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Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome.

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Abstract.

Soybean [*Glycine max* (L.) Merr.] cultivars show differences in their resistance to both the leaf scorch and root rot of sudden death syndrome (SDS). The syndrome is caused by root colonization by *Fusarium virguliforme* (ex. *F. solani* f. sp. *glycines*). Root susceptibility combined with reduced leaf scorch resistance has been associated with resistance to *H. glycines* HG Type 1.3.6.7 (race 14) of the soybean cyst nematode (SCN). In contrast, the *rhg1* locus underlying resistance to Hg Type 0 was found clustered with three loci for resistance to SDS leaf scorch and one for root infection. The aims of this study were to compare the inheritance of resistance to leaf scorch and root infection in a population that segregated for resistance to SCN and to identify the underlying quantitative trait loci (QTL). 'Hartwig', a cultivar to partially resistant to SDS leaf scorch, *F. virguliforme* root infection and SCN HG Type 1.3.6.7 was crossed with the partially susceptible cultivar 'Flyer'. Ninety two F5-derived recombinant inbred lines (RILs) and 144 markers were used for map development. Four QTL found in earlier studies were confirmed. One contributed resistance to leaf scorch on linkage group (LG) C2 (Satt277; P = 0.004, R^2 = 15%). Two on LG G underlay root infection at R8 (Satt038; P= 0.0001 R^2 = 28.1%; Satt115; P= 0.003 R^2 = 12.9%). The marker Satt038 was linked to *rhg1* underlying resistance to SCN Hg Type 0. The fourth QTL was on LG D2 underlying resistance to root infection at R6 (Satt574; P= 0.001, R^2 = 10%). That QTL was in an interval previously associated with resistance to both SDS leaf scorch and SCN Hg Type 1.3.6.7. The QTL showed repulsion linkage with resistance to SCN that may explain the relative susceptibility to SDS of some SCN resistant cultivars. One additional QTL was discovered on LG G underlying resistance to SDS leaf scorch

measured by disease index (Satt130; P= 0.003 R^2 = 13%).The loci and markers will provide tagged alleles with which to improve the breeding of cultivars combining resistances to SDS leaf scorch, root infection and SCN HG Type 1.3.6.7.

Introduction

Among the top four loss causing diseases of soybean [*Glycine max* (L.) Merrill.], worldwide were the root rot and leaf scorch called Sudden Death Syndrome (SDS; Wrather et al. 1996; 2003). Over a five year period 1999-2004 average losses around 1% or 0.9 million Mg per harvest, worth $190 million a year, were reported. The syndrome was accurately predicted to intensify and spread over the next 20 years (Scherm and Yang, 1996). Improved genetic resistance in germplasm releases will be key to containing soybean losses to SDS (Gibson et al. 1994; Kazi et al, 2007).

SDS was shown to be caused by the blue-pigmented soil borne fungus *Fusarium virguliforme* (Aoki et al. 2003; ex. *Fusarium solani* (Mart.) Sacc. f. sp. *glycines*; Fsg; Roy 1997). *F. virguliforme* is a member of an evolutionary group known as the "*F. solani* complex" that colonize a wide variety of habitats and hosts (Gray et al. 1999; O'Donnell, 2000). They are serious pathogens of many crops. Analysis in North America showed that only *F. virguliforme* prompted the symptoms of SDS on soybean but in South America two separate species, *F. tucumaniae* and *F. virguliforme*, were both responsible for SDS (Aoki et al. 2003; Covert et al. 2007).

The genetics of resistance to SDS is complex. Stephens et al. (1993) reported that a single dominant gene, *Rfs* controls SDS resistance in 'Ripley' soybean in greenhouse conditions. In contrast, the 'Essex' by 'Forrest' (ExF) population (Hnetkovsky et al. 1996; Chang et al. 1996; Kassem et al. 2006) showed that the SDS resistance was conditioned by several quantitative trait loci (QTL). By 2007, more than twenty detections of QTL for resistance to SDS have been reported among eight different recombinant inbred line (RIL) populations (Supplementary Table 1). By assigning QTL

detected in overlapping intervals to the effect of a single locus, the QTL may be assigned to as few as 12 *qRfs* loci on nine linkage groups (LGs) including A2, C2, D2, F, G, I, J, L and N. The map of ExF showed three (Kassem et al. 2006) or four QTL (Iqbal et al. 2001) that mapped to LG G and one on each of LGs C2, F, J, I, L and N (*qRfs*1 to *qRfs* 9).

Some QTL for resistance to SDS have been confirmed and suffixed *cqRfs*- (Triwitayakorn et al. 2005; Lightfoot 2008). The confirmed QTL either mapped to a similar location in separate populations or were mapped for a second time in near isogeneic lines (NILs) derived from RILs segregating across regions that encompassed the QTL. The confirmed QTL include C2 (Hnetkovsky et al. 1996; Njiti et al. 1998; 2002), one on D2 (Lightfoot et al. 2001; Farias-Neto et al. 2007), three all on G (Prabhu et al. 1999; Iqbal et al. 2001; Njiti et al. 2002), J (Sanitchon et al. 2004; Kassem et al. 2006) and N (Njiti et al. 2002; Hashmi 2004). The ExF QTL on F and I (Iqbal et al. 2001) were not yet confirmed by association in a second population by late 2007. Similarly not confirmed to date were the QTL found on A2 in Ripley by 'Spencer' (Hashmi 2004; Farias-Neto et al. 2007); L in 'Minsoy' x 'Noir 1' (Njiti and Lightfoot 2006) and H in ExF grown in Argentina (Bashir, 2007).

Some cultivars of soybean have a dual resistance to SDS leaf scorch and root infection by the causal organism, *F. virguliforme* that was consistent in both field and greenhouse (Njiti et al. 1997; 2001; 2003; Hartman et al. 1997). Among dually resistant lines are Forrest, 'Hartwig', 'Jack', Ripley and several commercial lines. Most of the dually resistant lines are also resistant to *Heterodera glycines* HG Type 0 (race 3) of the soybean cyst nematode (SCN). Subsequently, linkage and pleiotropy with loci underlying

resistance to SDS have been detected at the SCN resistance locus *rhg1* but not *Rhg4* (Meksem et al. 1999; Triwitayakorn et al, 2005; Ruben et al. 2006).

In contrast, cultivars that show root susceptibility to *F. virguliforme* combined with SDS leaf scorch resistance (like 'Pyramid', 'Fayette' and 'LS92-1920') have been associated with resistance to *H. glycines* HG Type 1.3.6.7 (race 14) of SCN (Gibson et al. 1994) across a wide collection of germplasm. Consequently, repulsion linkage and/or pleiotropy is expected with loci underlying resistance to SDS at loci that underlie resistance to Hg Type 1.3.6.7 (Webb et al. 1995; Lightfoot et al. 2001; Schuster et al. 2001; Concibido et al. 2004).

Preliminary separation of loci underlying root and leaf resistance used near isogeneic lines (NILs) to show a single root resistance locus in Forrest (*cqRfs1*, requested to be renamed *cqSDS-003*) was about 10 cM from cq*Rfs2/rhg1* gene cluster that separately conferred partial resistance to SCN and SDS leaf scorch (Njiti et al. 1998; Meksem et al. 1999; Triwitayakorn et al. 2005; Supplementary Table 1). The other loci on G (*cqRfs2*; or *cqSDS-002*) the locus *Rhg4* on LG A2 and the locus on C2 (*cqRfs4*; or *cqSDS-004*) were shown to have no effect on root infection (Njiti et al. 1998; Triwitayakorn et al. 2005).

The cultivar Hartwig was resistant to both leaf scorch and root rot (Wrather et al. 1995; Njiti et al. 1997; 2001; Mueller et al. 2003) and HG Type 1.3.6.7 (race 14) of SCN. Therefore, Hartwig might contain superior alleles underlying a combined SCN and SDS resistance. Cultivar Flyer was susceptible to SCN and both leaf scorch and root rot of SDS (Njiti et al. 1997; 2001). Recombinant inbred lines were developed from the cross of Flyer by Hartwig (FxH), released (Kazi et al. 2007) and used for preliminary QTL

detection (Prabhu et al. 1999). A locus for resistance to root infection (*Rfs1*) was detected on LG G in the same interval as *rhg1* but not *Rhg4* in ExF (Prabhu et al. 1999; Supplementary Table 1).

The mechanisms underlying resistance to root infection by *F. virguliforme* appear to include the increases in the abundance of transcripts encoded by stress- and defense-related genes (Iqbal et al. 2005). The response, over time, prevents the inhibition of cellular transcription found in susceptible roots. In turn, the *F. virguliforme* genome encodes several pathogenicity factors found in other plant pathogenic species within the section Martiella of the genus *Fusarium* (Dr. K. Meksem, SIUC, personal communication; and Dr. S. Covert, University of Georgia, personal communication 2007). These general plant pathogen responses might underlie the association between resistance to SCN and SDS. However, other mechanisms of resistance do operate. For example, since the pathogen is active in lignin degradation (Lozovaya et al. 2005), plant processes related to isoflavonoid production (Iqbal et al. 2003; Lozovaya et al. 2004), lignin deposition or modification (Triwitayakorn et al. 2005) might help prevent infection.

Mechanisms for leaf scorch development were expected to include infection rate and pathogen load (Njiti et al. 1997; 1998; Lightfoot et al. 2007). However, there is evidence that genotypes with root resistance in the absence of sufficient leaf scorch resistance alleles show unusually high leaf scorch indices (Triwitayakorn et al. 2005). Involved in the leaf scorch are at least 4 different toxins (Baker and Nemec 1994; Jin et al. 1996; Ji et al. 2006; Dr. M. Bhattacharryya, Iowa State University, personal communication 2007). Production, excretion, translocation, uptake and metabolism of the

toxins are all stages at which plant genetic diversity might act. SCN infestion might indirectly alter toxin responses by weakening the plants or altering translocation.

To explore the genetic relationship between root and leaf resistance to SDS and known loci for resistance to SCN this paper reports the identification of QTL underlying the inheritance of resistance to leaf scorch and root infection from a SCN Hg Type 0 and 1.3.6.7 resistant cultivar.

Materials and Methods

Plant Material

The genetic material used in this study consisted of 92 FxH F_5-derived recombinant inbred lines (RILs; Yuan et al. 2002). The population was advanced from the $F_{5:7}$ to the $F_{5:14}$ from 1997-2005. Seeds were released in 2006 (Kazi et al. 2007). Hartwig was resistant to the leaf scorch of SDS in nearly every replicate plot at all locations and the roots also appeared to be resistant to infection by *F. virguliforme* (Gibson et al, 1994; Wrather et al. 1995; Njiti et al. 1997; 2001; Mueller et al. 2003). However, the SDS resistance of Hartwig was partial and could be defeated by heavy fungal infestations (Njiti et al. 2001; Lightfoot et al. 2007). Hartwig was strongly resistant to most HG Types of SCN (Anand 1992; Niblack et al. 2003). Flyer was susceptible to most SCN HG Types and partially susceptible to SDS (McBlain et al. 1990; Gibson et al. 1994; Njiti et al. 1997; 2001; Yuan et al. 2002; Kazi 2005). Roots of Flyer did not appear to be resistant to infection by *F. virguliforme* (Njiti et al. 2001). However, Flyer was not completely susceptible to SDS (Gibson et al. 1994).

SDS Disease Evaluation

In 1997 fifty lines were selected in four groups based on the genotype at *rhg1* and *Rhg4* judged by DNA markers (Prabhu et al. 1999). Selected from the larger population were 12 lines with genotype H/H, 11 with H/F, 9 with F/H and 18 with F/F (at Satt038/BLT65). Selection was necessary because the root infection assay is labor intensive and because segregation distortion was observed at the *rhg1* locus in FxH. The lines were planted in SDS infested environments at Ullin (U) and Ridgway (R). The lines were chosen to reduce the cost of root infection assays. For disease rating RILs were

planted in a randomized complete block design (RCBD), 2-row plots and two replications. Disease incidence (DI), disease severity (DS) and root infection severity (IS) were measured. However, sufficient leaf scorch symptoms (DI and DS) to distinguish among genotypes did not develop due to insufficient rainfall during the growing seasons 1997-1999. In contrast, the IS was sufficient to separate genotypes at both locations (Prabhu et al. 1999) in 1997.

In 2000 the population was again planted at the ARC (Carbondale, IL) and Ullin in SDS infested fields. Severe leaf symptoms developed that allowed DI, and DS to be measured and disease index (DX) to be calculated. Measurements of SDS DX and IS followed Njiti et al. (1998) as modified by Triwitayakorn et al. (2005). To provide accurate scores of SDS leaf scorch, adjustment to maturity dates of individual lines was critical (Hnetkovsky et al. 1996; Njiti et al. 1997). Therefore, the days after planting to maturity were measured for each line from growth stages R5 to R8 (Fehr and Caviness 1977). SDS leaf scorch DI was rated 0% (no disease) to 100% (death of all plants). Scores were taken within the R5 to R6 and R6 to R7 transitions and was interpolated to the estimated R6 by linear regression (Hnetkovsky et al. 1996; Njiti et al. 1996). SDS leaf scorch DS was rated between 1 and 9, where 1=0-10% chlorosis or 1-5% necrosis and 9=premature death of plants and was adjusted to the R6. DX was calculated as DI*DS/9 after the maturity adjustments.

The IS was the mean percentage (0-100) of taproot slices with detectable *F. virguliforme* evident on restrictive media (Prabhu et al. 1999). The IS was measured in taproots recovered at both the R6 and R8 stages of growth (Njiti et al. 1997; 1998; 2003; Prabhu, 1999; Triwitayakorn et al. 2005) and was determined from 100 slices per

genotype per plot per location (36,800 slices were scored from RILs during the experiment). Several traits including seed yield in non-infested locations and resistance to SCN HG Type classifications for the RILs were as recorded as described in Yuan et al. (2002).

DNA Marker Analysis

DNA was extracted and used for microsatellite amplifications as in Yuan et al. (2002) with the following modifications. More than 350 BARC-Satt markers with either di- or tri-nucleotide repeat microsatellite markers from all 20 LGs were selected for polymorphism tests. Most (250) of the BARC-Satt markers were chosen to be spaced at 10 cM intervals from the soybean genetic map (Song et al. 2004). In addition, 140 SIUC-BES-SSR primers from the build 2 MTP BES clones (Shultz et al. 2006ab; 2007) were chosen to be spaced at 10,000 kbp intervals from the soybean physical map (Shultz et al. 2006ab; 2007). Amplification reactions for RILs were performed after Shultz et al. (2007) with no modifications.

Heritability Estimation

The heritability (h^2) estimates, a ratio of genotypic variation over phenotypic variation of SDS, were calculated using variance components obtained through ANOVA as described in Fehr (1987). Due to the low frequency of heterozygosity at the $F_{5:11}$, the genetic variance is almost entirely an additive and additive x additive interaction. Therefore the heritability estimate was considered narrow sense. All correlations were calculated using the PROC CORR function of SAS (SAS Institute, Cary, NC).

Construction of the Genetic Linkage Map

A linkage map was created using MAPMAKER/EXP 3.0 (Lander et al. 1987). Map distances between linked markers were calculated in centimorgans (cM, Haldane units) to construct a linkage map (heterogenous lines were excluded). The recombinant inbred line (RI-selfing genetic model) was used. The $\log_{10}$ of the odds ratio (LOD) for grouping markers (threshold) was set at 3.0, maximum distance was 50 cM. A maximum likelihood map was computed with error detection. The microsatellite markers used in this study have been mapped (Song et al. 2004) in other soybean populations that form a composite map. Therefore, most markers were anchored on the LGs on the basis of the locations expected from the composite map. Conflicts among the positions of linked markers in FxH were resolved in favor of experimental evidence when the maps generated at LOD 3.0 disagreed with the composite map of Song et al. (2004) because most markers do have homeologous loci in soybean (Shultz et al. 2006a).

Construction of QTL Maps

A. Single Point Analysis

For line mean comparisons, the data were subjected to analysis of variance (ANOVA; SAS Institute Inc., Cary, NY), with mean separation by LSD as described by Njiti et al. (1998). Markers were compared with SDS response measures by the F-test of ANOVA. The heterogeneous lines were excluded.

For SDS DX a significant difference ($P < 0.005$) was considered to be a preliminary indication of an association between a marker and a QTL for the trait in

question. A value of $P \leq 0.0005$ was suggested by an approximate Bonferroni correction ($P<0.05/100$) for the set of about 100 independent (unlinked or >10 cM apart) DNA markers (from the 144 mapped). However, at genomic regions where gaps between adjacent markers were greater than 10 cM in the map associations $0.005>P>0.0005$ were accepted as a potentially significant association. If the interval was large or was flanking a single marker the uncorrected P value of <0.05 was accepted. Precedents with first-pass mapping of other quantitative traits (Hnetkovsky et al. 1996, Chang et al. 1996; Njiti et al. 2002) have shown these criteria to be valid during the later saturation mapping of the intervals that were inferred at marginal P values (Njiti et al. 1998; Meksem et al. 2001; Yuan et al. 2002; Triwitaykorn et al. 2005; Ruben et al. 2006).

B. Interval Maps of QTL

The maps of all the linked markers and trait data were simultaneously analyzed with Mapmaker/QTL 1.1 using the F_2-backcross genetic model for trait segregation (Chang et al. 1996, Njiti et al. 2002). Putative QTL were inferred when LOD scores exceeded 2.0 at some point in each interval. LOD 2.0 was empirically determined to be equivalent (but not equal) to a single marker $P < 0.005$. The position of each QTL was inferred from the LOD peaks at individual loci detected by maximum likelihood tests at positions every 2 cM between adjacent linked markers.

C. Composite Interval Maps of QTL

For more accurate location of QTL among sets of linked markers, the composite interval map (CIM) function of WinQTL Cartographer (version 2.5) was used (Jansen and

Stam 1994; Basten et al. 2001). Following Kassem et al. (2006) a walk speed of 2 cM and the forward regression method were selected. QTL were inferred when LOD score peaks exceeded 2.0 for the traits studied, considering a $P < 0.05$ corrected for the use of about 100 independent markers. To confirm linkage, experiment-wise threshold was calculated from 1,000 permutations of each genotype marker against the phenotype in the population.

Results

Polymorphism and Linkage

One hundred and forty four markers (Supplemental Data Table 2) were found to be polymorphic within the Flyer x Hartwig (FxH) RIL population. Of those 104 were BARC- simple sequence repeats, 15 were BAC derived SSRs from different contigs and 23 were BAC derived SSRs from 11 contigs that contained loci syntenic with *rhg1* (3) or *Rhg*4 (8) and 2 SCARs were from genes in the loci (*rhg1* TMD1 and *Rhg4* BLT65). For IM just 61 loci mapped to 15 different LGs (Song et al. 2004) that encompassed just 534 cM (382 cM for CIM). Therefore, weakly linked markers (between LOD 1.5 to 2.9), unlinked markers and single marker ANOVAs were important for sampling genomic regions during QTL detection (see Supplemental Data Table 2). Assuming 10 cM as a distance for QTL detection by an unlinked or flanking marker, the fifteen LGs and the 81 unlinked markers would allow the detection of QTL associated with SDS resistance over about 1,971 cM using single point analysis.

Frequency distributions of SDS mean DX

DX at two locations (R00 and ARC00) showed similar severity with uni-modal and relatively normal ($P<0.01$) distribution so data were pooled and means used for further analyses. The distribution of mean DX was positively skewed (1.32) towards resistance. The distribution was continuous and had a significant kurtosis (0.88) that reflected a peaked distribution (Figure 1). The mean, R6 adjusted, DX distribution ranged from negative 15.1 to positive 56.4%. The DX for Flyer was 31.5% and for Hartwig was 0%. The three most resistant and seven most susceptible lines were significant ($P < 0.05$) transgressive segregants. The lines with negative DX after adjustment to the R6 maturity date (less than Hartwig) were all lines that matured earlier than Hartwig.

Frequency distribution of IS

Mean IS at two locations across two years among the 50 recombinant inbred lines selected from FxH92 were used for QTL detection (Prabhu et al. 1999). The R6 and R8 data were not pooled for mapping because examination of both mean values and rank correlations across sampling dates, replicates and locations showed significant differences related to the temporal development of resistance (Table 1; Njiti et al. 1997; Iqbal et al. 2005). The frequency distribution of IS was continuous, not normal, kurtosis varied in direction and scale (Figure 2). The IS ranged from 3.3 to 84.7%. IS for Flyer ranged from 24-70% and for Hartwig 16-42%. Six lines were significantly more resistant than Hartwig and eight were significantly more susceptible than Flyer.

Heritability estimates

The heritability estimates for mean SDS DX was 80%. This high value reflected the concordance between locations and severity of SDS. The heritability estimate for mean IS at R6 was 56% and mean IS at R8 was 49%. The lower values reflected the different severities at the locations and sampling dates, particularly the low severity at R6 at Ridgway (Supplementary Table 2). However, within R stage the genotype x environment (GxE) interaction was not significant and was used as the justification to use the mean data (Prabhu et al. 1999).

Correlations among traits

The correlation method was used to measure the relationships between SDS and the SCN and seed yield of Yuan et al. (2002). The DX scores for each genotype at each location were highly correlated (R = 0.99). Also, SDS resistance as measured by mean IS at R8 and mean DX were correlated ($R = 0.37$) suggesting mean DX was a trait only partly dependent on mean IS (Supplementary Figure 1). Rank correlations between DX and IS also showed partial dependency of DX on IS. Among environments the correlations between IS and DX also varied. Consequently selection of the top 10 lines for root resistance by mean IS recovered only five of the best ten lines for mean DX and vice versa. Therefore, separate selection for both traits will be necessary to improve germplasm for resistance to SDS (Lightfoot et al. 2007).

There was no correlation with mean seed yield in non-infested locations (Yuan et al. 2002) and any SDS trait. Therefore, in this population neither leaf scorch nor IS resistance genes caused significant reductions in seed yield in non-infested locations. This was an important result because it suggested that in most genotypes the presence of

genes conferring resistance to leaf scorch, and to root IS were not associated with any deleterious effects on seed yield. In contrast, resistance to IS at R8 among the ten most resistant lines was significantly associated with more yield depression than at IS-R6 or DX.

SCN responses for lines in this population were measured previously (Yuan et al. 2002) with the AP3 isolate of HG Type 0 (race 3) and the AP 14 isolate of HG Type 1.3.6.7 (race 14). Root infection measured as IS at R6 and R8 were both strongly correlated with SCN HG Type 0 resistance among the FxH RIL population ($R = 0.71$ to 0.75) whereas SDS DX was weakly correlated ($R = 0.31$).The correlations of resistances to SDS and SCN in Hartwig may reflect both the close linkage (2-3 cM) of *qRfs1* to *rhg1*and the clustering (less than 0.25 cM) between *qRfs2* and *rhg1* found in resistant cultivars Forrest (Triwitayakorn et al. 2005; Ruben et al. 2006) and Pyramid (Njiti et al 2002).

The correlations of resistance to mean IS metrics with responses to SCN HG Type 1.3.6.7 (race 14) was significant but less strong ($R = 0.27$ for ISR6; 0.43 for ISR8) but were significant. SDS DX was not significantly associated ($R = -0.06$ DX). The association between susceptibility to SDS and SCN race 14 resistant germplasm reported previously in cultivars derived from PI88788 (Gibson et al. 1994; Njiti et al. 2002) was evident in lines that derived from Hartwig. Therefore, the recombination events between loci conditioning SCN Hg Type 1.3.6.7 and resistance to SDS may be useful for breeding dually SDS and SCN resistant cultivars.

Consistent with the correlations between SDS and SCN scores the best line judged by DX (FxH13) ranked fourth by IS at R6, was HG Type 0 resistant and partially

resistant to HG Type 1.3.6.7. However, the best ranked line with HG Type 1.3.6.7 resistance (FxH33) was also best ranked by IS at both R6 and R8 but ranked fifteenth by DX. In view of the correlations resistance to *F. virguliforme* infection may be co-inherited with both resistance to SCN Hg Type 1.3.6.7 reproduction and susceptibility to SDS leaf scorch.

Significant genomic regions for SDS mean DX

Two regions significantly associated with resistance to leaf scorch were detected based on the markers used. One region was detected on LG C2 that was associated with mean SDS DX across two environments (Table 2). The region on LG C2 (Figure 3) of about 13 cM between the microsatellite markers BARC_Satt277 ($P = 0.004$, $R^2 = 14.8\%$) and BARC_Satt079 ($P = 0.003$, $R^2 = 9\%$) encompassed the QTL detected by CIM. The interval had a peak-LOD score of 2.7 and explained about 24.1% of the total variation in SDS DX. The region derived the beneficial allele from Flyer that reduced DX by about 20%. The locus was significant for DX, DI and DS at both locations ($0.001 < P < 0.04$). The locus was located between 108 and 118 cM on the composite map and therefore may be *cqRfs4* (Supplementary Table 1) the same locus that was detected with a beneficial allele from susceptible parents Essex; crossed with Forrest (ExF94; Hnetkovsky et al. 1996); and 'Douglas'; crossed with Pyramid (PxD90; Njiti et al. 2002).

The second locus underlying SDS DX variation was detected by BARC_Satt130 (Table 2). The marker did not have any significantly linked marker in the FxH RIL set. Satt130 was significantly associated with mean DX ($P = 0.003$, $R^2 = 12.9\%$) and DX, DI and DS ($0.003 < P < 0.04$) at each location. The locus identified derived the beneficial allele from Hartwig. The common allele of Satt130 was normally found on

LG G at 20 cM on the composite map (Song et al. 2004; Figure 3). However, in FxH the marker was not part of LG G and was not linked to Satt038, Satt324 or Satt275 of the composite map flanking markers mapped in FxH nor in any of the markers from other LGs. Therefore, in the FxH population, Satt130 may identify either *cqRfs1* (Supplementary Table 1), a new locus on LG G or a paralog of the marker found on the composite map located on a yet unknown linkage group (Shultz et al. 2006a).

The amount of variation in SDS DX explained by the markers was significant. However, the two regions jointly contributed only about 31% of the total variation compared to trait heritability across locations of 80%. Therefore, both markers more closely linked to the QTL and additional loci for resistance to SDS leaf scorch remain to be discovered in this population.

Significant genomic regions for mean IS at R6 and R8

A QTL for resistance to root infection for the R6 sampling was identified by BARC_Satt574 ($P = 0.001$, $R^2 = 10\%$) that derived the beneficial allele from Flyer and reduced IS by about 13% (Table 2). The linked (15 cM) marker BARC_Sat_001 ($P = 0.005$, $R^2 = 6.1\%$) was also found associated with mean IS at R6 (Figure 3). The markers were weakly associated with leaf scorch metrics DX, DI and DS and their means ($0.01 < P < 0.04$) in each location. The interval had a peak-LOD score of 3.0 and explained about 25% of the variation in SDS IS by CIM (Table 2). The locus was located on LG D2 of the composite map between 87 and 92 cM and so is likely to be *cqRfs11* (Supplementary Table 1) found in Pyramid by Lightfoot et al. (2001). The locus may also be the same as cqSDS001 reported by Farias-Neto et al. (2007).

Satt038_2, the single marker that identified the QTL on LG G reported by Prabhu et al. (1999; Table 2) was here placed between Satt309 and Satt610 by Mapmaker. That location was not the usual position for Satt038_1 on the composite map but was the location expected for *cqRfs1* (Supplementary Table 1). The marker was strongly linked (LOD > 3.0) to Satt309 (4.5 cM), TMD1 (5.0 cM), Satt 275 (8 cM) and Satt163 (9 cM). Those markers also showed significantly skewed segregation ratios away from the expected 1:1 ratio, with Hartwig alleles in the minority as had Satt038_2 in a larger population (Prabhu et al. 1999). However, Satt038_2 and Satt610 did not show skewed segregation ratios in the RILs selected for FxH92. None of the markers except Satt038_2 was significantly associated with IS or any measure of leaf scorch resistance. The skewed segregation ratios of all the markers (except Satt038_2, for which selection had been applied) may have caused real QTL to marker associations to be missed. Alternately, the association of Satt038_2 with IS at R8 may be an error caused be selection.

At Ridgway in 1997, a second region on LG G (Figure 3) for resistance to root infection was identified. IS at the R6 sampling at Ridgway identified a QTL linked to BARC_Satt115 ($P = 0.01$, $R^2 = 6.4\%$). It derived the beneficial allele from Hartwig that reduced IS by about 7% (Table 2). The marker was not associated with leaf scorch metrics DX, DI and DS at any location or their means. The linked markers Satt427 Satt566 and Satt352 were weakly associated with the IS at R6 trait. The interval had a CIM peak-LOD score of 3.6 and explained about 38.5% of the total variation in SDS IS-R6 (Table 2). The locus was located on LG G of the composite map between 43 and 51 cM and probably was *qRfs3* (Supplementary Table 1) described previously (Chang et al. 1997; Iqbal et al. 2001; 2005).

The amount of variation in SDS IS explained by the two QTL underlying IS at R6 was significant; the two regions jointly contribute about 40% of the total variation compared to a trait heritability of 56%. In addition, only one QTL was identified with R8 IS data. Therefore, markers more closely linked to the QTL rather than additional loci for resistance to root infection by the SDS causal pathogen may remain to be discovered in this population.

Discussion

The FxH linkage map detected only two QTL for SDS DX, one with beneficial allele from Hartwig (on LG G) and one from Flyer (on LG C2). The number of QTL was less than the three found in PxD (Njiti et al. 2002) and the eight found in ExF (Kassem et al. 2006). It is possible that because Flyer is not as susceptible to SDS as Essex and Douglas some QTL are fixed in the FxH population that segregate in ExF and PxD. Considering the SDS root IS QTL, Hartwig contributed two QTL for resistance on G (Figure 3) and Flyer contributed a cqSDS001-like locus on LG D2; even though Flyer was more susceptible to infection by *F. virguliforme* than Hartwig. In Ripley by Spencer and Pyramid by Douglas the SDS resistant parent provided the beneficial allele at the equivalent position (Lightfoot et al. 2001; Farias-Neto et al. 2007). The locus on D2 was in the same interval as a locus for resistance to Hg Type 1.3.6.7 from PI88788 (Schuster et al, 2001); Pyramid (Lightfoot et al. 2001); and PI437654 (Webb et al. 1995). Therefore, this genomic region may explain the negative association between resistance to SCN HG Type 1.3.6.7 and resistance to SDS in soybean germplasm (Gibson et al.

1994). The identification of recombination events in this region that separate the negative association will be important for germplasm improvement.

In earlier studies where R6 and R8 data were pooled, about half of the cultivars tested showed root resistance, suggesting single-gene inheritance (Njiti et al. 1997; Prabhu et al., 1999; Njiti et al. 2003). In this study either bi- or tri-geneic inheritance for root resistance was detected. That knowledge will significantly increase the ability to breed for increased root resistance. Molecular methods for detecting and quantifying the pathogen in the root will provide effective tools for germplasm testing at different developmental stages (Achenbach et al. 1996; Li and Hartman 2003) because the inheritance of resistance to infection was shown to be significantly affected by developmental stage in FxH. Further, the measurements of SDS by DX and IS were shown to be very different in heritability, trait distribution, trait correlation and selection based on rank. DX was a poor indicator of root resistance whether by value or rank (Njiti et al. 1997). Therefore, efficient breeding strategies should make selections by both DX and either IS or Hg type rating for the identification of the most resistant cultivars.

Perhaps the most surprising result was the absence of a set of QTL for resistance to SDS leaf scorch (*qRfs2*) clustered around or pleiotropic to *rhg1* . The region was well populated with markers (Supplementary Table 1; Triwitaytakorn et al. 2005; Ruben et al. 2006). Therefore, Forrest and Hartwig differ significantly in this region, despite sharing the same allele of the receptor like kinase at *rhg1* (Ruben et al., 2006). This result also argues against pleiotropy between *rhg1* and *Rfs2* postulated by Triwitayakorn et al. (2005). Perhaps the location of the functional SCN and linked or pleiotropic SDS resistance QTL may have shifted to the loci paralagous to *rhg1* or even

the non-paralagous D2 locus (Shultz et al. 2006ab; Afzal and Lightfoot 2007; Lightfoot 2008). In this case NILs recombinant in the D2 region around Satt574 may help identify candidate genes using the genome framework at SoyGD (Supplementary Figure 2; Shultz et al. 2006a) and data from the DOE soybean genome sequencing project.

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Figure Legends

Figure 1. Frequency distributions of mean DX among 92 RIL from the FxH cross. DX values were adjusted to the R6 by linear regression so some values are negative. Range mid-point values are given each range encompassed 7.85 DX units. The population mean DX was shown on the upper right. Flyer (F) and Hartwig (H) mean scores were arrowed. ARC'00 and U'00 were the environments used with sufficient leaf symptom development.

Figure 2. Frequency distributions of IS in taproots of soybean during 1997 (97). IS was scored at R6 from Ridgway (A) and Ullin (B) and at R8 from Ridgway (C) and Ullin (D) among 92 RILs from the FxH cross. Range midpoint values are given. The population mean IS was shown on the upper right. The ranges into which 'Flyer' (F) and Hartwig' (H) mean IS scores fall were arrowed. R6 was the full-pod reproductive development stage and R8 was the harvest maturity stage of soybean plant when samples were taken.

Figure 3. Locations of the QTL found in the Flyer by Hartwig population on linkage groups C2, D2 and G for SDS mean DX (black arrows) and SDS IS (black stippled arrows). Also shown are QTL for resistance to SCN (grey stippled arrows) and date of maturity (grey solid arrow). The size of the arrow reflects the interval significantly associated by QTL Cartographer or Mapmaker at LOD > 2.0 or ANOVA at $P < 0.001$.

Supplementary Figure 1: Correlation between leaf scorch measured as mean DX at the R6 and root infection measured as IS at the R8. Among the metrics used to measure leaf

and root SDS these two showed the closest correlation. The data was from different years. Only lines with IS scores are shown. The correlation was significant $P<0.05$ with 49 df.

Supplementary Figure 2. Gbrowse representation of the MTP clones in a portion of the soybean genome showing build 4 linkage group G from 1 to 10 Mbp encompassing *cqRfs1, cqRfs2, qRfs3* and *rhg1* (*cqSCN-001*) closely linked to Satt309. A 10 Mbp region with loci, QTL, clones, contigs, sequences and gene models was shown. Loci, or genetic map DNA markers, were shown as red arrow heads. QTL in the region were shown as blue bars. BAC clones were shown as the coalesced purple bar. Contigs were shown as green bars. Polyploid region contigs have ctg numbers greater than 8,000. Sequences from MTP BAC ends were shown as black lines. Related gene annotations were shown as purple lines (the 5 most probable Blastx hits at $P < e\text{-}5$ were listed). ESTs mapped to MTP BACs were shown as golden bars and annotated with master plate address and gene model (if known) below the bar and EST name above the bar. Clicking on EST or MTP clones would bring up the gene index number. MTP4 clones were annotated below the bar with MTP and the MTP plate address. MTP2 clones can be identified as they have BES and EST hits shown. BES-SSR markers were shown as green lines below the MTP clones at (http://soybeangenome.siu.edu/cgi-bin/gbrowse/soybeanv4).

Table 1: Mean square values from analysis of variance on IS and DX among 50 FxH recombinant inbred lines. IS and DX were measured in two locations with two replications per location.

Source	df	F-test devisor	Mean squares R6	Mean squares R8
IS				
1 Location	1	4	1809	10,933
2 Rep (loc.)	2	4	1290**	455
3 RILs	49	5	286	617**
4 Loc. x RILs	49	4	275	236
5 Error	98		226	241
DX				
1 Replication	1	3	34	
2 RIL	49	3	451***	
3 Error	49		179	

F-test divisor = Error term for F-test

Rep(Loc) = Replications within location

*** significant at P<0.001

Table 2. Intervals with the flanking markers by CIM (LOD; QTL variation) and single markers by ANOVA probability (P) and Variance (R^2) values associated with SDS mean DX (DXmn) and mean IS at the R6 and R8 stages in the Flyer by Hartwig (RIL) population. DX was measured at Ullin (U) and at the Agronomy Research Center (ARC) in 2000. IS was measured at Ullin and Ridgway (R) in 1997. Allelic means were shown along with standard errors of the means (SEM).

LG	Marker	Trait	P	R^2 (%)	LOD[a]	QTL var.[b]	Mean ± SEM for RILs with Allele from	
Locus name(s)[d]	Interval						Flyer	Hartwig
C2	Satt277	DXmn	0.004	14.8	2.1	19.0	10.9±2.2	30.3± 5.6
cqRfs4	Satt079	DXmn	0.003	9.0	2.2	8.2	13.2±2.7	24.5±4.8
	QTL	DXmn	-	-	2.7	24.1	-	-
D2	Satt574	IS R6mn	0.001	10	2.2	10.2	34.3±3.0	47.3±3.2
cqRfs11	Sat_001	IS R6mn	0.003	6.1	2.4	12	36.9±3.0	52.1±3.9
	QTL	IS R6mn	-	-	3.0	25.2	-	-
G								
cqRfs1	Satt038_2	IS R8mn	0.0003	28.1	-	-	28.1±1.9	41.6±1.9
qRfs13	Satt130	DXmn	0.003	12.9	-	-	12.0±2.5	27.4±5.1
cqRfs3	Satt115	IS R6 R97	0.01	6.4	2.50	16	24.7±1.4	17.6±2.6
cqRfs3	Satt427	IS R6 R97	0.001	15	2.8	17	25.4±1.8	15.5±1.9
cqRfs3	QTL	IS R6 R97	-	-	3.6	38.5	-	-

a. LOD: Log of the probability of a locus being present; LOD threshold was 2.0
b. Amount of variability in the infection explained by the marker loci based on MapMarkerQTL1.1
c. SEM: Mean+SD/√N; where N was the number of each of allele
d. QTL associated with resistance to root infection. QTL detected in common intervals in separate populations or derived NILs were considered confirmed and suffixed with c under Soybean Genetics Committee recommendations from 2000-2006 (http://soybase.agron.iastate.edu/nomenclature/QTL.html). QTL designations cqSDS00# were applied for.

Supplementary Table1: Previously reported SDS QTL found in different RIL populations by linkage group.

Linkage Group	Soybase QTL Name (Linked Markers) [a]	Populations	Probable Locus Names[b]	References
A2	SDS11-1 (Satt 187)	Ripley x Spencer	*qRfs7*	Hashmi, 2004
C2	SDS 1-1 (0005-250)	Essex x Forrest	*cqRfs4*	Hnetkovsky et al. 1996
	SDS 4-2 (K455_1)	Essex x Forrest	*cqRfs4*	Njiti et al. 1998
	SDS 7-5 (Satt 371)	Essex x Forrest	*cqRfs4*	Iqbal et al. 2001
	SDS 8-2 (Satt 307)	Pyramid x Douglas	*cqRfs4*	Njiti et al. 2002
	SDS 8-3 (Satt 316)	Pyramid x Douglas	*qRfs9*	Njiti et al. 2002
	SDS 9-2 (Satt277)	Flyer x Hartwig	*cqRfs4*	Kazi, 2005 (this work)
D2	SDS11-2 (Satt 528)	Ripley x Spencer	*cqSDS001*	Farias-Neto et al. 2007
	SDS 9-1 (Satt574)	Flyer x Hartwig	*qRfs11* [c]	Kazi, 2005 (this work)
	SDS 2-3 ($OZ19_{468}$)	Pyramid x Douglas	*qRfs11*	Lightfoot et al. 2001
F	SDS10-1 (Satt 160)	Essex x Forrest	*qRfs10*	Kassem et al. 2006
G	SDS 6-1 (Bng 122_1)	Essex x Forrest	*cqRfs1* [c]	Meksem et al. 1999
	SDS 3-1 (0G13_490)	Essex x Forrest	*cqRfs3*	Chang et al. 1996
	SDS 6-2 (Satt 309)	Essex x Forrest	*cqRfs2*	Meksem et al. 1999
	SDS8-1 (Satt309)	Pyramid x Douglas	*cqRfs1*	Njiti et al. 2002
	SDS 8-4 (Satt038)	Pyramid x Douglas	*cqRfs2*	Njiti et al. 2002
	SDS 7-1 (Satt 214)	Essex x Forrest	*cqRfs2*	Iqbal et al. 2001
	SDS 7-2 (Satt 309)	Essex x Forrest	*cqRfs2*	Iqbal et al. 2001
	SDS 7-3 (Satt 570)	Essex x Forrest	*cqRfs1* [c]	Iqbal et al. 2001
	SDS 7-4 (OEO2_1000)	Essex x Forrest	*cqRfs3*	Iqbal et al. 2001
	SDS 4-1 (0I03-512)	Essex x Forrest	*cqRfs1* [c]	Njiti et al. 1998
	SDS 4-3 (Bng122_1)	Essex x Forrest	*cqRfs1* [c]	Njiti et al. 1998
	SDS 5-1 (Satt 038)	Flyer x Hartwig	*cqRfs1* [c]	Prabhu et al. 1999
	SDS 8-1 (Satt 163)	Pyramid x Douglas	*cqRfs2*	Njiti et al. 2002
	SDS9-3 (Satt427)	Flyer x Hartwig	*cqRfs3*	Kazi, 2005 (this work)
I	SDS 7-6 (Satt 354)	Essex x Forrest	*qRfs5*	Iqbal et al. 2001
J	SDS10-2 (Satt 285)	Essex x Forrest	*qRfs8*	Kassem et al. 2006
	SDSna(Satt183)	GC87018-12-2B-1 x GC89045-13-1	*qRfs8*	Sanitchon et al. 2004

L	SDS12-1 (Satt 187) SDS12-1 (Satt 187)	Minsoy x Noir 1 Ripley x Spencer	*qRfs12* *qRfs12*	Njiti and Lightfoot 2006 Hashmi, 2004
N	SDS 2-7 (0C01-650) SDS 8-3 (Satt 080) qSDS002 (Satt 631)	Essex x Forrest Pyramid x Douglas Ripley x Spencer	*qRfs6* *qRfs6* *cqRfs6* [d]	Chang et al.1996 Njiti et al. 2002 Hashmi, 2004

[a] QTL numbers are from Soybase 2004-2006 under the rules of the Soybean Genetics Committee 200-2006, where assigned.

[b] From 27 QTL detections there were 15 QTL counting each detection in a separate population once. Assuming QTL detected in common intervals in separate populations represents the alleles there were 11-12 loci.

[c] QTL associated with resistance to root infection. QTL detected in common intervals in separate populations or derived NILs were considered confirmed and suffixed with c under Soybean Genetics Committee recommendations from 2000-2006 (http://soybase.agron.iastate.edu/nomenclature/QTL.html).

[d] QTL designations cqSDS00# applied for

Supplementary Table 2: Locations of the markers mapped in FxH in the soybean composite map (Song et al. 2004). BARC markers were indicated S***###, BES-SSRs were given as clone names. Composite map locations for BES-SSRs when positions were known were estimated and given as whole integers. LG signifies linkage group. LG Q was queue at SoyGD, a collection of unanchored BACs and contigs. Encompass provides an estimate of the genome over which QTL might be detected in single point analysis for each group of linked markers and each unlinked marker.

Marker	LG	cM	Type	Encompass	Total
Satt276	A1	17.162	SSR		
H100B10a	A2	41	BES-SSR		
Satt385	A1	64.736	SSR		
Satt545	A1	71.391	SSR		
Satt599	A1	85.579	SSR	88	
A2D8	A2	48	BES-SSR		
BLT65	A2	49	RFLP-SCAR		
Satt424	A2	60.585	SSR		
Satt089	A2	87.566	SSR		
Satt437	A2	107.051	SSR		
Satt158	A2	115.245	SSR		
Satt421	A2	115.928	SSR	88	
Satt509	B1	32.51	SSR		
Satt197	B1	46.393	SSR		
Sct_026	B1	78.127	SSR		
Sat_95	B1	81.312	SSR		
Satt583	B1	84.189	SSR		
Satt359	B1	102.556	SSR	90	
Satt318	B2	70.12	SSR		
Satt534	B2	87.592	SSR		
Satt560	B2	97.924	SSR	47	
Satt396	C1	24.106	SSR		
Satt399	C1	76.228	SSR		
Satt476	C1	80.623	SSR		
Satt195	C1	84.809	SSR	80	
Satt520	C2	42.365	SSR		
Satt291	C2	45.757	SSR		
Satt305	C2	69.665	SSR		
Satt450	C2	89.305	SSR		
Satt363	C2	98.071	SSR		
Satt286	C2	101.754	SSR		
Satt277	C2	107.592	SSR		
Satt079	C2	117.868	SSR		
Satt202	C2	126.236	SSR	104	

Satt184	D1a	17.517	SSR	
H67I21a	D1a	31	BES-SSR	
Satt342	D1a	48.144	SSR	
Satt532	D1a	49.071	SSR	
Satt502	D1a	49.835	SSR	
B09L01	D1a	64.522	BES-SSR	67
Satt141	D1b	72.887	SSR	
Satt290	D1b	73.346	SSR	
Satt604	D1b	74.208	SSR	
Satt005	D1b	75.292	SSR	
Satt537	D1b	75.674	SSR	
Satt579	D1b	75.939	SSR	
Satt282	D1b	76.103	SSR	
Sat_89	D1b	76.283	SSR	
Satt428	D1b	77.349	SSR	25
Satt514	D2	85.686	SSR	
Satt528	D2	86.336	SSR	
Satt082	D2	87.249	SSR	
Satt574	D2	87.666	SSR	
Satt543	D2	88.02	SSR	
Satt488	D2	89.199	SSR	
Sat_001	D2	92.123	SSR	
Satt301	D2	93.709	SSR	
Sat_86	D2	118.662	SSR	53
Satt573	E	35.794	SSR	
Satt151	E	44.925	SSR	
Satt553	E	67.924	SSR	52
Satt146	F	1.918	SSR	
Satt343	F	3.043	SSR	
Satt569	F	3.351	SSR	
Sat_39	F	27.872	SSR	
Satt160	F	33.185	SSR	
Satt510	F	71.412	SSR	
B02k20	F	77	BES-SSR	95
Satt163	G	0	SSR	
Satt038_1	G	1.840	SSR	
Satt275	G	2.201	SSR	
TMD1	G	4	Rhg1_SSR	
Satt309	G	4.534	SSR	
Satt038_2	G	9	SSR	
Satt610	G	10.923	SSR	
Satt570	G	12.737	SSR	
Satt130	G	23.096	SSR	
Sat_131	G	31.326	SSR	
Satt324	G	33.263	SSR	
B09L01	G	35	BES-SSR	
Satt115	G	43.782	SSR	
Satt566	G	49.905	SSR	
Satt352	G	50.527	SSR	
Satt427	G	51.689	SSR	

Satt594	G	52.938	SSR		
Satt564	G	57.322	SSR		
B30O12	G or D2	77	BES-SSR		
Satt191	G	96.572	SSR	106	
Satt353	H	8.48	SSR		
Satt442	H	46.947	SSR		
Satt181	H	91.116	SSR		
Satt434	H	105.735	SSR	100	
Satt239	I	36.935	SSR		
Satt270	I	50.108	SSR		
Satt292	I	82.777	SSR	66	
Satt431	J	78.838	SSR	40	
Satt539	K	1.798	SSR		
Sat_87	K	4.853	SSR		
Satt242	K	14.354	SSR		
Satt102	K	30.283	SSR		
Satt137	K	36.991	SSR		
Satt167	K	45.738	SSR		
Satt337	K	47.377	SSR		
Satt326	K	49.525	SSR		
Satt260	K	80.119	SSR	100	
Satt232	L	10.345	SSR		
Sct_010	L	59.523	SSR		
Satt006	L	92.001	SSR	80	
Satt631	N	26.135	SSR		
Satt159	N	27.132	SSR		
Satt009	N	28.52	SSR		
Satt530	N	32.848	SSR		
Satt237	N	74.987	SSR	70	
Satt358	O	5.44	SSR		
Satt420	O	49.711	SSR		
Satt478	O	71.104	SSR	80	1431
B14O11	A1 or D1a		BES-SSR	20	
H32G20b	A1, A2 or J		BES-SSR	20	
B03k03a	Q	nd	BES-SSR	20	
B04I02	Q	nd	BES-SSR	20	
B08D14	Q	nd	BES-SSR	20	
B01I14	Q	nd	BES-SSR	20	
B10O02	Q	nd	BES-SSR	20	
B11O06	Q	nd	BES-SSR	20	
B12B12	Q	nd	BES-SSR	20	
B13L17	Q	nd	BES-SSR	20	
B14B13	Q	nd	BES-SSR	20	
B14G13	Q	nd	BES-SSR	0	
B14L17a	Q	nd	BES-SSR	20	
B15I12a	Q	nd	BES-SSR	20	
B15I12b	Q	nd	BES-SSR	0	
B15L06	Q	nd	BES-SSR	20	
B17P05	Q	nd	BES-SSR	20	
B36E04a	Q	nd	BES-SSR	20	

B36E04b	Q	nd	BES-SSR	0	
B50N01a	Q	nd	BES-SSR	20	
B50N01b	Q	nd	BES-SSR	0	
B54L22a	Q	nd	BES-SSR	20	
B55I16b	Q	nd	BES-SSR	20	
B61P08a	Q	nd	BES-SSR	20	
B61P08b	Q	nd	BES-SSR	20	
B79C07a	Q	nd	BES-SSR	20	
B79C07b	Q	nd	BES-SSR	0	
H07E10a	Q	nd	BES-SSR	20	
H14J18a	Q	nd	BES-SSR	20	
H32P20a	Q	nd	BES-SSR	20	
H99L10a	Q	nd	BES-SSR	20	
H99L10b	Q	nd	BES-SSR	0	
H99P11a	Q	nd	BES-SSR	20	1971