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Mapping QTLs for plant height and flowering time in a Chinese summer planting soybean RIL population

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Abstract Soybean is a primary source of plant oil and protein and has a high nutritional value. Plant height (PH) and flowering time (FT) are two important agronomic traits in breeding programs for soybean. In this study, we mapped QTLs associated with PH and FT in three environments using a population with determinate growth including 236 recombinant inbred lines (NJZY-RIL) derived from a cross between two summer planting varieties, *ZXD* and *NN1138-2*. A high-density genetic map with 3255 SLAF-markers was constructed that spanned 2144.85 cM of the soybean genome with an average marker distance of 0.66 cM. Altogether, six QTLs controlling PH and eleven QTLs controlling FT were mapped using mixed-model-based composite interval mapping and composite interval mapping methods. *qPH-1-1* and *qFT-15-2* were two novel main effect QTLs identified in this study; *qFT-6-2*, *qFT-15-2*, *qFT-16-1*, *qPH-1-1*, *qPH-15-1* and *qPH-16-1* were consistently detected across environments and by the two mapping methods. Two pairs of QTLs, *qFT-15-2* and *qPH-15-1* as well as *qFT-16-1* and *qPH-16-1*, which were located in the same marker interval on chromosomes 15 and 16, respectively, were found to have close linkage or pleiotropy. These results may increase our understanding of the genetic control of PH and FT in soybean and provide support for implementing marker-assisted selection in developing soybean cultivars with high yield and early maturity in summer planting regions.

Keywords Soybean · Plant height · Flowering time · SLAF-seq · High-density genetic map · QTL

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Introduction

Soybean is a primary source of plant oil and protein for humans due to its high nutritional value (Wilcox 2004). However, the soybean yield per unit is relatively lower than that of cereal crops, such as maize. Molecular marker-assisted selection (MAS) for the breeding of high-yield varieties is an alternative to keep pace with the increasing global demand for soybean products. To date, new molecular marker systems based on next-generation sequencing (NGS) have quickly emerged. Among these, single nucleotide polymorphisms (SNPs) possess the most abundant DNA variation compared with amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers. Due to the completion of the whole genome sequencing of soybean *cv. Williams* 82 (Schmutz et al. 2010) and the rapid development in sequencing technology, highly polymorphic SNP markers are beginning to be used in soybeans for large-scale genotyping and high-density genetic map construction (Hyten et al. 2008; Song et al. 2016). Specific length amplified fragment sequencing (SLAF-seq) is a developed, high-resolution strategy for the large-scale de novo discovery and genotyping of SNPs based on NGS technology. This technology has greater genotyping accuracy and relatively lower sequencing cost. SLAF-seq technology has been used in several studies; for example, Zhang et al. (2013) constructed the first high-density genetic map for *sesame*, Huang et al. (2013) constructed a draft genome of the kiwifruit *Actinidia chinensis,* and Qi et al. (2014) reported a high-density genetic map for soybean. These new maps can facilitate efficiency in the identification of quantitative trait loci (QTLs) associated with important agronomic traits.

The development of cultivars with suitable maturity and plant type is the basic objective of soybean breeders. As an important plant architecture trait, plant height (PH) is considered one of the main yield-related traits in crops, and there are many studies regarding its genetics and QTL composition in soybean (Wilcox and Sediyama 1981; Cooper 1981, 1985; Ablett et al. 1989; Lee et al. 1996a, b, 2015; Mansur et al. 1996; Orf et al. 1999; Chapman et al. 2003; Zhang et al. 2004; Panthee et al. 2007; Liu et al. 2013). To date, at least 180 QTLs controlling PH have been reported (http://www.soybase.org/). Soybean stem growth habit is a major factor affecting PH and is controlled by two major genes, *Dt1* and *Dt2* (Bernard 1972). In *Dt1Dt1* genetic backgrounds, *Dt2Dt2* and *dt2dt2* genotypes produce semi-determinate and indeterminate stem termination phenotypes. However, in *dt1dt1* genetic backgrounds, the phenotype is determinate. Of these loci, *dt1* has a much greater effect on stem growth habit. There is a positive correlation between PH and maturity, and there is a similar correlation between maturity and flowering time (FT) (Lee et al. 1996b, 2015; Zhang et al. 2015). In general, the stem in plants with indeterminate growth continues to grow for a long period, even after flowering, while stem growth in plants with determinate growth is terminated when flowering begins or soon after. Thus, FT is considered important in determining the final PH of determinate cultivars.

FT is great interest to agriculture, as the regulation of FT is crucial for enabling crops to adapt to a particular growing region. FT is also positively correlated with PH in the determinate background (Lin et al. 1988; Curtis et al. 2000). It is difficult to breed a determinate variety with a tall PH and early flowering. Since the first QTL experiment on growth stage traits was reported 25 years ago (Keim et al. 1990), more than 70 loci distributed on 16 soybean chromosomes have been detected using different populations (Mansur et al. 1993, 1996; Orf et al. 1999; Tasma et al. 2001; Zhang et al. 2004; Yamanaka et al. 2005; Kong et al. 2014; Zhou et al. 2015). Ten major genes, *E1* through *E9* and *J* [*E1* and *E2* (Bernard 1971), *E3* (Buzzell 1971), *E4* (Buzzell and Voldeng 1980), *E5* (Mcblain and Bernard 1987), *E6* (Bonato and Vello 1999), *E7* (Cober and Voldeng 2001), *E8* (Cober et al. 2010), *E9* (Kong et al. 2014) and *J* (Ray et al. 1995)], have been reported to control the time of flowering and maturity. Among them, five cloned genes, *E1* to *E4* and *E9,* were located on Chr.06 (LG C2), Chr.10 (LG O), Chr.19 (LG L), Chr.20 (LG I), and Chr.16 (LG J), respectively (Cregan et al. 1999; Cober and Voldeng 2001; Abe et al. 2003; Cober et al. 2010; Xia et al. 2012; Kong et al. 2014). *E7*, *E8*, and *J* mapped to Chr.06 (LG C2), Chr.04 (LG C1) and Chr.04 (LG C1), respectively (Cober and Voldeng 2001; Cairo et al. 2002; Lu et al. 2015). *E6* has not been mapped, and *E5* might not be a unique locus (Dissanayaka et al. 2016).

The genetic maps used in most studies mentioned above for mapping QTLs associated with PH and FT

in soybean were constructed with only hundreds of RFLP, AFLP and/or SSR markers and therefore were of relatively low resolution (Mansur et al. 1993, 1996; Orf et al. 1999; Tasma et al. 2001; Zhang et al. 2004; Yamanaka et al. 2005). In the Chinese Huang-Huai River Valley, soybean is always planted in early June after wheat harvest and matures by the end of September or early October. The cultivar in this region has a nearly determinate stem and early flowering and maturity. However, little is known about the genetics of PH and FT in these genotypes. The objective of our study was to map QTLs for PH and FT in a recombinant inbred line (RIL) population with determinate growth using a high-density genetic map based on SLAF markers and then dissect the genetic basis of these two traits in summer soybean.

Materials and methods

Plant materials

An RIL population composed of 236 lines developed from a cross between *ZXD* (Maturity group III) and *NN1138-2* (Maturity group IV) was used in this study. *NN1138-2* is an elite cultivar characterized by high yield. *ZXD* is a landrace characterized by high protein content and good tolerance to flooding, among other traits. The RIL population used here (NJZY-RIL) was developed by 7 cycles of single seed descent (SSD) from an F_2 population at Jiangpu Experimental Station of Nanjing Agricultural University in Nanjing, Jiangsu Province.

Experimental design and collection of phenotypic data

NJZY-RIL and its two parents were grown in a randomized complete block design with three replications and one row per plot (10-cm plant spacing, 50-cm row spacing and 1.0-m row length; one plant per hill) from approximately June-October in three environments: Jiangpu Experiment Station, Nanjing, Jiangsu Province, in 2012 and 2014 (JP12 and JP14) and Fengyang Experiment Station, Chuzhou, Anhui Province, in 2012 (FY12). Field management was performed under normal conditions. The data for PH and FT in 2013 were inaccurate due to water logging at the seedling stage and the need to replant some lines. Accordingly, we did not use the data in 2013.

FT was calculated as the number of days from germination to the first bloom (R1, 50% of the plants in a plot had an open flower at one of the top nodes with a fully expanded leaf) (Fehr et al. 1971; Fehr and Caviness 1977; Orf et al. 1999). For the PH measurement, three individuals were randomly selected from the middle of each row, and the length between the cotyledon node and the peak of the main stem was measured. The measurement was averaged over the three individuals across three replications.

Population phenotypic data analysis

Statistical analyses, including the frequency distribution of the PH and FT phenotypic data, the mean of the RIL population, the coefficient of variation (CV), the broad-sense heritability (h^2), and the analysis of variance (ANOVA), were conducted using the SAS PROC UNIVARIATE, PROC GLM and PROC CORR programs (SAS Institute Inc. 2011a, b). The h^2 for PH and FT was estimated using the following equation:

$$h^2 = \sigma_g^2 / \left(\sigma_g^2 + \sigma_{ge}^2 / n + \sigma_e^2 / nr \right)$$

where σ_g^2 is the genotypic variance, σ_{ge}^2 is the variance due to the genotype-by-environment interaction, σ_e^2 is the error variance, n is the number of environments, and r is the number of replications within an environment (Nyquist and Baker 1991).

Genotyping and construction of genetic linkage map

SLAF-seq was used to genotype a total of 236 individuals and the two parents. Approximately 1 g of fresh leaves obtained from each plant were used to extract the genomic DNA using the cetyltrimethylammonium bromide method (Doyle 1990). SLAF library construction and high-throughput sequencing were performed as described by Sun et al. (2013). Then, all polymorphic SLAF markers were filtered four times and further quality assessed as described by Sun et al. (2013). A SLAF with less than three SNPs and an average depth of each sample above 3 was considered a high-quality SLAF marker. Parental homozygous

markers were used to construct a high-density genetic map.

After genotyping the 236 RILs, a high-density genetic map including 20 linkage groups (LGs) was constructed using High Map software (Liu et al. 2014); the Kosambi mapping function (Kosambi 1943) was used to calculate the map distances in cM from the recombination frequencies. MapChart v2.2 was used to draw the linkage map (Voorrips 2002). Chromosomes were named using Gm and the chromosome number; for example, Gm01 represents the first chromosome. Genotypes at the *Dt1* loci in *ZXD* and *NN1138-2* were identified by DNA sequencing according to Liu et al. (2010).

QTL mapping for PH and FT in multiple environments

Both mixed-model based composite interval mapping (MCIM) and composite interval mapping (CIM) methods were used to reveal the effects of the QTLs of PH and FT. Generally, more genetic effects were analyzed in MCIM than in CIM, whereas only additive effects were analyzed in CIM. QTL Network software v2.2 and MCIM were used to identify main additive effect QTLs, epistatic QTLs (AA), and genotype-by-environment interaction effects (additive by environment [AE] and AA by environment [AAE]) using MCIM (Wang et al. 1999; Yang et al. 2008; Xu et al. 2012). The CIM method of WinQTLCart version 2.5 (Wang et al. 2007) was also used to detect the main additive QTLs to identify stable QTLs expressed in different environments.

While QTL mapping was performed using the MCIM method, one- and two-dimensional genome scanning for QTLs was performed using a 10-cM testing window, a 0.1-cM walk speed and a 10-cM filtration window. The F thresholds for significant QTLs of each trait were determined by a 1000-permutation test at a 95% confidence level. When QTL analysis was performed by CIM, the window size, the working speed and the control marker number were set at 10, 1 and 5 cM, respectively. Model 6 (standard Model) in CIM was used to identify QTLs for each trait in each environment. Permutation tests of 1000 runs at a significance level of $P = 0.05$ were used to determine the LOD threshold for declaring whether the presence of a QTL in a certain chromosomal region was significantly associated with a target trait (Churchill and Doerge 1994).

If the confidence intervals of QTLs detected for the same trait in different environments overlapped and had the same sign of additive effects, then they were accepted as the same QTL. We followed the nomenclature suggested by McCouch et al. (1997) to name the QTLs detected in our study.

Results

Phenotypic evaluations of PH and FT

The phenotypic performance of the parents and the RILs is presented in Table 1 and Fig. S1. *ZXD* and *NN1138-2* showed a significant difference in PH but did not greatly differ from each other in FT in all environments. However, a continuous distribution and transgressive segregation were observed for the two traits in the RIL population in all environments. In addition, the kurtosis and the skewness (absolute

Table 1 Statistical analysis of flowering time (FT) and plant height (PH) in the parents and the NJZY-RIL population grown in different environments

Trait	Environment	Year	Location	Parents		RILs						
				ZXD	NN1138-2	Mean	SD	Range	Skewness	Kurtosis	CV (%)	h^2 (%)
FT (days)	JP12	2012	Jiangpu	39.7	39.8	40	1.6	31.0–44.7	−0.7	1.8	4	87.1
	FY12	2012	Fengyang	54.0	54.0	54.4	2.4	48.3–61.0	0.4	−0.4	4.4	
	JP14	2014	Jiangpu	45.7	47.0	46.6	2.7	35.0–54.5	0.8	0.2	5.8	
PH (cm)	JP12	2012	Jiangpu	80.6	56.9	65.9	11.9	45.2–135.4	0.9	1.6	17.5	84.0
	FY12	2012	Fengyang	85.2	66.2	76.4	14.8	47.9–136.2	0.8	0.5	19.4	
	JP12	2014	Jiangpu	84.7	69.7	83.9	11.3	57.6–123.6	0.2	0.1	13.5	

value) were less than 1 for both traits, except in the JP12 environment. This result indicates that PH and FT are quantitative traits and implies the existence of respective loci in the two parents controlling FT and PH.

The Pearson correlation coefficients (r) of PH and FT were moderately positively correlated in all environments: 0.32 in JP12, 0.52 in FY12, and 0.45 in JP14 ($P < 0.01$).

The h^2 of the traits was high for PH (0.84) and FT (0.87), which was consistent with the results of previous studies (Lee et al. 1996a). The ANOVA results indicated significant differences in genotype, environment and genotype-by-environment (Table S1), and the mean square (MS) value for the genotype-by-environment interaction was less than that of the genotype.

Marker genotyping and genetic map construction

The RIL population was genotyped using SLAF-seq technology. The average coverage for each SLAF marker was 101.85× for the parents and 3.05× for the RIL lines. In total, we obtained 103,845,237 point reads from the two parents and all of the lines. The reads were then mapped to the reference soybean genome (*cv. Williams* 82). The reads that could be mapped to a single locus were considered effective SLAFs. In this study, we obtained 71,888 SLAFs, of which 5333 were polymorphic. The polymorphic rate of these SLAFs was 7.4%. Ultimately, a total of 3279 SLAFs were used for high-density linkage map construction after filtration and quality assessment.

Using High Map software, a high-density genetic map was constructed. The 3255 SLAF markers were grouped into 20 LGs. The total genetic distance of this map was 2144.85 cM. The average distance between adjacent markers was 0.66 cM. The mean LG length was 107.24 cM. The LG containing the maximum number of markers was Gm18, with 338 SLAF markers and a length of 140.37 cM. The LG with a minimum number of markers was Gm12, with 48 SLAF markers and a length of 74.90 cM. The number of markers on each chromosome was consistent with its physical length; the longest chromosome was Gm18, and the shortest chromosome was Gm12 (Schmutz et al. 2010). In addition, we found that approximately 96.27% of the intervals between adjacent markers were shorter than 5 cM. Detailed map information is presented in Fig. 1, Table S2 and Figs. S2–S5. According to the DNA sequencing at the

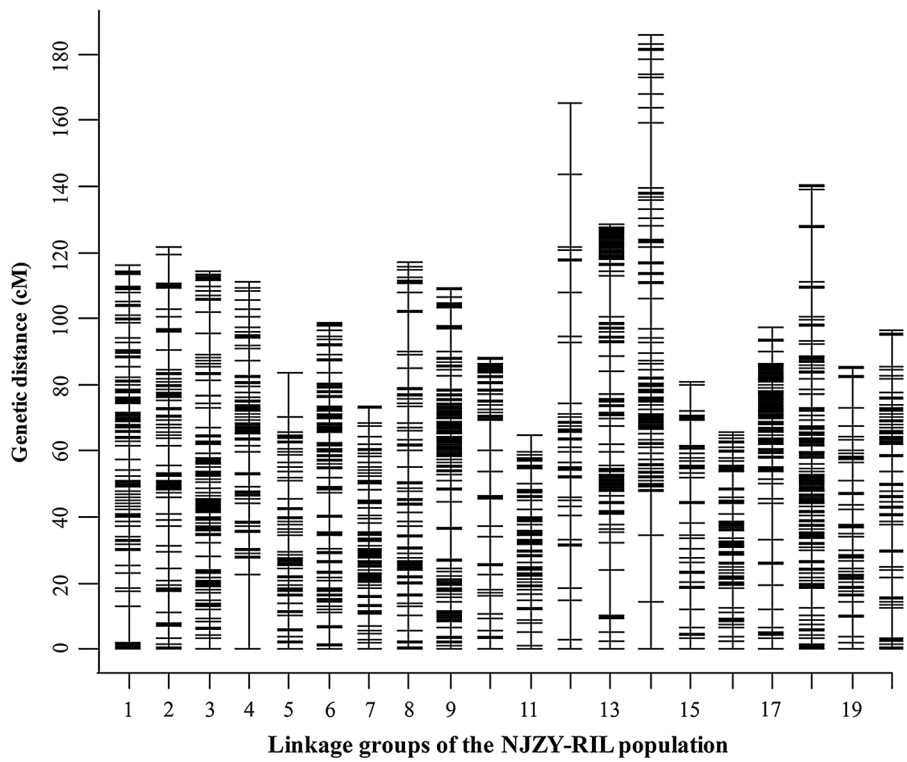

Fig. 1 Distribution of markers in 20 linkage groups in the NJZY-RIL population. The *black bars* in each linkage group represent mapped SLAF-seq markers. The linkage group number is shown on the *x-axis*, and genetic distance is shown on the *y-axis* (cM as unit). A detailed map is presented in the supplementary materials (Table S2; Figs. S2–S5)

Dt1 locus, both *ZXD* and *NN1138-2* were of the *dt1* type at the *Dt1* locus. This finding suggests that the difference in PH was not caused by the *Dt1* locus; the genetic background of NJZY-RIL was the determinate growth habit.

The main additive effect QTLs identified by MCIM for PH and FT

In the NJZY-RIL population, a total of 6 main additive effect QTLs for PH distributed on six LGs were identified by MCIM and explained 2.28 to 16.12% of the phenotypic variance (PV) (Table 2). *qPH-1-1* (named by a combination of trait and chromosome) was the largest additive effect QTL associated with PH ($a = -5.12$) and explained 16.12% of the PV. *qPH-16-1* and *qPH-15-1* were the second and third major QTLs for PH and explained 13.95 and 9.48% of the PV, respectively. The other three QTLs were *qPH-7-1*, *qPH-9-1* and *qPH-19-1*, which explained 2.10, 2.28 and 3.10% of the PV, respectively. In addition, the positive alleles of *qPH-1-1*, *qPH-7-1* and *qPH-19-1* came from *ZXD*, while those of *qPH-9-1*, *qPH-15-1* and *qPH-16-1* came from *NN1138-2*.

Ten QTLs were detected by MCIM on eight chromosomes associated with FT. The phenotypic variation explained by the detected QTLs ranged from 1.38 to 12.92% (Table 2). *qFT-16-1* could be the major QTL because of its high additive effect ($a = 0.82$), and it explained 12.92% of the PV. *qFT-15-2* was another major QTL for FT that explained 11.39% of the PV. *qFT-6-2* explained 6.33% of the PV. Other QTLs explained less than 5% of the PV. The additive effects of *qFT-5-1*, *qFT-6-1*, *qFT-6-2,* and *qFT-8-1* were negative, suggesting that the positive alleles came from the male parent, *ZXD*. In contrast, *qFT-2-2*, *qFT-14-1*, *qFT-15-1*, *qFT-15-2*, *qFT-16-1*, and *qFT-18-1* had positive additive effects, and the positive alleles came from the female parent, *NN1138-2*.

Epistasis and QTL-by-environment interaction

A total of 3 pairs of epistatic QTLs were identified for PH and FT (Table S3). Two pairs of epistatic QTLs involving four loci on three chromosomes were identified for PH. Pair 1 was composed of two additive QTLs, *qPH-1-1* on Chr.01 and *qPH-16-1* on Chr.16, and explained 1.3% of the PV for PH. Pair 2, *qPH-6-1* and *qPH-16-2,* had no additive effect and explained 2.7% of the PV. One pair of epistatic QTLs identified for FT was composed of two non-additive QTLs, *qFT-2-1* and *qFT-16-2,* and explained 1.4% of the PV.

In this study, additive QTL-by-environment interactions were relatively weak and explained 0.6% and 0.4–1.0% of the phenotypic variation in PH and FT, respectively (Table 2).

The main additive effect QTLs identified by CIM and a comparative analysis of the main additive effect QTLs detected by CIM and MCIM

A total of ive QTLs associated with PH were detected in at least one of the three environments using CIM based on WinQTLCart (Table 3). *qPH-7-1* and *qPH-19-1* were detected in a single environment; *qPH-1-1*, *qPH-15-1*, and *qPH-16-1* were detected in all environments (Fig. 2). Comparing the main additive effect QTLs identified by the two programs, *qPH-1-1*, *qPH-7-1*, *qPH-15-1*, *qPH-16-1,* and *qPH-19-1* were identified both by WinQTLCart and QTLNetwork (Table 2).

For FT, five QTLs on Chr.03, Chr.06, Chr.15, and Chr.16 were identified in at least one environment using CIM analysis (Table 3). *qFT-6-2*, *qFT-15-2,* and *qFT-16-1* were detected in all environments (Fig. 2); *qFT-6-1* was detected in two environments; and *qFT-3-1* was detected in a single environment. Compared with the results of MCIM, all main effect QTLs were identified both by WinQTLCart and QTLNetwork, with the exception of *qFT-3-1* (Table 2).

Discussion

Construction of a high-density genetic map based on SLAF-seq markers

QTL mapping has been used as an efficient approach to analyze quantitative traits in plants. The quality of genetic maps has a significant effect on the accuracy of QTL mapping, and increasing marker density can improve the resolution of genetic maps for a given mapping population (Gutierrez-Gonzalez et al. 2011; Zou et al. 2012). Therefore, it is feasible to construct high-density genetic maps and thereby improve the efficiency and accuracy of QTL mapping and MAS. In this study, we used 3279 high-quality SLAF markers to construct a high-density map. Ultimately, a total of

Table 2 Additive QTLs and QTL-by-environment interaction effect for flowering time and plant height as detected by MCIM in the NJZY-RIL population

QTL	Chr.	Flanking marker	Position (cM)	F[a]	Confidence interval (cM)	A[b]	h^2(A) (%)[c]	AE[d]	h^2(AE) (%)[e]	Reference[f]
Flowering time										
qFT-2-2	2	MARK24040-MARK60363	72.1	15.8	71.5–72.5	0.35***	2.31			13–4,13–5
qFT-5-1	5	MARK548154-MARK561057	22.2	10.9	20.3–24.2	−0.31***	1.88			Novel
qFT-6-1[g]	6	MARK780544-MARK767092	17.4	21.4	16.6–18.2	−0.45***	3.88			Novel
qFT-6-2[g]	6	MARK746221-MARK727369	66.9	22.8	66.9–67.1	−0.58***	6.33			5–1,12–2,8–1
qFT-8-1	8	MARK418778-MARK450439	86.3	10.7	82.6–86.6	−0.39***	2.91			13–1
qFT-14-1	14	MARK918931-MARK908219	107.2	7.7	106.0–109.8	0.29***	1.59			Novel
qFT-15-1	15	MARK818425-MARK812438	28.7	9.9	25.7–32.5	0.30***	1.69			Novel
qFT-15-2[g]	15	MARK829597-MARK835038	70.9	63.3	70.1–71.9	0.77***	11.39	−0.32***(AE1)/0.27**(AE3)	1.0/0.7	Novel
qFT−16-1[g]	16	MARK303192-MARK310155	19.2	54.5	19.2–19.7	0.82***	12.92	−0.36***(AE1)/0.21*(AE2)	1.2/0.4	13–7
qFT-18-1	18	MARK1018780-MARK1053790	98.6	8.4	97.2–100.0	0.27***	1.38			10–2
Plant height										
qPH-1-1[g]	1	MARK989081-MARK998454	25	50.3	23.0–27.3	−5.12***	16.12			Novel
qPH-7-1[g]	7	MARK840706-MARK839285	25	9.5	24.7–26.8	1.85***	2.10			3–3,2–1,6−5
qPH-9-1	9	MARK591384-MARK593133	98.1	9.5	96.9–98.4	−1.92***	2.28			Novel
qPH-15-1[g]	15	MARK829597-MARK835038	70.9	36	70.4–71.9	3.92***	9.48			26–10
qPH-16-1[g]	16	MARK294445-MARK277706	18.4	43.6	16.6–19.2	4.76***	13.95	1.4*(AE2)	0.6	5–9
qPH-19-1[g]	19	MARK139978-MARK115319	11.1	8.8	3.8–16.1	−2.25***	3.1			3–5,6–7,10−3

[a] The F value at the peak likelihood of the QTL; the thresholds were calculated from a 1000-permutation test ($P = 0.05$). For flowering time, the threshold was 7.6; for plant height, the threshold was 7.7

[b] Additive effect: positive values indicate that NN1138-2 contributed the allele for an increase in the trait value

[c] Phenotypic variance explained by additive QTL

[d] E1: JP12; E2: FY12; E3: JP14. AE: additive QTL-by-environment interaction effect

[e] Phenotypic variance explained by additive QTL-by-environment interaction effect

[f] The QTL name of the flowering time and plant height at soybase.org

[g] Indicates that this QTL can be detected using CIM

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3 Detection by CIM of QTLs associated with flowering time and plant height in the NJZY-RIL population grown in three different environments

QTL[a]	Chr.[b]	QTL peak position (cM)	Flanking marker	LOD[c]	1-LOD interval (cM)[d]	Additive effect[e]	R^2 (%)[f]	Env.[g]
Flowering time								
qFT-3-1	3	100.5	Mark179964-Mark168273	3.5	95.7−103.3	−0.49	4.2	FY12
qFT-6-1	6	16.6	Mark749562-Mark780544	6.3	16.4−17.4	−0.46	7.4	JP12
		16.6	Mark749562-Mark780544	3.6	16.2−18.2	−0.53	3.6	JP14
qFT-6-2	6	66.9	Mark727369-Mark763037	6.2	66.6−67.5	−0.49	8.4	JP12
		66.9	Mark742107-Mark729323	4.7	66.4−67.3	−0.54	5.3	FY12
		66.9	Mark727369-Mark763037	6.6	66.6−68.3	−0.74	7.2	JP14
qFT-15-2	15	70.1	Mark791715-Mark786950	16.3	69.6−70.7	1.06	20.3	FY12
		70.1	Mark791715-Mark786950	20.5	69.6−70.4	1.35	24.3	JP14
		71.9	Mark835038-Mark792948	7.2	70.7−72	0.51	8.5	JP12
qFT-16-1	16	18.4	Mark294445-Mark303192	17.8	16.5−19.8	1.11	22.0	FY12
		19.2	Mark277706-Mark310155	4.7	18.4−20	0.39	5.4	JP12
		19.2	Mark277706-Mark310155	12.4	18.4−19.8	0.99	12.8	JP14
Plant height								
qPH-1-1	1	22.5	Mark1001506-Mark989081	17.2	20.5−25.2	−5.71	24.6	JP14
		25.3	Mark998454-Mark979255	14.6	24.3−29.7	−6.58	18.9	FY12
		26.3	Mark959206-Mark955859	14.0	23.9−27.6	−5.52	20.1	JP12
qPH-7-1	7	21.5	Mark848352-Mark861629	3.5	18.4−22.5	2.90	3.8	FY12
qPH-15-1	15	70.9	Mark829597-Mark835038	11.1	70.2−71.4	4.50	13.8	JP12
		70.9	Mark829597-Mark835038	7.6	70.0−74.7	4.44	8.6	FY12
		70.9	Mark829597-Mark835038	8.5	70.2−71.3	3.67	10.2	JP14
qPH-16-1	16	18.4	Mark294445-Mark303192	14.8	16.2−19.3	6.46	18.9	FY12
		19.2	Mark277706-Mark310155	9.7	15.6−20.2	4.15	12.0	JP12
		19.2	Mark277706-Mark310155	8.4	18.4−20.4	3.66	10.2	JP14
qPH-19-1	19	3.0	Mark115986-Mark137888	4.1	0.0−3.8	−2.56	5	JP14

[a] QTLs detected in different environments at the same; adjacent or overlapping marker intervals were considered the same QTL

[b] Chr. Chromosome

[c] The log of odds (LOD) value at the peak likelihood of the QTL; LOD thresholds were calculated from a 1000-permutation test ($P = 0.05$). For flowering time, the threshold was 3.3, 3.5, and 3.4 in JP12, FY12, and JP14, respectively. For plant height, the threshold was 3.3, 3.4, and 3.4 in JP12, FY12, and JP14, respectively

[d] 1-LOD support confidence intervals (confidence interval length)

[e] The positive value indicates that *NN1138-2* contributed the allele for an increase in PH and FT, and negative additive effects indicated that *ZXD* contributed the allele for an increase in plant height and flowering time

[f] Phenotypic variance (%) explained by the QTL

[g] JP12, FY12, and JP14 represent the environments Jiangpu 2012, Fengyang 2012, and Jiangpu 2014, respectively

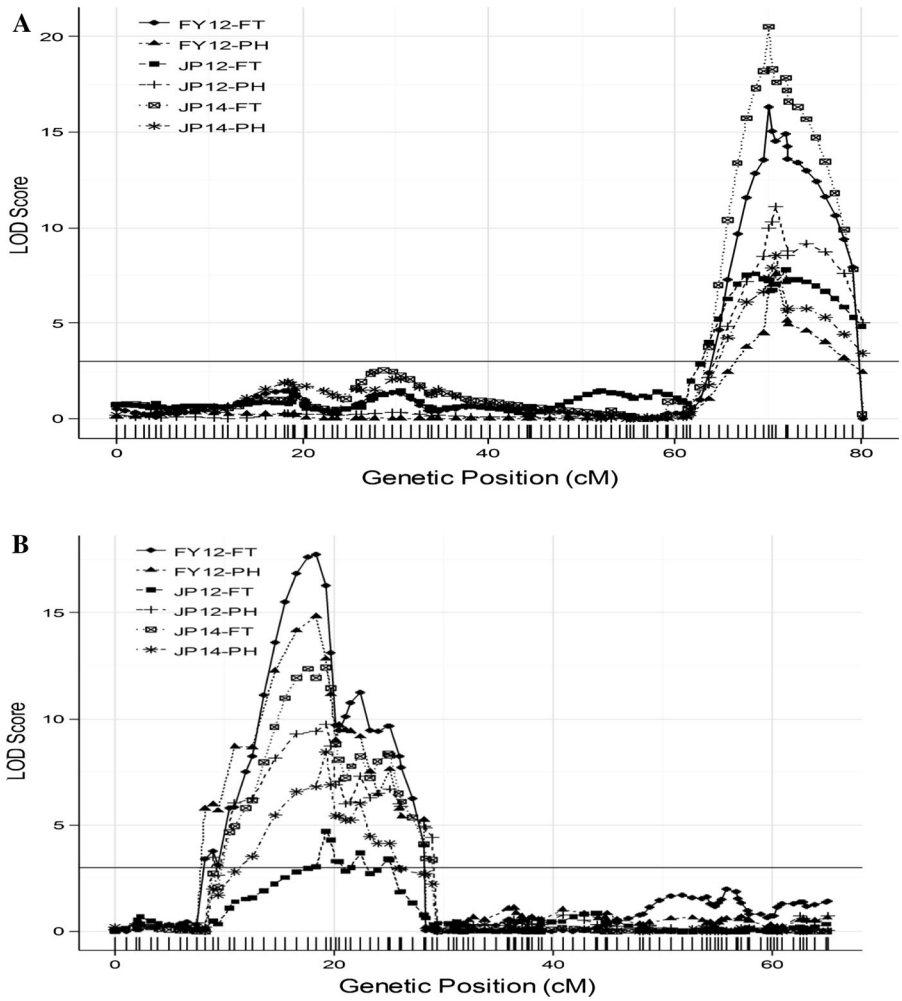

Fig. 2 Linkage maps of Chr.15 and Chr.16 for two locations associated with flowering time and plant height in the NJZY-RIL population. **a** Graphical representation of the loci of Chr.15 associated with plant height and flowering time, with LOD plots for three environments (JP12, FY12, and JP14). The *hatched lines* in the LOD plots indicate the LOD thresholds. **b** Graphical representation of the loci of Chr.16 associated with plant height and flowering time, with LOD plots for three environments (JP12, FY12, and JP14). The *hatched lines* in the LOD plots indicate the LOD thresholds

3255 SLAF markers were integrated into 20 LGs, and the average distance between adjacent markers was only 0.66 cM. This high-density genetic map could ensure that a molecular marker and QTL were tightly linked and provided a good foundation for analyzing quantitative traits.

QTLs for plant height in soybean

Because PH is an important yield-related trait in soybean, a series of associated QTLs/loci has already been reported. Liu et al. (2013) used an RIL population and detected 11 QTLs for PH; Lee et al. (2015) mapped six QTLs for PH with an SNP map; and Zhang et al. (2015) detected 27 loci for PH via a genome-wide association study (GWAS). Brummer et al. (1997) reported that in molecular-assisted breeding programs, breeders should use QTLs that are stable in multiple environments. In the present study, a total of six QTLs for PH were identified. *qPH-1-1*, *qPH-15-1,* and *qPH-16-1* could be considered the major and stable QTLs for PH because they could be detected by the two programs and in all environments with larger LOD values (14.0–17.2, 7.6–11.1 and 8.4–14.8, respectively) and explained more of the PV (18.9–24.6%, 8.6–13.8%, and 10.2–18.9%, respectively). Based on the high-density genetic map, the confidence interval for most of the QTLs was less than 5 cM, and each QTL had two or more closely linked markers (within 0–5 cM). These loci are favorable for the MAS of QTLs by soybean breeding programs.

qPH-15-1 and *qPH-16-1* have adjacent or physically overlapping QTLs, as reported by Sun et al. (2006) and Lee et al. (1996b), and might be the same

QTL. Because many QTLs associated with PH have been previously reported, it is difficult to identify novel QTLs associated with PH. Based on the QTLs listed in SoyBase (www.soybase.org), two novel QTLs, *qPH-1-1* and *qPH-9-1,* were identified in this study. Compared with the loci/genes that were already known, such as *Dt1* and *Dt2*, novel QTLs, particularly *qPH-1-1,* which was detected by two programs and in all environments and could be considered the major and stable QTL, in these summer soybean lines would add to the growing knowledge on the genetic control of PH.

Knowledge of epistasis and QTL-by-environment interaction is essential to understand the genetic architecture and the gene networks that underlie complex traits (Wurschum et al. 2011). When epistatic interactions are considered in a QTL mapping model, the precision of QTL mapping is greatly enhanced (Wang et al. 1999), which could help to accurately predict the phenotypic performance in MAS programs. In this study, two pairs of epistatic QTLs (*qPH-1-1* × *qPH-16-1*, *qPH-6-1* × *qPH-16-2*) and some additive QTL-by-environment interactions were detected by QTLNetwork (Table 2, Table S3). *qPH-6-1* and *qPH-16-2* did not display additive effects; these epistatic QTLs might be considered modifying genes that have no significant effects alone but might affect the expression of PH through epistatic interactions with other loci. In fact, we identified significant additive QTL-by-environment interactions using MCIM (Table 3), although the phenotypic variation for QTL-by-environment interactions was less than that for additive QTLs. This information might be useful for more accurately predicting the breeding value in MAS.

New QTLs for flowering time in soybean

FT is a topic of great interest in soybean breeding programs; it is crucial for the adaptation of crops to a particular growing region and may also affect other traits. In this study, using RILs derived from *ZXD* and *NN1138-2*, a total of 11 QTLs for FT were identified. Among these QTLs, *qFT-6-1* and *qFT-6-2* were located on the same chromosome as *E1* and *E7*. However, *qFT-6-1* and *qFT-6-2* might not be *E1* loci due to their physical position being far from known *E1* loci (Xia et al. 2012); *qFT-6-2* was located in the same physical range as reported in previous studies and might be the same QTL as *qFT-6-2* (Zhang et al. 2004). Corresponding to the physical location of the confidence interval of *qFT-16-1*, we found the *Arabidopsis* homolog *GmFT5a*. Previous studies have shown that *GmFT5a* controls the soybean photoperiod (Kong et al. 2010); *GmFT5a* might be a candidate gene for this QTL. Compared with ten major genes, the QTLs listed in SoyBase (www.soybase.org) have been reported to control the time of flowering and maturity. A few novel QTLs were identified for FT in this study, such as *qFT-5-1*, *qFT-6-1*, *qFT-14-1*, *qFT-15-1* and *qFT-15-2*. Compared with *E1* through *E9* and *J*, the novel QTLs, particularly *qFT-15-2,* which explained 11.39% of the PV, would add to the growing knowledge of the genetic control of FT. Among these QTLs, *qFT-6-2*, *qFT-15-2,* and *qFT-16-1* were detected by two programs and in all environments in this study and could thus be considered major and stable QTLs for further fine mapping and map-based cloning to elucidate the mechanisms of FT. Those loci might be useful in soybean breeding programs.

However, there are many possible FT genes in soybean (Kim et al. 2012). In many previous studies, most soybean materials used for QTL/gene mapping and cloning were from the spring planting type; some major loci, including *E1*, *E2*, and *E3*, play important roles in these lines (Xia et al. 2012; Kong et al. 2014; Lu et al. 2015; Zhang et al. 2015). However, in the NJZY-RIL population, most detected QTLs were novel, indicating the distinct genetic architecture of FT in these two summer soybeans. More diverse germplasms need to be used to reveal the genetic basis of FT in soybean.

Co-location of QTLs for soybean PH and FT

One locus can be related to more than one trait (Zhang et al. 2004); several QTLs of various traits can map to the same locus. In this study, two pairs of QTLs, *qPH-15-1* and *qFT-15-2* as well as *qPH-16-1* and *qFT-16-1*, were located in the same marker interval (Fig. 2). These loci were found to be related to the two agronomic traits PH and FT. This implies that *qFT-15-2* and *qFT-16-1* not only control FT but also may affect PH. This phenomenon is consistent with previous reports that found that the QTLs/genes of FT often impact other agronomic traits (Chapman et al. 2003; Kantolic and Slafer 2007; Li et al. 2008; Cober et al.

2010). Furthermore, QTLs clustered in the same LG could suggest moderate correlations among corresponding traits based on field data. Therefore, it is difficult to breed a determinate variety with a tall PH and an early FT. Due to the lines pyramiding different alleles and the additive effects of the QTLs controlling PH and FT from both parents (Tables 2, 3), we identified some lines with a tall PH and an early FT in the field experiments. Thus, we can produce offspring with desirable PH and FT by pyramiding different favorable alleles if we know the QTLs associated with PH and FT in summer soybean. These results would not only add to the growing knowledge of the genetic control of PH and FT but also provide useful information for understanding the molecular mechanisms of PH and FT.

In conclusion, the objective of this study was to detect QTLs for PH and FT, which are two important agronomic traits that should be considered in breeding programs. A high-density genetic map was constructed in this study. The main additive effect QTLs for PH and FT were detected, and the markers closely linked to each QTL were explored. Furthermore, a few novel QTLs, information about epistasis and the QTL-by-environment interaction, and the co-location of QTLs were obtained in this study. These results might be very useful for the fine mapping of soybean genes and provide support for implementing MAS for breeding high-yielding soybean.

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