1016/j.cropro.2015.01.017.

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

Iqbal MJ, K Meksem, VN Njiti, MA Kassem, and DA Lightfoot (2001) Microsatellite markers identify three additional quantitative trait loci for resistance to soybean sudden-death syndrome (SDS) in Essex · Forrest RILs. Theor Appl Genet 102: 187–192.

Kassem MA, J Shultz, K Meksem, Y Cho, AJ Wood, MJ Iqbal, and DA Lightfoot (2006) An updated 'Essex' by 'Forrest' linkage map and first composite interval map of QTL underlying six soybean traits. Theor Appl Genet 113: 1015–1026.

Kazi S, J Shultz, J Afzal, J Johnson, VN Njiti, and DA Lightfoot (2008) Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome. Theor Appl Genet 116: 967–977.

Lightfoot DA, VN Njiti, PT Gibson, MA Kassem, MJ Iqbal, and K Meksem (2005) Registration of Essex x Forrest recombinant inbred line (RIL) mapping population. Crop Sci 45: 1678–1681. doi:10.2135/cropsci2004.0279.

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

Lightfoot DA (2015) Two decades of molecular marker-assisted breeding for resistance to soybean sudden death syndrome. Crop Sci 55:1460–1484 (2015). doi: 10.2135/cropsci2014.10.0721.

Luckew A, L Leandro, MK Bhattacharyya, D Nordman, DA Lightfoot,

and S Cianzio (2013) Usefulness of 10 genomic regions in soybean associated with sudden death syndrome
resistance. Theor Appl Genet 126: 2391–2403. doi:10.1007/s00122-013-2143-4.

Matthews WJ, VN Njiti, PT Gibson, and MA Shenaut (1991) Inheritance of soybean SDS response in segregating F5 and F6 derived lines. Soy Genet Newsl 18: 102–108.

Meksem K, TW Doubler, K Chancharoenchai, VN Njiti, SJC Chang, AP Rao-Arelli, PE Cregan, LE Gray, PT Gibson, and DA Lightfoot (1999) Clustering among loci underlying soybean resistance to Fusarium solani, SDS and SCN in near-isogenic lines. Theor Appl Genet 99: 1131–1142.

Meksem K, P Pantazopoulos, VN Njiti, DL Hyten, PR Arelli, and DA Lightfoot (2001) 'Forrest' resistance to the soybean cyst nematode is bigenic: saturation mapping of the Rhg1 and Rhg4 loci. Theor. Appl. Genet. 103: 710–717.

Njiti VN, MA Shenaut, RJ Sutter, ME Schmidt, and PT Gibson (1996) Soybean response to soybean sudden-death syn¬drome: inheritance influence by cyst nematode resistance in Pyramid x Douglas progenies. Crop Sci 36: 1165–1170.

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

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

Song Q, DL Hyten, G Jia, CV Quigley, EW Fickus, RL Nelson, and PB Cregan (2013) Development and evaluation of SoySNP50K, a high-density genotyping array for soybean. PLoS ONE 8(1): e54985. doi:10.1371/journal.pone.0054985.

Srour AY, AJ Afzal, N Saini, L Blahut-Beatty, N Hemmati, DH Simmonds, W Li, M Liu, CD Town, H Sharma, PR Arelli, and DA Lightfoot (2012) The receptor like kinase transgene
from the Rhg1/Rfs2 locus caused pleiotropic resistances to soybean cyst nematode and sudden death syndrome. BMC Genom 13: 368–378. doi:10.1186/1471-2164-13-368.

Swaminathan S, NS Abeysekara, M Liu, SR Cianzio, and MK Bhattacharyya (2016) Quantitative trait loci underlying host responses of soybean to *Fusarium virguliforme* toxins that cause foliar sudden death syndrome. Theor Appl Genet 129 (3): 495-506.

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

Wen Z, R Tan, J Yuan, C Bales, W Du, S Zhang, MI Chilvers, C Schmidt, Q Song, PB Cregan, and D Wang (2014) Genome-wide association mapping of quantitative resistance to sudden death syndrome in soybean. BMC Genom 15: 809-817 doi: 10.1186/1471-2164-15-809.

Wrather JA and SR Koenning (2009) Effects of diseases on soybean yields in the United States 1996 to 2007. Plant Health Progr doi:10.1094/PHP-2009-0401-01-RS.

Yuan J, R Bashir, G Salas, H Sharma, AY Srour, and DA Lightfoot (2012) New approaches to selecting resistance or tolerance to SDS and fusarium root rot. J Plant Genom Sci 1:10–17.