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ORIGINAL PAPER

Mapping of quantitative trait loci for canopy-wilting trait in soybean (*Glycine max* L. Merr)

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Abstract Drought stress adversely affects [*Glycine max* (L.) Merr] soybean at most developmental stages, which collectively results in yield reduction. Little information is available on relative contribution and chromosomal locations of quantitative trait loci (QTL) conditioning drought tolerance in soybean. A Japanese germplasm accession, PI 416937, was found to possess drought resistance. Under moisture-deficit conditions, PI 416937 wilted more slowly in the field than elite cultivars and has been used as a parent in breeding programs to improve soybean productivity. A recombinant inbred line (RIL) population was derived from a cross between PI 416937 and Benning, and the population was phenotyped for canopy wilting under rain-fed field conditions in five distinct environments to identify the QTL associated with the canopy-wilting trait. In a combined analysis over environments, seven QTL that explained 75 % of the variation in canopy-wilting trait were identified on different chromosomes, implying the complexity of this trait. Five QTL inherited their positive alleles from PI 416937. Surprisingly, the other two QTL inherited their positive alleles from Benning. These putative QTL were co-localized with other QTL previously identified as related to plant abiotic stresses in soybean, suggesting that canopy-wilting QTL may be associated with additional morpho-physiological traits in soybean. A locus on chromosome 12 (Gm12) from PI 416937 was detected in the combined analysis as well as in each individual environment, and explained 27 % of the variation in canopy-wilting. QTL identified in PI 416937 could provide an efficient means to augment field-oriented development of drought-tolerant soybean cultivars.

Introduction

Drought affects soybean seed yield to some degree at almost all stages of growth (Boyer 1982). Inadequate and unpredictable rainfall has been reported to reduce yield by 36 % on average in North American soybean fields (Specht et al. 1999). Drought tolerance in plants can be complex

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and involve regulation of many metabolic pathways (Bartels and Sunkar 2005), as well as morphological and physiological traits (Jones et al. 1981). Mechanisms of drought tolerance in plants can be grouped into three broad categories as drought escape, dehydration avoidance, and dehydration tolerance (Carrow 1996). Dehydration tolerance is the ability of plants to withstand water deficit with low tissue water potential. In cropping situations, dehydration tolerance has little relevance, since water deficits resulting in plant dehydration will be sufficiently severe and survival of the drought by tolerance will still result in economically devastating losses for the farmer. Therefore, traits leading to dehydration avoidance rather than tolerance are much more useful.

The recently released North American soybean cultivars have a narrow genetic base, in that 80 % of the alleles found in public soybean cultivars released between 1947 and 1988 can be traced to only 13 ancestral lines (Gizlice et al. 1994; Carter et al. 2004). Drought tolerance has not been reported in the genetic base or in modern cultivars derived from them (Sneller and Dombek 1997). In contrast, the global genetic diversity available for soybean breeding is formidable. More than 20,000 accessions are preserved in the USDA soybean collection alone. Screening of this USDA collection has revealed the existence of a few exotic soybean accessions which possess drought tolerance (Carter et al. 1999).

One such plant introduction (PI) 416937, from Japan, exhibits slow wilting during drought (Sloane et al. 1990) and tolerance to soil aluminum toxicity (Bianchi-Hall et al. 1998; Villagarcia et al. 2001). Although PI 416937 was initially identified visually based on its slow canopy-wilting trait (Sloane et al. 1990), PI 416937 may possess more than one mechanism for drought resistance (King et al. 2009). Under moisture-deficit field conditions, PI 416937 is slower wilting than elite cultivars and its leaves possess lower solute potential, higher turgor and higher relative water content, which result in a relatively small yield reduction when compared with elite cultivars (Sloane et al. 1990). Later, Hudak and Patterson (1995) found that PI 416937 had a dense root surface area with higher number of root tips. Tanaka et al. (2010) found that PI 416937 possessed a lower stomatal conductance that conserved leaf water under drought conditions. Under vapor pressure deficit (VPD) of >2.0 kPa conditions, Fletcher et al. (2007) reported that PI 416937 exhibited a constant transpiration rate rather than a continually increasing rate with increasing VPD. PI 416937 showed no additional increase in transpiration rate at higher VPD, while two fast canopy-wilting cultivars continued to increase their transpiration rates as VPD was increased above 2.0 kPa. The transpiration response of PI 416937 under high VPD was associated with lower hydraulic conductance for water flow from leaf xylem into the guard cells (Sinclair et al. 2008). Ries et al. (2011) found that compared to several elite cultivars, PI 416937 had lower radiation use efficiency (RUE) values under water-replete conditions and that it had higher soil water content immediately prior to irrigation. The authors concluded that PI 416937 restricted crop growth and water loss when soil water was plentiful and then drew upon the saved soil moisture at the onset of drought. It was hypothesized that the lower hydraulic conductance of PI 416937 was a result of a unique population of aquaporins in PI 416937. To test this hypothesis, soybean plants were teated with aquaporin inhibitors, including $AgNO_3$ (Sadok and Sinclair 2009, 2010a). In contrast to other genotypes, PI 461937 had little or no decrease in transpiration rate when fed $AgNO_3$. It was concluded that PI 416937 did not have a population of aquaporins sensitive to silver, and that this deficiency resulted in a lower hydraulic conductance. Limited transpiration rate at high VPD allowed PI 416937 to conserve soil water for use later in the season to sustain growth during drought periods.

The application of QTL analysis allows the identification of chromosomal regions, conditioning the phenotypic variation in quantitative traits such as drought resistance traits, and identifies the desirable alleles at these QTL for use in marker-assisted selection (MAS). With the massive advancement in genomics knowledge about the physiological and functional aspects of traits and metabolic pathways controlling trait expression, the candidate gene approach has become a powerful technique to associate traits to functional genes. This approach can increase the precision of the genetic mapping and increase the accuracy of detecting QTL related to the trait of interest. A limited number of studies were conducted on QTL mapping of drought-related traits in soybean and these include yield under drought stress conditions (Du et al. 2009a, b; Specht et al. 1999), fibrous roots (Abdel-Haleem et al. 2011) and water use efficiency (WUE; Mian et al. 1996, 1998; Specht et al. 2001). Charlson et al. (2009) identified QTL controlling the variation in canopy wilting on Lg-A2 (Gm08), -B2 (Gm14), -D2 (Gm17) and -F (Gm13).

The objectives of this study were to: (i) evaluate the genetic variation in the canopy-wilting trait in a soybean RIL population developed from a cross of ‘Benning’ (US elite cultivar with fast canopy wilting) and PI 416937 (Japanese landrace with slow canopy wilting), (ii) determine the genetic basis of this trait, (iii) map QTL controlling the canopy-wilting trait, (iv) estimate the interactions between the detected QTL, if present and (v) elucidate genetic characteristics of the QTL to facilitate their use in MAS.

Materials and methods

Plant materials and phenotypic evaluation

A cross between Benning (PI 595645) and PI 416937 was made at the University of Georgia Plant Sciences Farm near Watkinsville, GA, USA. PI 416937 is a Maturity Group V Japanese landrace that phenotypically appears distinctly different from the ancestors of North American soybean cultivars. When compared with elite US cultivars (including Benning), it has a slower wilting canopy and a more fibrous root system (Pantalone et al. 1996a, b). Benning is a Maturity Group VII cultivar developed by University of Georgia (Boerma et al. 1997). The F_1 seeds were grown in the USDA/ARS Winter Nursery located near Isabella, Puerto Rico. The F_2 to F_5 generations were advanced by the single seed decent (SSD), where selection for unshattered pods, late maturity and resistance to bacterial pustule disease were practiced. The F_5 plants were harvested individually and used to develop F_5-derived RIL (Abdel-Haleem et al. 2011).

One hundred and fifty F_6-derived RIL, the two parents and check cultivars were tested for variation in the canopy-wilting trait at five environments: Arkansas Rice Research and Experimental Station at Stuttgart, AR during 2007(AR07) and 2009 (AR09); Sandhills Research Station at Windblow, NC, during 2009 (NC09) and 2010 (NC10); and Agriculture Experiment Station at Salina, KS during 2010 (KS10). Daily and maximum and minimum temperatures were recorded at each experimental site during the experimental seasons (2007–2010) (supplement table 1). At the Arkansas site, the RILs were planted as a single replicate on a Crowley silt loam soil as in 2007 and in a randomized complete block experimental design with three replications in 2009, under rain-fed conditions. Plots consisted of one row (AR07) and four rows (AR09) with an 80-cm spacing between rows. At the North Carolina site (NC09 and NC10), RILs were planted on a Candor sand soil in five sets each with 30 RILs plus the two parents, fast-wilting cultivar NC-Roy and slow-wilting breeding line N93-110-6 as checks in two replications. The plot size was three rows, 3 m in length with a 96-cm spacing between rows. At the Kansas site, the RILS were planted on a Hord silt loam soil type in sets within replications, with three replications, and each plot consisted of four rows with a 76-cm row spacing.

Under water deficient conditions, the canopy-wilting trait was rated as per King et al. (2009). Briefly, each plot was visually rated on a scale of 0 (no wilting) to 100 (plant death) at AR07, AR09 and KS10 sites, while at NC09 and NC10 the scale was 1 (no wilting) to 5 (plant death). The North Carolina data were converted to the 0–100 scale. Rating started at the R2–R5 stages of plant growth (Fehr et al. 1971) and recorded during two consecutive weeks during late August to early September of each year at each location. The data for the two ratings of each environment were averaged for each plot.

Statistical analyses

Since the AR07 environment was unreplicated, the data were included in the QTL analyses, but excluded from the statistical analyses. Because of differences in experimental designs among environments, analysis of variance was conducted separately for each replicated environment with SAS PROC GLM (Statistical Analysis System, SAS Institute 2001). The AR09 environment was analyzed as a randomized complete block experimental design with replicates and genotypes considered as random effects (Steel and Torrie 1980). The KS10 environment was analyzed as a set within replication design where replicates, genotypes and sets were treated as random effects. The North Carolina environments (NC09 and NC10) were analyzed over 2 years, where replicates, genotypes, sets and year were considered as random effects. To evaluate genotypes over all environments, least square means were calculated for each genotype in each environment separately and a combined analysis over environments was conducted (the model included genotypes and environments as random effects). In addition, an analysis of variance for combined eastern environments (NC09 and NC10) and western environments (AR09 and KS10) were conducted.

Heritability on RIL-mean base was calculated as $h^2 = (\sigma^2_{\mathrm{RIL}}/(\sigma^2_{\mathrm{RIL}} + \sigma^2_{\mathrm{ENV*RIL}}/e))$ for the combined environments (Nyquist and Baker 1991; Holland et al. 2003), where σ^2_{RIL} equaled the genetic variance among the genotypes, $\sigma^2_{\mathrm{RIL*ENV}}$ the variance of genotypes by environments interaction and e the number of environments.

QTL analysis

A total of 629 SSR markers were screened for polymorphism between Benning and PI 416937 using the protocol of Diwan and Cregan (1997). Genotypic data were collected on 276 SSR markers that were polymorphic between the parents. Linkage maps were constructed using Map Manager QTX (Meer et al. 2002) with a minimum logarithm of the likelihood-of-odds (LOD) score of 3.0 and a maximum recombination fraction of 0.25 as thresholds for detecting possible linkages and multipoint ordering of markers. Observed recombination frequencies were converted to map distance and expressed as centimorgans (cM) using Kosambi's mapping function (Abdel-Haleem et al. 2011).

Significant ($P > 0.01$) associations of SSR markers with the canopy-wilting scores were initially tested using single-factor analysis of variance (SF-ANOVA) of SAS (SAS Institute 2001) based on RILs means for each environment and least square means across environments. QTL analysis was performed with QTL Cartographer V 2.5 (Wang et al. 2007) using RIL least square means across environments and the multiple interval mapping (MIM) procedure of Kao et al. (1999) and Zeng et al. (1999). The MIM is a stepwise model adaptation procedure combined with an initial model selection of markers. The MIM was performed to test the presence of significant QTL and QTL effects including additive and epistatic effects by simultaneous analysis of the QTL in multiple regression models (Zeng et al. 1999) with the following steps: (i) composite interval mapping method (CIM) was employed to detect QTL and estimate the magnitude of their effects (Jansen and Stam 1994) using Model 6 of the Zmapqtl program module. A series of 1,000 permutations was run to determine the experiment-wise significant level at $P = 0.05$ of LOD Churchill and Doerge 1994). The genome was scanned at 2-cM intervals and the window size was set at 10 cM. Cofactors were chosen using the forward–backward method of stepwise regression; (ii) the pre-selected MIM model was optimized using the "optimize QTL positions" option; (iii) the whole genome was re-scanned searching for new main effect QTL and epistatic effect between main QTL using "search for new QTL" and "QTL interaction" options, respectively; (iv) the model was re-evaluated to fit all the significant main and epistatic QTL in MIM model using "test existing QTL" option; and (v) the MIM model with minimum Bayesian information criterion (BIC) was chosen, where $c(n) = \ln(n)$, and search walk speed was 1 cM. After identifying the best model, the main QTL effects, their total phenotypic variation and the proportion of the variation explained by each QTL of the model was estimated using the "summary" option.

Results

Phenotypic variation in canopy-wilting trait

PI 416937 had a lower wilting score than Benning in all environments except NC10 (Table 1). The narrow canopy-wilting scores range between parents in the studied environment could be related to the maturity date. For example in AR09, PI 416937 was 9.5 days earlier maturing than Benning (matured 16th October), while the population mean was similar to Benning (16th October) and ranged from 10th to 20th October. This was expected due to selection pressure for late maturity during population development. The RIL population showed significant ($P < 0.05$) genotypic variation in canopy-wilting scores and showed transgressive segregation beyond both parents (Table 1; Fig. 1). Canopy-wilting scores for the RILs across environments ranged from 24 to 47. The range within the RILs for slow canopy wilting was higher in NC09 and NC10 environments (Table 1).

Although ANOVA data are not shown, the effect of genotype was significant ($P < 0.001$) in all four replicated environments. At KS10, NC09 and NC10 environments, genotypes were planted in five sets. The variability between sets was significant ($P < 0.001$) at KS10, but not in NC09 or NC10 environments. The ANOVA for the combined data for NC09 and NC10 showed significant genotype × year interaction. The ANOVA for western environments (AR09 and KS10) indicated significant ($P < 0.001$) effects for genotypes and environment (data not shown). The combined ANOVA for the four environments indicated significant ($P < 0.001$) effect for genotypes and environments. The heritability estimate (based genotype means across four environments) for canopy-wilting score was 0.60.

Identification of canopy-wilting QTL

A linkage map was constructed with 276 informative markers (Abdel-Haleem et al. 2011). The linkage maps consisted of 20 linkage groups, which correspond to soybean's 20 chromosomes and collectively covered 2,169 cM or 94 % of the 2,276-cM soybean genome (based on the USDA consensus soybean linkage map; Song et al. 2004). This resulted in an average of 7.86 cM between adjacent SSR markers on the Benning × PI 416937 linkage maps (Abdel-Haleem et al. 2011).

Single-factor analysis of variance (SF-ANOVA) was employed to putatively identify QTL conditioning the canopy-wilting trait for each environment and across environments (Table 2). Multiple significant markers ($P < 0.01$) available within a chromosomal region were sorted based on their positions within each chromosome. The marker explaining the largest phenotypic variation (R^2) from the significant marker cluster was chosen as representative of QTL presence in that particular chromosomal region. When a marker was found to be significantly ($P < 0.01$) associated with canopy-wilting score in the combined data across environments, it was evaluated for significant associations in individual environments with significance level of $P < 0.05$. Even though AR07 environment was un-replicated, the data were included in the QTL analyses to access canopy-wilting QTL across a wider range of environments and determined the consistency of QTL detection across these environments. Fourteen SSR markers on 12 soybean chromosomes were identified as putative QTL associated with canopy wilting. Each of these

Table 1 Mean and range of canopy-wilting scores of the parents and the 150 RILs of the Benning × PI 416937 population in North Carolina 2009 (NC09) and 2010 (NC10), Kansas 2010 (KS10), Arkansas 2009 (AR09) and across environments

Genotype	NC09	NC10	KS10	AR09	Combined
Canopy-wilting score[a]					
Benning	37	43	42	36	40
PI 416937	34	51	37	34	39
Means of RILs	34	46	39	34	36
Range of RILs	10–57	28–74	31–48	25–40	24–47
LSD (0.05)	18	13	3	4	7

[a] Canopy-wilting rates were based on visually rating on scale 0 (no wilting) to 100 (plant death) at the R2–R5 stages of plant growth (Fehr et al. 1971); ratings were recorded during two consecutive weeks during late August to early September of each year at each location. The data for the two ratings of each environment were averaged

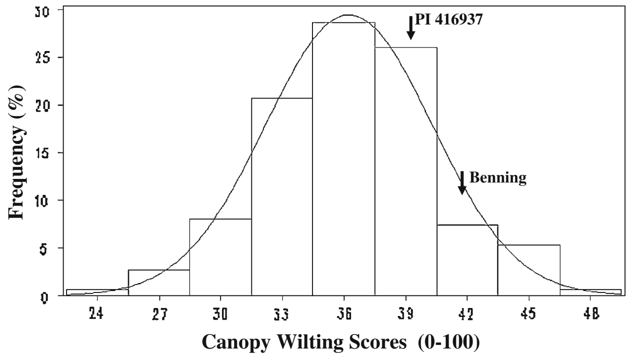

Fig. 1 Frequency distribution of canopy-wilting scores in the Benning × PI 416937 RIL population across environments

12 chromosomes had a single putative canopy-wilting locus, except Gm02 and Gm03 that had two putative QTL each (Table 2). The PI 416937 alleles contributed to reduction of wilting scores at 10 of these QTL, while the Benning alleles contributed to reduce canopy-wilting scores at four QTL. A total of 9, 8, 11, 9 and 7 significant SSR associated with slow canopy wilting were detected in the AR07, AR09, KS10, NC09 and NC10, respectively (Table 2). Of these putative QTL, three markers (Satt424 on Gm08, Satt302 on Gm12 and Sct_064 on Gm14) were identified in all individual environments as well in the combined analysis. The QTL on Gm08 and Gm14 inherited their alleles for slow canopy wilting from Benning. The QTL on Gm12 inherited its allele for slow canopy wilting from PI 416937.

The MIM model explained 75 % of the phenotypic variation in the combined analysis. Seven QTL were detected in the combined analysis at *qSW Gm02* (identified by Sat_254-Satt296 on Gm02), *qSW_Gm 04* (identified by Satt646 on Gm04), *qSW_Gm05* (identified by Satt276 on Gm05), *qSW_Gm12* (identified by Satt302 on Gm12), *qSW_Gm14* (identified by Satt066 on Gm14), *qSW_Gm17*, (identified by Satt135 on Gm17) and *qSW_Gm19* (identified by Satt462 on Gm19) (Table 3). At five of these QTL (*qSW_Gm02, qSW_Gm04, qSW_Gm05, qSW_Gm12* and *qSW_Gm19*), PI 416937 alleles reduced canopy-wilting scores, while at *qSW_Gm14* and *qSW_Gm17* the Benning alleles reduced the canopy-wilting scores. Individually, these QTL explained from 4 % (*qSW_Gm05* on Gm05) to 27 % (*qSW_Gm12* on Gm12) of the phenotypic variation in canopy-wilting score.

A QTL region was detected in all environments on chromosome 12 (Gm12), and this region was identified by Satt302 (Table 3; Fig. 2) with favorable alleles from PI 416937. The *qSW_Gm02* was detected in NC10 with favorable alleles from PI 416937; *qSW_Gm04* on Gm04 was detected in NC10 and AR07 environments with favorable alleles from PI 416937; *qSW_Gm05* was detected in NC09, AR 07 with favorable alleles from PI 416937; and *qSW_Gm19* was detected in AR09 and KS10 environments. The *qSW_Gm17* was detected in NC 09 and NC 10 with favorable alleles from Benning.

The identified slow canopy-wilting QTL were aligned to soybean linkage/QTL maps in Soybase (http://soybase.org/), to evaluate their effect on other agronomic traits. Based on SSR marker information, seven chromosomes were aligned with the canopy-wilting QTL identified (Fig. 3). For this analysis, we arbitrarily included QTL conditioning other traits if they were mapped within a bin that included the canopy-wilting QTL interval and ±5 cM beyond the canopy-wilting QTL boundary. About 84 QTL were reported for traits related to abiotic stresses, plant morphology and development, seed composition and nitrogen accumulation (Fig. 3). For example, on Gm12 near the canopy-wilting QTL identified by Satt302 (Fig. 2), there were three QTL for oil content (*cqoil-002, Oil 19-2* and *6-5*), two for protein content (*Prot 5-2* and *21-10*), two for plant height (*Pl_ht 17-12* and *13-4*), one for seed weight (*Sd_wt 13-8*), one for seed yield (*sd_yld 15-8*), three QTL for iron deficiency (*Fe-effic 4-3, 8-3* and *11-3*) and four QTL for isoflavone components *(Isoflv 1-3, Daidzein 2-2, Genistein 2-6* and *Glycitein 2-8*) (Fig. 3). On Gm14, there were five QTL for iron deficiency (Fe-*effic 3-1, 4-1, 8-1, 10-3* and *11-1*), two for seed weight (*Sd_wt 10-4* and *13-3*),

Table 2 SSR markers associated with the canopy-wilting trait based on single-factor analysis of variance (SF-ANOVA) for the Benning × PI 416937 RIL population in individual environments and across environments

Chrom(Lg)	Marker	Postion[a]	Combined		AR 07	AR 09	KS10	NC09	NC10	Favorable allele
			$P > F$	R^2 (%)	$P > F$	$P > F$	$P > F$	$P > F$	$P > F$	
Gm02(D1b)	Satt296	61.4	0.019	4	0.019	–	0.01	–	–	PI 416937
	satt041	91.3	0.005	6	0.002	–	0.003	–	–	PI 416937
Gm03(N)	Satt152	17.4	0.016	4	–	0.012	0.0001	0.007	–	PI 416937
	Sat_091	64.9	0.006	5	–	0.003	0.021	–	–	PI 416937
Gm04(C1)	Satt646	46	0.002	7	0.007	0.014	0.005	–	0.002	PI 416937
Gm05(A1)	Satt276	18.9	0.021	4	0.009	–	0.021	0.004	–	PI 416937
Gm08(A2)	Satt424	53.6	0.0001	11	0.0001	0.05	0.003	0.026	0.004	Benning
Gm12(H)	Satt302	78.9	0.0001	18	0.002	0.0001	0.0001	0.003	0.0001	PI 416937
Gm13(F)	Satt649	36.6	0.013	5	–	0.001	–	0.04	–	PI 416937
Gm14(B2)	Sct_064	77.8	0.0001	11	0.002	0.001	0.02	0.001	0.013	Benning
Gm17(D2)	Satt372	361	0.0001	10	–	–	–	0.0001	0.0001	Benning
Gm18 (G)	Sat_372	104	0.011	5	0.0001	–	0.051	–	0.01	PI 416937
Gm19(L)	Satt229	78.3	0.0001	10	0.002	0.0001	0.0001	0.036	–	PI 416937
Gm20(I)	Satt270	42.6	0.013	5				0.002	0.047	Benning

[a] Marker position in cM starts from the top of the designed chromosome based on 2004 UDSA Soybean-GmConsensus4.0 (http://soybeanbreederstoolbox.org/)

Table 3 QTL for the the slow-wilting canopy-wilting trait identified by multiple interval mapping (MIM) for Benning × PI 416937 RIL population based on RIL means across environments

QLT name[a]	Ch(LG)	Nearest[b] marker	Position[c]	CI (cM)[d]	LOD (cM)[e]	a[f]	R^2 (%)[g]	AR07[h] QTL	AR09 QTL	KS10 QTL	NC09 QTL	NC10 QTL
qSW-Gm02	Gm02(D1b)	Satt296	63.5	58.0–71.6	4.1	1.1	6	nd	nd	nd	nd	**
qSW-Gm04	Gm04(C1)	Satt646	36.9	33.3–40.9	7.0	1.4	9	**	nd	nd	nd	**
qSW-Gm05	Gm05(A1)	Satt276	8.0	0.0–14.4	2.6	0.9	4	**	nd	nd	**	nd
qSW-Gm12	Gm12(H)	Satt302	56.8	53.5–62.0	9.1	2.0	27	**	**	**	**	**
qSW-Gm14	Gm14(B2)	Satt066	74.2	68.1–81.3	5.2	−1.2	8	nd	nd	nd	nd	nd
qSW-Gm17	Gm17(D2)	Satt135	20.2	15.1–24.2	7.8	−1.6	13	nd	nd	nd	**	**
qSW-Gm19	Gm19(L)	Satt462	55.7	52.0–59.0	3.2	1.0	8	nd	**	**	nd	nd

[a] QTL name based on qTrait name-chromosome name

[b] Nearest marker to the QTL peak

[c] QTL position in cM starts from the top of the designed chromosome

[d] Confidence interval based on ±1 LOD

[e] LOD is the log-likelihood at QTL peak position, where LOD significance is based on type error I and $P < 0.01$

[f] Additive effects were calculated following multiple intervals mapping (MIM) by simultaneous analysis of the QTL in multiple regression models; positive sign indicates that favorable alleles were inherited from PI 416937 (*qSW-Gm02, qSW-Gm04, qSW-Gm05, qSW-Gm12, qSW-Gm19*), while negative sign indicates the Benning alleles (*qSW-Gm14 and qSW-Gm17)*

[g] Proportion of phenotypic variance explained by specific QTL

[h] Co-localization of detected QTL in specific environment, where nd refers to absence and ** presence of the QTL in Arkansas 2007 (AR07), Arkansas 2009 (AR09), Kansas 2010 (KA10), and North Carolina 2009 and 2010 (NC09 and NC10)

two for oil content and stearic fatty acid (*Oil 14-1* and *Stear 2-10*) and one *NitR5_1-3*, which conditions nitrogen accumulation during the R5 stage of seed development. QTL conditioning fatty acid and carbohydrate contents (*Palm 2-1 and Sucrose 1-1*, respectively) on Gm05 were detected near *qSW_Gm05*. Several QTL for abiotic stresses tolerance were detected near the canopy-wilting QTL. Canopy-wilting QTL were detected in the 'Jackson' × 'KS4892' population (Charlson et al. 2009), using the CIM method and data collected from Arkansas or North Carolina environments during 2000–2003. A canopy-wilting QTL was detected on Gm17 and inherited its positive

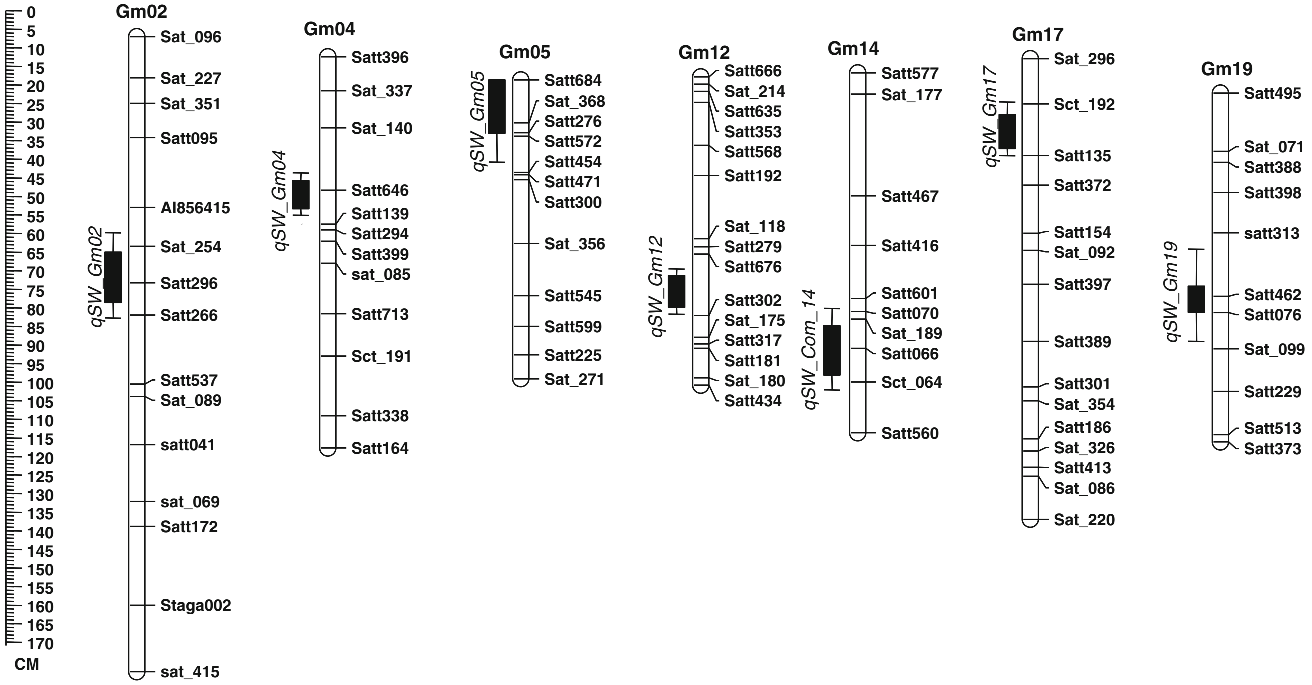

Fig. 2 Interval mapping for canopy-wilting score QTL in the Benning × PI 416937 RIL population based on individual environment and across environments. QTL nomenclature is in the form of qTrait-chromosome name. The length of the QTL bar indicates the LOD-1 confidence interval and QTL line is extended to LOD-2 confidence interval based on the maximum likelihood value

allele from KS4895; this QTL overlapped with *qSW_Gm17* in the Benning × PI 416937 population (Fig. 2). QTL for carbon isotope discrimination (CID), a trait that is related to the differences in transpiration efficiency under drought conditions (Specht et al. 2001), were located on Gm17 and Gm19. Leaf hydraulic conductance is another trait that measures transpiration efficiency under drought stress, and a QTL (*qSV_Gm12*) for low leaf hydraulic conductance is on Gm12 (Carpentieri-Pipolo et al. 2011). Iron efficiency uptake QTL were detected on Gm12 and Gm14 (Fig. 3). Recent studies reported association between drought resistance and isoflavone components contents (Gutierrez-Gonzalez et al. 2010). QTL for the isoflavones, daidzein, glycitein and/or genistein, were located near canopy-wilting QTL on Gm07 (*isolav 1-6* and *Daidzein 2-4*) and Gm12 *(Isoflv 1-3, Daidzein 2-2, Genistein 2-6* and *Glycitein 2-8)* (Fig. 3).

Discussion

The present study investigated the canopy-wilting trait in the Benning × PI 416937 population under rain-fed conditions and mapped responsive canopy- wilting QTL across five environments. The heritability on an entry mean for canopy wilting was 0.60 for the Benning × PI 416937 population when measured across four replicated diverse environments; the same heritability was reported in a different population (Charlson et al. 2009).

The canopy-wilting trait in soybean is complex, which was clear from the multiple QTL that were identified using single-factor and MIM methods. Seven main effect QTL were detected on seven chromosomes of soybean. Among these, a QTL located near Satt302 on Gm12, was identified in all environments plus the combined analysis across environments. This genomic location would be a good candidate for MAS for slow canopy wilting. The QTL at this genomic location has a major effect on canopy-wilting scores (R^2 accounted for 27 % of the phenotypic variation). The specificity of QTL within certain environments and its factors was reported before (Charlson et al. 2009), where environmental factors such as soil type, drought condition and water table level could be influential factors. These factors could alter the plant structure, which in turn could affect the plant response to water deficit.

Out of the identified QTL, PI 416937 alleles conditioned slower levels of canopy wilting at five QTL positions, while Benning provided the favorable alleles at two QTL positions. Similar allelic contributions by tolerant parent for slow canopy wilting was reported in KS4895 × Jackson (Charlson et al. 2009), where Jackson contributed the alleles for slower canopy wilting on chromosomes Gm13 and Gm14. Benning is one of the descendants of Jackson, and the positive alleles for canopy wilting could have been

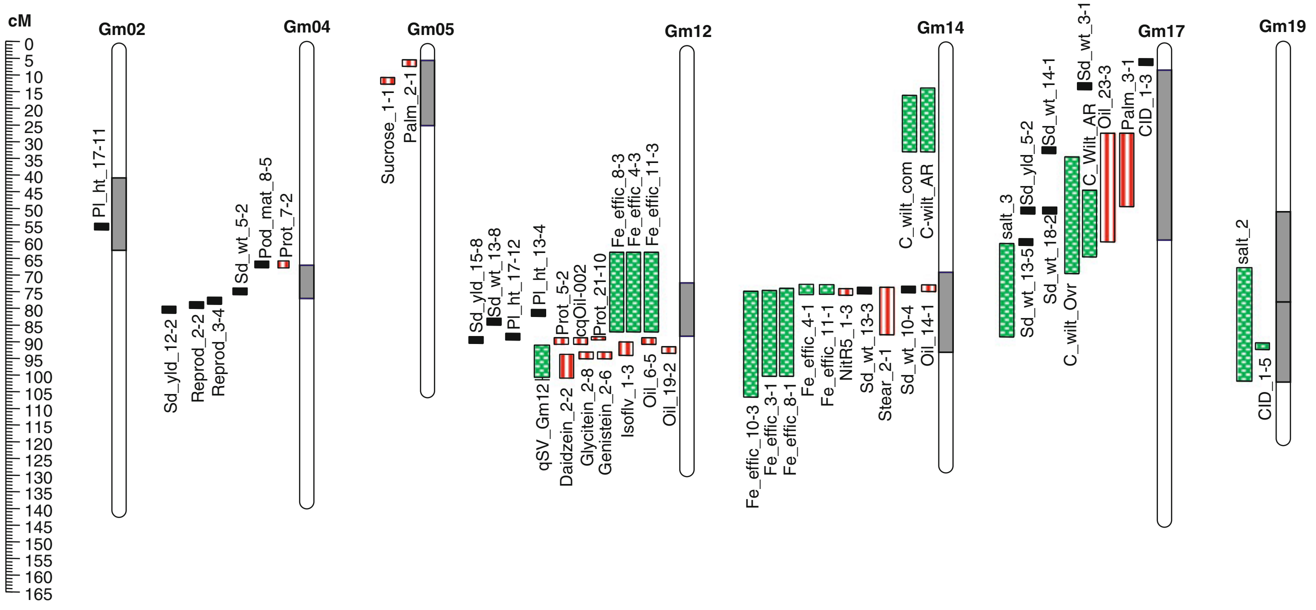

Fig. 3 Comparative QTL analysis of the canopy-wilting QTL detected in the Benning × PI 416937 population with coinciding QTL for abiotic stresses (🟩), plant morphology and development (⬛), and seed composition (🟥) found on Soybase (http://soybase.org/). These QTL were identified by alignment of both maps with common SSR markers

transferred to Benning via its pedigree relationship with Jackson. For example, Jackson's slow wilt alleles were detected at two QTL on Gm13 (Lg-F) and Gm14 (Lg-B2) (Charlson et al. 2009). In the present study, Benning contributed the positive allele for slower canopy wilting at *qSW_Gm14*. Based on SSR markers locations in the USDA consensus soybean linkage map (Song et al. 2004), these QTL were 40 cM apart. These two QTL may be same or not, as the studies differ with mapping populations, linkage map saturation and polymorphism information content.

Under water-deficit conditions, Benning exhibits a breakpoint in increasing transpiration rate as VPD increases, but unlike PI 416937 it has increasing transpiration rate at VPD above the breakpoint (Sadok and Sinclair 2010a). The transpiration rate of Benning decreased the most when treated with the silver aquaporin inhibitor of any genotypes tested by Sadok and Sinclair (2010b). Recently, Carpentieri-Pipolo et al. (2011) identified five QTL for silver nitrate response in Benning × PI 416937 population. One of these QTL was located on Gm12 and overlapped with *qSW_Gm12* (Fig. 3). Co-localizing a canopy wilting trait QTL with silver nitrate response QTL could help to explain soybean plant's ability to tolerate moisture stress. QTL associated with soybean seed yield under well-watered conditions were reported on Gm01 (Orf et al. 1999a), Gm04 (Yuan et al. 2002), Gm12 (Kabelka et al. 2004), and Gm17 (Orf et al. 1999b; Reyna and Sneller 2001). Co-localization of QTL for a specific trait and yield or yield components under drought stress could lead to improved yield via improving this trait (Babu et al. 2003; Lebreton et al. 1995). Drought stress during flowering and early pod expansion resulted in decreased pod set (Desclaux and Roumet 1996), which affected the number of seeds per pod and in return the seed weight and seed yield (Liu et al. 2003). Several QTL for seed weight were located in the same interval as canopy-wilting QTL on Gm04, Gm12, Gm14 and Gm17 (Hoeck et al. 2003; Maughan et al. 1996; Orf et al. 1999a; Specht et al. 2001).

QTL for oil content and quality and canopy wilting were located in the same genomic regions on Gm02, Gm05, Gm08, Gm12, Gm14 and Gm17 (Csanadi et al. 2001; Fasoula et al. 2004; Hyten et al. 2004; Lee et al. 1996; Li et al. 2002; Panthee et al. 2005). Seed protein QTL were located in the same genomic regions as canopy-wilting QTL on Gm04 and Gm12 (Lee et al. 1996; Orf et al. 1999a; Kabelka et al. 2004).

More than one of the abiotic stress factors could impact on plant growth at the same time, and the plant would need more than one mechanism to cope with them. For example, PI 416937 may possess more than one mechanism that could act together or separately in response to drought stress (Abdel-Haleem et al. 2011; Carpentieri-Pipolo et al. 2011; Hudak and Patterson 1995; King et al. 2009; Ries et al. 2011; Tanaka et al. 2010). Association among chromosomal locations harboring canopy-wilting QTL and iron efficiency QTL at Gm12 and Gm14 were observed. The partial closure of stomata by PI 416937 allows conservation of soil water for use late in the season to sustain seed growth. This can explain the association among canopy wilting, carbon isotope discrimination (CID, Specht et al.

2001) and salt stress (Lee et al. 2004; Tuyen et al. 2010) QTL on Gm17 and Gm19.

Conclusions

The exotic line, PI 416937, has positive alleles for important traits such as drought resistance and high seed yield, which can be introduced into elite cultivars to improve soybean productivity. In the present study, seven QTL for canopy-wilting trait were identified under drought stress in soybean across several different environments ranging from North Carolina to Kansas. Some of the canopy-wilting QTL were co-localized with fibrous rooting QTL as well as other morpho-physiological traits which eventually affect a soybean plant's ability to tolerate moisture stress. One of the canopy-wilting QTL, *qSW_Gm12*, identified in over environments, and explained 27 % of the variation in canopy-wilting score, was identified in all the individual environments. This locus could ease the challenge in introgression of favorable alleles for slow canopy wilting into an elite cultivar while not replacing large regions of the recipient genome with chromosomal segments of an unadapted donor parent. Marker-assisted backcrossing to combine fibrous roots and canopy-wilting alleles from PI 416937 into elite cultivars could increase the plant's ability to produce stable seed yield under drought stress conditions. The value of these traits to reduce yield loses in water-deficit environments warrants evaluation.

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