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Common loci underlie field resistance to soybean sudden death syndrome in Forrest, Pyramid, Essex, and Douglas

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Abstract Soybean [*Glycine max* (L.) Merr.] sudden death syndrome (SDS) caused by *Fusarium solani* f. sp. *glycines* results in severe yield losses. Resistant cultivars offer the most-effective protection against yield losses but resistant cultivars such as 'Forrest' and 'Pyramid' vary in the nature of their response to SDS. Loci underlying SDS resistance in 'Essex'× Forrest are well defined. Our objectives were to identify and characterize loci and alleles that underlie field resistance to SDS in Pyramid×'Douglas'. SDS disease incidence and disease severity were determined in replicated field trials in six environments over 4 years. One hundred and twelve polymorphic DNA markers were compared with SDS disease response among 90 recombinant inbred lines from the cross Pyramid×Douglas. Two quantitative trait loci (QTLs) for resistance to SDS derived their beneficial alleles from Pyramid, identified on linkage group G by BARC-Satt163 (261-bp allele, P=0.0005, R^2=16.0%) and linkage group N by BARC-Satt080 (230-bp allele, P=0.0009, R^2=15.6%). Beneficial alleles of both QTLs were previously identified in Forrest. A QTL for resistance to SDS on linkage group C2 identified by BARC-Satt307 (292-bp allele, P=0.0008, R^2=13.6%) derived the beneficial allele from Douglas. A beneficial allele of this QTL was previously identified in Essex. Recombinant inbred lines that carry the beneficial alleles for all three QTLs for resistance to SDS were significantly ($P \leq 0.05$) more resistant than other recombinant inbred lines . Among these recombinant inbred lines resistance to SDS was environmentally stable. Therefore, gene pyramiding will be an effective method for developing cultivars with stable resistance to SDS.

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Introduction

Soybean sudden death syndrome (SDS) caused by *Fusarium solani* f. sp. *glycines* (Roy 1997), reduces crop yields in the USA and South America (Gibson et al. 1994; Wrather et al. 1997). However, cultivars with quantitative partial resistance to SDS are protected against yield loss in *F. solani* infested field plots (Gibson et al. 1994; Njiti et al. 1998b; Prabhu et al. 1999).

Quantitative partial resistance to SDS is determined by a few major QTLs (Hnetkovsky et al. 1996; Njiti et al. 1996; Chang et al. 1997) and at least one represents a qualitative locus during fine mapping (Meksem et al. 1999). SDS resistance is difficult to determine in the field since disease expression is often environmentally sensitive, sporadic, and patchy (Gibson et al. 1994; Rupe et al. 1994; Rupe and Gbur 1995; Njiti et al. 1996). However, by careful selection of environments with uniform SDS incidence, and the selection of parental cultivars with stable and durable disease resistance, (such as Forrest) in replicated field tests, SDS resistance can be accurately determined in progeny populations (Gibson et al. 1994; Hnetkovsky et al. 1996).

Field tests for resistance to SDS are time-consuming and costly. The greenhouse assays for SDS resistance are faster and cheaper but do not always accurately predict field resistance (Stephens et al. 1993; Torto et al. 1996). Therefore, molecular markers linked to QTLs that condition a significant portion of variability in field resistance to SDS in many cultivars and across most environments would be valuable selection tools for plant breeders.

Field resistance to SDS among recombinant inbred lines derived from Essex × Forrest (E×F) is conditioned by a QTL cluster on linkage group G (1G) (Meksem et al. 1999) and three unlinked QTLs on linkage groups G (2G), C2 and N (Hnetkovsky et al. 1996; Chang et al.

1997). The SDS QTL cluster on linkage group G was shown to have a strong allelic difference in near-isogenic line populations derived from E×F (Njiti et al. 1998a; Meksem et al. 1999) and a recombinant inbred line population derived from Flyer×Hartwig during marker-assisted selection (Prabhu et al. 1999). The Essex-derived QTL on C2 (Hnetkovsky et al. 1996) had a weak allelic difference in NILs (Njiti et al. 1998b). The Forrest-derived QTL on linkage group N was shown to be composed of two distantly linked loci by fine mapping (unpublished).

Linkage group G contains a cluster of disease resistance genes that includes the *rhg1* gene for soybean cyst nematade (SCN) race-3 resistance (Rao-Arrelli et al. 1992; Webb et al. 1995; Concibido et al. 1996; Chang et al. 1997), the *rft* locus that reduces leaf scorch and the *rfs1* locus that causes soybean root resistance to *F. solani* infection (Meksem et al. 1999). The source of the cluster of genes underlying SDS and the root infection resistance QTL in Forrest derives from Hill via 'Dyer', but its origin is not clear among ancestors of 'Hill'. However, the gene cluster predates introgression of SCN resistance from Peking since all Peking cultivars are very susceptible to SDS in the field. Most adapted soybean cultivars that are resistant to SCN race 3 are also partially resistant to SDS leaf scorch but very few are resistant to root infection (Gibson et al. 1994). A gene that is closely linked to SCN resistance (*rft*) may increase resistance to SDS leaf scorch in many cultivars (Meksem et al. 1999). This linkage can be broken since several cultivars without SCN resistance have been reported to have partial resistance to SDS (Gibson et al. 1994).

Pyramid derives SCN race 14 resistance from PI 88788 and SCN race-3 resistance from Peking and/or PI 88788. PI 88788, and Peking cultivars are very susceptible to SDS (Gibson et al. 1994). The source of SDS resistance alleles in Pyramid is unknown; however, there is no root resistance to *F. solani* infection. There was a significant interaction of SDS response with the environment in Pyramid×Douglas (P×D) recombinant inbred lines that was attributed to the presence of SCN race 14 in the material (Njiti et al. 1996). In contrast, resistance to SDS in ExF recombinant inbred lines was stable across the environment (Hnetkovsky et al. 1996).

The present study was undertaken to identify and characterize loci and alleles that underlie field resistance to soybean sudden death syndrome in P×D. The implications for the use of DNA markers for the selection of improved SDS resistance in soybeans are considered.

Material and methods

Plant material

Ninety recombinant inbred lines derived from Pyramid (partially resistant to SDS, susceptible to *F.solani* root infection, resistant to SCN races 3 and 14; Myers and Schmidt 1988)×Douglas (susceptible to SDS, susceptible to *F. solani* root infection and, susceptible to SCN; Nickell et al. 1982) were used in this study. The cross was made at Southern Illinois University at Carbondale in 1985. The F_2 plants from the cross were inbred to the F_6 generation using the single-pod descent method (Brim 1966) in fields with no history of SDS symptoms. In 1988, 131 randomly selected F_6 plants were individually harvested. In 1989, 90 F_6-derived lines of similar maturity (late maturity group IV) with sufficient seeds (n=1,200) for field replicated testing were retained for use in the genetic study. Plant material for DNA extraction was collected from 20 seedlings per line at the $F_{6:10}$.

Field test

The lines were evaluated for SDS resistance by leaf scorch in six environments over a period of 4 years. A partially balanced simple-lattice design was used at each environment (Gomez and Gomez 1984). There were 90 lines, eight repetitions of each parent, and four checks, totaling 110 entries comprising a 10×11 rectangular lattice. Each plot consisted of two rows 0.75-m apart and 3-m long with about 17 plants per meter of row.

SDS disease scoring

SDS disease incidence (DI) and disease severity (DS) were rated weekly as described by Njiti et al. (1996). The last score before and the first score after the R6 (full pod) growth stage (Fehr et al. 1971) were used to standardize DI and DS to the R6 growth stage (Njiti et al. 1996). The trait data were used for QTL analysis after being normalized by arcsine transformation to increase variability among the genotypes with a low score (resistant). Thereby, variability was equalized across the progeny population. Both the mean DI across environments and the DI means for individual environments were analyzed.

Morphological markers

The recombinant inbred lines were scored for three morphological markers. Flower color was scored as white, purple or mixed, pubescence was scored as gray, tawny or mixed, and growth habit was scored as indeterminate or determinate.

RFLP markers

Polymorphic loci were detected and screened as described by Hnetkovsky et al. (1996) except that hybridization buffer was purchased (Amersham Corporation, Arlington Heights, Ill.) and supplemented with polyethylene glycol 8000 at 50 g l^{-1}. The mapped RFLP loci are reported using the nomenclature of Pfeiffer et al. (1995).

RAPD markers

The amplification reactions were performed after Williams et al. (1990) with 300 separate primers from kits A, B, C, D, E, F, G, H, I, J, O, R, S, W and X from Operon Technologies Inc. (Alameda, Calif.). DNA was amplified as described previously (Hnetkovsky et al. 1996) except that two thermal cyclers were used (Savant TC49, New York, and Perkin Elmer 480, Foster City, Calif.) to verify the RAPD patterns. All of the RAPD markers reported were amplified with the Stoffel fragment. RAPD markers associated with SDS resistance were amplified independently on three or more separate occasions to assure reproducibility. The RAPD loci are reported using the nomenclature of Michelmore et al. (1991).

Microsatellite markers

Microsatellite markers were generated and scored as described by Akkaya et al. (1995).

AFLP marker analysis

Soybean genomic DNA used for AFLP and microsatellite analysis was extracted and purified using the Qiagen Plant Easy DNA Extraction Kit (Qiagen, Hilden, Germany). Template DNA was prepared by digestion with the restriction enzymes *Eco*RI and *Mse*I.

AFLP analysis was performed as described by Vos et al. (1995) with minor modification (we omitted the streptavidin bead-selection step). PCR reactions were performed with *Eco*RI- and *Mse*I-digested DNA ligated to two sets of primers. Primers within the *Eco*RI set all included the sequence 5´-GAC TGC GTA CCA ATT C. Primers of the *Mse*I set contain the sequence 5´-GAT TCC TGA GTA A.

Detecting loci associated with quantitative resistance

To detect genomic regions associated with SDS resistance, the recombinant inbred lines were classified as Douglas (D) type or Pyramid (P) type for each marker. The relatively few heterogeneous lines (3 to 12 lines per marker) were excluded for RFLP, microsatellite markers but could not be identified or excluded from the RAPD data. Marker data were compared with SDS disease response scores (DI and DS) for each environment and the mean of five environments by one-way analysis of variance (ANOVA) performed with SAS (SAS Institute Inc., Cary, N.C.; Wang et al. 1994). The probability of association of each marker with each trait was determined and a significant association was declared if $P \leq 0.005$.

Detecting interactions between quantitative resistance loci

Selected pairs of markers were analyzed by the two-way ANOVA PROC GLM procedure to detect non-additive interactions between the unlinked QTLs (Lark et al. 1995). Non-additive interactions between markers which were significantly associated with SDS response were excluded when $P > 0.05$. Selected groups of markers were analyzed by multi-way ANOVA to estimate joint heritabilities for traits associated with multiple QTLs. Joint heritability was determined from the R^2 term for the joint model in multi-way ANOVA.

Mapping quantitative resistance loci

Mapmaker-EXP 3.0 (Lander et al. 1987) was used to calculate map distances [centimorgans (cM), Haldane units] between linked markers and to construct a linkage map. The recombinant inbred line (ri-self) genetic model was used. The $\log_{10}$ of the odds ratio (LOD) for grouping markers was set at 2.0, the maximum distance was 30 cM. Conflicts were resolved in favor of the highest LOD score after checking the raw data for errors. Marker order within groups was determined by comparing the likelihood of many map orders. A maximum-likelihood map was computed with error detection. Groups were assigned to linkage groups by anchored microsatellite and RFLP markers (Shoemaker and Specht 1995; Cregan et al. 1999).

The map and disease data were simultaneously analyzed with Mapmaker/QTL 1.1 (Paterson et al. 1988) using the F_2-backcross genetic model for trait segregation (after Webb et al. 1995; Chang et al. 1996, 1997; Hnetkovsky et al. 1996). Putative QTLs were inferred when LOD scores exceeded 2.0 at some point in each interval since this was found empirically to be equivalent to a single marker with $P < 0.005$, the criterion used in the one-way ANOVA. The positions of the QTLs were inferred from the interval peak LOD score.

Results

Polymorphism and linkage

The present report summarizes the data from 112 loci. Eight loci were identified by RFLP markers, 34 loci were identified by RAPD bands, 55 loci were identified by SSR bands, 13 loci were identified by AFLP bands and two loci by morphological markers (flower color and growth habit). In total 51 loci mapped to ten coherent linkage groups encompassing 1,662 cM. These ten linkage groups together with the 61 unlinked markers would allow the detection of QTLs associated with SDS resistance up to about 2,094 cM given mean intervals of about 20 cM, although this is likely to be an overestimation. The 2,094 cM compares with a recombination distance for the soybean genome of nearly 3,000 cM within 20 linkage groups (Shoemaker and Specht 1995; Chang et al. 1997; Cregan et al. 1999). The distances and orders of markers in linkage groups agreed with those reported (Cregan et al. 1999; Meksem et al. 1999). The $F_{6:10}$ lines were heterogeneous at 6% of loci scored by co-dominant markers compared to the 3 to 15% expected.

Analysis of agronomic traits

The heritability of SDS response ranged from 0.68 to 0.83 for individual environments and was 0.75 across environments (Njiti et al. 1996). The description of agronomic traits for this population has been reported in detail by Njiti et al. (1996). The pyramid SDS disease index was lower than that for Douglas in all six environments.

SCN race-3 resistant recombinant inbred lines (n=43) had reduced susceptibility to SDS in some environments, while recombinant inbred lines with both SCN race-3 and race-14 resistance (n=29) showed an increased susceptibility to SDS in some environments. The SDS-SCN race-14 interaction contributed to the significant genotype by environment interaction in the SDS response that was observed in this population (Njiti et al. 1996).

DNA markers associated with disease incidence

A one-way analysis of variance detected four independent genomic regions on three linkage groups and one unlinked locus with significant ($P \leq 0.005$) effects on SDS disease incidence. Molecular linkage groups G, N and C2 each contain one QTL for resistance to SDS.

A region on linkage group G (Fig. 1) about 4 cM from BARC-Satt163 was found to contain a major QTL for SDS disease incidence (P=0.0005, R^2=16%) that derived the beneficial allele (216 bp) from Pyramid. The interval had a peak LOD score of 5.4 and explained about 25% of the total variation in SDS disease incidence (Table 1) in the P×D recombinant inbred line population. The linked marker BARC-Satt038 (221-bp allele

Table 1 DNA markers and intervals most likely to be associated with the SDS QTLs for mean disease incidence (DI) across six environments. L.G: molecular linkage group; a: LOD indicates how much more probable the data are to have arisen assuming the presence of a locus than assuming its absence; LOD threshold=2.0; b: amount of variation in the phenotype explained by the DNA marker using Map-Maker QTL, †: allele sizes were determined from acrylamide-gel electrophoresis and were not confirmed by sequencing the alleles

DNA marker	Allele size† (bp)		L.G.	R^2	*P*>F	LOD[a]	QTL var.[b]	Mean DI (%)±SEM for RILs with alleles from	
	Douglas	Pyramid						Douglas	Pyramid
BARC-Satt163	298	261	G	16.0	0.0005	5.4	24.4	51.5±1.8	38.6±3.2
BARC-Satt309	146	130	G	8.5	0.007	1.6	8.0	51.7±1.9	42.1±2.8
BARC-Satt038	223	221	G	12.3	0.0011	1.5	8.1	52.9±1.8	41.2±2.9
BARC-Satt307	292	285	C2	13.6	0.0008	2.4	14.0	36.6±4.2	50.1±1.8
BARC-Satt316	222	237	C2	9.1	0.007	2.2	13.8	59.6±1.8	38.7±4.4
BARC-Satt080	177	230	N	15.6	0.0009	2.7	14.9	54.9±3.8	41.3±2.0
BARC-Satt387	252	241	N	10.2	0.0043	1.8	11.0	54.6±3.7	43.3±2.0
OG01	–	596	UL	17.2	0.0005	2.3	13.2	50.0±2.6	40.2±2.3

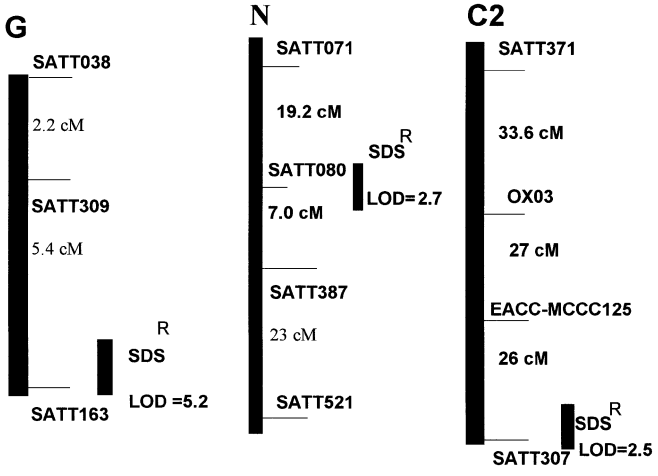

Fig 1 Locations of DNA markers and three QTLs conditioning soybean sudden death syndrome response. The QTLs were assigned to linkage groups G, N and C on the soybean genetic map (Shoemaker and Specht 1995). Genetic distances were from the recombinant inbred-line function of MAPMAKER/EXP 3.0. The estimated position of the QTLs is shown based on interval mapping using MAPMAKER/QTL 1.1. The QTL LOD score is the peak LOD score of the interval showing association with the sudden death syndrome

from Pyramid, 2.2 cM) and BARC-Satt309 (130-bp allele from Pyramid, 7.6 cM) were weakly associated with SDS resistance.

The QTL for resistance to SDS on linkage group N (Fig. 1), identified by BARC-Satt080 (230 bp fragment, *P*=0.0008, R^2=16%), derived the beneficial allele from Pyramid (Table 1). The linked (7 cM) marker (BARC-Satt387) was also strongly associated with DI. The interval had a peak LOD score of 2.7 and explained about 15% of the total variation in SDS disease incidence (Table 1) in the P×D recombinant inbred line population.

A QTL for resistance to SDS was detected on Linkage group C2 (Fig. 1) by BARC-Satt307 (*P*=0.0008, R^2=13.6%) derived the beneficial allele from Douglas (292 bp allele, Table 1). The interval had a peak LOD score of 2.5 and explained about 23% of the total variation in SDS disease incidence (Table 1) in the P×D recombinant inbred line population. Another region on linkage group C2 identified by BARC-Satt316 was weakly associated (*P*=0.007, R^2=9%) with SDS resistance. However, the amount of variation in DI explained by the marker was below the cut off for the QTL (Table 1).

One unlinked locus identified by OG01 was significantly (*P*=0.0005) associated (*P*=0.0001, R^2=13%) with disease incidence and derived the beneficial allele (596-bp DNA fragment) from Pyramid. All the QTLs for SDS DI were significantly associated with SDS disease severity (*P*≤0.007). The QTLs explained from 9% to 14% of the total variation in DS.

SDS disease pressure was moderate to severe in all environments except at Cora in 1991 where disease pressure was very low (Njiti et al. 1996). The QTL on Linkage group G identified by BARC-Satt163 was significantly associated with SDS disease incidence at Villa Ridge in 1990 and 1994 (Table 2). The linked markers BARC-Satt038 and BARC-Satt309 were also significantly associated with DI at Villa Ridge in 1990 and 1994 (Table 2). The association between this genomic region and the disease mean across environments was a reflection of the strong association of the region with the SDS phenotype in these specific environments. The region identified by BARC-Satt080 and the linked marker BARC-Satt387 on linkage group N was significantly associated with DI at Pulaski in 1991 only (Table 2). The region identified by BARC-Satt316 was associated with DI at Pulaski in 1991 and at Villa Ridge in 1994 (Table 2).

There was a significant (*P*=0.002, R^2=10.6%) association between growth habit and SDS disease incidence. The determinate-type (Douglas-type) had a higher disease incidence mean (52.5%) compared to 41.7% for the indeterminate-type (Pyramid-type).

Interaction among loci contributing to SDS resistance

A three-way analysis of variance detected a significant interaction (*P*≤0.05) among the three marker loci con-

Table 2 Markers and intervals showing significant association with SDS disease incidence (DI) at specific environments. L.G: molecular linkage group; a: LOD indicates how much more probable the data are to have arisen assuming the presence of a locus than assuming its absence, LOD threshold=2.0; b: amount of variation in the phenotype explained by the DNA marker using Map-Maker QTL

DNA marker	LG	Env.	R2	*P*>F	LOD[a]	Var.[b]	DI means with±SEM QTL alleles from	
							Douglas	Pyramid
BARC-Satt163	G	V90	20.4	0.0001	7.4	32.2	50.9±4.5	22.8±4.9
		V94	17.7	0.0003	6.5	29.3	72.5±3.5	45.3±6.6
BARC-Satt309	G	V90	32.1	0.0001	6.3	28.5	60.6±4.3	24.2±3.9
		V94	18.7	0.0001	3.8	18.1	76.5±2.9	48.7±5.3
BARC-Satt038	G	V90	27.8	0.0001	4.9	23.2	58.7±4.2	25.0±4.3
		V94	25.2	0.0001	4.6	22.6	78.4±2.8	46.0±5.4
BARC-Satt307	C2	V94	12.2	0.0016	2.2	13.2	43.7±8.4	68.6±3.4
		P91	18.4	0.0001	3.2	18.1	29.0±3.8	55.2±3.7
BARC-Satt316	C2	V94	7.8	0.0135	1.9	11.1	68.2±3.4	48.6±8.8
		P91	12.7	0.0013	2.4	14.2	52.1±3.6	30.7±4.0
OG01	UL	V94	14.2	0.0020	2.1	12.2	75.8±3.7	50.9±5.1
		P91	17.1	0.0005	2.6	16.7	55.4”4.9	38.6±3.7
BARC-Satt080	N	P91	26.5	0.0001	4.8	24.6	64.1±6.1	34.8±3.0
BARC-Satt387	N	P91	21.7	0.0001	4.2	22.0	64.6±5.5	37.6±3.1

Table 3 Interaction amongst three loci contributing to sudden death syndrome resistance in Pyramid×Douglas with beneficial alleles from Pyramid (P) (two loci) and Douglas (D) (one locus). p: derives beneficial allele (P) for SDS resistance from Pyramid. d: derives beneficial allele (D) for SDS resistance from Douglas

DNA markers			Disease incidence						Mean
Satt163[p]	Satt080[p]	Satt307[d]	Villa Ridge 90	Villa Ridge 94	Pulaski 1991	Cora 1991	Cora 1992	Ridgway 1993	
P	P	D	8.9	13.3	23.2	2.2	33.2	67.9	24.8
P	P	P	8.6	46.9	49.1	4.7	32.7	82.0	37.5
P	D	D	56.2	74.8	51.3	21.3	40.7	80.0	54.0
P	D	P	13.8	47.2	63.0	7.0	32.7	80.0	40.6
D	P	D	34.1	87.7	20.6	8.5	42.2	82.4	45.9
D	P	P	60.6	71.5	38.9	10.3	39.0	74.5	49.1
D	D	P	58.6	86.6	75.6	14.7	61.7	90.8	64.7
LSD (0.05)			**23.5**	**22.6**	**23.5**	**11.1**	**22.7**	**16.7**	**12.7**

tributing most strongly to SDS resistance in this population (Table 3). Eight allelic class combinations were expected but only seven were observed. The allelic classes BARC-Satt163 Douglas (D), BARC-Satt080 Douglas and BARC-Satt307 Douglas were missing. A significant deviation (P=0.001, χ^2=12.8) from the expected genetic ratio of one Pyramid (P): one Douglas allele was observed at the BARC-Satt307 locus.

Recombinant inbred lines with the beneficial alleles (BARC-Satt163 P BARC-Satt080 P, and BARC-Satt307 D) for all three major SDS QTLs identified in this population were more resistant across environments than lines with any other allelic combinations (Table 3). The QTLs on linkage groups G and N had the major contribution to resistance in the recombinant inbred lines. Recombinant inbred lines that accumulated only one or none of the beneficial alleles from these QTLs were more likely to show a higher degree of susceptibility and experience inconsistent response across environments (Table 3). Jointly, the three QTLs explained about 60% of the total variation in the SDS disease phenotype in the PxD recombinant inbred line population.

Discussion

Three SDS resistance QTLs were identified in the PxD recombinant inbred line population. The QTL on linkage group G was identified by BARC-Satt163. BARC-Satt163 maps 0 to 5 cM from BARC-Satt038, BARC-Satt309 and BARC-Satt122, that each can detect the 1G SDS resistance QTL in E×F or Flyer×Hartwig (F×H) (Prabhu et al. 1999). We conclude that the G SDS resistance QTL in P×D may be the same as the 1G QTL in E×F and F×H (Chang et al. 1997; Meksem et al. 1999; Prabhu et al. 1999). However, the QTL may be displaced by error in the phenotypic data and does not include root resistance to *F. solani* infection.

The marker A071T_1 maped about 25 cM from the SDS-resistant QTL reported in ExF. A071T_1 is expected to be 33 cM from BARC-Satt387 (7 cM from BARC-Satt080) (Shoemaker and Specht 1995) that identified the QTL on Linkage group N in P×D. We conclude that the SDS resistance QTL on linkage group N in PxD may be the same QTL that was previously reported in E×F (Hnetkovsky et al. 1996).

The QTL for resistance to SDS on Linkage group C2 identified by BARC-Satt307 in Pyramid×Douglas maps

to the same region as the QTL for resistance to SDS in E×F on linkage group C2 identified by K455D (Shoemaker and Specht 1995; Hnetkovsky et al. 1996). As was the case in ExF, the C2 QTL for resistance to SDS in P×D derived the beneficial allele from the SDS susceptible parent, Douglas.

The heritability of SDS disease incidence in Pyramid×Douglas has been estimated at 75% (Njiti et al. 1996). Since only 60% of the total variation in SDS disease phenotype was explained by these three QTLs, more genomic regions affecting SDS resistance may yet be detected in this population. An analysis with 90 lines and 112 markers is unlikely to detect all SDS resistance genes segregating in the progeny population. Further only loci with a relatively large effect, a simpler structure, and with close linkage to markers, were more-likely to be detected. However, the three QTLs detected here seemed to provide field resistance when all were present.

The SDS resistance QTLs detected in this population were expected to be stable across environments (Hnetkovsky et al. 1996; Chang et al. 1997). However, the QTLs were only effective in some environments in the broader P×D recombinant inbred line population. The significant G×E interaction in this recombinant inbred line population was previously reported (Njiti et al. 1996).

A three-way analysis of variance indicated that the pyramiding of resistance alleles will increase resistance, possibly via gene interaction and/or gene complementation (Huang et al. 1997). The absence of one or more beneficial alleles resulted in less resistance and more environmental instability.

The absence of one allelic class combination in the three-way interaction was most likely due to the distorted segregation ratio observed in BARC-Satt307. The Douglas allele was disfavored at this locus. Scoring errors, nonrandom population or zygotic selection (Webb et al. 1995; Prabhu et al. 1999) may account for the skewed segregation ratio.

A subset of three QTLs present in Pyramid also contributes to SDS resistance in Forrest and Hartwig where five QTLs were detected. The present results suggest that the significant GxE that was previously reported in the P×D population (Njiti et al. 1996) was probably a function of the low overall resistance in the recombinant inbred line population determined by the accumulation of beneficial alleles from few (three) major resistance loci. There were only 12 recombinant inbred lines with beneficial alleles from all three loci in the P×D recombinant inbred line population. The E×F recombinant inbred lines were evaluated in the same environments as the P×D recombinant inbred lines. Therefore more E×F recombinant inbred lines may have accumulated the critical number of beneficial alleles required for stable resistance. Additive gene action, allele strength (Hnetkovsky et al. 1996; Chang et al. 1997 Meksem et al. 1999) or root resistance (Njiti et al. 1997) may have contributed to the environmental stability observed in the ExF recombinant inbred lines.

QTLs for resistance to SDS in PxD were less effective compared to E×F at Ridgway in 1993. This may have been due to the interaction of resistant alleles with other unknown loci. High inoculum concentration has been found to breakdown SDS resistance (Gray and Achenback 1996).

The original sources of SDS resistance in Forrest or Pyramid cultivars are not known. Forrest is more resistant to SDS than both of its immediate parents, suggesting that they each provided alleles that combined to give Forrest a higher level of resistance. While no SDS data is available on ‘J74–5’, an immediate parent of Pyramid, the other immediate parent, ‘Franklin’ and ‘Bedford’, a sister line of J74–5, however, has a lower SDS resistance than Pyramid suggesting that gene pyramiding (Huang et al. 1997) occurred during the development of Pyramid.

The finding that the determinate (Douglas-type) recombinant inbred lines were significantly more susceptible than the indeterminate (Pyramid-type) recombinant inbred lines may be a function of the number of beneficial alleles accumulated by these genotypes. However, disease severity data from the greenhouse was previously found to be associated with a gene that controls determinacy in soybean (unpublished data of the authors). The ability to grow after SDS symptoms become apparent would tend to reduce disease severity in indeterminate cultivars. Determinacy has also been found to underlie resistance to *F. solani* in other crops (Gray et al. 1999).

The results from this study suggest that SDS resistance QTLs are effective in multiple genetic backgrounds and stable across environments. Cultivars with resistance to SDS can be developed via gene pyramiding.

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