



# A new dominant locus, *E11*, controls early flowering time and maturity in soybean

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**Abstract** Flowering time, as an important ecological trait related to photoperiod response, maturity, and final yield, is a complex trait conferred by multiple genes. To further elucidate the genetic mechanism for the flowering time, quantitative trait loci (QTLs) related to the flowering time and maturity were identified utilizing specific-locus amplified fragment sequencing (SLAF-Seq) technology. In total, we identified three QTLs on chromosomes 5, 6, and 7 from a recombinant inbred line (RIL) population of 171 individuals derived from a cross between Minsoy and Archer soybeans. Of these QTLs, one new QTL on chromosome 7, called *E11*, was simultaneously detected in an ~1.03 Mb region from the F<sub>6</sub> and F<sub>8</sub> generations of the RIL population, and accounted for ~15% of the total phenotypic variation over 2 years. The gene symbol *E11e11* had been approved by the soybean genetic committee. The segregation patterns observed in residual heterozygous lines

(RHLs) at the *E11* locus revealed that early flowering was controlled by a single dominant gene. The gene was fine-mapped to an ~138 kb interval, including 11 genes based on the reference genome. Through amino acid sequence analysis, three most likely candidate genes, *Glyma.07 g048500*, *Glyma.07 g049000*, and *Glyma.07 g049200*, were identified. The phenotypes detected from two near-isogenic lines (NILs) revealed that NILs for *E11* allele significantly promoted the flowering time and maturity than NILs for the *e11* under the long-day (LD) conditions. These results suggest that *E11* is a new flowering time gene that will be valuable in improving our understanding of the mechanism for the flowering time and molecular breeding.

**Keywords** Quantitative trait locus · *E11* · Flowering time · Maturity · Soybean

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## Introduction

Soybean [*Glycine max* (L.) Merrill] is a facultative short-day plant that is very sensitive to photoperiod, but cultivars can grow across the world in a wide range of latitudes from 50° N to 35° S (Watanabe et al. 2012). This is attributed to the rich genetic variability in the genes or loci conditioning the photoperiod of the flowering time. A number of major genes and QTLs have been selected during the breeding process. Thus far, 11 major genes controlling the flowering time and maturity have been reported in soybean: *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), *E10* (Samanfar et al. 2017), and *J* (Ray et al. 1995).

Of the 11 major loci, only *E6* and *J* were reported to control the flowering time under short-day (SD) conditions (Li et al. 2017). Moreover, *J* was identified as the ortholog of the *Arabidopsis* gene *EARLY FLOWERING 3* (Lu et al. 2017). The other nine genes were detected under long-day (LD) conditions. *E1* has been cloned using a map-based approach and identified as a legume-specific transcription factor with a putative nuclear localization signal and a B3 DNA-binding domain (Xia et al. 2012). *E2* is an ortholog of the *Arabidopsis* flowering gene *GIGANTEA* (Watanabe et al. 2011). Both *E1* and *E2* play core functions in the photoperiod pathway. *E3* and *E4*, which have been confirmed as *phytochrome A* homologs, control the flowering time by upregulating *E1* and *E2* (Liu et al. 2008; Watanabe et al. 2009). *E9* and *E10* are homologs of *FLOWERING LOCUS T* and play function downstream of *E1* and *E2* (Kong et al. 2014; Zhao et al. 2016; Samanfar et al. 2017; Lu et al. 2017). In cultivated soybeans, there are at least three dysfunctional alleles and one hypomorph allele at the *E1* locus (Xia et al. 2012; Tsubokura et al. 2014), one dysfunctional allele at the *E2* locus (Watanabe et al. 2009), three dysfunctional alleles and one hypomorph allele at the *E3* locus (Xu et al. 2013), and six dysfunctional alleles at the *E4* locus (Tsubokura et al. 2013). The various allelic combinations at the *E1*, *E2*, *E3*, and *E4* loci greatly contribute to the wide adaptability of soybeans (Xu et al. 2013; Tsubokura et al. 2014). However, these allelic variations generate some, not all, of the variations in the flowering time among soybean cultivars. Thus, the detection of new QTLs and genes associated with the flowering time is necessary to improve soybean adaptation and productivity.

With the great development in next-generation sequencing (NGS), it provides a powerful method for

single-nucleotide polymorphism (SNP) discovery and genotyping in large population, including restriction-site associated DNA tag sequencing (RADseq, Baird et al. 2008), genotyping-by-sequencing (GBS, Elshire et al. 2011), and specific-locus amplified fragment sequencing (SLAF-seq, Sun et al. 2013). One of these, SLAF-seq performs specifically a predesign experiment to evaluate restriction enzymes and sizes of restriction fragments using the soybean reference genome sequence, which improves its overall efficiency (Sun et al. 2013). Therefore, SLAF-seq is widely used for the construction of high-density genetic mapping for QTLs.

As mentioned above, flowering and maturity processes in soybeans are not fully understood. Many QTLs controlling the flowering time are still not reported or cloned. The objectives of this study are as follows: (1) to detect more QTLs associated with the flowering time, (2) to fine-map one major locus, *E11*, and to identify the candidate gene(s). We hope that the results of this study will promote a better understanding of the mechanisms controlling the flowering time and identify the tightly linked molecular marker for *E11* that will be helpful in molecular breeding.

## Materials and methods

### Field experiments

Genetic material consisting of recombinant inbred lines (RILs) was developed by a single-seed descendent method (Fehr 1987) from the cross between Minsoy (PI27890) and Archer (PI54687) (called MA population). The F<sub>6</sub> and F<sub>8</sub> seeds for each RIL were developed by single seed descent, and the seeds of the parental lines were sown in the field under natural day length conditions at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China (45°43' N, 126°45' E), during the month of May in 2016 and 2017. The RHL segregating families for the *E11* locus (Family #13 and Family #131) were selected from RILs and planted on 9 May 2017; their progenies were planted in a line on 14 May 2018. Hybrids and lines were sown with a single seed every 20 cm in 5 m rows, spaced 60 cm apart. All trials received standard cultural practices to control insects and weeds (Lu et al. 2017).

## Construction of high-density genetic maps

Total DNA was extracted from leaf tissue of MA population plants and the two parents using the CTAB extraction method. The parent plants were sequenced on an Illumina HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA), and 125-bp paired-end reads with insert sizes of around 350 bp were generated. Sequencing libraries were generated as described by Cheng et al. (2015). The MA population was genotyped using SLAF sequencing. The sequencing data of the 171 RILs were analyzed following previously reported methods (Sun et al. 2013; Li et al. 2014). Briefly, low-quality reads (quality score < 20e) were filtered out, and then raw reads were sorted for each RIL according to duplex barcode sequences (Biomarker, Beijing, China). After the barcodes and the terminal 5-bp positions were trimmed from each high-quality read, clean reads from the same sample were mapped onto soybean genome sequences (Wm82.a2.v1) using SOAP software (Li et al. 2008). Sequences mapped to the same position. The SNP of each SLAF locus was detected. The SNP loci of each SLAF locus were detected between parents, and SLAFs with more than three SNPs were filtered out first. Alleles of each SLAF locus were then defined according to parental reads with sequence depth > 30-fold, while for each offspring the reads with sequence depth > tenfold were used to define alleles. Genotype scoring was then performed using a Bayesian approach to further ensure genotyping quality. High-quality SLAF markers for genetic mapping were filtered following reported criteria (Kong et al. 2018).

As for bin mapping, markers with the same genotype were divided into bin markers using a Perl script. Based on their physical position, the markers were divided into 20 chromosomes (Chr), and HighMap software (Liu et al. 2014) was used to order the markers in every linkage group. A linkage map for MA RILs was constructed containing 5074 SNP markers and covered 3588.94 cM.

## Screening of Indel markers

Indel markers were developed in this study on the basis of resequencing data from the Minsoy and Archer. Three types of software, SAMtools (v1.7) (Li 2011), Pindel (v1.0) (Ye et al. 2009), and Soapindel (v2.1) (Li et al. 2013), were used for the discovery of indel markers. To optimize the Indels, we trained the support vector machine

(SVM) filter by simulative data and identified Indels with quality scores > 200. High-quality Indel markers contrasting among parents were selected for QTL analysis, fine-mapping, and NILs genotype identification. We selected eight Indels to map the *E11* in the R<sub>6</sub> (2016) and R<sub>8</sub> (2017) populations and constructed linkage maps with Map Manager QTXb2.0 (Manly et al. 2001). Eleven indel markers were used for fine-mapping. Sixteen Indels were used to develop NILs.

## QTL analysis

For the flowering time, QTLs in different environments were detected by multiple-QTL model (MQM) mapping using the MapQTL5 package (Van Ooijen 2004). The LOD threshold for declaring significant QTLs was determined using a permutation test (PT) with a significance level of  $p < 0.05$  ( $n = 1000$ ).

## Fine-mapping

We genotyped 11 indels markers flanking the genes from ID7079 to ID7088 in the progeny of RIL families #13 ( $n = 271$ ) and #131 ( $n = 109$ ) and detected some recombinant plants. The QTL genotypes of recombinants and nonrecombinant control plants were determined by comparing their graphical genotypes constructed with Indel markers. Phenotypic segregation patterns in the progeny of the recombinants were determined by GraphPad Prism 6 Software (Berkman et al. 2018).

## The candidate genes for *E11* locus

The candidate genes for *E11* loci were categorized; the function of which was determined using the Phytozome database (<https://phytozome.jgi.doe.gov/pz/portal.html>). The Blast2GO 4.0 (BioBam Bioinformatics S.L., Valencia, Spain) or Phytozome database was used to determine the GO ID of candidate genes. Default parameter settings were employed.

## Phenotype statistics

Days to flowering and maturity were recorded at the R1 stage (days from emergence to first open flower appeared on 50% of the plants) and the R8 stage (days from emergence to 95% of pods exhibiting mature colors) (Fehr et al. 1971). Chi-square ( $\chi^2$ ) tests were conducted to detect segregation distortion. A one-way

analysis of variance (ANOVA) was used to detect significant marker-phenotype associations between polymorphic DNA markers and days to the flowering time and maturity.

## Results

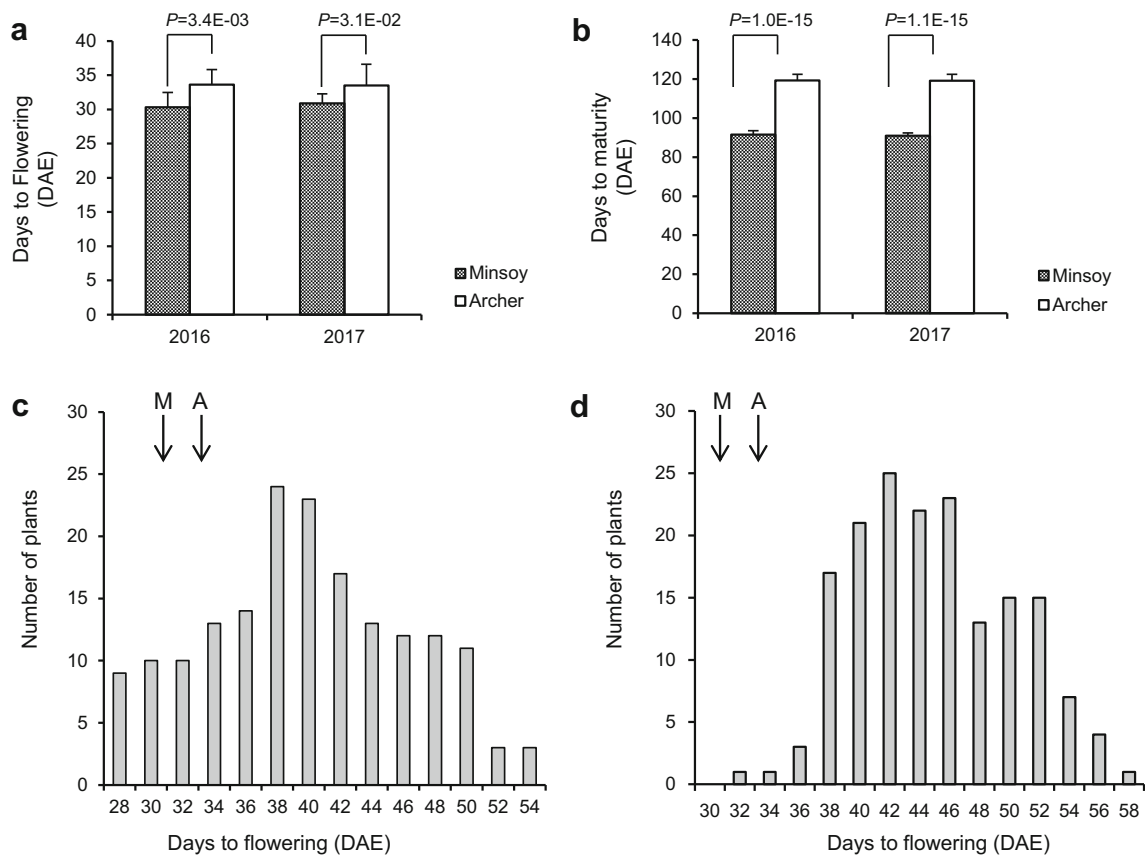
### Phenotypic analysis of flowering time in soybean RIL populations

In order to find new QTLs associated with the flowering time, we grew the RIL population of 171 individuals derived from the cross between Minsoy and Archer in Harbin during 2016 and 2017. Minsoy flowered averagely  $\sim 30.6$  days after emergence (DAE) and matured 91 DAE, while Archer flowered  $\sim 33.6$  DAE and matured 119 DAE on average (Fig. 1a, b). Frequency

distributions of the flowering time for different years in the segregating populations of the cross are presented in Fig. 1c, d. The  $F_6$  and  $F_8$  RIL populations showed a continuous and normal distribution, with days to flowering ranging from 28 to 54 DAE in  $F_6$  RILs during 2016 and from 32 to 58 DAE in  $F_8$  RILs during 2017. These results indicated that the flowering time was mainly controlled by genetic factors and inherited in a quantitative manner. Moreover, transgression segregations were observed in the RIL population, suggesting that of the two parents might contain recessive genes that affect the late-flowering trait.

### QTL mapping for the flowering time using high-density genetic map

Orf et al. (1999) analyzed RIL populations derived from the cross between Minsoy and Archer and found that



**Fig. 1** Phenotype of Minsoy, Archer, and RILs in the yield. **a** Flowering time of the parents in 2016 and 2017. **b** Maturity of the parents over 2 years. **c** Segregation of flowering time in the MA  $F_6$  (2016) population. **d** Segregation of flowering time in the MA  $F_8$

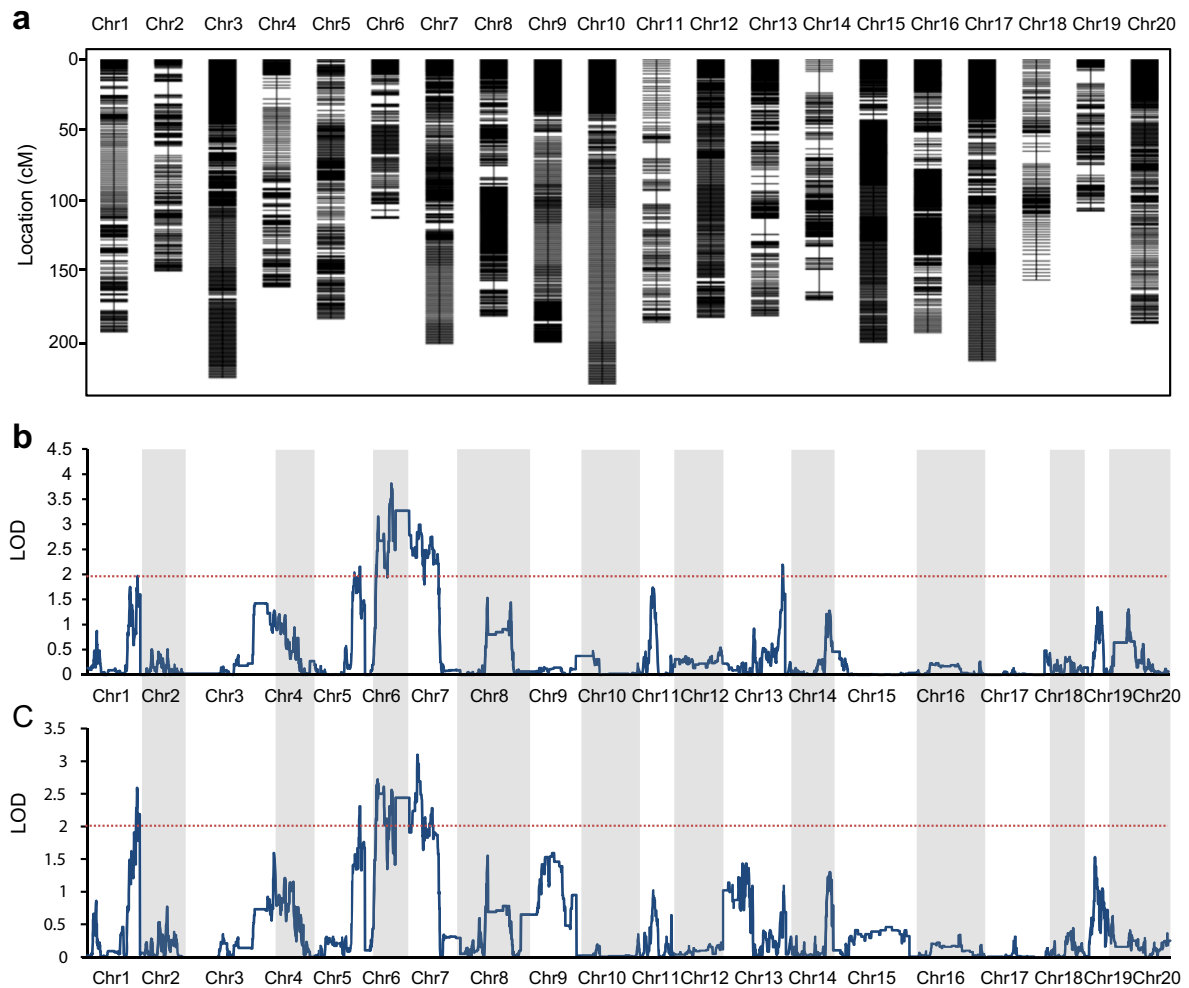
(2017) population. Arrows indicate the days to flowering of the parent soybeans, Minsoy (M) and Archer (A). X-axis represents number of plants flowering at the same time. Y-axis represents days to flowering (DAE)

four QTLs for the flowering time were located on linkage group (LG) C2 (Chr6), LG M (Chr7), and LG L (Chr19). The QTL on Chr6 most likely corresponds to *E1*, which account for 31% of the observed variation in the flowering time; this is in accordance with previous findings that *E1* has the largest effect on soybean flowering (Cober et al. 1996; Liu et al. 2007; Xia et al. 2012). The second QTL on Chr7 can explain 26% of the observed variation, which is a new QTL for the flowering time.

A high-density genetic map comprising of 5074 SLAF markers was used for QTL mapping (Fig. 2a) in the study. Based on 1000 permutations, a LOD score of 2.0 was used as the threshold for declaring the presence of an additive QTL. Using this genetic map and R1 data

in 171 lines, three flowering time QTLs (*qTOF5*, *qTOF6.1*, and *qTOF7*) were consistently identified over 2 years (2016 and 2017). Three separate QTLs (*qTOF1*, *qTOF6.2*, and *qTOF13*) were located on Chr1, 6, and 13, respectively, and only appeared for 1 year (Table 1; Fig. 2b; Fig. 2c).

Of these QTLs, one major QTL on Chr6 (*qTOF6.1*) was detected over the 2 years and accounted for 9.8% of the observed variation and had the highest LOD score (3.81) in  $F_8$  RILs (Table 1). The genotypes for Minsoy and Archer soybeans were determined using allele-specific DNA markers for the *E1*, *E2*, *E3*, and *E4* genes (Supplement Table S1). The genotypes for Minsoy and Archer are *E1e2e3E4* and *e1<sup>as</sup>e2E3E4*, respectively. The difference of the *E1* genotype between parents



**Fig. 2** Genetic map of RIL populations and QTL mapping for flowering time. **a** Genetic map of the MA RIL population constructed with HighMap software. Chr, chromosome. **b** QTLs of the

flowering time trait (R1) using 2017 data. **c** QTLs of the flowering time trait (R1) using the 2016 data

**Table 1** The QTLs for flowering time in the RIL population in 2016 and 2017

Year	QTL-LG	Position (marker/cM)	LOD	Pve%	Additive effect
2017	<i>qTOF6.1</i>	Block5536/100.17	3.81	9.8	-1.58
	<i>qTOF7</i>	Block6096/32.53	2.99	7.7	1.41
	<i>qTOF13</i>	Block11785/168.97	2.19	5.7	-1.21
	<i>qTOF5</i>	Block4452/162.19	2.15	5.6	-1.2
2016	<i>qTOF7</i>	Block5994 /26.33	3.1	8	1.86
	<i>qTOF6.2</i>	Block5361 /41.02	2.72	7.1	-1.75
	<i>qTOF6.1</i>	Block5536/100.17	2.56	6.7	-1.7
	<i>qTOF1</i>	Block535 /181.03	2.59	6.7	-1.7
	<i>qTOF5</i>	Block4452/162.19	2.31	6	-1.61

further indicates that the QTL on Chr 6 is the *E1* gene, which is consistent with the findings of Orf et al. (1999).

For the flowering time, another major QTL on Chr7 (*qTOF7*, named *E11*) was repeatedly identified on soybean Chr7 (Fig. 2b; Fig. 2C). This QTL accounted for 8% and 7.7% of the observed variation in F<sub>6</sub> and F<sub>8</sub> RILs, respectively, with the highest LOD scores (3.1 and 2.99, respectively; Table 1). Thus far, there are no known flowering time genes located on Chr7 in soybean. Therefore, *E11* is a new locus for the flowering time and should be subject to further characterization.

#### A single gene on *E11* locus

To map *E11* more precisely, eight Indel markers on Chr7 were used, contrasting between parents, were used on the basis of resequencing data (Supplement Table S2). The highest LOD scores (4.91 and 5.21) were detected near ID7088 in R<sub>6</sub> (2016) and ID7079 in R<sub>8</sub> (2017), implying that *E11* might locate between ID7079 and ID7088 that are 1.03 Mb apart. In F<sub>6</sub> and F<sub>8</sub>, this QTL accounted for 15.2% and 15.0% of the total phenotypic variance in the flowering time, respectively. The additive effect of the MA allele was 2.81 d in the F<sub>6</sub> population and 2.05 d in the F<sub>8</sub> population. Using these two markers, two RHL

families, #13 and #131, were found to be heterozygous at markers ID7088 and ID7079, which suggests that these two families might segregate at the *E11* locus. Family #13 possessed homozygous *E1* allele with 271 progenies and family #131 possessed homozygous *e1<sup>as</sup>* allele with 109 progenies (Supplement Table S1). In 2017, we grew the 271 plants of family #13 and 109 plants of family #131 in Harbin to test the segregation patterns of the flowering time and maturity, and both exhibited a bimodal distribution for the flowering time and maturing time (Supplement Figs. S1, S2).

The results revealed that the individuals of family #13 were clearly grouped into two groups: an early or intermediate flowering ( $n = 199$ ) group and a late-flowering ( $n = 72$ ) group. When they were separated at 43 DAE, the observed frequencies fit a monogenic 3:1 ratio ( $\chi^2 = 0.36$ ,  $p = 0.55$ ). A similar result was obtained for early maturity ( $n = 176$ ) and late maturity ( $n = 60$ ); the observed frequencies fit a 3:1 ratio ( $\chi^2 = 0.02$ ,  $p = 0.88$ ; Table 2). In the population of family #131, with separation at 41 DAE, plants were grouped into two groups: an early-flowering ( $n = 79$ ) group and a late-flowering ( $n = 30$ ) group. The observed frequencies fit a monogenic 3:1 ratio ( $\chi^2 = 0.37$ ,  $p = 0.54$ ). While the maturity was grouped into either early maturity ( $n = 79$ ) or late maturity ( $n = 26$ ) by separation at

**Table 2** Goodness of fit to a 3:1 ratio of days to flowering and maturity in two RHLs RHL families #13 and #131

Crosses	Number of plants	Phenotype	Early phenotype progenies	Late phenotype progenies	$\chi^2$	<i>P</i> value
#13	271	Flowering	199	72	0.36	0.55
	236	Maturity	176	60	0.02	0.88
#131	109	Flowering	79	30	0.37	0.54
	105	Maturity	79	26	0.003	0.96

**Table 3** Goodness of fit to a 1:2:1 ratio of days to flowering in two F<sub>3</sub> progeny derived from two RHLs#13 and #131

Code	Number of plants	Homozygous early flowering	Segregation	Homozygous late flowering	$\chi^2$	<i>P</i> value
F <sub>3</sub> -#13	236	59	121	56	0.23	0.89
F <sub>3</sub> -#131	105	27	53	25	0.09	0.96

127 DAE; the observed frequencies also fit a 3:1 ratio ( $\chi^2 = 0.003$ ,  $p = 0.96$ ; Table 2). These F<sub>2</sub> segregation tests demonstrate that the single *E11* locus was segregated for the flowering time and maturity in both families #13 and #131, implying that the dominant *E11* allele might condition the early flowering time and early maturity.

To confirm whether the single *E11* locus conditions early flowering and maturity, we further planted the F<sub>3</sub> progenies of #131 and #13 F<sub>2</sub> plants in the field in 2018 and evaluated their segregation patterns in the flowering time and maturity. The results revealed that in family #13, 59 of 236 rows showed homozygous early flowering, while 121 rows segregated for the flowering time, and another 56 rows were homozygous for late flowering. In family #131, 27 of 105 rows produced only early-flowering plants and were considered to be homozygous for the early-flowering allele, while 53 segregated for the flowering time, and another 25 were late-flowering plants and were considered to be homozygous for the late-flowering allele. These segregation ratios for families #13 and #131 fit a 1:2:1 ratio (F<sub>3</sub>-#13:  $n = 236$ ,  $\chi^2 = 0.23$ ,  $p = 0.89$ ; F<sub>3</sub>-#131:  $n = 105$ ,  $\chi^2 = 0.09$ ,  $p = 0.96$ ; Table 3). A similar result was observed in the maturing time as well and fits a 1:2:1 ratio (F<sub>3</sub>-#13:  $n = 236$ ,  $\chi^2 = 0.09$ ,  $p = 0.96$ ; F<sub>3</sub>-#131:  $n = 105$ ,  $\chi^2 = 0.31$ ,  $p = 0.85$ ; Table 4). These results strongly suggest that a single dominant gene, *E11*, controls early flowering time, and maturity under natural LD environments.

#### Fine-mapping of *E11* locus

According to the mapping results, *E11* was mapped between ID7079 and ID7088 on Chr7 (Fig. 3). To

define the *E11* gene, 11 polymorphic Indel markers (Supplement Table S4) were further genotyped to screen the recombinants from two families, #13 and #131. Based on the segregation patterns observed in the progenies of family #13, five recombinants were detected (#13-131-5, #13-249, #13-188, #13-121, and #13-67). Combined with the flowering time detected during 2017, which was confirmed based on the segregation patterns in the progeny during 2018, an interval between markers M3 and M8 was delimited (Fig. 4a). In family #131, four recombinants were found (#131-105, #131-20, #131-62, and #131-102). The flowering time and segregation patterns helped identify the interval between markers M1 and M7 (Fig. 4b). Integrated with the above intervals, *E11* was delimited to an interval of ~138 kb between markers M3 and M7 (Fig. 4c). Additionally, markers M4, M5, and M6 co-segregated with days to flowering. Overall, these results confirm that the candidate gene, *E11*, is located in a ~138 kb region.

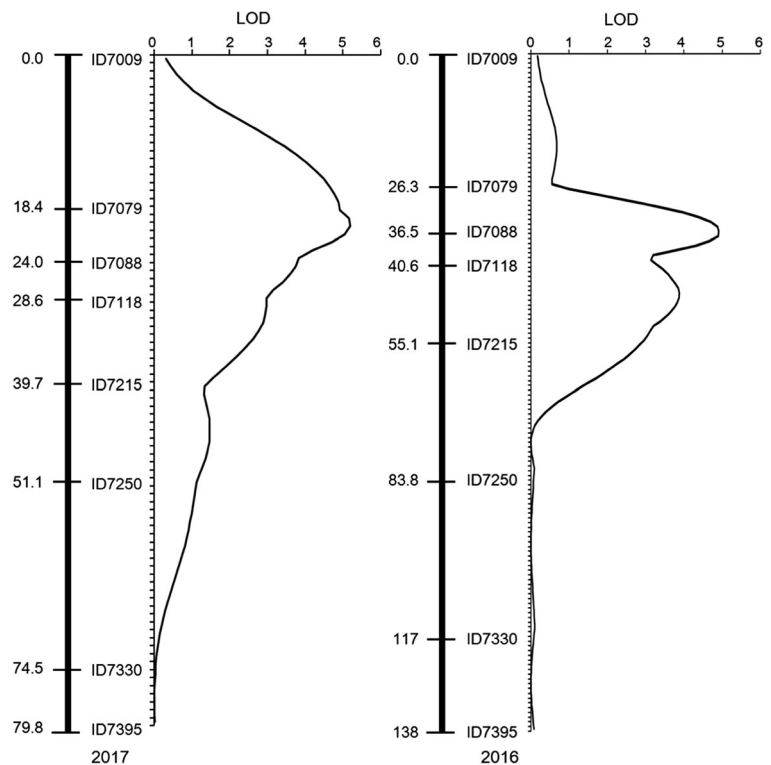
*Glyma.07 g4850*, *Glyma.07 g049000*,  
and *Glyma.07 g049100* as candidates for *E11*

According to the Williams, 82 genome sequence and 11 genes are located in ~138 kb region (Fig. 4c; Table 5). Specifically, there are three genes for O-methyltransferase I (*Glyma.07 g048700*, *Glyma.07 g048800*, and *Glyma.07 g048900*), one gene for pyrophosphorylase I (*Glyma.07 g048300*), one gene for protein kinase superfamily protein (*Glyma.07 g048400*), one gene for 2-oxoglutarate (2OG), and Fe(II)-dependent oxygenase superfamily protein (*Glyma.07 g048600*), one gene for

**Table 4** Goodness of fit to a 1:2:1 ratio of days to maturity in two F<sub>3</sub> progeny derived from two RHLs #13 and #131

Code	Number of plants	Homozygous early flowering	Segregation	Homozygous late flowering	$\chi^2$	<i>P</i> value
F <sub>3</sub> -#13	236	60	119	57	0.09	0.96
F <sub>3</sub> -#131	105	28	53	24	0.31	0.85

**Fig. 3** Genetic mapping of the *E11* locus. The *E11* gene was initially delimited near ID7088 or ID7079 using the  $F_6$  and  $F_8$  RIL populations in **a** 2016 and **b** 2017

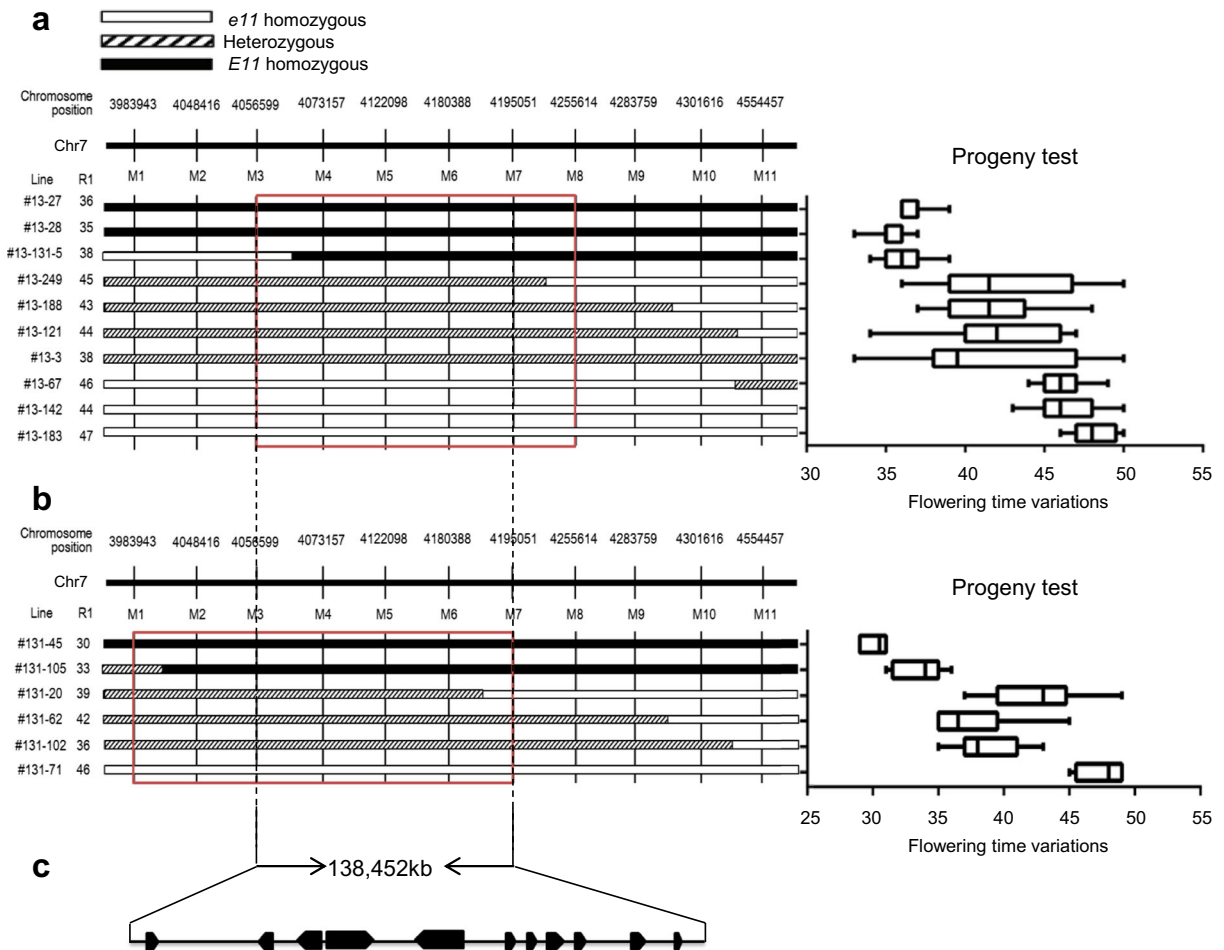


photosystem I P subunit (*Glyma.07 g049000*), one gene for metal tolerance protein A2 (*Glyma.07 g049200*), one gene encoding an AP2/B3-like transcriptional factor (*Glyma.07 g048200*), one gene for Homeodomain-like superfamily protein (*Glyma.07 g048500*), and one encoding basic helix-loop-helix (bHLH) DNA-binding superfamily protein (*Glyma.07 g049100*). According to the theory of transcriptional networks that mainly control the flowering time (Fornara et al. 2010; Rychel et al. 2019), *Glyma.07 g048300*, *Glyma.07 g048400*, *Glyma.07 g048600*, *Glyma.07 g048700*, *Glyma.07 g048800*, and *Glyma.07 g048900* do not participate in flowering regulation. Sequence analysis of the remaining five genes based on resequencing data of Minsoy and Archer, we found that *Glyma.07 g048500*, *Glyma.07 g049000*, and *Glyma.07 g049200* differed in CDS between the two parents. Therefore, *Glyma.07 g48500*, *Glyma.07 g049000*, and *Glyma.07 g049200* were considered as candidate genes of the *E11* locus. Functional characterization of these genes should be performed to further validate their functions on the soybean flowering time and maturity in future studies.

*E11* results in early flowering and maturity

We confirmed the association between the genotype at *E11* and the flowering time using NILs for the Minsoy allele (NIL-*E11*) and the Archer allele (NIL-*e11*). In all of the segregating families (namely,  $F_7$  and  $F_8$ ), the average flowering time trait was homozygous for the *E11* allele (such as #13–27 and #13–28) was earlier than those of plants homozygous for the *e11* allele (such as #13–142 and #13–183) in the yield, suggesting that the *E11* allele behaved as a dominant allele (Fig. 4). Two NILs derived from the heterozygous plant (#13–249 and #131–20) at *E11* (Supplementary Fig. S3). The flowering and maturity time under LD conditions were significantly different ( $p < 0.001$ ) between the NIL-*E11* and NIL-*e11* alleles in all sets of NILs tested; plants that were homozygous for the NIL-*E11* allele from Minsoy flowered on average 9–10 days earlier than those that were homozygous for the NIL-*e11* allele from archer in the field condition. The maturity date of NILs for *E11* allele was on average 12–15 day earlier than NILs for *e11* allele (Fig. 5). In conclusion, the results demonstrate that the NIL-*E11* allele significantly promoted earlier flowering time and maturity, confirming that *E11* plays a central role in flowering regulation.





**Fig. 4** Fine mapping of the *E11* gene. **a** Graphical genotypes of soybean recombinants carrying crossovers. White bars represent the homozygote for the *e11* allele from Archer, black bars represent the homozygote for the *E11* allele from Minsoy, and cross-hatched bars represent the heterozygote. Fine mapping of *E11* gene on Chr7 using the recombinants from #13. Five recombinants (#13-131-5, #13-249, #13-188, #13-121 and #13-67), and four non-recombinant control plants were genotyped at 11 Indels. Chromosome positions of markers were from the soybean reference genome, indicated at the top of the markers. Days to flowering of the recombinants were evaluated in the LD conditions. The phenotypic segregation of family #13 is shown in right

boxplot format. The interquartile region, median, and range are indicated by the box, the bold vertical line, and the horizontal line, respectively. The *E11* gene was delimited between M3 and M8 on Chr7 (red frame). **b** Fine mapping of the *E11* gene on Chr7 using the recombinants from family #131. The information of chart is as above Fig. 4a. With  $F_2$  recombinants from family #131, we were able to delimit the *e11* gene between M1 and M7 in Chr7. **c** The *E11* gene was located between M3 and M7 in Chr7 according to Fig. 4a and Fig. 4b. Eleven open reading frames (arrows) were predicted in a genomic region of ~138 kb delimited between M3 and M7

## Discussion

In this study, we described the molecular dissection of an important QTL (named *E11*), for the flowering time under LD conditions by the cross between early-maturing soybean cultivars. Genetic analysis of the flowering time in residual heterozygous lines (#13 and #131) segregated only for *E11* and possessed the homozygous *E1* genotype, revealing that *E11* controls the

flowering time and maturity regardless of *E1*. As is known, *E1* has the largest effect on soybean flowering under LD conditions (Cober et al. 1996; Liu et al. 2007; Xia et al. 2012; Tsubokura et al. 2014).

Minsoy and Archer soybeans were used as parents in this study, and the *E1* genotype of the two parents is different (Supplement Table S1), but their flowering time has no obvious difference (Fig. 1a), which suggests that there may be undiscovered genes that regulate the

**Table 5** Predicted genes within ~138 kb region of *E11* locus in the reference Willimas 82 sequence

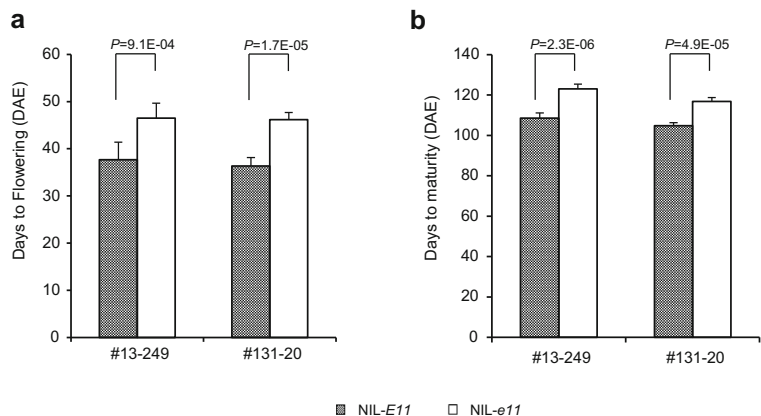
Genes and markers	Chromosome position	Gene ID	Arabidopsis ortholog gene	Phytozome functional annotations
Gene1	4,061,864–4,064,513	Glyma.07G048200	AT2G46870.1	AP2/B3-like transcriptional factor family protein
Gene2	4,086,892–4,090,342	Glyma.07G048300	AT1G01050.1	pyrophosphorylase 1
Gene3	4,096,301–4,102,195	Glyma.07G048400	AT3G61960.1	Protein kinase superfamily protein
Gene4	4,102,968–4,114,174	Glyma.07G048500	AT1G01060.4	Homeodomain-like superfamily protein
Gene5	4,123,806–4,139,591	Glyma.07G048600	AT4G10490.1	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
Gene6	4,146,598–4,148,813	Glyma.07G048700	AT5G54160.1	O-methyltransferase 1
Gene7	4,151,161–4,153,449	Glyma.07G048800	AT5G54160.1	O-methyltransferase 1
Gene8	4,156,669–4,160,634	Glyma.07G048900	AT5G54160.1	O-methyltransferase 1
Gene9	4,162,544–4,164,995	Glyma.07G049000	AT2G46820.1	photosystem I P subunit
Gene10	4,176,470–4,179,911	Glyma.07G049100	AT2G46810.1	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
Gene11	4,187,619–4,192,503	Glyma.07G049200	AT3G58810.1	metal tolerance protein A2

flowering time. The RIL population of 171 individuals was derived from crossing Minsoy and Archer soybeans in order to clone new genes for the flowering time. Although the two parents were early-maturing soybean cultivars, significant transgression segregations were observed in RILs outside of the parents over the course of 2 years (Fig. 1c, d). The F<sub>6</sub> and F<sub>8</sub> populations showed a continuous and normal distribution, which indicates that more than two genes control the flowering time in this RIL population. Using SLAF-genetic mapping and the corresponding flowering dates, we mapped three stable QTLs (*qTOF5*, *qTOF6.1*, and *qTOF7*) on Chr5, 6, and 7, one QTL (*qTOF13*) on Chr13 during 2017, and two QTLs (*qTOF1* and *qTOF6.2*) on Chr1 and Chr6 during 2016 (Table 1; Fig. 2). These flowering time loci will contribute to a better understanding of

flowering time regulation in soybeans. Additionally, these results suggest that the crosses did not limit parents with large differences in phenotypes when a population was developed.

Of these QTLs, *qTOF6.1* and *qTOF7* were also reported on by Orf et al. (1999), while other QTLs (*qTOF1*, *qTOF5*, *qTOF6.1*, and *qTOF13*) were not detected. Flowering time QTLs in the same region as the major QTL, *qTOF6.1*, was previously detected in many studies, which accounted for the higher ratio of the observed variation in different populations (Mansur et al. 1993; Orf et al. 1999; Githiri et al. 2007). The region near *qTOF6.1* also harbors the *E1* gene. Combined with the difference of *E1* genotype between parents, we further revealed that *qTOF6.1* was *E1* gene. This finding of *E1* implies that our RIL population could

**Fig. 5** Phenotypes of the NIL-*E11* and NIL-*e11* under LD conditions: **a** flowering time and **b** maturity



be further researched in order to study other new genes that may also control flowering time.

The *qTOF7* locus was called *E11*, which is the second major site other than *E1* that largely influenced flowering time in the MA population. The gene symbol of *E11e11* had been approved by the soybean genetic committee. This QTL was consistently detected in different populations (Orf et al. 1999; Liu et al. 2018), indicating its importance in regulating soybean flowering time. Hence, this QTL was selected for detailed analysis in the present study. Based on the vital role of *E1* under LD conditions, two RHLs were selected for cloning the *E11* gene; family #13 had the *E1* allele and family #131 had the *e1<sup>as</sup>* allele, which aimed to remove the effect of *E1* on flowering time.

The segregation patterns observed in RHLs for *E11* revealed that early flowering was controlled by a single dominant gene. The gene was fine-mapped to an ~138 kb interval. In this region, there are 11 annotated genes, including six genes for function enzymes (*Glyma.07 g048300*, *Glyma.07 g048400*, *Glyma.07 g048600*, *Glyma.07 g048700*, *Glyma.07 g048800*, and *Glyma.07 g048900*), three genes encoding transcription factors (*Glyma.07 g048200*, *Glyma.07 g048500*, and *Glyma.07 g049100*), one gene encoding photosystem I P subunit (*Glyma.07 g049000*), and one gene encoding metal tolerance protein A2 (*Glyma.07 g049200*) (Table 5). Through amino acid sequence analysis, three candidate genes were identified: *Glyma.07 g48500*, the best BLAST hit to LATE ELONGATED HYPOCOTYL (*LHY*); *Glyma.07 g049000*, a homologous gene of *CURVATURE THYLAKOID 1B* (*CURT1B*); and *Glyma.07 g49200*, an ortholog of metal tolerance protein 3 (MTP3) in *Arabidopsis*.

Previous studies on homologous genes suggest that *Glyma.07 g48500* is most likely a candidate gene for *E11*. In *Arabidopsis*, loss-of-function mutant, *lhy-1*, flowered earlier than wild-type plants under SD and LD conditions, exhibiting an effect independently of photoperiod (Schaffer et al. 1998; Spensley et al. 2009). Meanwhile, *LHY*, *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), and *TIMING OF CAB EXPRESSION1* (*TOC1*) formed core oscillator of the circadian clock (Alabadi et al. 2001; Cao et al. 2017; Xu et al. 2018). As one of the core members in regulating circadian clock, mutation of *LHY* disrupted circadian clock regulation of gene expression and leaf movements, causing dwarfing of plants and reduction in yields (Mizoguchi et al. 2002). These

investigations suggest that *LHY* in soybeans may be important in the regulation of flowering time and maturity. However, the *CURT1B* protein was previously located in thylakoids and designated as a “thylakoid membrane phosphoprotein of 14 kD” (TMP14), which is a novel submit of plant photosystem I (Hansson and Vener 2003; Armbruster et al. 2013). Additionally, the MTP3 protein contributes to basic cellular zinc tolerance and controls zinc partitioning, particularly under conditions with high rates of zinc influx into the root symplasm. Moreover, MTP3–GFP fusion proteins are localized to the vacuolar membrane when expressed in *Arabidopsis* (Arrivault et al. 2006).

As an additional effort to examine the specific effects of *E11*, we compared phenotypes of the two NILs with a separation of genotypes between M3 and M7 (Supplement Fig. S3). The results showed that NIL-*E11* plants significantly promoted flowering time and maturity as opposed to NIL-*e11*, confirming that the *E11* gene could greatly control flowering time and maturity in soybeans under LD conditions (Fig. 5). In previous studies, *E11* was reported to be related to flowering time and maturity date, as well as yield-related traits, such as seed weight and seed number (Orf et al. 1999; Wang et al. 2004; Panthee et al. 2007; Han et al. 2012). Meanwhile, we found that the yield and grains of NIL-*E11* plants were lower than NIL-*e11* plants (data not presented). As is known, many major genes and QTLs for flowering time often influence agronomic traits other than flowering time and maturity, such as plant height and yield (Cober and Morrison 2010). Taken together, *E11* was characterized as having a major role in the regulation of flowering time and maturity, which can be important for soybean breeding.

For the flowering time, dominant alleles at *E6*, *E9*, and *J* promote early flowering (Kong et al. 2014; Li et al. 2017). This study demonstrated that *E11* was similar to *E9*, such that the dominant allele promoted early flowering under LD conditions. Additionally, *J* and *E6* were characterized as having a role in the promotion of flowering under induced SD conditions, differing from *E11*, which promotes flowering under LD conditions. Further research is needed to confirm the effects of *E11* on the regulation of flowering time. The new early flowering gene, *E11*, will prove useful for the molecular breeding of early maturity and the stable productivity of soybean under high latitude environments.

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#### Compliance with ethical standards

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

**Ethical standards** The authors declare that this study complies with the current laws of the countries in which the experiments were performed.

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