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Identification of QTL for increased fibrous roots in soybean

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Abstract Drought stress adversely affects soybean at various developmental stages, which collectively results in yield reduction. Unpredictable rainfall has been reported to contribute about 36% to variation of yield difference between the rain-fed and irrigated fields. Among the drought resistance mechanisms, drought avoidance in genotypes with fibrous roots was recognized to be associated with drought resistance in soybean. Plant introduction PI416937 was shown to possess fibrous roots and has been used as a parent in breeding programs to improve soybean productivity. Little information is available on relative contribution and chromosomal location of quantitative trait loci (QTL) conditioning fibrous roots in soybean. To identify the genomic locations and genetic bases of this trait, a recombinant inbred line population was derived from a cross between PI416937 and 'Benning'. To detect associated QTLs, phenotypic data were collected and analyzed for 2 years under rain-fed field conditions. The selective genotyping approach was used to reduce the costs and work associated with conducting the QTL analysis. A total of five QTLs were identified on chromosomes Gm01 (Satt383), Gm03 (Satt339), Gm04 (Sct_191), Gm08 (Satt429), and Gm20 (Sat_299), and together explained 51% of the variation in root score. Detected QTLs were co-localized with QTLs related to root morphology, suggesting that fibrous roots QTL may be associated with other morpho-physiological traits and seed yield in soybean. Genetic dissection of the fibrous roots trait at the individual marker loci will allow for marker-assisted selection to develop soybean genotypes with enhanced levels of fibrous roots.

Introduction

Higher plants have evolved multiple, interconnected strategies that enable them to survive abiotic stresses. However, these strategies are not well understood in most crops. Among these, drought is the major abiotic stress that generally reduces yield to less than half of that possible under ideal growing conditions (Boyer 1982). Drought affects soybean seed yield to some degree at almost all growth stages. When the plant suffers a water deficit, its turgor pressure approaches zero, cells begin to collapse, membranes suffer damage, proteins and enzymes are denatured and their structure is altered, and finally the cells die. Under water deficit conditions, a soybean plant reduces its metabolism to survive the stress and then resumes its activities when the stress is removed, which affects the seed yield as a final plant product (Korte et al. 1983; Dornbos and Mullen 1992). Inadequate and unpredictable rainfall has been reported to contribute about a 36% yield difference between the rain-fed and irrigated soybean fields (Specht et al. 1999).

The mechanisms of drought resistance in plants can be grouped into three categories as drought escape, drought

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avoidance, and drought tolerance (Carrow 1996). The escape mechanism maximizes the plant's ability to complete its life cycle before a serious deficit occurs. This mechanism involves rapid physiological development characters such as early flowering and early maturity, and remobilization of pre-anthesis assimilates to seed. The avoidance mechanism is characterized by the ability to maintain relatively high tissue water potential, despite a shortage of soil moisture. Plants that utilize the avoidance mechanism survive by balancing the maintenance of turgor and the reduction of water loss, through increased rooting depth, an efficient root system, and increased hydraulic conductance. This results in increased water availability or reduced water loss through reduced stomatal conductance, reduced absorption of radiation by leaf rolling or folding, and reduced evaporation surface. Drought tolerance is the ability of plants to withstand water-deficit with low tissue water potential. The mechanisms of drought tolerance include maintenance of turgor through osmotic adjustment, increased plasticity in the cell, and decreased cell size and desiccation tolerance by protoplasmic resistance. Drought resistance is a complex trait, and plants may employ more than one mechanism at a time to resist drought. Resistant plants could contain a combination of different characters including morphological, physiological, and biochemical characters to reduce plant damage (Jones et al. 1981).

Secondary traits evaluated to assess drought tolerance include morphological and/or physiological traits such as yield components, leaf area, leaf wilting, flowering time, anthesis period, and root morphology (Richards 1996). A secondary trait should be genetically associated with seed yield in the target environment, highly heritable, genetically variable, inexpensive and rapid to measure, and observed at or before flowering; (Monneveux and Ribaut 2006). Canopy wilting (slow wilting) has been used as secondary trait to predict drought resistance in soybean (Charlson et al. 2009).

Several studies indicated that deeper rooting might be the underlying mechanism of drought resistance for the tolerant soybean genotypes, which is positively related to soybean yield under drought stress (Brown et al. 1985; Cortes and Sinclair 1986; Hudak and Patterson 1995). The root system is particularly important in response to drought stress since plant exposure to limited soil water occurs in the root zone first (Hoogenboom et al. 1987). Root plasticity is another trait associated with drought resistance. A deep and thick root system provided access to water deep in the soil profile in upland rice (Oryza sativa) (Fukai and Cooper 1995). On the other hand, a fibrous root system has been reported to be more efficient for water absorption in soybean because of the increase in surface area and the number of root tips (Hudak and Patterson 1995; Pantalone et al. 1996a).

Genetic diversity in soybean root traits has been reported for root elongation, root nodule formation, fibrous root, and root mass in response to various abiotic/biotic stresses such as aluminum toxicity (Bianchi-Hall et al. 1998; Villagarcia et al. 2001), flooding (Bacanamwo and Purcell 1999), soybean cyst nematode (Heterodera glycines) (Concibido et al. 1994; Miltner et al. 1991), iron deficiency (Charlson et al. 2005; Lin et al. 1997; Wang et al. 2008), manganese deficiency (Kassem et al. 2004), and phosphorus deficiency (Li et al. 2005; Zhang et al. 2009a, b). Exotic soybean accessions have been found to possess greater drought tolerance than elite cultivars and to have unique root characteristics when evaluated in drought stress environments (Hudak and Patterson 1995). In the field studies under moisture deficit field conditions PI416937 possessed a slower wilting canopy than an elite cultivar and its leaves possessed lower solute potential, higher turgor, and higher relative water content which resulted in a relatively small yield reduction when compared to the elite cultivar (King et al. 2009; Sloane et al. 1990). The fibrous root morphology of PI416937 was reported to have a positive association with seed protein concentration and was shown to be a heritable trait indicating its potential use in breeding programs for enhancing seed yield under drought conditions (Pantalone et al. 1996a).

Screening soybean for root traits in breeding populations is tedious and expensive due to difficulties of measuring root characteristics under field conditions. This has resulted in relatively limited genetic studies of soybean root morphology under field conditions. The application of QTL analysis allows the identification of chromosomal regions conditioning the phenotypic variation in quantitative traits such as root morphology and identifies the desirable alleles at these QTLs for use in marker assisted selection (MAS). Previous QTL mapping of drought related traits in soybean includes yield under drought stress conditions (Du et al. 2009b; Specht et al. 1999), slow canopy wilting (Charlson et al. 2009), water use efficiency (WUE; Mian et al. 1996, 1998; Specht et al. 2001), iron deficiency (Charlson et al. 2005; Lin et al. 1997; Wang et al. 2008), manganese deficiency (Kassem et al. 2004), phosphorus deficiency (Li et al. 2005; Zhang et al. 2009a, b), aluminum tolerance (Bianchi-Hall et al. 2000), and salt tolerance (Lee et al. 2004). Molecular markers can be also used to obtain genetic information about root traits, including location of the loci, inheritance of positive alleles, the components of genetic variance, pleiotropic effects of the loci, and epistasis. Following this strategy, high-throughput screening methods have been used in rice and maize (Price and Tomos 1997; Zhang et al. 2001; Tuberosa et al. 2002). No studies have been published on fibrous roots QTL in soybean even though the trait holds potential for yield

improvement in rain-fed environments. The objectives of this study were to identify QTL(s) associated with the fibrous root trait in a soybean recombinant inbred line (RIL) population developed from a cross of 'Benning' (few fibrous roots) and PI416937 (many fibrous roots) and to elucidate genetic characteristics of the QTL(s) to facilitate their use in MAS.

Materials and methods

Plant materials and phenotypic evaluation

A cross between cultivar Benning (PI595645) and PI416937 was made during the summer of 1996 at the University of Georgia Plant Sciences Farm near Watkinsville, GA. PI416937 is a Japanese landrace that phenotypically appears distinctly different from the ancestors of North American soybean cultivars. When compared with elite US cultivars it has a slower wilting canopy and a more fibrous root system (Pantalone et al. 1996a, b; King et al. 2009). 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 during the winter of 1996–1997. The F_2–F_5 generations were advanced by the single seed decent (SSD) method in Georgia and Puerto Rico. The F_5 plants were harvested individually, and the F_5-derived lines were grown as plant rows at the University of Georgia Plant Sciences Farm during the summer of 1999. At harvest, one plant per row was selected to represent each family depending on uniform maturity and non-shattering. In 2000, a total of 240 F_6 plants were grown as individual plant rows. At maturity each row was individually harvested.

In 2001 the 240 F_6-derived RILs were planted in hill plots thinned to six plants per hill in a randomized complete block experimental design with nine replications at the University of Georgia Plant Sciences Farm (an Appling coarse sandy loam soil) under rain-fed conditions. Prior to planting 107 kg ha^{-1} P_2O_5 was added to the soil. Nematodes, insects, and weeds were controlled using commercial chemicals as needed. At the R4 growth stage (Fehr et al. 1971), plants of each hill were tied into a bundle and the roots were removed from the soil with a mechanical peanut inverter. Roots of the six plants in each hill were visually rated according to Pantalone et al. (1996a) on scale of 1–8; where 1 is when the roots are course and contain very few fibrous roots and 8 is when the hill contains mostly fibrous roots (Fig. 1).

In 2009, 128 of the original 240 RILs were selected based on to their high or low fibrous roots score in 2001. The 128 RILs and parents Benning and PI416937 each repeated three times were planted in hill plots thinned to six plants per hill at the University of Georgia Plant Sciences Farm, (in a Cecil coarse sand loam soil) in a randomized complete block design experimental design with eight replications. Prior to planting 94 kg ha^{-1} P_2O_5 and 46 kg ha^{-1} K_2O were applied to the soil. Nematodes, insects and weeds were controlled using commercial chemicals as needed. Root harvesting and scoring followed the same methods used in 2001 experiment.

Statistical analyses

The data from the 2001 and 2009 experiments were analyzed using SAS PROC GLM as randomized complete block experimental designs with replicates and RILs considered as random effects (Statistical Analysis System, SAS institute 2001). Analysis of the data across years was conducted by SAS PROC MIXED using restricted maximum likelihood (REML) method as randomized complete block experimental design with replicates, years, and RILs considered as random effects (Steel and Torrie 1980).

Heritabilities on an entry-mean basis was calculated as $h^2 = (\sigma^2_{RIL}/(\sigma^2_{RIL} + \sigma^2_e/r)$ for 2001 and $h^2 = \sigma^2_G/[(\sigma^2_{RIL} + (\sigma^2_{RIL*Y}/y) + (\sigma^2_e/y)]$ for the combined 2001 and 2009 data (Nyquist 1991; Holland et al. 2003); where σ^2_{RIL} equaled the genetic variance among the RILs, σ^2_{RIL*Y} represented the variance of RILs by year interaction, σ^2_e the variance of experimental error, y the number of years, and r the number of replications. Because the 2001 and 2009 data were unbalanced, the value for r was computed as the harmonic mean of the 2001 and 2009 number of replications (Holland et al. 2003).
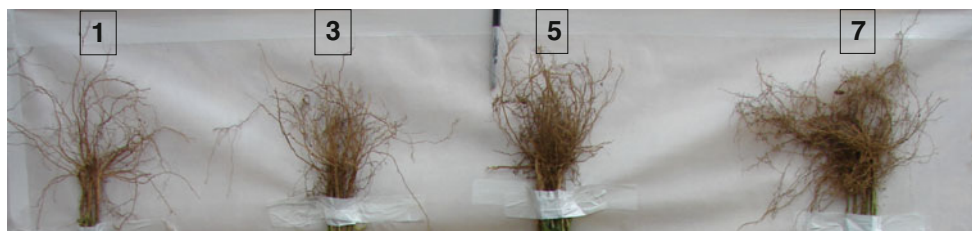
Fig. 1 The examples of roots in various root score categories; where 1 = few fibrous roots and 8 = many fibrous roots

DNA markers, map construction and QTL analysis

DNA from each of the F_6-derived lines and the two parents was extracted from the first trifoliolate leaves of greenhouse-grown plants using a CTAB (Hexadecyltrimethylammonium acid) procedure modified from Keim et al. (1988). PCR reactions were based on the protocol of Diwan and Cregan (1997) with slight modifications. The reactions were performed in a dual 384-well or a 96-well GeneAmp PCR System 9700 (PE-ABI, Foster City, CA). Data were collected on 276 SSR markers, that were polymorphic between the parents. The primers were labeled with fluorescent dyes. A loading sample for each lane of a gel was prepared with loading mixture (2.0 µl of deionized formamide, 2.0 µl of loading buffer, 0.3 µl of Genescan Rox-500) and 1–2 µl of the PCR products. DNA was denatured at 95°C for 5 min, prior to separation on a 4.8% (w/v) polyacrylamide gel with 1× TAE (Tris acetate EDTA) running buffer using ABI PRISM 377 DNA Sequencer (PE-ABI, Foster City, CA). The marker fragments were analyzed with GeneScan and scored with Genotyper software (PE-ABI, Foster City, CA).

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 using the Kosambi's mapping function.

Significant associations of the markers with the fibrous root trait were tested using single-factor analysis of variance (SF-ANOVA) of SAS (SAS Institute 2001) based on RIL means for each year separately and least square means across years. QTL analysis was performed using QTL Cartographer 2.5 (Wang et al. 2007) using genotypic 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 QTLs and QTL effects including additive and epistatic effects by simultaneous analysis of the QTLs in multiple regression models (Zeng et al. 1999) with the following steps: (1) the initial model included representatives of significant markers that associated with the variation in fibrous roots using SF-ANOVA. To select representatives, the significant markers were sorted by chromosomes and position within each chromosome, then one marker with largest phenotypic variation from each significant marker cluster was chosen as representative of this chromosomal region, (2) the pre-selected MIM model was optimized using the "optimize QTL positions" option,

then (3) 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; (4) model was re-evaluated to fit all the significant main and epistatic QTLs in MIM model using "test existing QTLs" option, (5) 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 (BIC-M0 = 185.1993), the 5 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

SSR linkage map of Benning × PI416937

A total of 629 SSR markers were screened for polymorphism between Benning and PI416937, and 281 markers (44.6%) were polymorphic. A linkage map was constructed with 276 informative markers (Supplemental Fig. 1). The linkage maps consisted of 20 linkage groups which corresponds to soybean's 20 chromosomes and collectively covered 2169 cM or 94% of the 2276 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 × PI416937 linkage maps (Supplemental Table 1). Among the 20 linkage groups, chromosome Gm13 included the most linked markers with 20 while chromosome Gm15 had the fewest with nine linked markers. In general, the SSR marker order and distance between adjacent markers were similar to those on the soybean genetic linkage consensus map of Song et al. (2004) and SoyBase (http://soybase.org/; Supplemental Table 1).

Fibrous roots variation of the parents and RILs

In 2001 PI416937 averaged significantly ($P < 0.05$) higher fibrous roots score (7.2 vs. 4.5) than Benning (Table 1). The root scores of the 240 RILs ranged from 3.4 to 7.2. The scores for the 240 RILs were normally distributed and showed transgressive segregation with some of the RILs producing less fibrous roots than Benning (Fig. 2, Table 1). None of the RILs produced more fibrous roots than PI416937. The entry-mean heritability for selection based on means of nine replications was 0.74.

A selective genotyping approach was employed to identify the RILs for inclusion in the 2009 experiment. Based on the 2001 mean root scores, 64 RILs were selected for their low root scores and 64 RILs were selected for their

Table 1 Mean fibrous root scores of the parents and the 240 RILs in 2001 and the 128 selected RIL in 2009 and across years

Genotype	2001 Score	2009 Score	Combined Score
Benning	4.5	3.6	3.8
PI416937	7.2	5.6	6.4
Mean of RILs	5.5	4.5	5.0
Range of RILs	3.4–7.2	3.4–5.6	3.7–6.2
LSD (0.05)	1.0	2.0	2.0
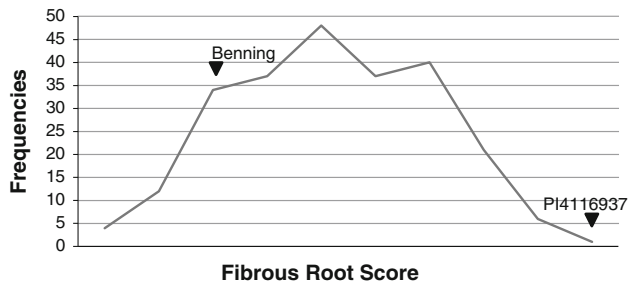
Fig. 2 Phenotypic frequencies of fibrous root score in 240 RILs of Benning × PI416937 population in 2001

high root scores. In 2009 both parents averaged few fibrous roots than in 2001 (Table 1). PI416937 had an average root score of 5.6 compared to a root score of 3.6 for Benning. The range of root scores (3.4–5.6) among the 128 RILs was similar to the two parents and none of the RILs expressed transgressive segregation.

The combined analysis of variance of root score over years showed significant ($P < 0.01$) effects for year, genotype, and the genotype by year interaction. The 2001/2009 root score means for the 128 RILs were distributed in a bimodal fashion (data not shown). This bimodal distribution was expected based on the selective genotyping

approach used in 2009. When the genotype by year mean squares was used as the error term (random-effect model), there were significant ($P < 0.05$) differences for the 2-year means among the 128 RILs and between the parents, Benning and PI416937. Benning averaged a root score of 3.8 compared to a score of 6.4 for PI416937. The range in root scores among the 128 RILs (3.6–6.2) was similar to the range between their parents. The RILs did not show transgressive segregation for root score. The entry-mean heritability estimate (based on selection of the 2-year means) was 0.37 (data not shown).

QTL analysis

Single-factor analysis of variance (SF-ANOVA) was employed to putatively identify QTL conditioning the fibrous roots trait for each year and across years. If a marker was significantly ($P < 0.01$) associated with root scores for the combined data across years, it was then tested for significance ($P < 0.05$) in 2001 and 2009. The rationale for using the less stringent probability level for the single-year analysis was to test the consistency of QTL detection across years. Nine SSR markers on nine soybean chromosomes identified putative QTLs associated with root scores based on 2-year means (Table 2). Of these nine putative QTLs, three (Satt429 on Gm08, Satt383 on Gm01, and Satt339 on Gm03) were identified in both the 2001 and 2009 experiments. Five putative QTLs (Sct_191 on Gm04, Sat_227 on Gm02, Satt568 on Gm12, Sat_229 on Gm20, and Satt196 on Gm09) were significant ($P < 0.05$) only in the 2001 environment when 240 RILs were evaluated. Satt383 and Sat_345, which are located ~11 cM apart on Gm01, are likely associated with the same fibrous roots QTL. The remaining QTL on Gm19 (Sat_071) was the only putative QTL identified based on the 2-year data and not found significant ($P > 0.05$) in either the 2001 or 2009

Table 2 SSR markers associated with the enhanced fibrous root trait based on single-factor analysis of variance (SF-ANOVA) for the Benning × PI416937 RIL population in 2001, 2009 and across years

Chromosome	Maker	2001/2009		2001		2009	
		R^2	a	R^2	a	R^2	a
Gm01	Satt383	9.5****	−0.35	5.8**	−0.453	7.6***	−0.248
Gm02	Sat_227	7.9***	−0.316	7.0**	−0.496	–	–
Gm03	Satt339	10.2****	−0.368	7.7**	−0.525	5.6**	−0.212
Gm04	Sct_191	6.7**	−0.312	12.6****	−0.702	–	–
Gm08	Satt429	13.5****	−0.435	9.4***	−0.6	8.5***	−0.269
Gm09	Satt196	6.1**	−0.276	5.5**	−0.431	–	–
Gm12	Satt568	7.6***	−0.316	4.7*	−0.408	–	–
Gm19	Sat_071	5.3**	0.26	–	–	–	–
Gm20	Sat_299	76.0**	0.298	9.6***	0.585	–	–

*, **, ***, **** Significance levels at 0.05, 0.01, 0.001, 0.0001, respectively

environment. These putative fibrous roots QTLs explained from 5.3 to 13.5% of the phenotypic variation in 2001/2009 root scores (Table 2). The PI416937 alleles increased fibrous rooting at all the putative QTLs with the exception of the QTL identified by Sat_299 on Gm20 (Table 3).

The SSR marker and root data were then analyzed by the MIM model of QTL-Cartographer. The MIM method utilizes multiple marker intervals simultaneously to identify QTLs. This method fits all QTLs into the model and has the capacity for the evaluation of QTL epistasis. Based on the RIL least square means across environments, the MIM model identified five QTLs at LOD ≥ 2.5; *Q_root_Gm08* (flanked with Satt228 and Satt429 on Gm08), *Q_root_Gm04* (flanked with Satt713 and Sct_191 on Gm04), *Q_root_Gm01* (flanked with Satt179 and Satt383 on Gm01), *Q_root_Gm20* (flanked with Sat_420 and Sat_299 on Gm20), and *Q_root_Gm03* (flanked with Satt339 and Sat_091 on Gm03). The LOD scores of the detected QTLs ranged from 2.62 to 5.71 (Table 3, Fig. 3). The phenotypic variation for the fibrous root trait explained by each of these QTLs ranged from 7.3% for *Q_root_Gm04* to 13.5% for *Q_root_Gm03*, and the total phenotypic variation explained by model was 51.1%. At four of these QTLs (*Q_root_Gm08*, *Q_root_Gm04*, *Q_root_Gm01* and *Q_root_Gm03*) the PI416937 alleles increased the root scores, while at *Q_root_Gm20* the Benning allele increased the root score (Table 3).

In order to gain insight into the potential effect the five fibrous roots QTLs may have on other soybean traits, we conducted a comparative QTL analysis. The genomic locations of the five fibrous roots QTLs were aligned on the soybean linkage/QTL maps in Soybase (http://soybase.org/). The soybean linkage/QTL maps of chromosomes Gm01, Gm03, Gm04, Gm08, and Gm20 were aligned with the fibrous root QTL intervals by use of the SSR markers

defining the genomic locations of the fibrous root QTL (Table 3). For this analysis we arbitrarily included QTLs conditioning other traits if these trait QTLs were mapped within a bin that included the fibrous root QTL interval ±5 cM beyond the two markers that defined the fibrous root QTL boundary. A total of 35 QTLs[e] were reported for traits related to abiotic stresses, plant morphology and development, seed composition, and nitrogen accumulation (Fig. 4). In addition, there were three QTLs for seed yield near *Q_root_Gm01* and *Q_root_Gm03*, and two QTLs for seed weight near to *Q_root_Gm01* and *Q_root_Gm20*.

On Gm08 near the fibrous roots QTL, *Q_root_Gm08*, there were two QTLs for length of reproductive period (R3_1-3 and R7_1-7), one for maturity (Pod_mat_13-1), and one for leaf water loss (LWL). Data from an unpublished study with this RIL population indicated that this region of the chromosome Gm08 did not contain QTL conditioning maturity. The LWL QTL was reported to map in this region by Du et al. (2009b) in a different population.

The fibrous root QTL *Q_root_Gm04* mapped to the same region as QTLs for maturity, seed protein and oil content, plant height, seed abortion (Tischner et al. 2003), and water use efficiency. The plant height (Pl_ht 5-4), maturity (Pod_mat_6-6), water-use efficiency (WUE_2-3), and protein (Prot_4-3) QTLs were originally mapped in a 'Young' × PI416937 F4-derived population (Mian et al. 1996, 1998). The PI416937 alleles at these QTLs resulted in later maturity, more efficient water use, higher protein content, and taller plants than the Young alleles. The WUE_2-2 QTL was mapped in an S100 × Tokyo population and mapped to the same genomic location as the WUE_2-3 QTL.

The trait QTLs associated with the *Q_root_Gm01* fibrous root QTL were NitR7_1-2 which conditions

Table 3 QTLs for the enhanced fibrous root trait identified by multiple interval mapping for Benning × PI416937 RIL population based on the combined 2001/2009 data

Chromosome	QTL name	Marker interval[a]	QTL position[b]	LOD score[c]	Additive effect[d] (*a*)	R^2(%)[e]	Favorable alleles[f]
Gm01	*Q_root_Gm01*	**Satt383**-Satt580	50.5	2.6	−0.14	7.5	PI416937
Gm03	*Q_root_Gm03*	**Satt339**-Sat_091	57.3	5.3	−0.23	13.5	PI416937
Gm04	*Q_root_Gm04*	Satt713-**Sct_191**	77.2	3.6	−0.16	7.3	PI416937
Gm08	*Q_root_Gm08*	Satt228-**Satt429**	149.6	5.7	−0.23	12.8	PI416937
Gm20	*Q_root_Gm20*	Sat_420-**Sat_299**	77.8	3.3	0.17	7.6	Benning

[a] Flanking markers interval with the nearest marker in bold

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

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

[d] Additive effects were calculated following multiple intervals mapping (MIM) by simultaneous analysis of the QTLs in multiple regression models

[e] Proportion of phenotypic variance explained by specific QTL

[f] Favorable alleles were assigned according to the fibrous root, where fibrous roots is preferred over the tap roots

Fig. 3 Interval mapping fibrous root score QTLs in the Benning × PI416937 RIL population based on the combined 2001/2009 data. QTL nomenclature is in the form of Q-trait-chromosome-position. Length of QTL bar indicates the LOD-1 confidence interval and QTL line is extended to LOD-2 confidence interval drops from the maximum likelihood value
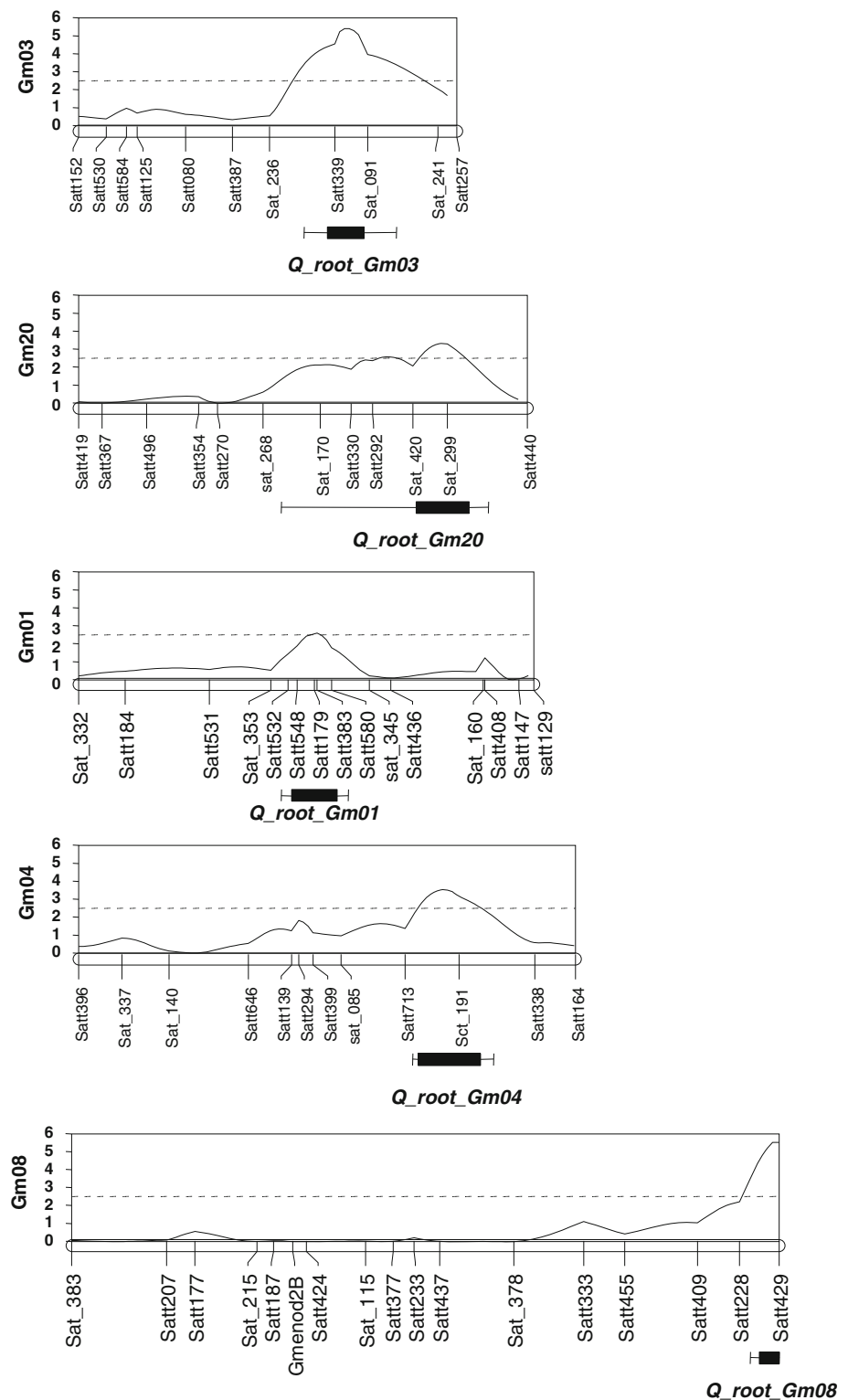
nitrogen accumulation during the R7 stage of development, oil content (Oil_13-2), plant height (Pl_ht_17-10), and resistance to race 2 of SCN nematode (SCN_race2). In the interval of the *Q_root_Gm20* QTL, the Cnpy_wdth_1-3 QTL was found which conditions canopy width. A total of two drought tolerance QTLs were found outside of the interval of *Q_root_Gm20*, one for leaf water loss (LWL) (at 15 cM away) and wilt coefficient (wilt Coe) (22 cM away). Located near the *Q_root_Gm03* fibrous root QTL were four iron efficiency uptake QTLs, a salt tolerance QTL, two protein-related QTLs (Beta_conglycinin_1-1 and Prot_21-9), and a plant height QTL.
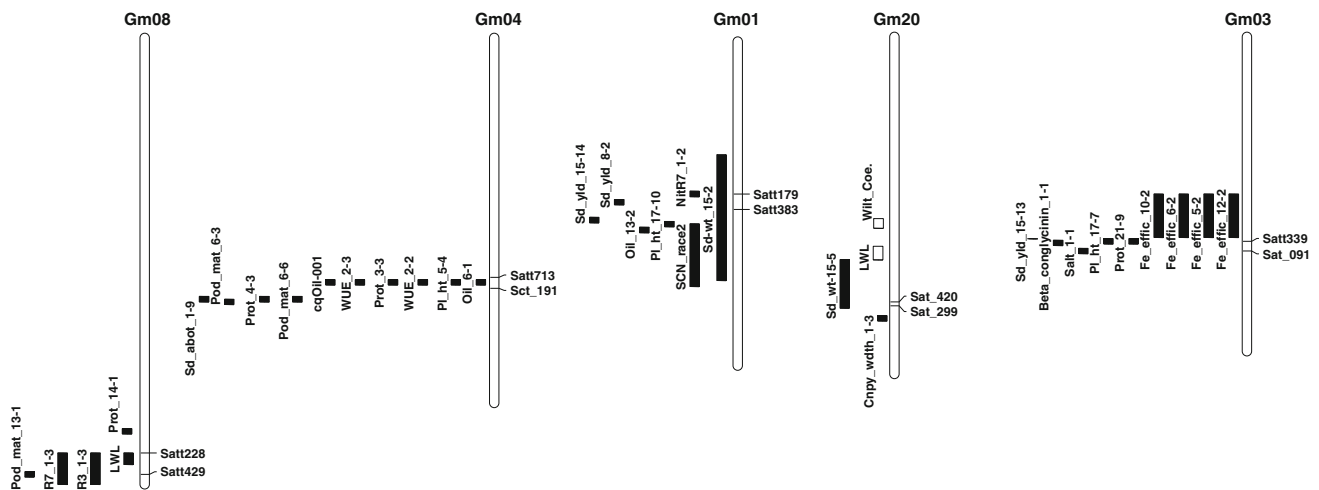
Fig. 4 Comparative QTL analysis of the fibrous root QTLs detected in the Benning × PI416937 population with coinciding QTLs found on Soybase. These QTLs were identified by alignment of both maps with common SSR markers (http://soybase.org/)

Discussion

Drought affects soybean growth and seed yield to some degree at almost all growth stages. Balancing water loss by modifying soybean root morphology is one of the drought avoidance mechanisms. Screening plant root morphology in large breeding populations is tedious and expensive due to difficulties of measuring root characteristics in the field and the relatively low heritability of the trait. Pantalone et al. (1996b) reported an entry-mean heritability for fibrous roots score of 0.39 (selection based on means across 2 years and 2 locations). In the present study we found a similar entry-mean heritability of 0.37 based on selection of 2-year means (data not shown), but this heritability estimate must be considered somewhat biased based on non-random selection of RILs for inclusion in the 2009 experiment by selective genotyping. The QTL analysis of this population identified five fibrous root QTLs, *Q_root_Gm08*, *Q_root_Gm04*, *Q_root_Gm01*, *Q_root_ Gm20,* and *Q_root_Gm03*. These QTLs can be employed in a MAS program and which should overcome many of the previous limitations in the selection of soybean genotypes with modified root morphology.

These five QTLs were found to explain from 7 to 15% of the phenotypic variation in fibrous roots scores. None of these QTLs is considered a major fibrous root QTL. This was unexpected considering the unique fibrous roots system of PI416937 when compared to other soybean genotypes. We are unaware of other soybean genotypes with PI416937's level of fibrous roots or even genotypes with intermediate levels of fibrous roots. This may be due to their lack of existence or due to the limited sampling of soybean germplasm for root morphology. The identification of four fibrous root QTLs where the PI416937 alleles

conditioned greater levels of fibrous roots and one QTL where Benning contained the allele for more fibrous roots provides breeders the opportunity to develop soybean lines with intermediate levels of fibrous roots and to evaluate the agronomic value of these modified rooting patterns.

To minimize the population size and maximize genetic information gained from this study, a selective genotyping (SG) method was used in 2009 to select RILs from the tails of whole population (Lander and Botstein 1989). Selective genotyping has been used to study single traits related to biotic/abiotic stresses in several plant species including stripe rust in barley (Vales et al. 2005), salt tolerance in tomato (Foolad et al. 2001), *sclerotinia* midstalk rot resistance in sunflower (Micic et al. 2005), phosphorus deficiency in rice (Ni et al. 1998), and drought in maize (Hao et al. 2009) and rice (Subashri et al. 2009). The present findings indicate that SG can be used in identifying QTL associated with fibrous root traits as well as other traits where population size is one of the limiting factors.

QTLs associated with soybean seed yield under well-watered conditions were reported on Gm01 (Kabelka et al. 2004; Orf et al. 1999) and Gm03 (Kabelka et al. 2004). Measuring the fibrous root trait is destructive and yield could not be conducted in the same experiment. Tuberosa et al. (2002) noticed overlap between weight of the adventitious seminal roots in hydroponics with QTLs for grain yield under both well-watered and water-stressed conditions. Co-localization of QTLs for a specific trait and yield under drought stress could lead to improved yield via improving root traits (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 turn the seed weight and yield (Liu et al. 2003). Liu

et al. (2004) suggested that soybean root signals control pod growth under drought conditions.

Soybean protein and oil concentrations are affected by environmental stresses (Bellaloui and Mengistu 2008; Carrera et al. 2009; Dornbos and Mullen 1992; Specht et al. 2001). Nitrogen fixation activity has also been shown to be highly sensitive to moisture deficits, where drought depressed its activity resulting in reduced N_2 accumulation rate, protein content, and plant growth (King and Purcell, 2001; Streeter 2003; Sinclair et al. 2007). QTLs for oil content and root score in the same genomic regions were reported on, Gm04 (Lg-C1) (Fasoula et al. 2004; Lee et al. 1996) and Gm01 (Lg-D1a) (Hyten et al. 2004). Seed protein QTLs were located in the same genomic regions as fibrous root QTL on Gm08 (Vollmann et al. 2002), Gm04 (Brummer et al. 1997; Lee et al. 1996), and Gm03 (Kabelka et al. 2004). A QTL for β-conglycinin (β-subunit; 48 kDa) was reported on chromosome Gm03 (Panthee et al. 2004a). Samarah et al. (2006) indicated that severe drought during seed development delayed the appearance of both the basic unit of glycinin and β-subunit of β-conglycinin. It is known that environmental stresses, including drought, modifies protein expression, where some proteins are increased (expressed) and others are decreased (suppressed). A previously detected QTL for nitrogen accumulation on Gm01 at reproductive stages R7 (Panthee et al. 2004b) coincided with the genomic locations of the root QTL *Q_root_Gm01* suggesting that the fibrous root system of PI416937 could increase root surface area resulting in greater nodule numbers and that could enhance the N_2 fixation (Pantalone et al. 1999).

Chromosomal locations harboring fibrous root QTLs were also found to be associated with QTLs for different abiotic stresses. Mian et al. (1998) mapped a QTL for water use efficiency (WUE 2-3) to the same genomic location as *Q_root_Gm04* using progeny from the Young × PI416937 population. The positive allele at QTL WUE 2-3 was also inherited from PI416937. Although not located within ±5 cM of the *Q_root_Gm20* QTL interval, a leaf wilting coefficient (Wilt-Coe) QTL was reported within 22 cM of *Q_root_Gm20*, as well as a leaf water loss (LWL) QTL that was located within 15 cM (Fig. 4). Leaf water loss QTLs were also located near the *Q_root_Gm08* and *Q_root_Gm20* (Du et al. 2009a). The similar genomic location of QTLs for water use efficiency, leaf water loss, and fibrous roots is interesting given the obvious potential for each of these traits to contribute to drought tolerance.

King et al. (2009) found that PI416937 may possess more than one mechanism for drought resistance. PI416937 was initially identified based on its slow canopy wilting trait (Sloane et al. 1990). Fletcher et al. (2007) found that PI416937 may conserve soil moisture by limiting transpiration during periods of high evaporative demand by

reaching its maximum transpiration rate at a vapor pressured deficit (VPD) of 2.0 kPa. PI416937 showed no additional increase in transpiration at higher VPD while two fast canopy wilting cultivars continued to increase their transpiration rates as VPD was increased above 2.0 kPa.

Four QTLs on Gm03 (Fe_effic_5-2, Fe_effic_6-2, Fe_effic_10-2, and Fe_effic_12-2) were reported to be associated with the iron deficiency, a trait that depends on the iron absorption by soybean roots (Lin et al. 1997, 2000). Salt tolerance is another trait that is likely impacted by the root morphology. A salt tolerance QTL was previously detected and mapped in the same genomic location as *Q_root_Gm03* (Lee et al. 2004). *Q_root_Gm01* and a seed yield QTL has been mapped to the same interval in a population of 'BSR 101' × LG82-8379 (Kabelka et al. 2004). The LG82-8379 breeding line was selected from a cross between two exotic accessions, PI 68508 and FC 04007B.

North American soybean cultivars have a narrow genetic base (Gizlice et al. 1994), where 80% of the alleles found in public soybean cultivars released between 1947 and 1988 were derived from 13 ancestral lines. The exotic line, PI416937 has positive alleles for important traits such as drought resistance and high seed yield, which can be introduced into elite germplasms to improve soybean productivity. The results suggest that the identified fibrous root QTLs may be associated with other morpho-physiological traits which eventually affect a soybean plant's ability to tolerate moisture stress. Clustering of QTLs for various abiotic and nitrogen/seed protein-related traits near these five fibrous roots QTLs could be pleiotropic effects of the same gene affecting these related traits, or this chromosomal region has tightly linked genes responsible for the variation in these traits. The challenge is introgression of these favorable alleles into an elite cultivar while not replacing large regions of the recipient genome with chromosomal segments of an unadapted donor parent. The effect of combining the favorable alleles at different fibrous roots loci into one background would be a straightforward application of MAS. Backcrossing the fibrous roots alleles from PI416937 into elite cultivars would allow the effective evaluation of the fibrous root traits and its ability to produce stable seed yield under drought conditions.

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