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Identification of QTLs underlying water-logging tolerance in soybean

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Abstract

Soil water-logging can cause severe damage to soybean [*Glycine max* (L.) Merr.] and results in significant yield reduction. The objective of this study was to identify quantitative trait loci (QTL) that condition water-logging tolerance (WLT) in soybean. Two populations with 103 and 67 $F_{6:11}$ recombinant inbred lines (RILs) from A5403 × Archer (Population 1) and P9641 × Archer (Population 2), respectively, were used as the mapping populations. The populations were evaluated for WLT in manually flooded fields in 2001, 2002, and 2003. Significant variation was observed for WLT among the lines in the two populations. No transgressive tolerant segregants were observed in either population. Broad-sense heritability of WLT for populations 1 and 2 were 0.59 and 0.43, respectively. The tolerant and sensitive RILs from each population were selected to create a tolerant bulk and a sensitive bulk, respectively. The two bulks and the parents of each population were tested with 912 simple sequence repeat (SSR) markers to select candidate regions on the linkage map that were associated with WLT. Markers from the candidate regions were used to genotype the RILs in both populations. Both single marker analysis (SMA) and composite interval mapping (CIM) were used to identify QTL for WLT. Seventeen markers in Population 1 and 15 markers in Population 2 were significantly ($p < 0.0001$) associated with WLT in SMA. Many of these markers were linked to *Rps* genes or QTL conferring resistance to *Phytophthora sojae* Kaufmann and Gerdemann. Five markers, Satt599 on linkage group (LG) A1, Satt160, Satt269, and Satt252 on LG F, and Satt485 on LG N, were significant ($p < 0.0001$) for WLT in both populations. With CIM, a WLT QTL was found close to the marker Satt385 on LG A1 in Population 1 in 2003. This QTL explained 10% of the phenotypic variation and the allele that increased WLT came from Archer. In Population 2 in 2002, a WLT QTL was located near the marker Satt269 on LG F. This QTL explained 16% of the phenotypic variation and the allele that increased WLT also came from Archer.

Introduction

Soil water-logging injury is the result of excess soil moisture that adversely affects plant roots or a section of the shoot (Reyna et al. 2003). A classification of U.S. soils indicated that about 16% of the total territory of the United States suffers from excessive moisture (Boyer 1982). Many of these areas often experience water-logging or flooding due to soil properties such as poor internal or

surface drainage or high clay content. Other areas may experience waterlogged conditions due to cropping systems such as a rice [*Oryza sativa* L.] and soybean rotation. Flooded soils can severely affect plant growth and development. Most common symptoms of water-logging stress are leaf chlorosis, necrosis, stunting, defoliation, and plant death. Substantial yield reductions in soybean have been observed when excessive soil water occurs during the vegetative and reproductive stages of the plant (Scott et al. 1989, 1990; Oosterhuis et al. 1990; Van Toai et al. 1994; Linkemer et al. 1998; Reyna et al. 2003). Certain symptoms of water-logging injury such as leaf chlorosis and plant wilting are also symptoms of Phytophthora root rot, caused by *P. sojae*. Phytophthora root rot disease is favored by wet conditions and tends to be prevalent in the field with poor drainage.

The study of WLT in other crops has suggested that this trait is quantitatively inherited (Xu and Mackill, 1996; Setter et al. 1997; Sripongpangkul et al. 2000; Boru et al. 2001). Quantitative traits are generally difficult to assess, and often interact with different environments, making them difficult for breeders to manipulate. The identification of genomic regions affecting the tolerance of soybean to water-logging can be valuable for marker assisted selection. Van Toai et al. (2001) mapped a QTL for WLT in two northern soybean populations. 'Archer' was used as the source of WLT and marker Sat_064 on LG G was significantly associated with WLT in some but not all of the northern environments studied. Based on the linkage map by Song et al. (2004), Sat_064 is 57 cM distant to marker Satt472, which linked to the *Rps4* and *Rps6* genes (Demirbas et al. 2001) that confer resistance to *P. sojae*. Van Toai et al. (2001) pointed out that while the WTL QTL linked to marker Sat_064 was not related to the effects of *Rps4* gene the possibility of the QTL was related to effects of *Rps6* gene could not be ruled out. Reyna et al. (2003) conducted a study to evaluate the association of Sat_064 with WLT in southern environments using near isogenic lines from the crosses of 'A5403' × Archer and 'P9641' × Archer. No significant association between Sat_064 and WLT was found in their study.

Evaluation of soybean populations involving germplasm adapted to southern US locations offers the opportunity to confirm genetic markers previously reported to be associated with WLT and to identify new QTLs that confer tolerance to water-logging stress. The production of high-yielding soybean cultivars with improved tolerance to excessively wet soils will be beneficial for soybean producers to limit yield losses. The objective of this study was to map QTLs conditioning WLT in soybean in southern USA environments in two soybean populations.

Materials and methods

Population development

Southern elite cultivars A5403 [Maturity group (MG) 5.4] and P9641 (MG 6.4) were selected for their superior agronomic performance in southern USA environments, and were crossed with Archer (MG II, a northern USA cultivar with WLT) to develop genetic populations. Archer was shown to be tolerant to water-logging in northern USA environments (Van Toai et al. 2001). The crosses 'A5403 × Archer' and ''P9641 × Archer' were made in 1991. Recombinant inbred lines from these two populations were developed using the single pod descent breeding method for generation advancement. The plant populations were maintained without selection to the F_4 generation from 1992 to 1995. Since Archer is an early-maturing cultivar (blooming and maturing 25 to 35 days earlier than A5403 and P9641), individual plant selection at the F_4 generation for late maturity similar to that of the southern parents was performed in order to test the progeny lines in southern environments. Plants with the tendency of shattering and lodging were avoided while selecting for maturity. However, selection was not made for plant growth habit and there was no other unintentional selection due to biotic and abiotic stress. In 1996, two mapping populations with 103 and 67 F_6–derived RILs, respectively, were developed from the crosses of A5403 × Archer (Population 1) and P9641 × Archer (Population 2). The $F_{6:7}$ lines were grown and bulked in 1997. The $F_{6:8}$ RILs were grown and evaluated on the basis of casual waterlogging injury observations in 1998. The most tolerant and susceptible RILs were selected for bulked segregant analysis. All the RILs ($F_{6:9}$ and $F_{6:10}$) were grown and maintained as separate lines in bulk in 1999 and 2000.

Phenotypic evaluation for water-logging tolerance

Flooding experiments were conducted at the University of Arkansas Rice Research and Extension Center in Stuttgart, AR in 2001, 2002, and 2003 on a Calloway silt loam (Fine smectitic, hyperthermic, Typic Aldaqualf). Fields were managed in a rice and soybean rotation system (one year of rice followed by one year of soybean). Growing conditions were typical for soybean production during the years the three experiments were conducted. Temperatures were within the normal range throughout the three growing seasons. The average temperatures for the month of August, during which the flood treatment was applied and plant injury scores were taken, were 28 °C, 27 °C, and 27 °C for 2001, 2002, and 2003, respectively. The rainfall during the two weeks of treatment and the 2 weeks of observation after treatment in the 3 years of the experiment did not deviate from the norm for the location with an average of 45 mm for the month of August. Conditions tended to facilitate water-logging damage symptoms during the period when plots were flooded.

A full-season soybean production system managed in a rice-soybean rotation was used to evaluate the lines for WLT. Plantings were made on May 6, 2001, May 8, 2002, and May 12, 2003. Fertilizer was applied according to soil test recommendations. Plots were planted on a flat soil surface and later cultivated to facilitate furrow irrigation. Conventional herbicides were used for weed control. All plots were furrow-irrigated as needed until plants reached the R2 (full bloom) growth stage (Fehr and Caviness 1977). At the R2 stage, levees were constructed around each plot, and water was applied until it reached 7 to 12 cm deep. Water was maintained at that level until moderate canopy chlorosis and necrosis appeared about 10 to 14 days later. Then, water was allowed to drain from each plot. RILs and parents were visually rated based on the presence and frequency of foliar chlorosis and plant death 7 to 10 days after the flood was removed. A rating scale of 0 to 9 (0 being no damage and 9 being >90% dead plants) was used (Figure 1).

Lines from each population were evaluated in randomized complete blocks with two replications in one-row plots 6 m long and 80 cm apart. The southern parents for each population were included in the experiments for comparisons. Single-row plot with no borders was adequate for evaluating waterlogging tolerance based on canopy chlorosis since yield was measured for the experiment. Plots were arranged in tiers with 1.5 m between tiers which allowed easy harvest using a plot combine. Plots were not end-trimmed, but rogued before harvest to maintain pure seeds. A tank mix of Dual Magnum and Scepter herbicides

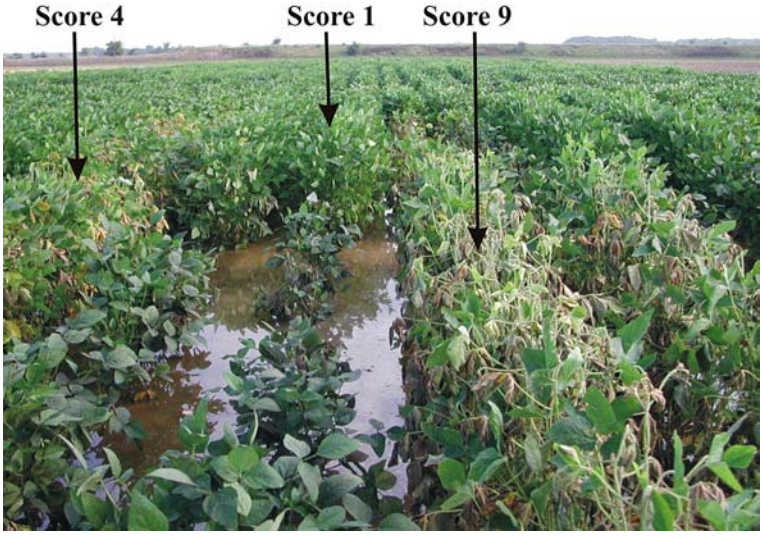

Figure 1. Visual ratings for water-logging injury of RIL mapping populations in the field. Injury ratings range from 0 to 9 with 0 being no damage and 9 being 90% or more of the plants dead.

were used for pre-emergence weed control for each of the 3 years, while Post Plus (2001), First Rate (2002), and Select + Flexstar (2003) herbicides were used for post emergence weed control. All herbicides were applied according to label specifications. Fertilizer (0-20-30) was applied at a rate of 36.7 kg ha^{-1} in each of the 3 years.

Statistical analyses

Analysis of variance (ANOVA) was performed with the GLM procedure of SAS (SAS 2000). The broad sense heritability for each population was estimated based on the ANOVA results and the expected mean squares using the equation $h^2 = (\frac{\sigma^2 g}{\sigma^2 e/RE + \sigma^2 ge/E + \sigma^2 g})$ (Fehr 1987), where h^2 is the heritability, σ^2_g is the genetic variance, σ^2_e is the experimental error (residual), R is the number of replications, E is the number of years, and σ^2_{ge} is the variance for the genotype × year interaction. Histograms for the distribution of progeny mean values were computed with Microsoft Excel software and tested using the UNIVARIATE procedure of SAS (SAS 2000). All analyses were carried out with a nominal 5% level of significance.

DNA extraction and genotyping with SSR markers

DNA of the $F_{6:11}$ RILs and their parents was isolated from the first trifoliate leaves of 10 greenhouse-grown seedlings by using the CTAB (hexadecyltrimethyl ammonium bromide) method described by Kisha et al. (1997). Genotyping of the DNA samples with SSR primers was performed according to Cregan and Quigley (1997). Briefly, the 15 μl polymerase chain reaction (PCR) mix contained 10 mM Tris–HCl (pH 8.4), 3.0 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Sigma-Aldrich, St. Louis, MO), 0.5 μM primer, 50 ng of genomic DNA, 1 unit of *Thermus aquaticus (Taq)* DNA polymerase, and sterile ddH_2O. The DNA fragments were amplified in a MJ Tetrad^{TM} thermal cycler (MJ Research, Waltham, MA). Cycling consisted of an initial denaturation of 4 min at 94 °C followed by 33 cycles of 25 s at 94 °C, 25 s annealing at 47 °C, and 25 s synthesis at 68 °C, and finally, an additional 5 min extension at 72 °C before cooling to 4 °C. The reactions were set up using a MultiProbe II Automated Liquid Handler from Packard (Downers Grove, IL). The PCR products were analyzed in 6% (w/v) non-denaturing polyacrylamide gel with a vertical sequencing system described by Wang et al. (2003). The SSR primer sequences were obtained from Dr. Perry Cregan at USDA-ARS at Beltsville, MA. The SSR primers were synthesized at the Genomics Technology Support Facility at Michigan State University.

A total of 912 SSR markers were screened for polymorphisms between the parents and two bulked DNA samples from each population. The tolerant bulk for Population 1 included the eight most flood-tolerant RILs and the sensitive bulk included the 13 most flood-sensitive RILs. The tolerant and sensitive bulks for Population 2 included the six most tolerant RILs and the seven most sensitive RILs, respectively. Only those lines that were consistently tolerant (injury score < 2.5) over years were selected to form the tolerant bulk. Likewise, only those lines that were consistently sensitive (injury score > 5) over years were used to form the sensitive bulk. Therefore, the number of RILs for the flood-sensitive bulks and the number of RILS for the flood-tolerant bulks were different in both populations. Markers that showed polymorphism between the two bulks and the two parents of each population were used to genotype the entire population. Single marker analysis was then carried out to identify those markers that are associated with WLT. Additional SSR markers that were linked to the WTL-associated markers according to the soybean linkage map (Song et al. 2004) and were bimorphic between the two parents were selected for further population genotyping.

Linkage map construction and QTL analysis

Linkage analysis was performed in both populations with JoinMap Version 3.0 (Van Ooijen and Voorrips 2001) and the Kosambi mapping function (Kosambi 1944), with a minimum LOD score of 4.0 and a maximum recombination fraction of 0.45. Linkage group names were assigned according to the soybean composite map (Song et al. 2004).

Both single marker analysis (SMA) and composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994) methods were used for QTL identification. Single marker analysis (SMA) was carried out by PROC GLM of SAS (SAS 2000)

with the following statistical model: $y_{ijklm} = \mu + E_i + R(E)_{ij} + M_k + F(M)_{kl} + E \times F(M)_{ikl} + \varepsilon_{ijklm}$, where y_{ijklm} is each observed phenotype, μ is the population mean, E_i is the effect of year (i = 1, 2, 3), $R(E)_{ij}$ is the effect of replication within year (j = 1, 2), M_k is the effect of marker genotype (k = 1, 2), $F(M)_{kl}$ is the effect of RILs within marker genotype (l = 1, …, 103 or 67), $E \times F(M)_{ikl}$ is the interaction between the effect of year and the effect of RILs within marker genotype, and ε_{ijklm} is residual error. The threshold for declaring a marker significant was chosen to be marker-wise $p < 0.0001$, which is approximately equal to an experiment-wise $p < 0.025$ in this study because less than 250 markers were bimorphic between the two parents of either population (see "Results" section below).

The CIM was carried out with QTL Cartographer (Basten et al. 1999) using model 6 of the Zmapqtl program with a window size of 10 cM. The linkage map constructed with JoinMap was used as the map input in the CIM analysis. Five markers outside the test window were used as cofactors to control background effects. The markers used as cofactors were selected with a backward and forward stepwise regression approach. The QTL analysis for Population 1 was performed with markers that were polymorphic between the bulks and the parents, and markers that were polymorphic only between the parents. An experiment-wise $\alpha = 0.10$ was used as the threshold to declare a putative QTL significant. The empirical threshold of the log-of-odds (LOD) score corresponding to experiment-wise $\alpha = 0.10$ was established by 1000 permutation (Churchill and Doerge 1994). For Population 1, the estimated LOD threshold for an experiment-wise $\alpha = 0.10$ was 2.2 for 2001, 2.5 for 2002, 2.2 for 2003, and 2.2 for the average over the 3 years. For Population 2, these thresholds were 1.9 for 2003 and 1.7 for 2001, 2002, and the average over the 3 years. The LOD plots were created with the software MapChart (Voorrips 2002) based on the output of QTL Cartographer.

Results

Variation for WLT among RILs was highly significant in both populations. Significant differences ($p < 0.05$) among years and a significant year × genotype interaction were observed for Population 1. Variation for WLT between years and year × genotype interactions were not significant in Population 2.

For Population 1, the average WLT score among RILs was higher than, but very close to, that of the non-tolerant parent A5403 (Table 1, Figure 2). For Population 2, all of the RILs were less tolerant to excessive water than was the parent P9641 (Table 1, Figure 2). The mean flood injury of Population 2 and its southern parent P9641 appeared to be stable across the 3 years, whereas Population 1 and its southern parent A5403 had lower injury ratings in 2002. The northern parent Archer for both populations was not included in the test due to its early maturity and poor adaptation to Arkansas growing conditions. Moderate heritabilities of WLT were observed in both populations (Table 1). There were no transgressive tolerant segregants in either population.

Two hundred and one SSR markers were parentally bimorphic in population 1. However, only 18 of the 201 parentally bimorphic markers were bimorphic between the two contrasting phenotypic bulks (data not shown). The 18 markers were used to genotype Population 1 and all 18 markers were bimorphic in the population. Eight of these 18 markers were significantly associated with WLT in SMA. Additional 35 markers linked to the eight significant markers were selected from the soybean linkage map (Song et al. 2004) to genotype Population 1. Therefore, a total of 53 markers were used to genotype population 1. Linkage analysis by JoinMap mapped 25 of the 53 markers into nine linkage groups (data not shown), which were sections of LGs A1, F, J, and

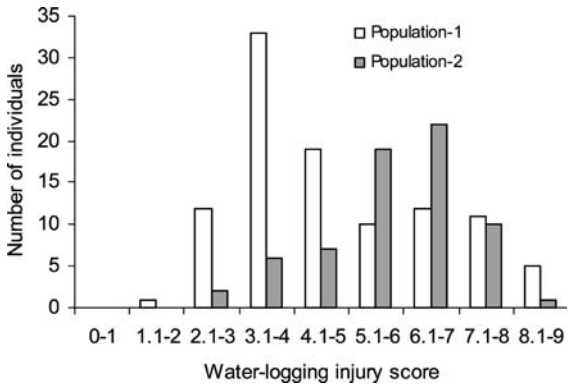

Figure 2. Frequency distribution of water-logging injury scores for RILs from two populations of A5403 × Archer (Population 1) and P9641 × Archer (Population 2).

Table 1. Broad sense heritabilities and mean water-logging tolerance scores for parents (Archer, A5403 and P9641) and populations plus estimates of heritability derived from A5403 × Arher (Population 1) and P9641 × Archer (Population 2) for tolerance to water-logging in each 2001, 2002, and 2003 at Stuttgart, AR.

Genotype and Parameter	2001	2002	2003	Average
Water-logging injury score *				
Archer **	–	–	–	–
A5403	6.0 (13)	1.7 (3)	4.2 (5)	3.9
(A5403 × Archer) RILs	4.6	3.7	5.5	4.6
h^2				0.59
Archer **	–	–	–	–
P9641	2.1 (8)	2.0 (3)	2.0 (5)	2.0
(P9641 × Archer) RILs	5.6	5.8	5.7	5.7
h^2				0.43

*Score based on 0 (no injury) to 9 (>90% dead plants).
**Tolerance scores not included for Archer due to its very early maturity and poor adaptation to southern US environments. Number in parenthesis denotes number of plots evaluated for parental lines.

N on the soybean linkage map (Song et al. 2004). The other 28 markers did not show linkage to any markers in the linkage analysis and, therefore, were not included in the CIM analysis.

Two hundred and twenty eight SSR markers were parentally bimorphic in Population 2 and only 30 of these markers showed bimorphism between the two bulks (data not shown). These 30 markers were used to genotype Population 2 and all markers were bimorphic in the population. Linkage analysis with JoinMap mapped nine markers into four linkage groups (data not shown), which were sections of LGs A1, F, J and N on the soybean linkage map (Song et al. 2004). The other 21 markers did not show linkage to any markers in the linkage analysis and were not included in the CIM analysis.

In the SMA, 17 and 15 markers were found significantly ($p < 0.0001$) associated with WLT in populations 1 and 2, respectively (Table 2). These markers were located on LGs A1, F, G, J, and N. Five markers, Satt599 on LG A1, Satt160, Satt269, and Satt252 on LG F, and Satt485 on LG N, were significantly associated with WLT in both populations (Table 2).

In the CIM analysis, a QTL for WLT was found close to the marker Satt385 on LG A1 in Population 1 in 2003. This QTL had a LOD score of 2.5 and explained 10% of the phenotypic variation for WLT (Figure 3). Parent Archer contributed the allele that significantly increased WLT for this QTL. A putative QTL (LOD = 1.6) was found close to the marker Satt569 on LG F in Population 1 in 2003 and this putative QTL explained 6% of the phenotypic variation (Figure 3). Parent Archer contributed the allele that increased WLT for this putative QTL. In Population 2 in 2002, a QTL with an LOD score of 2.0 was located near the marker Satt269 on LG F. This QTL explained 16% of the phenotypic variation for WLT (Figure 3) and the allele that significantly increased WLT came from Archer. A putative QTL (LOD = 1.5) was also identified in this same region on LG F for the average of WLT over the 3 years. Table 3 shows the most water-logging tolerant and sensitive RILs from populations 1 and 2 and their

Table 2. Markers significantly associated with water-logging tolerance revealed by single marker analysis of populations derived from A5403 × Archer (Population 1) and P9641 × Archer (Population 2) for tolerance to water-logging when grown at Stuttgart, AR.

Marker	Linkage Group	Position (cM)	Significance	
			Pop 1	Pop 2
Satt276	A1	17.2		**
Satt591	A1	31.1	**	
Satt155	A1	32.7	**	
Satt385	A1	64.7	**	
Satt545	A1	71.4	**	
Satt599	A1	85.6	**	**
Satt258	A1	95.5		**
Satt569	F	3.4	**	
Satt269	F	11.4	**	**
Satt252	F	16.1	**	**
Satt160	F	33.2	**	**
Satt516	F	44.4		**
Satt114	F	63.7	**	
Satt510	F	71.4	**	
Sct_188	F	85.3		**
Satt275	G	2.2		**
Satt610	G	10.9		**
Satt303	G	53.4		**
Satt612	G	80.4		**
Satt287	J	15.7	**	
Sct_046	J	24.1	**	
Satt406	J	38.2	**	
Sat_412	J	41.1		**
Sat_396	J	69.3	**	
Satt641	N	29.3		**
Satt485	N	38.1	**	**
Satt022	N	102.1	**	

**Significant at the less 0.0001 probability level, respectively. Linkage group names and relative position for the markers were assigned according to the soybean composite map (Song et al. 2004)

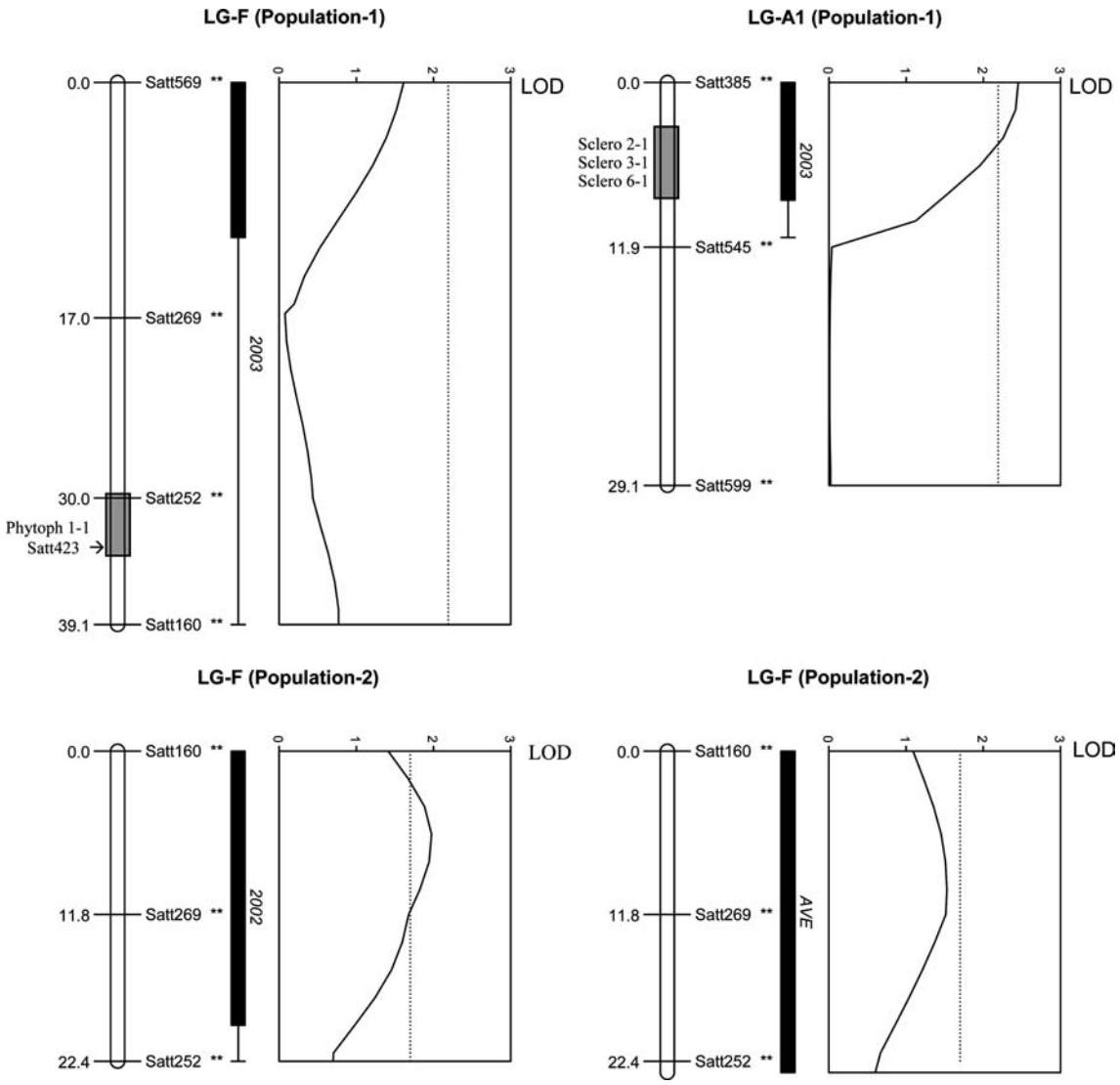

Figure 3. LOD score plots for the two regions on linkage groups A1 and F containing QTL for water-logging tolerance in populations derived from A5403 × Archer (Population 1) for 2003, and P9641 × Archer (Population 2) for 2002, and the average over years. The QTL bars are shown as 1-(thick bar) and 2-LOD (thin bar) support intervals generated automatically from the LOD graphs by MapChat. *, ** indicate the marker is significantly associated with water-logging tolerance at the 0.05 and 0.01 probability levels, respectively, based on single marker analysis.

genotypes at the marker loci in the QTL regions on linkage groups A1 and F. In general, the tolerant RILs carried most of the favorable alleles while the sensitive RILs carried most of the non favorable alleles (Table 3).

Discussion

Differences among RILs in both populations in response to water-logging were highly significant suggesting important genetic components are involved in WLT. Reaction of RILs in Population 2 and its southern parent appeared to be stable across different years, whereas population 1 and its southern parent were variable in different years indicating environmental factors in the expression of WLT.

The northern parent Archer, which is early maturing and not adapted to southern environments, was not evaluated along with the RIL

Table 3. Selected water-logging tolerant and sensitive RILs from Populations 1 and 2 and their genotypes at the marker loci in the QTL regions on linkage groups A1 and F.

Marker	Linkage Group A1			Linkage Group F				Injury score
	Satt385	Satt545	Satt599*	Satt569	Satt269*	Satt252*	Satt160*	
RIL 179	A	A	A	A	A	B	B	1.7
RIL 137	A	B	A	A	A	B	A	2.3
RIL 156	B	A	A	A	A	A	A	2.4
RIL 116	A	B	A	B	A	A	A	2.5
RIL 221	B	B	A	B	B	B	B	8.0
RIL 297	B	B	B	B	B	B	A	8.2
RIL 270	B	B	B	B	B	B	B	8.3
RIL 350	U	U	A	B	A	A	A	2.3
RIL 322	U	U	B	B	A	A	A	2.8
RIL 364	U	U	A	A	B	B	A	7.9
RIL 312	U	U	A	A	B	B	B	8.1

Favorable alleles from Archer are indicated A, non-favorable alleles from other parents, A5403 and P9641, are indicated B, and uncertain alleles (parentally monomorphic) are indicated U. Injury scores are the means of all measurements over 3 years and replicates. RIL numbers less than 300 are from Population 1 and RIL numbers greater than 300 are from Population 2.
*indicates the maker were significantly ($p < 0.0001$) associated with WLT in both populations in the single marker analysis

populations (Table 1). Based on previous studies by Van Toai et al. (1994), Archer would have had an injury score equivalent to 2.0 in our rating system. The alleles of the two QTLs identified in Population 1 that contributed to the increased WLT were both from Archer. The parent A5403 exhibited high scores of water-logging injury in our rating system and no QTLs for increased WLT were found in A5403. The parent Archer consistently possessed the QTL identified in Population 2 that contributed most to the increased WLT.

Marker Sat_064, located on LG G, was previously found to be associated with WLT in soybean (Van Toai et al. 2001). However, no such association was found in this study. Sat_064 did not show consistent associations with WLT across different northern USA environments in the study by Van Toai et al. (2001) nor across different southern USA environments in this study and in the study by Reyna et al. (2003).

In water-logged conditions, the environment is favorable for Phytophthora root rot caused by *P. sojae*. Plants with resistance to *P. sojae* would appear tolerant to water-logging when *P. sojae* was prevalent in the water-logged field. Soybean has both partial resistance and race-specific resistance to *P. sojae*. The partial resistance seems to be controlled by several genes (Walker and Schmitthenner 1984; Glover and Scott 1998). Two QTLs for partial resistance to *P. sojae* have been identified (Table 4). Eight genetic loci with fourteen genes (*Rps1a, Rps1b, Rps1c, Rps1d, Rps1k, Rsp2, Rps3a, Rps3b*, and *Rps3c*, *Rps4, Rps5, Rps6, Rsp7* and *Rps8)* are responsible for race-specific resistance to *P. sojae* (Table 4). Archer has two Phytophthora resistance genes, *Rps1k* and *Rps6*. These two genes were expected to segregate in the two mapping populations used in our study. Weng et al. (2001) mapped *Rps1a* to LG N between Satt159 (27.1 cM) and Satt009 (28.5 cM) on the soybean linkage map (Table 4). *Rps1k* is by definition allelic to *Rps1a* and is expected to be mapped at the same location as *Rps1a*. Satt641 (29.3 cM) on LG N that was significantly ($p < 0.0001$) associated with WLT in SMA (Table 2) is closely linked to *Rps1k*, suggesting the WLT might have been related to phytophthora resistance conferred by *Rps1k*. Demirbas et al. (2001) found that *Rps6* was linked to markers on LG G between 94 cM and 97 cM on the soybean linkage map. We identified four markers on LG G between 2 cM and 81 cM significantly ($p < 0.0001$) associated with WLT in the SMA (Table 2). Although the markers were not within the genomic region where markers linked to *Rps6* were found, the closest marker was only 15 cM away from the *Rps6* region. The possibility that the WLT was related to *Rps6* gene could not be ruled out.

The *Rps2* and *Rps3* resistant genes were not known to be present in any of the parents of the mapping populations used in this study. Nevertheless, markers linked to the *Rps2* and *Rps3* were

Table 4. *Rps* loci and QTLs underlying resistance to phytophthora root rot and their locations on the soybean composite map (Song et al. 2004)*.

Rps loci or QTL	Linkage Group	Flanking marker (position cM)	Reference
Rps1	N	Satt159–Satt009 (27.1 – 28.5)	C. Weng et al. 2001
Rps2	J	A233_1–A724_1 (83.2 – 84.9)	B.W. Diers et al. 1992; A. Demirbas et al. 2001
Rps3	F	A757_1–R045_1 (63.1 – 70.1)	B.W. Diers et al. 1992; A. Demirbas et al. 2001
Rps4	G	A586_2 (111.2)	B.W. Diers et al. 1992; A. Demirbas et al. 2001
Rps5	G (possibly)	T005_2 (81.5)	B.W. Diers et al. 1992; A. Demirbas et al. 2001
Rps6	G	Not defined	A. Demirbas et al. 2001
Rps7	N	Satt009–Satt125 (28.5 – 40.6)	C. Weng et al. 2001
Rps8	A2	Sat_040–Satt228 (118.6 – 154.1)	K.D. Burnham et al. 2003a
QTL	F	Satt252–Satt423 (16.1 – 20.6)	K.D. Burnham et al. 2003b
QTL	D1b	Satt266–Satt579 (59.6 – 75.9)	K.D. Burnham et al. 2003b

*Linkage group names, marker names, and marker positions are updated as shown on the soybean composite map (Song et al. 2004).

found significantly ($p < 0.0001$) associated with WLT in SMA. *Rps2* mapped to LG J between A233_1 (83.2 cM) and A724_1 (84.9 cM) on the soybean linkage map (Table 4). Sat_396 (69.3 cM) on LG J that was significantly ($p < 0.0001$) associated with WLT (Table 2) is less than 16 cM distant to the *Rps2* locus. *Rps3* mapped to LG F between A757_1 (63.1 cM) and R045_1 (70.1 cM) (Table 4). Satt114 (63.7 cM) and Satt510 (71.4 cM) on LG F that were significantly ($p < 0.0001$) associated with WLT (Table 2) are closely linked to *Rps3*. Further studies are needed to determine if different alleles at the *Rps2* and the *Rps3* loci were segregating in the populations and whether the WLT was associated with the two *Rps* loci.

A QTL for partial resistance to *P. sojae* was located on LG F between marker Satt252 and marker Satt423 (Burnham et al. 2003b; Table 4). Soybean variety Conrad was the source of the resistance allele (Burnham et al. 2003b). Satt252 was significantly ($p < 0.0001$) associated with WLT in both populations in our study (Table 2). Archer, the source of WLT, is not closely related to Conrad and is not known to carry the resistance allele of the QTL for partial resistance to *P. sojae*. Further research is needed to determine if the association of Satt252 with WLT is related to the QTL for partial resistance to *P. sojae*.

No markers on LG A1 have been found to be linked to any *Rps* genes heretofore. Seven markers on LG A1 between 17 cM and 96 cM (Table 2) were significantly ($p < 0.0001$) associated with WLT in the SMA in this study. The association of these markers with WLT is, therefore, not related to any known *Rps* genes. One of these markers, Satt545, was linked to resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary (Arahana et al. 2001) (Figure 3). The infections of *S. sclerotiorum* mainly occurred in the fields where cool, moist environmental conditions were consistently present. At the time when the two populations were evaluated for WLT in Arkansas, the temperature was too high for the infection to occur. No symptom of Sclerotinia stem rot, which is caused by *S. sclerotiorum*, was observed during the evaluation. Therefore, the WLT was not likely related to resistance to *S. sclerotiorum*.

The identification of genomic regions responsible for tolerance to water-logging in soybean will be useful for marker-assisted selection (MAS) to expedite the development of high-yielding WLT soybean cultivars. With MAS, it is possible to combine multiple favorable alleles into a single cultivar (Table 3). Cultivar selection for the commonly used rice-soybean rotation and marginal production areas prone to water-logging conditions will benefit from significant QTLs for WLT. Future research is needed to confirm these QTLs for WLT before using MAS to efficiently incorporate the trait into high-yielding cultivars.

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