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ORIGINAL ARTICLE

Confirmation of delayed canopy wilting QTLs from multiple soybean mapping populations

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Abstract

Key message **QTLs for delayed canopy wilting from five soybean populations were projected onto the consensus map to identify eight QTL clusters that had QTLs from at least two independent populations.**

Abstract Quantitative trait loci (QTLs) for canopy wilting were identified in five recombinant inbred line (RIL) populations, 93705 KS4895 × Jackson, 08705 KS4895 × Jackson, KS4895 × PI 424140, A5959 × PI 416937, and Benning × PI 416937 in a total of 15 site-years. For most environments, heritability of canopy wilting ranged from 0.65 to 0.85 but was somewhat lower when averaged over environments. Putative QTLs were identified with composite interval mapping and/or multiple interval mapping methods in each population and positioned on the consensus map along with their 95 % confidence intervals (CIs). We initially found nine QTL clusters with overlapping CIs on Gm02, Gm05, Gm11, Gm14, Gm17, and Gm19 identified from at least two different populations, but a simulation study indicated that the QTLs on Gm14 could be false positives. A QTL on Gm08 in the 93705 KS4895 × Jackson population co-segregated with a QTL for wilting published previously in a Kefeng1 × Nannong 1138-2 population, indicating that this may be an additional QTL cluster. Excluding the QTL cluster on Gm14, results of the simulation study indicated that the eight remaining QTL clusters and the QTL on Gm08 appeared to be authentic QTLs. QTL × year interactions indicated that QTLs were stable over years except for major QTLs on Gm11 and Gm19. The stability of QTLs located on seven clusters indicates that they are possible candidates for use in marker-assisted selection.

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Introduction

In North America over the last 60 years, soybean breeding has produced over 500 cultivars and increased yield by more than 25 % (Fox et al. 2013; Specht et al. 1999).

Currently, more than 50 soybean breeders evaluate a total of more than 2 million yield plots annually (T.E. Carter, Jr. and K.M. Matson, personal communication, 2015). A natural consequence of these intense breeding activities is that mating of relatives is common, which has led unavoidably to both increased relatedness among modern soybean cultivars and reduced genetic diversity (Carter et al. 2004). This effect has been accentuated in soybean by the relatively small genetic base upon which North America soybean breeding rests (a dozen major founding ancestors, Gizlice et al. 1994), such that the mating of relatives and loss of diversity are more common than it would otherwise be. Currently, the average pedigree relatedness among modern cultivars is the equivalent of half-sibs. This level of co-ancestry among cultivars is sufficient to impede breeding progress in many cases (Gizlice et al. 1993; Hyten et al. 2006; Manjarrez et al. 1997).

The substantial relatedness among North American cultivars suggests that introgressing agronomically important alleles from outside the mainstream of applied soybean breeding could increase genetic diversity and also improve soybean yield of cultivars flowing though the plant breeding pipeline. One approach for introgression of new diversity into applied breeding programs is the identification of soybean types that have stress tolerance. The limited studies available at present suggest that drought tolerance is a relatively rare trait among North American soybean cultivars, and that improvement of this important trait could be addressed by identifying tolerant types in the USDA/ARS Soybean Germplasm Collection (Purcell and Specht 2004). Over 18,000 exotic soybean accessions are preserved and available for this purpose.

Drought is a primary limitation to soybean yield (Purcell and Specht 2004; Sinclair et al. 2010). Delayed canopy wilting was identified as a potential drought-tolerant trait with the discovery of delayed-wilting plant introduction (PI) 416937 in the early 1980s after screening several hundred soybean plant introductions collected in Asia (Carter et al. 1999; Sloane et al. 1990). In other research, a rare adapted population derived from the hybridization of U.S. cultivars KS4895 and Jackson was also identified as segregating for the delayed-wilting trait (Charlson et al. 2009). Physiological mechanisms related to delayed wilting have now been identified in multiple soybean genotypes (Sloane et al. 1990; Carter et al. 1999; Fletcher et al. 2007; King et al. 2009; Ries et al. 2012). Genetic studies of delayed wilting have identified QTLs, evaluated heritability, and reported relationships with other agronomic traits (Charlson et al. 2009; Du et al. 2009; Abdel-Haleem et al. 2012).

A practical application of QTL analysis in plant breeding for stress tolerance improvement is the use of QTLs for marker-assisted selection (MAS) to discard undesirable drought-sensitive genotypes early in the breeding process so that those breeding lines most likely to perform well under stress are targeted for subsequent phenotypic evaluations. A current limitation to the use of QTL for delayed wilting in selection is that QTL mapping is generally not precise enough for efficient MAS. Resolution has been hampered by relatively small RIL population sizes and low map density in many case studies. An additional limitation is that QTL confirmation in multiple populations is relatively rare at present, even though it is a prerequisite to reliable MAS for drought tolerance traits.

Additional mapping studies and more densely populated genetic maps are required to precisely map QTLs for delayed wilting, confirm major QTL with large effects, and ultimately identify the causal genes. The primary objective of our research was to confirm and identify QTLs for delayed wilting that were in common from at least two independent mapping populations. The confirmation of QTLs from different populations for delayed wilting is a key step in developing a strategy for MAS.

Materials and methods

Population materials

Five populations were evaluated in this research: (1) 93705 KS4895 × Jackson, (2) 08705 KS4895 × Jackson, (3) KS4895 × PI 42410, (4) A5959 × PI 416937, and (5) Benning × PI 416937. Population size, number of polymorphic markers, and length of the genetic map for each population are summarized in Table 1. For the remainder of the manuscript, these populations will be referred to, respectively, as: 93K×J, 08K×J, K×PI, A×PI, and B×PI. The 93K×J population (Hwang et al. 2013, 2014a, b) was the same population evaluated for wilting as in a previous study (Charlson et al. 2009), with the addition of five additional RILs for genotypic evaluation and six simple sequence repeats (SSRs) and 491 additional single nucleotide polymorphisms (SNPs). KS4895 (PI 595081) is a maturity group (MG) IV cultivar developed in Kansas (Schapaugh and Dille 1998). Jackson (PI 548657) is an MG VII cultivar developed by the USDA-ARS in North Carolina (Johnson 1958). The 08KxJ population was developed as a confirmation population of the 93K×J population. Both the 93K×J and the 08K×J populations and the K×PI population were generated with the purpose of observing differences in N_2 fixation and nodule traits among RILs (Hwang et al. 2013, 2014b). PI 424140 is an MG IV accession from South Korea (USDA National Genetic Resources Program 2014a). A5959 is an MG V cultivar developed by Monsanto (St. Louis, MO 63167, USA). The AxPI population was developed specifically for evaluating canopy wilting since parental lines represent extreme phenotypes for

Table 1 Summary of mapping populations for canopy wilting study

Population	Abbreviated name	Number of RILs	Number of polymorphic markers		Length of genetic map (cM)	Average distance between markers (cM)
			SSRs	SNPs		
93705 KS4895 × Jackson	93K×J	97	171	491	4218.6	6.37
08705 KS4895 × Jackson	08K×J	168	37	511	2089.7	3.81
KS4895 × PI 424140	K×PI	103	22	530	3250.5	5.89
A5959 × PI 416937	A×PI	103	0	948	2970.2	3.13
Benning × PI 416937	B×PI	150	276	0	2169.0	7.86

canopy wilting (King et al. 2009). Benning is MG VII cultivar that was developed by University of Georgia (Boerma et al. 1997). PI 416937 is an MG VI accession from Japan (USDA National Genetic Resources Program 2014b).

The F_2 seeds in each population were bulk-threshed from F_1 plants and progenies at the F_2 generation were advanced by the single seed decent method (Brim 1966). Each plant at the F_5 generation (or the F_6 generation for the BxPI population) was individually threshed to generate the F_5-derived (or F_6-derived) RILs. RILs of all populations, except for the AxPI, were selected with similar maturity during generation advancement.

Field trials and phenotyping for canopy wilting

Table 2 summarizes when and where the five mapping populations were evaluated along with the number of replications and number of rating dates each year. Trials were conducted under rainfed conditions at the Arkansas Rice Research and Extension Center near Stuttgart, AR (34°28′39.5″N, 91°25′12″W) on a Crowley silt loam, at the Sandhills Research Station near Windblow, NC (35°12′07.9″N, 79°40′55″W) on a Candor sand, and/or at the Agriculture Experiment Station near Salina, KS (38°50′26″N, 97°36′40″W) on a Hord silt loam. All evaluations used a randomized complete block design except for the AxPI population in 2012 and 2013. In 2012 and 2013, we used a balanced incomplete block design, grouping genotypes of similar maturity within each block. Wilting evaluations for all populations were conducted between R2 and beginning R5 (Fehr and Caviness 1977). At the Stuttgart and Salina locations, wilting was rated from 0 (no wilting) to 100 (plant death) (King et al. 2009). At Windblow, wilting was rated on a scale of 1–5 and converted to the 0–100 scale as described by Abdel-Haleem et al. (2012). Plots at Stuttgart consisted of either two or four rows, with rows that were 80 cm apart and 4.5 m in length. At Windblow, plots consisted of three rows, 96 cm apart and 3.1 m in length. At Salina, there were four-row plots, 76-cm apart, and 4.5 m in length. The BxPI population was evaluated at Stuttgart, AR (2007, 2009), Salina, KS (2010), and Windblow, NC (2009, 2010) as described by Abdel-Haleem et al. (2012).

Statistical analysis

The SAS 9.3 (2013) software package (SAS Institute Inc., Cary, NC, USA) or R (3.0.1) was used for randomization, ANOVA, least square means (LS means), heritability, phenotypic correlation, and parental independent *t* test. The PROC MIXED or GLM procedures of SAS were used for ANOVA and estimation of heritability and LS means. Year, replicate, RIL, maturity, wilting rating date, interactions between two factors, and interactions among three factors were treated as random effects. The LS means of RILs for each year and wilting rating date were used for QTL analysis. The heritability was estimated on a progeny-mean basis (Knapp et al. 1985) across environments or using expected mean squares (EMS) within a year.

Genotyping populations

Detailed descriptions of genotyping the 93K×J (Hwang et al. 2013, 2014a, b) and B×PI (Abdel-Haleem et al. 2012) populations have been reported previously. For the 08KS×J and K×PI populations, DNA from a bulk sample of $F_{5:6}$ young leaves was extracted using a Maxwell 16 automated machine (Promega, Madison, WI, USA), and DNA concentration was estimated by absorbance at 260 and 280 nm with a spectrophotometer. Polymorphic SSR markers were screened by the size of two parental amplicons using an ABI 3730 XL sequencer (Applied Biosystems, Foster City, CA, USA). The Illumina GoldenGate Assay with the BeadStation 500G (Illumina, Inc., www.illumina.com) was used to screen polymorphic SNPs using the 1536-SNP USLP version 1.0 array (Hyten et al. 2010). The genotype calls for each SNP were performed with Illumina GenomeStudio SNP analysis software (www.

Table 2 Population statistics for delayed canopy wilting in recombinant inbred line mapping populations, including parental test for significance, and heritability (h^2)

Population	Year	Location	Replications	Rating dates	Mean	Range	Parent test[a]	h^2
93K×J	Average[b]	Average	–	–	38.0	0.0–100.0	–	0.58
93K×J	2000	Stuttgart, AR	3	1	42.6	0.0–100.0	–	0.84
93K×J	2002	Windblow, NC	3	1	42.0	12.5–62.5	–	0.30
93K×J	2003	Stuttgart, AR	3	3	34.8	20.0–65.0	ns	0.77
08K×J	Average[c]	Average	–	–	34.6	15.0–60.0	–	0.75
08K×J	2012	Stuttgart, AR	2	1	26.0	15.0–35.0	ns	0.76
08K×J	2013	Stuttgart, AR	2	2	38.8	25.0–60.0	ns	0.66
K×PI	2013	Stuttgart, AR	2	1	38.0	20.0–55.0	ns	0.81
A×PI	Avg. 2010/2011[d]	Stuttgart, AR	–	–	33.0	20.0–65.0	–	0.52
A×PI	2010	Stuttgart, AR	3	1	36.0	20.0–65.0	**	0.81
A×PI	2011	Stuttgart, AR	3	1	30.8	20.0–45.0	**	0.70
A×PI	Avg. 2012/2013[d]	Stuttgart, AR	–	–	32.0	15.0–50.0	–	0.78
A×PI	2012	Stuttgart, AR	3	1	26.0	15.0–35.0	*	0.78
A×PI	2013	Stuttgart, AR	3	1	38.0	25.0–50.0	***	0.84
B×PI	Average	Average	–	–	36.0	24.0–47.0	ns	0.60
B×PI	2007	Stuttgart, AR	1	4	27.2	15.0–39.5	–	–
B×PI	2009	Stuttgart, AR	3	2	36.0	25.0–40.0	ns	0.71
B×PI	2009	Windblow, NC	2	3	34.0	10.0–57.0	ns	0.40
B×PI	2010	Salina, KS	3	3	39.0	31.0–48.0	**	0.86
B×PI	2010	Windblow, NC	2	3	46.0	28.0–74.0	ns	0.63

Within each population and year, rating was conducted on one date unless otherwise noted. Wilting ratings were based on a scale from 0 (no wilting) to 100 (severe wilting and plant death)

[a] Parent test indicates independent t test between two parent group means. Significance is indicated when parental means were different from at least one of the rating dates. There was no parental test in 2000 and 2002 in the 93705 KS4895 × Jackson population

[b] All environment was defined as the data pooled from years, wilting rating dates, and location

[c] All environment was defined as the data pooled from years and wilting rating date

[d] Experiments in 2010 and 2011 used a randomized complete block design while experiments in 2012 and 2013 used an incomplete block design (to account for difference in maturity), and hence, combined analyses were grouped by the experimental designs

illumina.com) based on array-based fluorescence emission. In addition, the 93K×J population was genotyped with eight Non-USLP version 1.0 markers using a KASP reaction (K-Bioscience, Hoddesdon Herts, UK) (Hwang et al. 2013, 2014b). The endpoint genotyping of Roche LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA) was used to interpret reaction results for these eight SNPs.

The AxPI population was genotyped at the Monsanto company using a proprietary set of 3,072 SNPs on the Illumina GoldenGate platform with the BeadStation 500G (Illumina, Inc., www.illumina.com). The position of each proprietary SNP marker was then converted to those at the public soybean consensus genetic map (Version 4.0) based on common reference markers.

Genetic map construction

Population-specific maps were created for each of the five mapping populations. The BxPI population had considerably fewer markers than the other populations, and the Kosambi mapping function (Kosambi 1944) was used for the genetic map that was described previously for this population (Abdel-Haleem et al. 2012).

For the other populations, linkage grouping was tested with the function, from Linkage Groups in the R/qtl library (Broman et al. 2003) in R (3.0.1). Initial linkage groups (LGs) were established using a minimum logarithm of odds (LOD) criterion of 6 and a maximum recombination fraction of 0.372 cM, which is equal to 50 cM in terms of the Kosambi mapping function. The increment of recombination fraction or decrement of LOD criterion was performed to check if unlinked markers or sub-LGs were rejoined to match the known chromosome number.

Potential genotyping errors were investigated before construction of genetic maps in the R/qtl library (Broman et al. 2003). Segregation distortion was evaluated to test 1:1 Mendelian segregation at each locus (excluding the residual heterozygous/heterogeneous RILs) using the adjusted

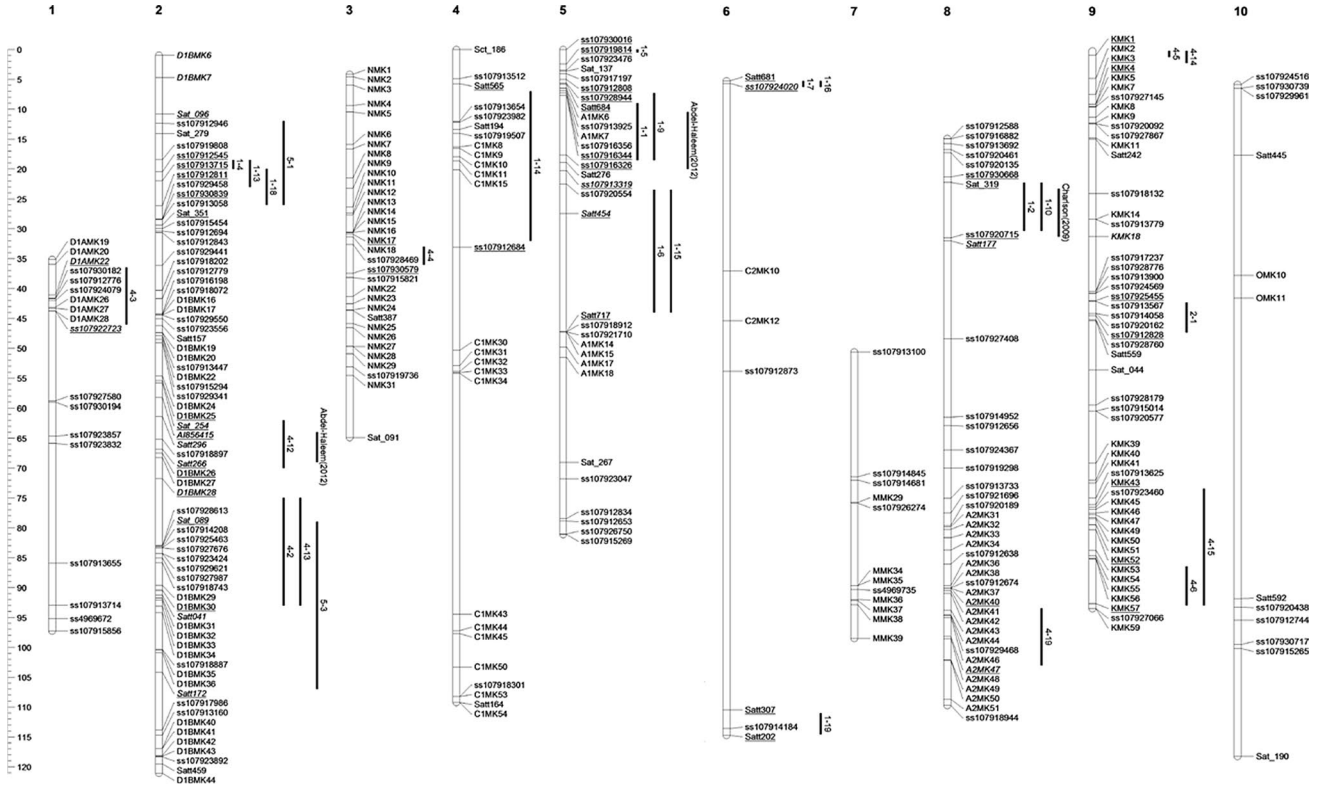

Fig. 1 QTL mapping for canopy wilting for chromosomes 1–10. QTLs from five mapping populations and previous studies (Charlson et al. 2009; Abdel-Haleem et al. 2012) were projected onto the soybean consensus version 4.0 map with confidence intervals of QTLs. *Bars* in *black* indicate confidence intervals of QTLs. *Markers* in *italic* were not used as cofactors. *Underlined markers* indicate flanking markers of confidence intervals of QTLs

Bonferroni type 1 error ($\alpha = 0.05$/total of polymorphic markers) in the χ^2 goodness-of-fit test ($df = 2$). Lines with a large number of crossovers, duplicate lines with identical genotypes for most markers, anomalous lines with monomorphic marker data (Abdel-Haleem et al. 2013), switch of A or B allele codes, and erroneous genotypes at 0.01 % of genotyping error rate were investigated and eliminated if necessary. Lastly, the genetic map was constructed in the R/qtl library (Broman et al. 2003).

The Kosambi mapping function was used to determine the genetic map distance. The recombination fraction value between a pair of markers was estimated by maximum likelihood with the Expectation–Maximization (EM) algorithm (Lander et al. 1987). The default iteration maximum number was 10,000, and 0.000001 was used as the tolerance value. A genotyping error rate of 0.01 % was assumed for the estimation of recombination fraction values. The marker order was tested with the likelihood ratio test (LRT) with a window size of 3 comparing to that of the soybean consensus map (version 4.0; Hyten et al. 2010). The genetic map in Figs. 1 and 2 was drawn by MapChart (Voorrips 2002).

QTL analysis and mapping

The WinQTLCartographer version 2.5.010 was used for single marker analysis (SMA) (Kearsey and Hyne 1994) and composite interval mapping (CIM) (Zeng 1994). We estimated parameters in the QTL model, assuming that canopy wilting followed a normal distribution (Abdel-Haleem et al. 2012; Charlson et al. 2009), using the maximum likelihood approach (Weller 1986) and the EM algorithm (Meng and Rubin 1993). Single-factor ANOVA was used to determine if polymorphic markers were significantly ($P < 0.05$) associated with canopy wilting, and significant markers were used as cofactors in the standard CIM model (model 6, WinQTLCartographer, v. 2.5.010). The CIM procedure used the cofactors to identify control markers using a forward and backward stepwise selection ($\alpha = 0.05$). The selected control markers were used to control the genetic background noise as covariates in the CIM model. The genome walk speed was 1 cM with a window size of 1 cM. A permutation test (1000 times) (Churchill and Doerge 1994) was used to determine an empirical genome-wise threshold for LRT and to identify a QTL.

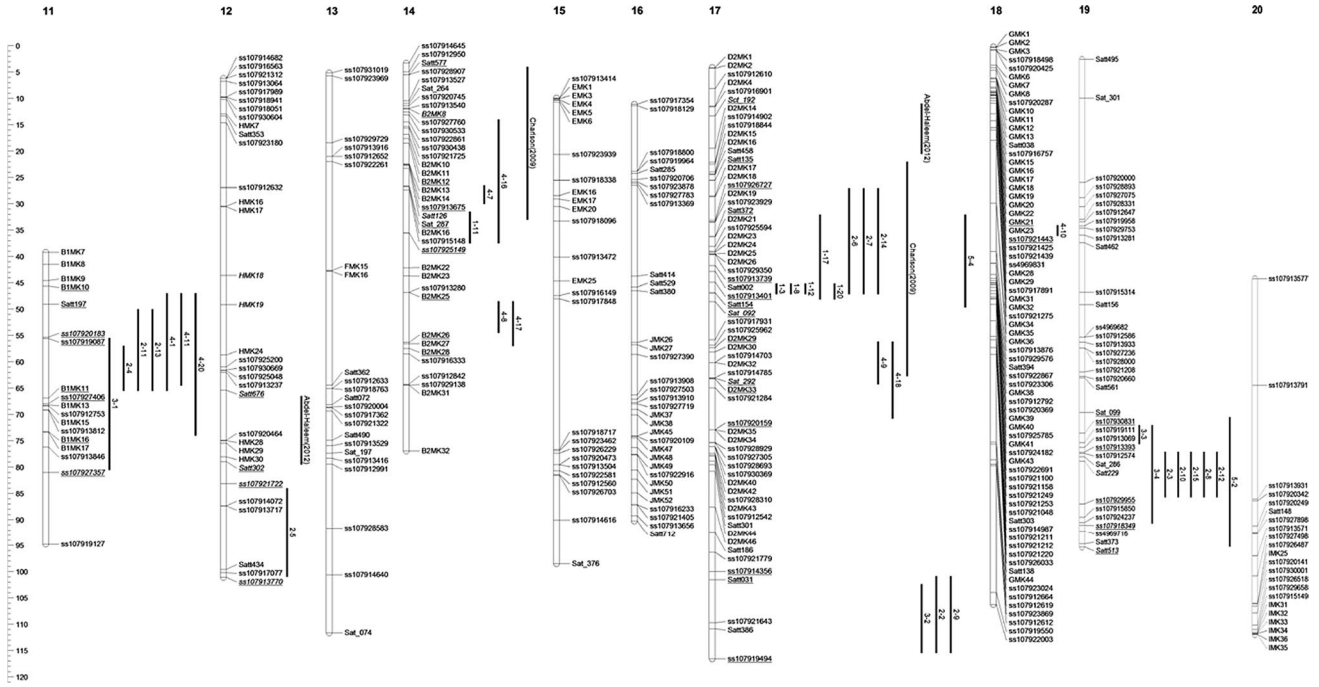

Fig. 2 QTL mapping for canopy wilting for chromosomes 11–20. QTLs from five mapping populations and previous studies (Charlson et al. 2009; Abdel-Haleem et al. 2012) were projected onto the soybean consensus version 4.0 map with confidence intervals of QTLs. *Bars* in *black* indicate confidence intervals of QTLs. *Markers* in *italic* were not used as cofactors. *Underlined markers* indicate flanking markers of confidence intervals of QTLs

We evaluated the interaction between a QTL and year (H_o: QTL × year = 0; H_a: QTL × year ≠ 0) using the joint-mapping module of multiple-trait analysis in WinQTLCartographer (v. 2.5.010) (Chung et al. 2003; Hwang et al. 2013). Multiple-trait analysis (Jiang and Zeng 1995) can be used to determine if an association between traits is due to pleiotropy or to closely linked QTLs. Likewise, the expression of a trait in different environments can be considered as distinct traits and analyzed using a similar procedure. In the model we used for this analysis, the effect of QTL × year was added to the previous CIM model. In each permutation test, canopy wilting was both randomized independently for each year and was randomized jointly over years to determine the LRT. The genome-wise threshold values for each year and across years (the 'joint trait') were generated from 1000 permutations. If an LOD value of the joint trait at a QTL position was greater than the threshold value, we concluded that the QTL was not stable across years. Two wilting ratings in early September in both 2003 and 2013 were used for multiple-trait analysis of 93K×J and 08K×J populations.

In addition to CIM, we used two different multiple interval mapping (MIM) QTL models: WinQTLCartographer (v.2.5.010) and QTL Network (v. 2.0, Yang et al. 2008). In WinQTLCartographer (v.2.5.010), the MIM procedure used the stepwise model procedure of Kao et al. (1999) in which QTLs from the CIM model were used in an initial MIM model. This pre-selected model was iteratively optimized to find the maximum likely QTL positions, new main QTL effects, and epistasis between main QTLs. To increase precision, the genome walk speed and window size were set at 1 cM. Two criteria, the maximum likelihood value and the Bayesian information criterion (BIC), $c(n) = 3*\ln(n)$, were evaluated between a present model and previous model to fit the best model as determined by an LOD profile. The wilting data for each environment were used to determine possible QTL × QTL interactions.

In QTL Network (v. 2.0), the MIM model first uses QTLs that were identified in the CIM model (Zeng 1994). Then, significant marker intervals were identified via a marker pair selection (Piepho and Gauch 2001) in a one-dimension genome scan. Next, a two-dimension genome scan considered all possible significant interactions between marker intervals regardless of whether or not loci were in a QTL region. Finally, possible interactions between a locus and year were tested. An F test was executed at all stepwise model selection procedures. The Bayesian method using Gibbs sampling (Wang 1994) as a type of Markov Chain Monte Carlo (MCMC) was used to estimate parameters in the model without the consideration for the distribution of canopy wilting. For each sequential model in one- and two-genome scans, a permutation test (1000 times) (Churchill and Doerge 1994) was applied

for new coefficient terms in the model (Yang et al. 2007) to determine the empirical experiment-wise false-positive rate. A genome-wise threshold value of 0.05 was used for the best model selection for each sequential model based on an *F* test. Wilting data from two dates in early September in 2003 and 2013 for both the 08K×J and 93K×J populations were used to determine possible QTL × QTL interactions and QTL × year interactions. Multiple-trait analysis and MIM analysis in QTL Network were not used in the B×PI population.

In the B×PI population, Abdel-Haleem et al. (2012) previously reported delayed-wilting QTLs using the same MIM model in WinQTLCartographer (v.2.5.010) as described above with LS means across environments but with less stringent criteria [BIC, $c(n) = \ln(n)$]. In the present research, instead of considering the average response of RILs over environments (Abdel-Haleem et al. 2012), we determined the wilting response of RILs from the B×PI population in individual environments (Table 2). For each environment, wilting was rated multiple times, and for our analysis we determined the LS means of wilting for RILs over rating dates for each environment. Herein, we report the QTLs identified for individual environments.

QTLs were originally mapped with 95 % CIs using their respective genetic maps. To project CIs of QTLs from each mapping population onto the soybean consensus map (version 4.0), flanking markers, which covered 95 % CIs for QTLs, were identified that were in common for each population-specific map and with markers in the consensus map. Based on this information, QTLs with 95 % CIs in each genetic map were simply projected onto the soybean consensus map. In the BxPI population (Abdel-Haleem et al. 2012), CIs were estimated as the LOD values ±1 deviation.

A simulation study was conducted to identify QTLs that might be false positives using the qtl Design library in R (Broman et al. 2003; Sen et al. 2007). The simulation predicts the minimum detectable QTL effect and the phenotypic variation for a QTL effect (R^2), and these metrics were compared with the observed values to evaluate the possibility that QTLs were false positives. Inputs for the simulation were the observed genetic variance, error variance, the number of replications, sample size, recombination fraction value, and statistical power value in the CIM model. Average linkage distance was used as the recombination fraction value between adjacent flanking markers from the genetic map of each population. A statistical power value of 0.8 was assumed to evaluate the sensitivity of the null hypothesis. If either the QTL effect or R^2 of a QTL from the observed data was greater than the simulated value, we concluded that the QTL was not a false positive. However, if either the QTL effect or R^2 of a QTL was substantially less than the observed values, we concluded that the QTL could be a false positive.

Results

Analysis of canopy wilting data

ANOVA was performed by year and across years (data not shown). Genotype (RIL), year, and interaction (genotype × year) effects were significant in most mapping populations except that year and genotype × year effects were not significant in the 08K×J population. Rating date, genotype, and interaction (date × genotype) effects in both the 93K×J and 08K×J populations were significant in 2003 and 2013. The RILs differed significantly ($P < 0.05$) for maturity date each year in the A×PI population, but they did not differ significantly across multi-year environments. The interaction effect among year, maturity, and genotype was significant in the A×PI population (data not shown). The phenotypic correlation coefficient between canopy wilting and maturity date in the A×PI was −0.38 ($P < 0.001$) in 2010. On any given rating date, the earlier-maturing lines (with an early maturity date) would be at a more advanced physiological stage than later-maturing lines, and wilting in these lines tended to be more severe. Hence, wilting tended to be more severe on a given rating date for early maturing RILs.

Canopy wilting scores generally ranged from 15.0 to 65.0 for all populations although there was a greater range of extreme values in the 93K×J population (Table 2). The differences between parental means were only significant in the A×PI (2010, 2011, 2012, 2013) and B×PI (2010) populations (Table 2). The distribution of canopy wilting among genotypes extended beyond the parental values, and for the 08K×J, K×PI, A×PI, and B×PI populations the means of the parental genotypes were significantly different ($P < 0.05$) from the population extremes (data not shown). Together this indicates the possibility of transgressive segregation. The grand mean of canopy wilting from all populations was 35, and population means were close to mid-parent means (data not shown).

The heritability for canopy wilting across multiyear environments ranged 0.52–0.78 (Table 2). Previous studies reported that the heritability for canopy wilting ranged from 0.50 to 0.60 (Charlson et al. 2009; Abdel-Haleem et al. 2012). King et al. (2009) demonstrated that the ranking of canopy wilting among genotypes was relatively consistent across years and rating dates within a single location when linear regressions ($R^2 = 0.72$–0.98) among all rating date combinations were compared. With two exceptions, canopy wilting within an environment was highly heritable ($h^2 > 0.63$). Heritability was considerably lower at Windblow, NC in 2002 for the 93K×J population ($h^2 = 0.30$) and in 2009 for the BxPI population ($h^2 = 0.40$).

Genetic map construction

Table 1 provides a summary of the different mapping populations. The 93K×J population was originally genotyped with 165 SSRs as described by Charlson et al. (2009). An additional 497 informative markers were used to construct the genetic map of the 93K×J population in the present research. Genetic maps of these five populations covered most of the soybean genome, although start and end points on some chromosomes were not well covered. Average map distances between adjacent markers ranged from 3.1 (A×PI) to 8.6 (93K×J) cM. For all the populations, except the B×PI population, the total length of genetic maps was longer than that of the soybean consensus version 4.0 map (2241.3 cM). In part, this was because we relaxed the stringency when constructing the genetic maps, thereby allowing all linkage groups to be directly associated with specific chromosomes. Had the stringency been increased, flanking markers with large recombination frequencies would have been separated into sub-linkage groups, and the total length of the genetic map would have been decreased (Hwang et al. 2013, 2014a). The marker order of each genetic map was compared to that of the soybean consensus version 4.0 map order. Marker order for all the genetic maps was generally similar to the consensus map although there were minor differences in marker order on a given chromosome (data not shown).

QTL analysis by population

With the exception of the BxPI population, all markers that were significantly ($P < 0.05$) associated with wilting (i.e., cofactors) and QTLs from these populations were projected onto the soybean consensus map (Figs. 1, 2). Highly significant markers ($P < 0.001$) from the SMA were located near the maximum likely QTL positions. Other significant markers ($P < 0.05$) were located near QTL positions or within CIs of QTLs.

A total of 20 putative QTLs were identified in the 93K×J population, but only 10 of these QTLs appeared to be unique based on overlapping 95 % CIs (Table 3; Figs. 1, 2). Seven QTLs on Gm02, Gm05, Gm06, Gm08, and Gm17 were identified with the CIM model with R^2 values ranging from 0.11 to 0.43 and with additive effects from 1.55 to 8.68 units. Two QTLs on Gm04 and Gm14 were identified with the MIM model with R^2 values ranging from 0.08 to 0.64. Two QTLs, which were close to markers BARC-044481-08709 (Gm05) and Satt681 (Gm06), had large R^2 values ranging from 0.34 to 0.64 in CIM and MIM models. However, since these QTLs were identified between flanking markers with large gaps, R^2 values for these QTLs may have been overestimated (Darvasi et al. 1993). In the present research, no QTL was identified from wilting ratings in 2002 at Sandblow, NC while previous research (Charlson et al. 2009) found a QTL in 2002 on Gm13 near Satt362 using the same phenotypic data and a subset of molecular markers used in the current research. Excluding the two QTLs in large gaps on Gm05 and Gm06, a QTL on Gm17 accounted for the highest phenotypic variation ($R^2 = 0.14$–0.22) with the highest additive effect (1.78–8.73 units) across environments. All alleles contributing to delayed canopy wilting, except for a QTL on Gm17, were from Jackson.

The stability of QTLs across years (2000, 2002, and data for the third wilting date in 2003) was evaluated with multiple-trait analysis for the 93K×J population. LOD values for QTL positions did not exceed the threshold value of the joint trait, indicating that interactions between QTLs and years were not significant (data not shown). Additive effects for QTL positions in each year had the same sign. However, the magnitudes of additive effects in 2000 were greater than those of other years. These results indicated that most QTLs seemed to be stable and had useful effects over years. There was no significant epistasis among QTLs in MIM models. However, there was a significant interaction between a pair of loci in the MIM model of QTL Network although these loci were not QTLs. Two loci, which were located near two markers, BARC-026065-05240 and BARC-010353-00615 on Gm02 and Gm09, respectively, had a negative interaction effect of 0.11 units ($P < 0.0001$).

There were a total of 15 putative QTLs in the 08KxJ population on Gm09, Gm11, Gm12, Gm17, and Gm19, and six of these QTLs appeared to be unique based on their overlapping CIs (Table 4; Figs. 1, 2). QTLs in the CIM model accounted for phenotypic variation ranging from 0.07 to 0.29 with additive effects ranging from 0.95 to 3.23 units. For the MIM model, R^2 values ranged from 0.09 to 0.28 and additive effects ranged from 1.05 to 2.10 units. A QTL, which was located near BARC-026069-05243 on Gm19, had the highest R^2 value (0.25–0.29) and additive effect (1.85–2.10 units) in both models across environments. Alleles of all QTLs conditioning delayed wilting, except for a QTL on Gm17, were from Jackson as was found for the 93K×J population.

Multiple-trait analysis was performed across years (2012 and the second wilting rate date in 2013) for the 08KxJ population. Two QTLs on Gm11 and Gm19 had significant QTL × year interactions, indicating that these QTLs were not stable across years (data not shown). The additive effect for the QTL on Gm11 had a different sign among traits (i.e., 2012, 2013, and joint trait), and the magnitudes of additive effects for a QTL on Gm19 were very different. It appeared that other QTLs were stable and had additive effects with the same sign and similar magnitude. In the MIM model of QTL Network, a QTL on Gm11 also had a significant interaction with year ($P < 0.001$). These results support the conclusion that a QTL on Gm11 was not stable across years. There was significant epistasis between two

Table 3 QTL marker information for canopy wilting in the 93705 KS4895 × Jackson population

QTL model	QTL name	Chromosome	Year	Score date[a]	Nearest marker	SNP # submitted in NCBI	R^2	QTL effect[b]	Favorable allele[c]	Nearest marker position[d]	LOD	$P > \chi^2$ or $P > F$[e]	Confidence interval[f]	
													Flanking markers	
													Left	Right
CIM	1-1	Gm05	2000	–	Satt684	–	0.16	−7.33	Jackson	5.9	5.7	0.000000	ss107928944	ss107916326
	1-2	Gm08	2000	–	BARC-045199-08906	ss107920715	0.15	−7.13	Jackson	31.2	4.5	0.000015	ss107930668	ss107920715
	1-3	Gm17	2000		BARC-	ss107913401	0.2	8.68	KS4895		7.3	0.000000	ss107913739	ss107913401
				–	024449-04894		2			44.7				
	1-4	Gm02	2003	3	BARC-032525-08992	ss107913715	0.12	−1.60	Jackson	22.0	3.6	0.000019	ss107912545	ss107912811
	1-6	Gm05	2003	4	BARC-044481-08709	ss107920554	0.34	−2.90	Jackson	22.5	4.1	0.000017	ss107913319	Satt717
	1-7	Gm06	2003	2	Satt681	–	0.43	−1.88	Jackson	5.0	22.9	0.000000	Satt681	ss107924020
	1-8	Gm17	2003	3	BARC-024449-04894	ss107913401	0.14	1.70	KS4895	44.7	4.2	0.000017	ss107913739	ss107913401
MIM	1-9	Gm05	2000	–	Satt684	–	0.14	−6.73	Jackson	5.9	5.0	0.000002	ss107912808	ss107916326
	1-10	Gm08	2000	–	BARC-045199-08906	ss107920715	0.14	−7.78	Jackson	31.2	5.0	0.000002	ss107930668	ss107920715
	1-11	Gm14	2000	–	BARC-015539-02002	ss107915148	0.08	−5.75	Jackson	27.4	3.5	0.000019	ss107913675	ss107925149
	1-12	Gm17	2000	–	BARC-024449-04894	ss107913401	0.21	8.73	KS4895	44.7	7.2	0.000000	ss107913739	ss107913401
	1-13	Gm02	2003	3	BARC-032525-08992	ss107913715	0.20	−1.93	Jackson	22.0	3.8	0.000020	ss107912545	ss107930839
	1-14	Gm04	2003	2	BARC-030765-06943	ss107913654	0.11	−1.10	Jackson	12.0	3.8	0.000020	Satt565	ss107912684
	1-15	Gm05	2003	4	BARC-044481-08709	ss107920554	0.46	−3.35	Jackson	22.5	2.8	0.000202	ss107913319	Satt717
	1-16	Gm06	2003	2	Satt681	–	0.64	−2.33	Jackson	5.0	5.4	0.000000	Satt681	ss107924020
	1-17	Gm17	2003	3	BARC-024449-04894	ss107913401	0.17	1.78	KS4895	44.7	3.5	0.000019	Satt372	Satt154
MIM	1-18	Gm02	All	All	BARC-063263-18286	ss107929458	–	−2.38	Jackson	28.4	–	0.000048	ss107913715	Sat_351
	1-19	Gm06	All	All	BARC-010777-00746	ss107914184	–	−1.23	Jackson	113.1	–	0.048664	Satt307	Satt202
	1-20	Gm17	All	All	BARC-024449-04894	ss107913401	–	3.93	KS4895	44.7	–	0.000000	ss107913739	Satt154

Note that there were no QTLs identified in this population in 2002 at Windblow, NC

[a] Numbers 2, 3, and 4 indicate the second, third, and fourth wilting rating dates in 2003. In the MIM model of QTL Network, only the fourth wilting date in 2003 was used for QTL analysis across years

[b] QTL effects were estimated as a half of differences between the average effects of two parental alleles on the maximum likely QTL positions in the genetic map of the 93705 KS4895 × Jackson population

[c] Favorable parental alleles were defined as the one giving the low wilting score on the maximum likely QTL positions in the genetic map of the 93705 KS4895 × Jackson population

[d] Nearest markers were positioned based on the soybean consensus version 4.0 map presenting in Fig. 2

[e] Since LOD asymptotically follows χ^2 distribution, P values were used as another way to show LOD criteria. In the MIM model of QTL Network, P values in F distribution were used instead of LOD

[f] The LOD values with ±1 deviation were approximately used to indicate 95 % confidence intervals of the maximum likely QTL positions in the genetic map of the 93705 KS4895 × Jackson population. Two flanking markers indicate markers, which include confidence intervals of the maximum likely QTL positions

Table 4 QTL marker information for canopy wilting in the 08705 KS4895 × Jackson population

QTL model	QTL name	Chromosome	Year	Score date[a]	Nearest marker	SNP number submitted in NCBI	R^2	QTL effect[b]	Favorable allele[c]	Nearest marker position[d]	LOD	$P > \chi^2$ or $P > F$[e]	Confidence interval[f]	
													Flanking markers	
													Left	Right
CIM	2-1	Gm09	2012	–	BARC-029275-06136	ss107913567	0.09	−3.23	Jackson	41.8	3.6	0.000019	ss107925455	ss107912828
	2-2	Gm17	2012	–	Satt386	–	0.09	1.12	KS4895	110.4	4.0	0.000018	ss107914356	ss107919494
	2-3	Gm19	2012	–	BARC-026069-05243	ss107912574	0.25	−1.90	Jackson	76.8	8.7	0.000000	ss107913393	ss107929955
	2-4	Gm11	2013	2	BARC-032817-09052	ss107919087	0.16	−2.13	Jackson	55.6	6.3	0.000000	ss107919087	ss107927406
	2-5	Gm12	2013	2	BARC-039237-07479	ss107913770	0.09	−1.50	Jackson	101.1	3.3	0.000020	ss107921722	ss107913770
	2-6	Gm17	2013	1	BARC-035383-07190	ss107913739	0.06	0.95	KS4895	41.6	3.2	0.000020	ss107926727	ss107913401
	2-7	Gm17	2013	2	BARC-035383-07190	ss107913739	0.07	1.38	KS4895	41.6	3.3	0.000020	ss107926727	ss107913401
	2-8	Gm19	2013	1	BARC-026069-05243	ss107912574	0.29	−2.15	Jackson	76.8	12.7	0.000000	ss107913393	ss107929955
MIM	2-9	Gm17	2012	–	Satt386	–	0.09	1.05	KS4895	110.4	3.3	0.000020	ss107914356	ss107919494
	2-10	Gm19	2012	–	BARC-026069-05243	ss107912574	0.25	−1.85	Jackson	76.8	7.5	0.000000	ss107913393	ss107929955
	2-11	Gm11	2013	2	BARC-032817-09052	ss107919087	0.14	−1.93	Jackson	55.6	4.7	0.000014	Satt197	ss107927406
	2-12	Gm19	2013	1	BARC-026069-05243	ss107912574	0.28	−2.10	Jackson	76.8	9.7	0.000000	ss107913393	ss107929955

Table 4 continued

QTL model	QTL name	Chromosome	Year	Score date[a]	Nearest marker	SNP number submitted in NCBI	R^2	QTL effect[b]	Favorable allele[c]	Nearest marker position[d]	LOD	$P > \chi^2$ or $P > F$[e]	Confidence interval[f]	
													Flanking markers	
													Left	Right
MIM	2-13	Gm11	All	All	BARC-032817-09052	ss107919087	–	−0.55	Jackson	55.6	–	0.016972	Satt197	ss107927406
	2-14	Gm17	All	All	BARC-035383-07190	ss107913739	–	0.93	KS4895	41.6	–	0.000023	ss107926727	ss107913401
	2-15	Gm19	All	All	BARC-026069-05243	ss107912574	–	−1.05	Jackson	76.8	–	0.000067	ss107913393	ss107929955

[a] Numbers 1 and 2 indicate the first and second wilting rating dates in 2013. In the MIM model of QTL Network, only the second wilting date in 2013 was used for QTL analysis across years

[b] QTL effects were estimated as a half of differences between the average effects of two parental alleles on the maximum likely QTL positions in the genetic map of the 08705 KS4895 × Jackson population

[c] Favorable parental alleles were defined as the one giving the low wilting score on the maximum likely QTL positions in the genetic map of the 08705 KS4895 × Jackson population

[d] Nearest markers were positioned based on the soybean consensus version 4.0 map presenting in Fig. 2

[e] Since LOD asymptotically follows χ^2 distribution, P values were used as another way to show LOD criteria. In the MIM model of QTL Network, P values in F distribution were used instead of LOD

[f] The LOD values with ±1 deviation were approximately used to indicate 95 % confidence intervals of the maximum likely QTL positions in the genetic map of the 08705 KS4895 × Jackson population. Two flanking markers indicate markers, which include confidence intervals of the maximum likely QTL positions

Table 5 QTL marker information for canopy wilting in the KS4895 × PI 424140 population

QTL model	QTL name	Chromosome	Year	Nearest marker	SNP # submitted in NCBI	R^2	QTL effect[a]	Favorable allele[b]	Nearest marker position[c]	LOD	$P > \chi^2$ or $P > F$[d]	Confidence interval[e]	
												Flanking markers	
												Left	Right
CIM	3-1	Gm11	2013	BARC-041167-07925	ss107913846	0.22	−2.50	PI 424140	76.2	3.2	0.000020	ss107920183	ss107927357
	3-2	Gm17	2013	BARC-048855-10738	ss107921643	0.10	1.68	KS4895	109.3	3.3	0.000020	Satt031	ss107919494
	3-3	Gm19	2013	BARC-065769-19741	ss107930831	0.11	−2.23	PI 424140	73.0	3.4	0.000020	ss107930831	ss107913393
MIM	3-4	Gm19	2013	BARC-035235-07156	ss107919111	0.20	−2.85	PI 424140	74.8	4.3	0.000017	ss107930831	ss107918349

[a] QTL effects were estimated as a half of differences between the average effects of two parental alleles on the maximum likely QTL positions in the genetic map of the KS4895 × PI 424140 population

[b] Favorable parental alleles were defined as the one giving the low wilting score on the maximum likely QTL positions in the genetic map of the KS4895 × PI 424140 population

[c] Nearest markers were positioned based on the soybean consensus version 4.0 map presenting in Fig. 2

[d] Since LOD asymptotically follows χ^2 distribution, P values were used as another way to show LOD criteria. In the MIM model of QTL Network, P values in F distribution were used instead of LOD

[e] The LOD values with ±1 deviation were approximately used to indicate 95 % confidence intervals of the maximum likely QTL positions in the genetic map of the KS4895 × PI 424140 population. Two flanking markers indicate markers, which include confidence intervals of the maximum likely QTL positions

Table 6 QTL marker information for canopy wilting in the A5959 × PI 416937 population

QTL model	QTL name	Chromosome	Year	Nearest marker	SNP # submitted in NCBI	R^2	QTL effect[a]	Favorable allele[b]	Nearest marker position[c]	LOD	$P > \chi^2$ or $P > F$[d]	Confidence interval[e]	
												Flanking markers	
												Left	Right
CIM	4-1	Gm11	2010	B1MK11	–	0.29	−3.48	PI 416937	66.9	6.9	0.000000	B1MK10	ss107927406
	4-2	Gm02	2011	D1BMK29	–	0.16	−1.93	PI 416937	89.6	5.2	0.000002	D1BMK28	D1BMK30
	4-3	Gm01	2012	D1AMK26	–	0.13	−1.53	PI 416937	43.1	4.2	0.000017	D1AMK22	ss107922723
	4-4	Gm03	2012	NMK19	ss107930579	0.10	−1.33	PI 416937	32.6	3.5	0.000019	NMK17	ss107930579
	4-5	Gm09	2012	KMK2	–	0.11	−1.38	PI 416937	4.5	4.0	0.000018	KMK1	KMK3
	4-6	Gm09	2012	KMK54	–	0.16	1.65	A5959	84.2	5.4	0.000000	KMK52	KMK57
	4-7	Gm14	2013	B2MK13	–	0.09	−1.30	PI 416937	22.4	3.6	0.000019	B2MK12	ss107913675
	4-8	Gm14	2013	B2MK25	–	0.15	1.65	A5959	46.8	5.2	0.000002	B2MK25	B2MK26
	4-9	Gm17	2013	D2MK30	–	0.13	1.48	A5959	57.9	5.0	0.000002	D2MK29	D2MK33
	4-10	Gm18	2013	GMK23	–	0.09	−1.25	PI 416937	9.5	3.7	0.000020	GMK21	ss107921443
MIM	4-11	Gm11	2010	B1MK11	–	0.39	−3.90	PI 416937	66.9	5.4	0.000000	B1MK10	B1MK11
	4-12	Gm02	2011	D1BMK26	–	0.18	2.08	A5959	67.5	4.0	0.000018	D1BMK25	D1BMK26
	4-13	Gm02	2011	D1BMK29	–	0.19	−2.00	PI 416937	89.6	5.6	0.000000	D1BMK28	D1BMK30
	4-14	Gm09	2012	KMK2	–	0.11	−1.40	PI 416937	4.5	3.1	0.000020	KMK1	KMK3
	4-15	Gm09	2012	KMK54	–	0.15	1.53	A5959	84.2	3.9	0.000018	KMK43	KMK57
	4-16	Gm14	2013	B1MK15	ss107913675	0.10	−1.53	PI 416937	22.6	3.7	0.000020	B2MK8	ss107925149
	4-17	Gm14	2013	B2MK26	–	0.16	2.03	A5959	56.3	5.7	0.000000	B2MK25	B2MK26
	4-18	Gm17	2013	D2MK33	–	0.19	1.88	A5959	63.0	6.1	0.000000	D2MK29	ss107920159
MIM	4-19	Gm08	All	A2MK42	–	–	1.03	Asgrow5959	90.5	–	0.000000	A2MK40	A2MK47
	4-20	Gm11	All	B1MK11	–	–	−0.98	PI 416937	66.9	–	0.000002	B1MK10	B1MK16

[a] QTL effects were estimated as a half of differences between the average effects of two parental alleles on the maximum likely QTL positions in the genetic map of the A5959 × PI 416937 population

[b] Favorable parental alleles were defined as the one giving the low wilting score on the maximum likely QTL positions in the genetic map of the A5959 × PI 416937 population

[c] Nearest markers were positioned based on the soybean consensus version 4.0 map presenting in Fig. 2

[d] Since LOD asymptotically follows χ^2 distribution, P values were used as another way to show LOD criteria. In the MIM model of QTL Network, P values in F distribution were used instead of LOD

[e] The LOD values with ±1 deviation were approximately used to indicate 95 % confidence intervals of the maximum likely QTL positions in the genetic map of the A5959 × PI 416937 population. Two flanking markers indicate markers, which include confidence intervals of the maximum likely QTL positions

Table 7 QTL marker information for canopy wilting in the Benning × PI 416937 population

QTL model	QTL name	Chromosome	Year	Location[a]	Nearest marker	SNP # submitted in NCBI	R^2	QTL effect[b]	Favorable allele[c]	Nearest marker position[d]	LOD	$P > \chi^{2e}$	Confidence interval[f]	
													Flanking marker	
													Left	Right
MIM	5-1	Gm02	2009	NC	Sat_096	–	0.06	−2.26	Benning	10.8	3.1	0.000200	Sat_096	Sat_351
	5-2	Gm19	2009	AR	Satt229	–	0.16	1.13	PI 416937	78.3	5.9	0.000000	Sat_099	Satt513
	5-3	Gm02	2010	KS	Satt041	–	0.06	0.75	PI 416937	91.3	2.5	0.001300	Sat_089	Satt172
	5-4	Gm17	2010	KS	Satt154	–	0.07	−0.97	Benning	46.8	3.9	0.000018	Satt372	Sat_092

[a] Wilting was evaluated at Windblow NC (2009 and 2010), Stuttgart AR (2009), and Salina KS (2010) as described by Abdel-Haleem et al. (2012)

[b] QTL effects were estimated as a half of differences between the average effects of two parental alleles on the maximum likely QTL positions in the genetic map of the Benning × PI 416937 population

[c] Favorable parental alleles were defined as the one giving the low wilting score on the maximum likely QTL positions in the genetic map of the Benning × PI 416937 population

[d] Nearest markers were positioned based on the soybean consensus version 4.0 map presenting in Fig. 2

[e] Since LOD asymptotically follows χ^2 distribution, P values were used as another way to show LOD criteria

[f] The LOD values with ±1 deviation were approximately used to indicate 95 % confidence intervals of the maximum likely QTL positions in the genetic map of the Benning × PI 416937 population. Two flanking markers indicate markers, which include confidence intervals of the maximum likely QTL positions

QTLs close to markers BARC-032817-09052 and BARC-035383-07190 on Gm11 and Gm17, respectively, that had a negative interaction effect of 0.58 units ($P < 0.013$).

A total of four putative QTLs were identified with the CIM or MIM model in the KxPI population, three of which appeared to identify unique loci based on their CIs (Table 5; Figs. 1, 2). The R^2 values ranged from 0.10 to 0.22 with additive effects ranging from 1.68 to 2.85 units. The allele for a QTL on Gm11 that was derived from PI 424140 had the largest R^2 value (0.22) although this QTL was identified between flanking markers with a relatively large gap (about 35 cM), which may have overestimated the effect.

We identified 20 putative QTLs in the AxPI population on Gm01, Gm02, Gm03, Gm08, Gm09, Gm11, Gm14, Gm17, and Gm18, and 12 of these appeared to be unique QTLs based on CIs (Table 6; Figs. 1, 2). The R^2 values of these QTLs in the CIM model ranged from 0.09 to 0.29. A QTL on Gm11 located near B1MK11 had the highest R^2 value in the CIM (0.29) and MIM (0.39) models; however, this QTL was also identified between flanking markers with a large gap (35 cM) with the allele conditioning delayed wilting coming from PI 416937. The additive effect in the CIM model ranged from 1.25 to 3.48 units. Two QTLs on Gm02 and Gm08 were identified with MIM models, and these QTL alleles were from PI 416937.

Multiple-trait analysis for wilting was conducted across years (2010, 2011, 2012, and 2013) for the AxPI population. Most QTLs were stable over years although the magnitudes of additive effects for QTL positions were variable (data not shown). The LOD value of a QTL on Gm11 exceeded the threshold value of the joint trait, indicating that the interaction between this QTL and year was significant. There were no significant interactions among QTLs in MIM models. However, two pairs of loci had significant interactions in the MIM model of QTL Network although these loci were not identified as QTLs. These loci were close to D1BMK7 and KMK14 on Gm02 and Gm09, respectively, and had a positive interaction effect of 1.25 units ($P < 0.0001$). Another two loci were located near MMK18 and JMK29 on Gm12 and Gm16, respectively, and these loci had a negative interaction effect of 1.00 unit ($P < 0.0001$).

The BxPI population was evaluated in five environments, and seven QTLs were reported based on RIL values averaged over environments as reported by Abdel-Haleem et al. (2012). When considering QTLs in individual environments, 25 putative QTLs were identified on Gm01, Gm02, Gm03, Gm04, Gm05, Gm07, Gm08, Gm12, Gm13, Gm17, and Gm19 (Supplemental 1). Based on overlapping CIs, five of these QTLs were identified in two or more environments, giving a total of 17 unique QTLs. Eleven of these 17 QTLs received the favorable allele from PI 416937. Of the five QTLs from multiple environments, a QTL on Gm12 was found in five environments and had R^2 values ranging from

Table 8 Summary of QTL clusters for delayed canopy wilting identified from previously published reports and from current research with mapping populations 93705 KS4895 × Jackson (93K×J), 08705 KS4895 × Jackson (08K×J), KS4895 × PI 424140 (K×PI), A5959 × PI 416937 (A×PI), and Benning × PI 416937 (B×PI)

Chromosome	R^2 range[a]	Approximate position[b] (cM)	Populations contributing to QTL clusters	Parent(s) contributing favorable allele	Comments
Gm02	0.06–0.12	10.8–28.4	93K×J, B×PI	Benning, Jackson	
Gm02	0.06–0.18	63.5–67.5	A×P, Abdel-Haleem et al. (2012)	A5959, PI 416937	
Gm02	0.06–0.19	89.6–91.3	B×PI, A×PI	PI 416937	
Gm05	0.04–0.16	5.9–8.0	93K×J, Abdel-Haleem et al. (2012)	PI 416937, Jackson	
Gm08	0.05–0.15	30.5–31.2	93K×J, Charlson et al. (2009), Du et al. (2009)	Jackson, Nannong 1138-2	
Gm11	0.14–0.39	66.9–76.2	08K×J, K×PI, AxPI	PI 416937, Jackson, PI 424140	Significant QTL × year interaction
Gm14	0.08–0.12	22.6–27.4	93K×J, A×PI, Charlson et al. (2009)	Jackson, PI 416937	Potential false positive
Gm17	0.06–0.22	41.6–63.0	93K×J, 08K×J, A×PI, B×PI, Charlson et al. (2009)	KS4895, A5959, Benning	
Gm17	0.09–0.10	109.3–110.4	08K×J, K×PI	KS4995	
Gm19	0.11–0.29	73.0–78.3	08K×J, K×PI, B×PI	PI 416937, Jackson, PI424140	Significant QTL × year interaction

[a] Range of R^2 values determined from different mapping populations, years, locations, and scoring dates as described in Tables 3, 4, 5, 6 and 7

[b] Approximate positions are based on the range of nearest markers from the different populations. For more specific locations, refer to Tables 3, 4, 5, 6 and 7

0.10 to 0.21 and with additive effects between 1.2 and 3.0 units. The other QTLs that we found in multiple environments were located on Gm04, Gm05, 17 and 19. Four QTLs on Gm02, Gm17, and Gm19 co-segregated with QTLs from other populations (Table 7; Figs. 1, 2).

QTL analysis across populations

There were nine QTL clusters on Gm02, Gm05, Gm11, Gm14, Gm17, and Gm19 that had overlapping CIs from at least two different populations (Fig. 1, 2). Table 8 summarizes the approximate position, populations from which QTLs were identified, and parents contributing favorable alleles for these nine QTL clusters and a tenth QTL cluster on Gm08 that was identified using previously published information on delayed wilting (Abdel-Haleem et al. 2012; Charlson et al. 2008; Du et al. 2009). There were three QTL clusters on Gm02. Near the top of Gm02 (~22 cM), there were QTLs from the 93K×J population and the B×PI population, with the favorable alleles being contributed from Jackson and Benning. At about 67 cM on Gm02, QTLs were present from the A×PI population and from a QTL identified by Abdel-Haleem et al. (2012) in the B×PI population, and the favorable alleles were contributed by A5959 and from PI 416937. Towards the bottom of Gm02 (~89 cM), QTLs were identified from the A×PI population and the BxPI population, and PI 416937 contributed the favorable allele from both populations. Near the top of Gm05 (~6 cM), there were QTLs from the 93K×J population and from a QTL identified by Abdel-Haleem et al. (2012) from the B×PI population. The favorable alleles at this cluster were from Jackson and PI 416937.

On Gm08, there was a QTL from 93K×J population (31.2 cM, Table 3) and a QTL reported by Charlson et al. (2009) (21.9 cM) from a subset of the 93K×J population. Additionally, near this same position there was a QTL for wilting coefficient reported by Du et al. (2009) from a Kefeng1 × Nannong 1138-2 population. Although Du et al. (2009) did not provide sufficient information to project this QTL onto the soybean consensus map, it was located between flanking markers, Satt589 and BE820148 (30.5 and 31.2 cM on the soybean consensus map), that overlapped with QTLs for slow wilting identified by Charlson et al. (2009) and from the 93K×J population in the present research. Because QTLs at this position were found from the 93K×J population and from the Kefeng1 × Nannong 1138-2 population, we consider this a likely wilting QTL cluster. The favorable alleles for the QTL cluster on Gm08 were from Jackson and Nannong 1138-2.

A QTL cluster on Gm11 (~55 cM) consisted of individual QTLs from the 08KxJ, KxPI, and AxPI populations (Fig. 2; Table 8), with favorable alleles being contributed from Jackson, PI 424140, and PI 416937. There was one QTL cluster on Gm14 with individual QTLs reported from

the 93K×J and A×PI populations, and this QTL cluster had overlapping CI with a QTL previously reported for slow wilting by Charlson et al. (2009). The favorable alleles were from Jackson and PI 416937.

On Gm17, there were two QTL clusters with overlapping CIs. The cluster on Gm17 located at about 45 cM had QTLs from 93K×J, 08K×J, A×PI, and B×PI; in addition Charlson et al. (2009) found a QTL at this position, and Abdel-Haleem et al. (2012) reported a QTL just outside of this region. The favorable alleles at this cluster were from KS4895, A5959, and Benning, all of which would be considered the sensitive parent. The second QTL cluster on Gm17 was located near the bottom of the chromosome (~109 cM); individual QTLs at this cluster were from the 08K×J and K×PI populations with the favorable alleles originating from KS4895 in both populations.

On Gm19, there was one QTL cluster located at about 77 cM with QTLs originating from the 08K×J, K×PI, and B×PI populations. The favorable alleles for this cluster were from Jackson, PI 424140, and PI 416937.

Discussion

Confirmation of QTLs for canopy wilting

The genetic maps for the five populations we evaluated had average map distances between adjacent markers ranging from 3.8 to 7.9 cM (Table 1). Xu et al. (2005) reported that marker density less than 10 cM between flanking markers containing QTLs greatly improved QTL detection power and precision of CIs. Most QTLs were identified within dense flanking marker intervals; the exceptions to this were QTLs on Gm05 and Gm06, which were near Satt681 and BARC-04481-08709 in the 93K×J population (Table 3).

Previous research by Charlson et al. (2009) and Abdel-Haleem et al. (2012) identified QTLs for slow wilting in the 93K×J and B×PI populations, respectively. The 08K×J population was created to serve as a confirmation population of the 93J×K population. Using CIM, Charlson et al. (2009) reported QTLs for slow wilting on Gm08, Gm13, Gm14, and Gm17, and of these QTLs, only the one on Gm17 was confirmed in the 08K×J population. However, QTLs reported by Charlson et al. (2009) on Gm08, Gm14, and Gm17 were identified in QTL clusters with QTLs from other populations. Abdel-Haleem et al. (2012) reported seven QTLs using MIM from the B×PI population (when averaged over environments) on Gm02, Gm04, Gm05, Gm12, Gm14, Gm17, and Gm19. We found that the QTLs on Gm02 and Gm05 identified by Abdel-Haleem et al. (2012) had overlapping CIs with QTL clusters that we identified from other populations.

To identify potential false-positive QTLs, we performed a simulation study using the qtl Design library in *R* (data not shown; Broman et al. 2003; Sen et al. 2007). This analysis predicts threshold R^2 values and minimum QTL effects that can be used as a criterion to identify false-positive QTLs. We evaluated the nine QTL clusters that originated from at least two independent populations plus the QTL cluster on Gm08 that was identified in the 93K×J population and by Charlson et al. (2009) and Du et al. (2009). Of the 10 QTL clusters, nine had similar additive effects and R^2 values with those from the simulation. An exception to this was for three QTLs in a QTL cluster on Gm14 from the 93K×J and B×PI populations that had lower R^2 values and QTL effects than the threshold values from the simulation. Lander and Kruglak (1995) determined that false-positive QTLs were more likely to increase as the number of genome scans increased (due to marker density and walk speed) although stringent threshold values were used. Therefore, the three QTLs in a cluster on Gm14 could be false positives even though these QTLs were identified using high LOD thresholds (LOD $\geq$ 3.5).

The identification of QTLs with overlapping CIs from at least two populations in different years gave us confidence that QTLs in nine QTL clusters were true QTLs. However, we were unable to determine the common nearest markers in these clusters because of differences in polymorphic markers for each population due to the diverse level of linkage disequilibrium (LD) in parents (Lande and Thompson 1990). Moreover, only SSRs were genotyped in previous mapping studies (Charlson et al. 2009; Du et al. 2009; Abdel-Haleem et al. 2012). From the perspective of MAS, although the nearest markers for QTLs were located close to these nine QTL clusters, it would be difficult to decide which markers could be used for MAS. Additionally, selecting a marker to use for MAS from different genetic backgrounds may be hindered due to epistasis or recombination (Reyna and Sneller 2001). Before use in MAS, it would be necessary to collect additional data about these QTLs in other environments. Finding the same nearest markers for QTLs from different populations through fine mapping would be helpful for validation. Another way for improving QTL resolution for MAS is by meta-analysis, which may identify consensus QTLs by narrowing down CIs of original mapping population studies.

Candidate traits related to canopy wilting

Ries et al. (2012) evaluated five fast-wilting and five slow-wilting genotypes under well-watered conditions for physiological mechanisms that might be associated with delayed canopy wilting, including carbon isotope discrimination as a measure of WUE, stomatal conductance, radiation use efficiency (RUE), and canopy temperature depression. In

controlled environments, transpiration of some delayed-wilting genotypes plateaus as vapor pressure deficit (VPD) increases to a VPD of about 2 kPa whereas transpiration of fast-wilting genotypes increases linearly as VPD increases (Fletcher et al. 2007). Further experimentation showed that the aquaporin inhibitor silver nitrate resulted in decreased transpiration of fast-wilting soybean genotypes but had no effect on the delayed-wilting genotype PI 416937 or three progeny lines derived from PI 416937 (Sadok and Sinclair 2010). The authors concluded that PI 416937 had a different population of aquaporins than fast-wilting genotypes, which resulted in a hydraulic restriction at high VPD values. These conclusions are consistent with the finding of Ries et al. (2012) that RUE of PI 416937 and several other delayed-wilting genotypes is generally less than fast-wilting genotypes.

Aquaporin gene families are found on all 20 chromosomes of soybean (www.soybase.org/). One aquaporin gene on Gm14 was linked to Satt126 (Yamanaka et al. 2001), which was one of the markers in a delayed-wilting QTL cluster on this chromosome. Carpentieri-Pipolo et al. (2011) mapped the transpiration response to the aquaporin inhibitor silver nitrate in the BxPI population that had also been mapped for delayed wilting (Abdel-Haleem et al. 2012). They found four QTLs conditioning differential transpiration response to silver nitrate. One of these four QTLs was localized at the QTL cluster for delayed wilting near the top of Gm05 (~6 cM, Fig. 1). Deep rooting ability could be a candidate trait for slow wilting (Ries et al. 2012; Hufstetler et al. 2007). Although a deep-rooting, slow-wilting genotype has not been characterized, PI 416937 does have a dense fibrous root system near the soil surface (Hudak and Patterson 1995). Additionally, the BxPI population has been mapped for the fibrous-rooting trait (Abdel-Haleem et al. 2011), but none of the fibrous-rooting QTLs were coincident with QTL clusters for slow wilting.

As mentioned previously, canopy wilting was more severe in early maturing lines from the AxPI population in 2010 ($r = -0.38$, $P < 0.001$), which provides evidence that wilting severity likely increases as maturity approaches. Although all the populations except the AxPI were selected for a narrow range of maturity, maturity still ranged from 5 to 10 days, and in the AxPI population maturity varied up to 20 days. Maturity QTLs previously identified on Gm11 (Gai et al. 2007; Zhang et al. 2004) and Gm19 (Specht et al. 2001) fell within the CIs of the QTLs clusters we identified for delayed wilting. It is noteworthy that the only population not having QTLs in either of these two QTL clusters was the 93K×J population, which also had the most narrow maturity range (~5 days). The *E3* gene, which has a major effect on flowering time and maturity (Molnar et al. 2003), is located within the CI of the QTL cluster on Gm19. Also, within the CI on Gm19 is the *Dt1* gene, which controls determinancy. It is likely, therefore, that maturity had a pleiotropic effect on wilting at these locations.

Conclusions

We identified QTLs and corresponding significant molecular markers for canopy wilting from five mapping populations. Additionally, epistasis among some QTLs was evident. Ten QTL clusters were found on Gm02, Gm05, Gm08, Gm11, Gm14, Gm17, and Gm19 based on the overlapping of 95 % CIs from at least two mapping populations including a QTL for slow wilting identified by Du et al. (2009). The results showed that QTLs in nine QTL clusters on Gm02, Gm05, Gm08, Gm11, Gm17, and Gm19 were likely true QTLs, but QTLs in a QTL cluster on Gm14 could be false positives (summarized in Table 8). These results open up the possibility for fine mapping that can then be applied to MAS. Further research, including expression QTL (eQTL) analysis, will be required to understand how genes for canopy wilting can interact with other genes forming a genetic architecture.

Author contribution statement SH designed the experiment and performed the statistical analysis. CAK, TEC, HAH, WS, and LCP conducted field experiments and collected phenotypic data. PC, CAK, HAH, ZL, KWM, and LCP developed the populations. JDR, PBC, ZL, and KWM collected the genotypic data. SH co-wrote the manuscript with LCP. JDR, TEC, PC, ZL, and KWM critically revised the manuscript. LCP coordinated and supervised the project. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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