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ORIGINAL PAPER

# Polygenic inheritance of canopy wilting in soybean [*Glycine max* (L.) Merr.]

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**Abstract** As water demand for agriculture exceeds water availability, cropping systems need to become more efficient in water usage, such as deployment of cultivars that sustain yield under drought conditions. Soybean cultivars differ in how quickly they wilt during water-deficit stress, and this trait may lead to yield improvement during drought. The objective of this study was to determine the genetic mechanism of canopy wilting in soybean using a mapping population of recombinant inbred lines (RILs) derived from a cross between KS4895 and Jackson. Canopy wilting was rated in three environments using a rating scale of 0 (no wilting) to 100 (severe wilting and plant death). Transgressive segregation was observed for the RIL population with the parents expressing intermediate wilting scores. Using multiple-loci analysis, four quantitative trait loci (QTLs) on molecular linkage groups (MLGs) A2, B2, D2, and F were detected ($P \leq 0.05$), which collectively accounted for 47% of the phenotypic variation of genotypic means over all three environments. An analysis of the data by state revealed that 44% of the observed phenotypic variation in the Arkansas environments could be accounted for by these QTLs. Only the QTL on MLG F was detected at North Carolina where it accounted for 16% of the phenotypic variation. These results demonstrate that the genetic mechanism controlling canopy wilting was polygenic and environmentally sensitive and provide a foundation for future research to examine the importance of canopy wilting in drought tolerance of soybean.

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## Introduction

Agriculture accounts for approximately 70% of water usage globally, and 40% of crop hectarage is grown on irrigated soils (IPCC 2001). With the increasing impact of global warming and rising human population, scientists expect an increased demand on water supply in the form of irrigation world-wide (IPCC 2001). Some agricultural regions where water supply is currently plentiful may experience decreases in water availability as a result of more frequent drought episodes, or they may become too arid for agricultural production. With these challenges to water availability, agriculturalists will need to adopt efficient management strategies that reduce the amount of water necessary for crops and/or increase a crop's efficiency for using water.

Drought-tolerant cultivars will be an important component of future water management strategies in agriculture,

but development of such cultivars is difficult. Plant–environment interactions often form complex barriers making drought tolerance difficult to identify and manipulate. Overcoming these barriers may require a holistic or team approach that incorporates physiology, molecular genetics, and crop management strategies into the plant breeding effort, and this approach was adopted in the present study.

Drought responses of crop plants are not well understood as they are genetically and physiologically complex. Crop response to water deficit often include physiological changes that minimize water loss, such as closing stomata and reducing leaf surface area by leaf rolling (O'Toole and Moya 1978). An additional response that has been given less attention is canopy wilting (Lawlar and Cornic 2002). Preliminary evidence (Carter et al. 1999, 2006; Sloane et al. 1990) indicates that soybean genotypes differ in how rapidly canopy wilting occurs under water-deficit stress and delayed-canopy wilting has agronomic benefit.

The mechanisms conferring canopy wilting differences among soybean genotypes are only partially understood. One mechanism determining genotypic differences in wilting appears to be related to soil moisture conservation even before drought stress becomes severe (Fletcher et al. 2007; King et al. 2009). When soil water is plentiful, some slower-wilting genotypes have the ability to maintain relatively lower transpiration rates compared to conventional cultivars, and thus do not deplete the soil-moisture reservoir as rapidly as they grow. Subsequently, as drought effect builds, sufficient soil moisture is available for slow-wilting genotypes to prolong transpiration and leaf turgor for several days compared to fast-wilting genotypes. Currently, genetic mechanisms controlling these physiological adaptations to drought are unknown. Therefore, identification of quantitative trait loci (QTLs) for canopy wilting will assist researchers in identifying those genes and their functions. This information then could be used for marker-assisted selection to identify genotypes with delayed wilting in response to soil-water deficits.

With the availability of a dense, genetic map of molecular markers for soybean (Choi et al. 2007; Song et al. 2004) and high-throughput molecular genotyping methods, it has become increasingly efficient to examine the quantitative nature of traits, such as canopy wilting with molecular markers and gene mapping. Because of the inherent difficulty in breeding for drought tolerance, the canopy wilting trait is an ideal candidate for marker-assisted selection in commercial and public breeding programs. Therefore, our objective was to elucidate the genetic mechanisms by determining the inheritance and genomic locations of QTLs associated with canopy wilting in soybean.

## Materials and methods

### Plant material

A population of 92 recombinant-inbred lines (RILs) consisting $F_3$- and $F_5$-derived soybean lines [*Glycine max*. (L.) Merr] was developed by single-seed descent from crosses between cultivars KS4895 (PI 595081) (Schapaugh and Dille 1998) and Jackson (PI 548657) (Hollowell 1958). Seed were bulked from $F_1$ plants and the resulting $F_2$ plants were advanced by single seed descent to the $F_3$ or $F_5$ generations. Seed from the individual $F_3$ or $F_5$ plants were bulked to develop $F_3$- or $F_5$-derived RILs. Wilting was evaluated for 79 $F_5$-derived lines; however, genotypic data was successfully obtained for only 76 of these lines for QTL analyses. To construct a genetic linkage map, genetic information collected for the 76 $F_5$-derived lines were combined with an additional 16 $F_3$-derived lines to increase map resolution.

Parental cultivars were chosen due to their differences in nitrogen fixation during drought: KS4895 is drought sensitive whereas Jackson is tolerant (Purcell et al. 1997). Although the parents did not differ in canopy wilting in response to water-deficit stress, the RILs in the mapping population demonstrated variation in canopy wilting during drought conditions. Subsequently, the population was investigated for the inheritance of canopy wilting.

### Evaluation of canopy wilting

Canopy wilting was evaluated in three environments for 79 $F_5$-derived lines, where $F_{5:8}$, $F_{5:9}$, and $F_{5:10}$ generations were used in 2000, 2002, and 2003, respectively. In 2000 and 2003, parents and RILs were evaluated at the University of Arkansas Rice Research and Experiment Station at Stuttgart, Arkansas (AR 2000 and AR 2003) and Sandhills Research Station at Windblow, North Carolina in 2002 (North Carolina).

The experiments were arranged in a randomized complete block design with three replications per environment. At the Arkansas location, four-row plots with 80-cm spacing between rows were planted on a Crowley silt loam soil, whereas at the North Carolina location, three-row plots with 96-cm spacing between rows were planted on a Candor sand soil.

Canopy wilting was evaluated visually for the center two rows at Arkansas and the center row at North Carolina. Rating was conducted once on each of two consecutive days for each environment between late-August and early-September during water-deficit stress coinciding with R2 to early R5 developmental period (Fehr and Caviness 1977). The rating day giving the greatest range of wilting values within each environment were used for analysis. Wilting

was rated using either a 0 (no wilting) to 5 (plant death) unit scale at Arkansas 2000 and North Carolina 2002 or 0 (no wilting) to 100 (plant death) unit scale at Arkansas 2003. For analysis, data from each environment were standardized to a common rating system of 0 to 100 units (0 = no wilting, 40 = moderate wilting, 60 = severe wilting, and 100 = plant death) (King et al. 2009).

Phenotypic data were analyzed with analysis of variance using PROC GLM (SAS 9.1; Cary, NC). All effects were considered random in the model, where each year by location combination was considered an environment. Because a significant ($\alpha = 0.05$) genotype × environment (G × E) interaction was detected using data collected from all environments, data from each environment were analyzed separately. Pearson's correlation coefficients ($r$) were calculated to determine the consistency of RIL wilting score means between individual environments (2000, 2002, and 2003) and states (Arkansas vs. North Carolina). Broad-sense heritability estimates ($H$) of canopy wilting on a genotypic-mean basis were calculated for combined data over all environments or by state using the variance components obtained from results of analysis of variance (Fehr 1987).

## DNA extraction and marker evaluation

DNA was extracted (Shultz et al. 2007) from the parents and RILs. Simple Sequence Repeats (SSR) marker primer sequences (SoyBase; http://soybase.org/resources/ssr.php) were tested for polymorphisms between the two parental lines. Using PCR, each marker was amplified with 35 cycles of a denaturation step (94°C for 30 s), an annealing step (46°C for 30 s) and primer extension (72°C for 30 s). Initially, polymorphic alleles for each marker were identified via separation using polyacrylamide gel electrophoresis (PAGE) stained with ethidium bromide and genotype rated visually. As newer, more sensitive technology became available, the remaining markers were screened using fluorescently labeled primers, and PCR products were separated by size using an ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA) at the USDA-ARS Midsouth Area Genomics Facility at Stoneville, MS. The products were analyzed using GeneMapper 3.7 (Applied Biosystems, Foster City, CA). A total of 562 SSR markers were tested on the parental lines, and 304 markers (54%) were found to be polymorphic. Only 165 SSR markers were used to develop the genetic map.

## Development of genetic linkage map

A genetic map was developed using the populations of $F_{3:7}$-(16 lines) and $F_{5:7}$ (76 lines) RILs. Genotypic analysis was conducted using DNA collected from five plants bulked within a RIL. To eliminate any markers demonstrating segregation distortion resulting from genotyping errors, which could result in false positives for an association between a marker and phenotype, the marker data were tested for 1:1 genotypic ratio by a $X^2$-test (i.e., 1 homozygous Jackson genotype (JJ): 1 homozygous KS4895 genotype (KK)) for each marker across RILs. This analysis was done on 183 SSR markers originally used to genotype the 92 RILs. Eighteen markers deviated significantly ($\alpha = 0.001$) from the expected segregation pattern across the population and were removed from subsequent analyses. Therefore, only homozygous loci for 165 SSR markers were used to examine the proportion of homozygosity of the RIL population and to develop the genetic linkage map.

The proportion of homozygosity was estimated as the sum of genotypes homozygous for KS4895 (KK) or Jackson (JJ) alleles for a given marker locus. Examining all 92 derived lines using the 165 SSR markers mentioned above, the mean proportion of homozygosity across marker loci within each of the subpopulations of $F_{3:7}$ and $F_{5:7}$ lines was similar at 91% and 94%, respectively. This value is similar to the 94% expected homozygosity for an $F_5$-derived population. Subsequently, the genotypic data from both $F_{3:7}$ and $F_{5:7}$ lines were combined for analysis. To construct the genetic linkage map, marker data were partitioned by molecular linkage group (MLG) as described by Song et al. (2004). Additionally increasing the number of lines increased the similarity of genetic distances between markers on our map relative to the public genetic map (analysis not shown) (Song et al. 2004).

MapMaker 3.0b (Lander et al. 1987) was used to determine linkage and estimate genetic distance using the Kosambi centimorgan function (Lander et al. 1987). Grouping of markers within MLG was first conducted with a LOD-score threshold of 3.0 to identify initial linkage groups. To combine these initial linkage groups within reported MLGs (Song et al. 2004), a more liberal LOD-score threshold value of 1.5 (Blair et al. 2003; Bouck et al. 2005) was used to estimate genetic distances between these linkage groups to form a composite linkage map for each MLG. Any markers not linked to any composite linkage group at LOD-score less than 1.5 with estimated genetic distance of 37.0 cM or greater (default value for MapMaker) were treated as single-marker linkage groups (Bouck et al. 2005). The genetic map in this study represented all 20 MLGs (Song et al. 2004) with 1,844 cM coverage and average genetic distance between markers of 20 cM.

## Identification of wilting QTLs

Three analyses were used to identify putative QTLs: single-marker analysis (SMA), multiple-loci analysis (MLA), and composite interval mapping (CIM) using genotype means over the appropriate environments and replications. Both

SMA and MLA were conducted using PROC GLM (SAS 9.1; Cary, NC). Single-marker analysis is a linear regression of wilting phenotype of the individuals, averaged over all observations, against each individual marker locus. Single-marker analysis was the first analysis performed to search for significant associations. Associations between markers and wilting score mean were considered significant at the $\alpha = 0.05$ level. Coefficients of determination ($R^2$) were obtained for each marker associated with wilting.

For MLA, single markers were chosen to represent specific chromosomal regions or loci. A chromosomal region was defined as a contiguous segment on the linkage map where several linked markers were significantly associated with wilting according to SMA. Significant ($\alpha = 0.05$) single markers with the greatest $R^2$-value from SMA were selected to represent a single chromosomal region. The selected markers then were used in multiple regressions. Using a step-wise backward regression, all selected markers were included as independent variables in the first analysis of variance. For each subsequent analysis, a single marker with the least value of Type III sum of squares was removed from the model. Analysis continued until only markers with significant ($\alpha = 0.01$) associations remained in the model. Values of $R^2$ were calculated for each retained marker.

The entire genetic map was scanned for QTLs by composite interval mapping with a walking speed of 1 cM using WinQTL Cartographer (Wang et al. 2007). A QTL was termed putative if it was detected using MLA and had a LOD-score greater than 3.0 indicated by CIM. A QTL was termed suggestive if it was detected by MLA and had a LOD-score between 2.0 and 2.99 indicated by CIM (Dong et al. 2005; Kassem et al. 2006). Both LOD-value thresholds were used to determine genetic range (cM) of each QTL across marker intervals.

## Results

### Phenotypic evaluation of canopy wilting

In the analysis of data pooled from all environments, the genotype, environment and genotype × environment interaction (G × E) effects were all significant ($P \leq 0.05$) (Table 1). An orthogonal partitioning of the G × E interaction indicated that the genotype x state interaction as well as the genotype × year interaction within Arkansas were also significant ($P \leq 0.05$). This result indicated that the G × E interaction detected in the overall analysis does not reflect a geographical bias. However, a significant ($r = 0.71$, $n = 79$, $P \leq 0.05$) correlation of genotypic means existed between years for the Arkansas data. Whereas a much weaker relationship for wilting was found between North Carolina and Arkansas genotypic means averaged over years ($r = 0.47$, $n = 79$, $P \leq 0.05$). This result indicated that although a G × E interaction was present, both within Arkansas and between North Carolina and Arkansas, agreement was better between the 2 years in Arkansas and suggests that the interaction within Arkansas may be due to differences in scale of genotypic differences rather than overall changes in genotype ranking. As such, we investigated QTL using means from Arkansas and using means from North Carolina.

**Table 1** Analysis of variance of canopy wilting

| Source of variation | Degrees of freedom | Mean squares | |
|---|---|---|---|
| Combined over three environments | | | |
| Rep (environment) | 6 | 605 | *** |
| Environment | 2 | 5,303 | * |
| Genotype | 78 | 557 | *** |
| Genotype × environment | 156 | 229 | *** |
| Error | 466 | 82 | |
| State | | | |
| Rep (state) | 4 | 577 | ** |
| State | 1 | 2,501 | ns |
| Genotype | 78 | 379 | *** |
| Genotype × state | 78 | 164 | * |
| Error | 547 | 129 | |
| Arkansas only | | | |
| Rep (year) | 4 | 866 | *** |
| Year | 1 | 8,259 | * |
| Genotype | 78 | 629 | *** |
| Genotype × year | 78 | 292 | *** |
| Error | 310 | 90 | |
| North Carolina only | | | |
| Rep | 2 | 85 | ns |
| Genotype | 78 | 91 | * |
| Error | 156 | 65 | |

Wilting scores were collected for 79 $F_5$-derived RILs at Arkansas in 2000 and 2003, and North Carolina in 2002. Analyses shown for data combined over all environments, where each year was considered an environment, by state, and within state

*ns* non-significant

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Broad-sense heritability estimates ($H$) varied among environments. Heritability of genotypic means was similar over all environments ($H = 0.46$, three environments, 3 reps) and among the two Arkansas environments ($H = 0.51$, two environments, 3 reps).

### Distribution of wilting score means within the RIL population

A normal distribution was observed for the overall genotypic means with a range in wilting scores from 29 to 64

units (Fig. 1) with a population mean of 40 units. The distribution range for genotypic means was greater at Arkansas (means combined over years) with scores from 19 to 68 units relative to North Carolina with a more narrow range of 29–54 units. Transgressive segregation occurred as the wilting scores of the parents in Arkansas 2003 were 32 units for KS4895 and 38 units for Jackson and similar to the population mean of 40 units.

### Identification of wilting QTLs

Because of the G × E interaction detected in this study, marker analysis was performed on the genotypic means for Arkansas and North Carolina separately as well as on overall genotypic means. Using SMA, 17 SSR markers were significantly ($P \leq 0.05$) associated with wilting when analyzed over all environments and using only Arkansas data. Only five markers were significant using only North Carolina data and only two of these markers (Satt362 and Satt072 on MLG F) were also significant for Arkansas (Table 2).

The MLA was conducted using 12 of the markers representing significant chromosomal regions identified by SMA (Table 2). Using data from the two Arkansas environments, four markers were significantly ($P \leq 0.01$) associated with wilting at Arkansas. The $R^2$-value for the model containing all four markers was 0.44 with individual $R^2$-values ranging from 0.07 to 0.16 (Table 2). The markers that were significant ($P \leq 0.01$) over all environments and within Arkansas were the same. Just one marker (Satt362 on MLG F) was significant in North Carolina, which was also significant for Arkansas.

Composite interval mapping detected four chromosomal intervals with LOD scores of 3.0 or greater (Table 2). Using the overall data or just Arkansas data, the QTL on MLGs B2 and D2 were significant with LOD scores >3.0, while the QTL on MLG F was significant (LOD > 3.0) using the North Carolina data and with the Arkansas data at a LOD score of 2.0 (Fig. 2). In addition, one marker (Sat_319) on MLG A2 was detected only for Arkansas with a LOD-score of 3.5. Lastly, Jackson contributed slow-wilting alleles for three of the four QTLs (MLG A2, B2, and F) whereas, KS4895 contributed a slow-wilting allele for the QTL on MLG D2 (Table 2).

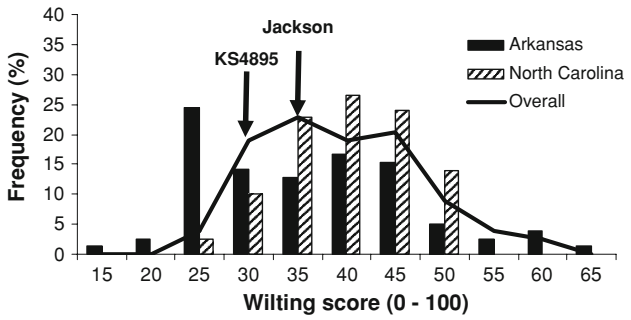


**Fig. 1** Distribution of wilting scores in the population of 79 $F_5$-derived RILs for the overall means across all three environments (Arkansas 2000 and 2003, and North Carolina 2002), Arkansas combined average (over years 2000 and 2003), and North Carolina. Wilting was evaluated using a 0–100 unit rating scale, where 0 (no wilting) to 50 (moderate wilting) to 100 (plant death). The wilting value means for each environment were approximately 40 units. Parents exhibited similar wilting scores of 32 units for KS4895 and 38 units for Jackson at Arkansas in 2003

## Discussion

Using our most stringent analyses, we detected four putative QTLs for canopy wilting on MLG of A2, B2, D2, and F. These results, along with the normal distribution of wilting phenotypes, indicate polygenic inheritance of wilting.

One QTL on MLG F was found in both states. Because the QTL on MLG F was identified in both environments, this QTL appears to have potential utility in marker-assisted selection for genotypes with reduced wilting over different environments. The environmental factors that influence delayed-canopy wilting, such as soil type and water-vapor deficits, are poorly understood. Therefore, identification of a single QTL associated with both environments is significant. Furthermore, the other QTLs we detected may also have utility across environments though more research will be needed to confirm this. Jackson was the genetic source of three of the four slow-wilting alleles. In breeding efforts to introgress the slow-wilting trait into commercial cultivars, Jackson would be a useful genotype to improve slow wilting in soybean.

In addition to environmental factors affecting whole plant physiological response to soil-water deficits, other agronomic traits may play a role in expression of the wilting phenotype. Therefore, we consulted SoyBase (www.soybase.org) for reported QTLs of agronomic importance genetically associated with the four wilting QTLs presented in this research. No QTLs were associated with the QTL on MLG A2, however, seed protein and oil content correspond to markers for wilting on MLGs B2, D2, and F (Diers et al. 1992; Hyten et al. 2004; Lee et al. 1996; Orf et al. 1999; Panthee et al. 2005). This observation may indicate that water-deficit stress may influence protein and oil concentrations of seed, or the genes controlling these traits may be located in similar chromosomal regions and no interaction exists between wilting and seed quality. Furthermore, because drought affects biomass production and yield, it is interesting to note that QTLs for seed weight and yield (Reyna and Sneller 2001) are genetically linked to markers associated with the wilting QTL on MLG D2.

Lastly, corn earworm (Rector et al. 2000) and Javanese root-knot nematode resistance [*Meloidogyne javanica*

**Table 2** Molecular markers that were associated with canopy wilting by single marker analysis (SMA), multiple-loci analysis (MLA), and composite interval mapping (CIM) using wilting data combined over all three environments (combined), within Arkansas (AR), and for North Carolina (NC)

| Molecular linkage group (chrom. #)[a] | SSR marker[b] | Slow-wilting allele[c] | $R^2$-value × 100 | | | | | | CIM | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SMA | | | MLA | | | LOD score[d] | | |
| | | | Combined | AR | NC | Combined | AR | NC | Combined | AR | NC |
| A1 (5) | **Satt684** | J | – | – | 10.8** | – | – | – | – | – | – |
| A2 (8) | **Sat_319** | J | 5.0* | 6.3 * | – | 12.6*** | 14.1*** | – | – | 3.5 | – |
| B2 (14) | Satt577 | J | 5.2* | 6.4* | – | 10.1*** | 11.7*** | – | 3.0 | 3.0 | – |
| | **Sat_264** | | 11.4** | 12.0** | – | | | | | | |
| | Sat_287 | | 5.5* | 6.2* | – | | | | | | |
| D1b (2) | Satt157 | J | 7.0* | 9.0** | – | – | – | – | – | – | – |
| | **Sat_351** | | 7.9* | 9.7** | – | | | | | | |
| D2 (17) | Satt372 | K | 10.7** | 10.7** | – | 15.1*** | 11.6*** | – | 4.5 | 4.3 | – |
| | **Satt002** | | 15.2*** | 15.5** | – | | | | | | |
| | Satt154 | | 11.6** | 11.9** | – | | | | | | |
| F (13) | **Satt362** | J | 6.5* | 5.0* | 13.3 ** | 9.0** | 6.8** | 15.5*** | – | 2.0 | 4.0 |
| | Satt072 | | 8.1* | 7.2* | 7.1 * | | | | | | |
| G_1 (18)[e] | **Satt303** | J | 8.3* | 10.0** | – | – | – | – | – | – | – |
| | Satt138 | | 6.6* | 7.6* | – | | | | | | |
| G_2 (18)[e] | **Satt038** | J | – | – | 5.7 * | – | – | – | – | – | – |
| J (16) | **Satt285** | K | 12.6** | 14.2** | – | – | – | – | – | – | – |
| K_1 (9)[e] | **Sat_044** | K | 13.6*** | 12.4** | – | – | – | – | – | – | – |
| | Satt559 | | 12.1** | 11.9** | – | | | | | | |
| K_2 (9)[e] | **Satt102** | J | 8.0* | 11.7** | – | – | – | – | – | – | – |
| O (10) | **Satt592** | K | – | – | 5.7* | – | – | – | – | – | – |

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; (–) non-significant

[a] Chromosome number designation as described at www.soybase.org

[b] Markers in bold denote markers used to represent each individual QTL in MLA

[c] Parent contributing slow-wilting alleles for a given QTL; KS4895 (K) or Jackson (J)

[d] The greatest Likelihood of Odds (LOD) score within a genetic interval is reported for values greater than 2.0

[e] Represent unique QTLs on the specified MLG (chromosome)

(Treub) Chitwood)] (Mienie et al. 2002) correspond to wilting QTLs on MLGs B2 and F, respectively. Neither corn earworm nor root knot nematode was observed in our experiments and it is unlikely that they affected our results. However, Rahi et al. (1988) demonstrated that tobacco plants infected by *M. javanica* have lower water-use efficiency than non-infected plants. Therefore, changes in water relations *in planta* may be associated with earworm or nematode infection and canopy wilting in soybean. Further research is needed to examine this potential relationship.

In addition to developing tools for marker-assisted selection, embarking on QTL studies will lead to discovering genes conferring the slow-wilting trait and their functions. For example, a gene (GM010) for a water channel protein, aquaporin, was previously mapped to the QTL region on MLG B2 (Yamanaka et al. 2001). Aquaporins are membrane-intrinsic protein channels important for maintaining cellular water status, stomatal opening, and $CO_2$ transport across mesophyll and chloroplast plasma membranes (Kaldenhoff and Fischer 2006). This aquaporin gene is a promising candidate for a role in water relations *in planta* and potentially associated with the slow-wilting trait. To the best of our knowledge, we are the first to report the inheritance for canopy wilting in soybean. Subsequently, the next steps in our research will be to confirm these QTLs in different genetic backgrounds and in different environments to evaluate the efficacy of these four QTLs in selecting slow-wilting genotypes. In addition, we plan to examine the physiological differences for drought tolerance traits (i.e. water-use efficiency and stomatal conductance) using the RILs representing the extremes in wilting.

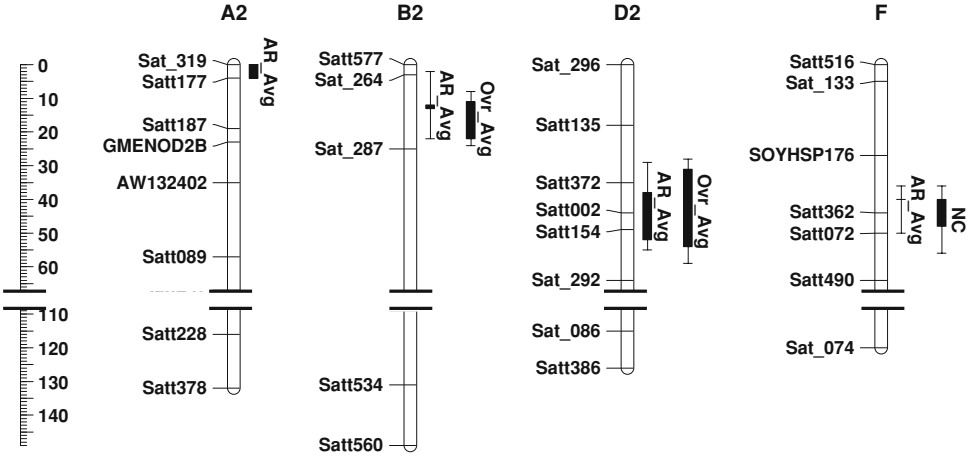


**Fig. 2** Location of wilting QTLs on the genetic linkage map. Results of composite interval mapping using data combined over all three environments (Ovr_Avg), over 2 years within Arkansas (AR_Avg), and from North Carolina (NC). Thin line indicates genetic regions associated with wilting at LOD = 2.0–2.9 and thick bar indicates LOD $\geq$ 3.0. Scale on the *left* indicates genetic distance in cM

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