**Quantitative Trait Locus Mapping of Soybean Maturity Gene E6**

Xiaoming Li, Chao Fang, Meilan Xu, Fengge Zhang, Sijia Lu, Haiyang Nan, Tong Su, Shichen Li, Xiaohui Zhao, Lingping Kong, Xiaohui Yuan, Baohui Liu,* Jun Abe,* Elroy R. Cober,* and Fanjiang Kong*

**ABSTRACT**

Soybean *Glycine max* (L.) Merr. sensitivity to photoperiod determines adaptation to a specific range of latitudes for soybean cultivars. When temperate-adapted soybean cultivars are grown in low latitude under short day conditions, they flower early, resulting in low grain yield, and consequently limiting their utility in tropical areas. Most cultivars adapted to low-latitude environments have the trait of delayed flowering under short day conditions, and this trait is commonly called long juvenile (LJ). In this study, the *E6* locus, the classical locus conditioning the LJ trait, was molecularly mapped on Gm04 near single-nucleotide polymorphism marker HRM101. Testcross, genetic mapping, and sequencing suggest that the *E6* and *J* loci might be tightly linked. Genetic interaction evaluation between *E6* and *E1* suggests that *E6* has a suppressive effect on *E1* and that the function of *E6* is dependent on *E1*. The tagging markers for *E6* are very useful for molecular breeding for wide adaptation and stable productivity of soybean under low-latitude environments. Molecular identification and functional characterization of the *E6* gene will greatly facilitate the understanding of the genetic and molecular mechanisms underlying the LJ trait.

**Soybean** *Glycine max* (L.) Merr. is an important crop for human consumption, for animal feed, and for use as biodiesel fuel. Time to flowering and maturity significantly affects soybean adaptation and grain yield (Cober and Morrison, 2010). Soybean is a short day (SD) plant, and SD results in early flowering, whereas long day (LD) delays flowering (Watanabe et al., 2012). Soybean, however, can grow over a wide range of latitudes, at
least from 50° N to 35° S (Cao et al., 2016). The wide adaptability of soybean has been created by natural variation in the major genes and quantitative trait loci (QTLs) controlling flowering time (Kong et al., 2014). In soybean, 11 maturity loci (E1–E10 and J) that control flowering time and maturity have been previously identified and characterized at the phenotypic and genetic levels (Bernard, 1971; Buzzell, 1971; Buzzell and Voldeng, 1980; McBlain and Bernard, 1987; Ray et al., 1995; Bonato and Vello, 1999; Cober and Voldeng, 2001; Cober et al., 2010; Kong et al., 2014; Samanfar et al., 2016). Among them, E1 was cloned by a map–based approach and assumed to be a legume–specific transcription factor that has a putative nuclear localization signal and a B3 distantly related domain (Xia et al., 2012); E2 was identified as an ortholog of the Arabidopsis GIGANTEA gene (Watanabe et al., 2011); and E3 and E4 were confirmed as PHYA homologs by a map–based cloning (Watanabe et al., 2009) and a candidate gene approach (Liu et al., 2008), respectively. The various allelic combinations at the E1, E3, and E4 loci condition soybean flowering time, as well as preflowering and postflowering photoperiod responses, and greatly contribute to the wide adaptability in soybean (Tsubokura et al., 2013; Xu et al., 2013; Jiang et al., 2014). In addition, two FLOWERING LOCUS T homologs, GmFT2a and GmFT5a, are involved in the transition to flowering, and these two FT homologs coordinately control flowering in soybean (Kong et al., 2010; Nan et al., 2014). The maturity genes E1, E2, E3, and E4 downregulate GmFT2a (E9) and GmFT5a expression to delay flowering and maturation under the LD condition, suggesting that GmFT2a and GmFT5a are the soybean flowering integrators and the major targets in the control of flowering (Kong et al., 2010; Thakare et al., 2011; Watanabe et al., 2011; Xia et al., 2012, Nan et al., 2014).

Soybean sensitivity to photoperiod determines the limits of the sowing period for a specific latitude and prevents adaptation to wider ranges of latitude. When soybean cultivars are grown under SD conditions, cultivars with sensitivity to photoperiod flower early, resulting in low grain yield and consequently limiting their growing area in SD environments (Destro et al., 2001). It is therefore important to understand the genetic control of delayed flowering time under SD environments. This trait was termed the long juvenile (LJ) trait (Parvez and Gardner, 1987; Sinclair and Hinson, 1992; Ray et al., 1995). The LJ trait, which delays flowering under SD conditions, has been identified in tropical soybean cultivars. The introduction of the LJ characteristic in soybean has made its cultivation possible in regions with latitudes lower than 15° S (Destro et al., 2001). The LJ trait plays a pivotal role in extending the range of adaptation of soybean cultivars to lower latitudes and to new management schemes with shifted sowing dates in tropical countries (Destro et al., 2001). It has been reported that the northward expansion of soybean production in South America, where more extensive research has been performed, is dependent on the LJ trait (Spehar, 1995). However, the genetic control mechanism for this trait remains elusive. Two genes, J and E6, were reported to play an important role in the LJ trait (Ray et al., 1995; Bonato and Vello, 1999). The single locus J has been identified in a number of crosses with PI 159925 (Ray et al., 1995). The single locus E6 was a natural mutation in cultivar Parana and produced the LJ cultivars Paranagoiana and SS-1 (Bonato and Vello, 1999). Recently, an F3 population resulting from a cross between conventional juvenile (CJ) line OT94-47 and the LJ line Paranagoiana exhibited a 15:1 early/late flowering ratio in 12-h photoperiods. A similar 15:1 ratio was observed in offspring of a cross between CJ line OT94-47 and the LJ line PI 159925 (Cober, 2011). These results suggested that the LJ trait is conditioned by two recessive alleles in PI 159925 and Paranagoiana (Cober, 2011). Other studies of LJ parents also suggested that recessive alleles at two or three loci control the LJ trait (Carpentieri-Pipolo et al., 2000, 2002). The J gene has been mapped to the soybean linkage group Gm04 between the simple sequence repeat (SSR) markers Sat_337 and Satt396, where the genetic distance between the J allele and the closest marker, Sat_337, is 0.7 cM (Cairo et al., 2002, 2009). Recently, J had been molecularly identified as an orthologue of Arabidopsis EARLY FLOWERING 3 (ELF3), and natural variations at J locus improved soybean adaptation in low-latitude regions and enhanced soybean yield (Yue et al., 2016; Lu et al., 2017). J depends genetically on the legume–specific flowering repressor E1, and J protein physically associates with the E1 promoter to downregulate its transcription, relieving repression of two important FT genes and promoting flowering under SD. (Lu et al., 2017). Using a different LJ cultivar from Thailand, a new major QTL, qFT-J2, conditioning the LJ trait was identified and mapped on Gm16, where the flowering gene GmFT2a is located, but the QTL near the J locus was not detected (Lu et al., 2015). This suggested that different genes and QTLs condition the LJ trait in different genetic backgrounds from different geographical regions.

Although variation in the J gene clearly plays important role in conferring the LJ trait and has been widely deployed in several major soybean production regions, the existence of many late-flowering lines from low-latitude regions that carry an apparently functional J allele suggests that, on a global scale, it is not the only locus responsible for this trait (Lu et al., 2017). The E6 locus, another classical LJ locus, plays important roles for soybean adaptation and yield improvement in low-latitude regions (Bonato and Vello 1999). In spite of the importance of the E6 for soybean adaptation and yield productivity in tropical regions, the genetic information regarding E6 is very
MATERIALS AND METHODS
Genetic Populations and Growth Conditions
To map the E6 locus, an F2 population (hereafter named PGH) of the cross between LJ line Paranagoiana (e6e6, PI 628880) and CJ line Harosoy (E6E6, PI 548573) was developed. An F2 population (hereafter named PGO) from a cross between Paranagoiana and OT94–47, developed previously, was also used for E6 mapping (Cober, 2011). To understand the genetic effect between J and E6 loci, a F3 population (hereafter named PGI) from the cross between two LJ lines, Paranagoiana (e6e6) and PI 159925 (jj), was developed.

The F2 populations PGH and PGI and the parental lines were sown in pots in growth cabinets under SD conditions (12 h light and 12 h dark). Each pot contained four plants for the two F2 populations. Days to flowering were recorded at the R1 stage (first open flower appeared) for each plant (Fehr et al., 1971). The R1 values reported for the three parents Paranagoiana, PI 159925, and Harosoy are the means from five plants. The R1 data from the cross of PGO were reported previously (Cober, 2011).

Molecular Analysis
DNA was extracted individually from leaves of plants, as described by Kong et al. (2010). Simple sequence repeat markers were selected from those designed and mapped by Cregan et al. (1999). Insertion and deletion (Indel) markers and single-nucleotide polymorphism (SNP) markers (Table 1) were developed in this study according to resequencing data from the parents Harosoy, PI 159925, and Paranagoiana. The SNP markers were developed and detected by the high-resolution melting (HRM) approach (Li et al., 2010). Briefly, HRM SNP marker analysis contained two rounds of polymerase chain reaction (PCR) amplification, the first round of PCR is to amplify the specific fragment containing the SNP site, and the second round of PCR was run in a LightScanner HR196 (Idaho Technology) to detect the SNP signal (Li et al., 2010). The whole-genome resequencing of Harosoy, PI 159925, and Paranagoiana and the Indel analysis using the software of SOAPIndel was conducted by BGI-Shenzhen, China, as described previously (Kong et al., 2014). The procedures for PCR and gel electrophoresis followed a standardized procedure, as reported earlier (Kong et al., 2014; Lu et al., 2016). Marker order and distance were determined by Map Manager Program QTxml (Lu et al., 2015) using the Kosambi function and a criterion of 0.001 probability (df = 1), and a genetic map was constructed. The multiple QTL model from MapQTL 5.0 (Van Ooijen, 2004), interval mapping from QTL IciMapping (Meng et al., 2015), and composite interval mapping 3.0 from Windows QTL Cartographer 2.5 (Wang et al., 2012) were used for QTL detection. A logarithm of odds (LOD) score of 3.0 was used as a minimum to declare the significance of a QTL in a particular genomic region. One thousand permutations at a 0.05 probability were conducted to identify the genomewide LOD score (Churchill and Doerge, 1994). Sequencing of E1 and J genes was performed according to previous reports (Xia et al., 2012; Lu et al., 2017). Genotyping of e1e1 and e1e0 alleles using their functional markers was conducted as described previously (Xu et al., 2013; Jiang et al., 2014).

RESULTS AND DISCUSSION
To study the E6 locus, two F2 populations named PGH and PGO from a cross between a Brazilian LJ cultivar, Paranagoiana (e6e6, Table 2), in which the E6 gene was originally identified (Bonato and Vello, 1999) and two CJ lines, Harosoy (E6E6) and OT94–47 (E6E6), (Cober, 2011) were used. Under SD conditions (12 h light and 12 h dark), large variations in flowering time (R1 stage) were observed in both F2 populations (Table 3). The variations of flowering time ranged from 27 to 61 d after emergence (DAE) in PGH and from 25 to 54 DAE in PGO (Table 3). We next constructed linkage maps of both crosses using different molecular markers (Table 1, Supplemental Table 1). In the F2 population of PGH, 162 polymorphic markers were identified between parents Paranagoiana and Harosoy. Twenty linkage groups were constructed and covered the genetic length of 1681 cM (Table 1). In the F2 population of PGO, 216 polymorphic markers were used to construct 20 linkage groups, which covered 2364 cM genetic length (Table 1). We used three approaches—multiple QTL mapping from MapQTL 5.0, interval mapping from QTL IciMapping, and composite interval mapping from WinQTL cartographer—to conduct whole–chromosome scans to identify consensus QTLs. In the F2 population of PGH, a major QTL conditioning flowering time under SD, qFT-C1, located on chromosome 4 (Gm04), was consistently identified by all three methods (Fig. 1a, Table 4). The same major QTL of qFT-C1 was also consistently identified by all three methods in the second F2 population, PGO (Fig. 1b, Table 4). In addition, a second QTL, qFT-C2, coinciding with E1, a major soybean maturity locus (Xia et al., 2012), was consistently detected in both crosses PGH and PGO (Fig. 1a

Table 1. Linkage groups obtained from four soybean F2 populations.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Population names</th>
<th>No. of F2 plants</th>
<th>No. of linked markers†</th>
<th>No. of linkage group</th>
<th>Total map length cM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paranagoiana × Harosoy</td>
<td>PGH</td>
<td>184</td>
<td>162</td>
<td>20</td>
<td>1681</td>
</tr>
<tr>
<td>Paranagoiana × OT94-47</td>
<td>PGO</td>
<td>58</td>
<td>216</td>
<td>20</td>
<td>2364</td>
</tr>
<tr>
<td>Paranagoiana × PI 159925</td>
<td>PGI</td>
<td>126</td>
<td>163</td>
<td>20</td>
<td>1987</td>
</tr>
</tbody>
</table>

† Markers for linkage map construction are listed in Supplemental Table 1.
Table 2. Soybean materials used in this study.

<table>
<thead>
<tr>
<th>Line name</th>
<th>Line ID</th>
<th>Genotype</th>
<th>Pedigree</th>
<th>Flower time (12 h light/12 h dark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paranagoiana</td>
<td>PI 628880</td>
<td>E1, E2, E3, E4, e6</td>
<td>Natural mutation from cultivar Parana</td>
<td>62a†</td>
</tr>
<tr>
<td>PI 159925</td>
<td>PI 159925</td>
<td>E1, E2, E3, E4, j</td>
<td>Landrace collected from Peru</td>
<td>47b</td>
</tr>
<tr>
<td>Harosoy</td>
<td>PI 548573</td>
<td>e1h, e2, E3, E4, J, E6</td>
<td>Mandarin (Ottawa) × 2/AK (Harrow)</td>
<td>28c</td>
</tr>
<tr>
<td>OT97-47</td>
<td>–</td>
<td>e1h, e2, e3, e4, J, E6</td>
<td>OT89-5/X2749-K1</td>
<td>27c</td>
</tr>
</tbody>
</table>

† Means within a column followed by the same letter are not significantly different (P = 0.01) according to Tukey’s honestly significant difference test.

Table 3. Statistical analysis of the flowering times of F₂ populations in short day environments.

<table>
<thead>
<tr>
<th>Population†</th>
<th>Flowering time (d after emergence)</th>
<th>Kurtosis‡</th>
<th>Skewness§</th>
<th>Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>PGH</td>
<td>27</td>
<td>61</td>
<td>36.6 ± 9.3a¶</td>
<td>−0.16</td>
</tr>
<tr>
<td>PGO</td>
<td>25</td>
<td>54</td>
<td>30.2 ± 6.9b</td>
<td>4.37</td>
</tr>
<tr>
<td>PGI</td>
<td>45</td>
<td>71</td>
<td>58.5 ± 6.2c</td>
<td>0.48</td>
</tr>
</tbody>
</table>

† PGH, Paranagoiana × Harosoy; PGO, Paranagoiana × OT94-47; PGI, PI 159925 × Paranagoiana.
‡ Kurtosis of the phenotypic trait.
§ Skewness of the phenotypic trait.
¶ Different lowercase letters (a, b, and c) indicate the extremely significant differences in short day environments (P < 0.01).

Fig. 1. Quantitative trait locus (QTL) mapping by multiple QTL mapping, implemented by MapQTL 5.0. Whole-chromosome scan of QTLs in three F₂ populations: (a) Paranagoiana × Harosoy, (b) Paranagoiana × OT94-47, and (c) PI 159925 × Paranagoiana. Red lines indicated the threshold of QTL detection. The detailed QTL information is indicated in Table 4. LOD, logarithm of odds.
and 1b, Table 4) under SD conditions. Only two major QTLS, qFT-C1 and qFT-C2, were consistently identified from two crosses, PGH and PGO, in which E6 and E1 loci were segregating. Sequencing of E1 genes in Paranagoiana and OT94-47 showed that Paranagoiana possesses the dominant allele of E1, whereas OT94-47 possesses the loss of function allele e6 and Harosoy possesses the recessive weak allele e6w (Xia et al., 2012) (Fig. 2). These results suggested that the QTL qFT-C2 corresponds to the E1 locus in both F2 populations PGH and PGO (Fig. 1a and 1b, Table 4). We therefore consider that the major QTL qFT-C1 on Gm04 conditioning flowering time under SD corresponds to the E6 locus.

Previously, the J locus had been mapped on Gm04 between SSR markers Sat_337 and Sat396 (Cairo et al., 2009; Yue et al., 2016; Lu et al., 2017), and the E6 locus was mapped in the same position in two F2 populations, PGH and PGO, in this study. This raised the question whether J and E6 are the same gene. To investigate this question, we generated a F2 population (named PGI) from a cross between Paranagoiana (e6e6) and PI 159925 (j), in which the J allele was original identified (Ray et al., 1995). The flowering time in F2 population PGI under SD segregated from 45 to 71 DAE (Table 3). We also generated 20 linkage groups by integrating 163 polymorphic markers in the F2 population PGI (Table 1, Supplemental Table 1).

Whole-chromosome QTL scans by the above mentioned three methods identified the same major QTL qFT-C1 located in Gm04 (Fig. 1c, Table 4). The LOD scores were from 5.61 to 7.77, and the allele from Paranagoiana had an additive effect of 4.26 to 8.86 over that from PI 159925 (Table 4), which suggests that the allele from Paranagoiana delayed flowering under SD condition, in contrast with the allele from PI 159925. Since E6 and J loci were comapped in the same position on Gm04 and J was the Arabidopsis flowering gene ELF3 (Yue et al., 2016; Lu et al., 2017), this suggests that E6 might be the J (ELF3) gene. We therefore sequenced the J gene in Paranagoiana, but there are no sequence polymorphisms between Paranagoiana and Harosoy. These results suggested that E6 and J might be different genes but tightly linked together. In addition, a second QTL qFT-D1b on Gm02 conditioning the LJ trait was also identified by QTL IciMapping (Table 4), confirming that the LJ trait is a quantitative trait and conditioned by multiple loci (Carpentieri-Pipolo et al., 2000, 2002). Identification of the responsible genes of E6 and qFT-D1b loci will facilitate the understanding of molecular mechanisms underlying LJ trait.

E1 is the legume-specific transcription factor and is the core soybean flowering suppressor that downregulates two soybean FLOWERING LOCUS T genes, FT2a (E9) and FT5a (Xia et al., 2012). Two major QTLs for

Table 4. Identification of main-effect quantitative trait loci (QTLs).

<table>
<thead>
<tr>
<th>Model†</th>
<th>Population‡</th>
<th>QTL name</th>
<th>Chromosome</th>
<th>Marker or interval§</th>
<th>Position¶</th>
<th>LOD#</th>
<th>R²††</th>
<th>A‡‡</th>
<th>Threshold§§</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQM</td>
<td>PGH</td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>HRM101–ID04101</td>
<td>30.4</td>
<td>30.38</td>
<td>68.80</td>
<td>9.49</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qFT-C2</td>
<td>Gm06</td>
<td>E1</td>
<td>28.5</td>
<td>3.76</td>
<td>21.25</td>
<td>4.18</td>
<td></td>
</tr>
<tr>
<td>PGO</td>
<td></td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>HRM101–ID04106</td>
<td>26.2</td>
<td>5.73</td>
<td>37.10</td>
<td>5.98</td>
<td>3.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qFT-C2</td>
<td>Gm06</td>
<td>E1</td>
<td>56.6</td>
<td>3.27</td>
<td>25.30</td>
<td>4.46</td>
<td></td>
</tr>
<tr>
<td>PGI</td>
<td></td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>ID04090–ID04101</td>
<td>31.5</td>
<td>5.61</td>
<td>45.40</td>
<td>8.86</td>
<td>2.96</td>
</tr>
<tr>
<td>IM</td>
<td>PGH</td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>HRM101–ID04101</td>
<td>32.0</td>
<td>37.02</td>
<td>51.60</td>
<td>9.67</td>
<td>8.71</td>
</tr>
<tr>
<td></td>
<td>PGH</td>
<td>qFT-C2</td>
<td>Gm06</td>
<td>E1-ID06095</td>
<td>29.0</td>
<td>13.22</td>
<td>17.04</td>
<td>3.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGO</td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>BAR04007–HRM101</td>
<td>18.8</td>
<td>16.32</td>
<td>44.67</td>
<td>9.37</td>
<td>4.13</td>
</tr>
<tr>
<td></td>
<td>PGO</td>
<td>qFT-C2</td>
<td>Gm06</td>
<td>E1</td>
<td>59.2</td>
<td>5.94</td>
<td>27.14</td>
<td>5.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGI</td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>ID04101–ID04134</td>
<td>40.5</td>
<td>7.50</td>
<td>44.90</td>
<td>4.26</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>PGI</td>
<td>qFT-D1b</td>
<td>Gm02</td>
<td>ID02182–ID02230</td>
<td>58.0</td>
<td>4.94</td>
<td>13.47</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>CIM</td>
<td>PGH</td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>HRM101–ID04101</td>
<td>27.2</td>
<td>16.30</td>
<td>55.10</td>
<td>8.22</td>
<td>4.62</td>
</tr>
<tr>
<td></td>
<td>PGH</td>
<td>qFT-C2</td>
<td>Gm06</td>
<td>E1-ID06095</td>
<td>29.7</td>
<td>5.87</td>
<td>26.45</td>
<td>3.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PGO</td>
<td>qFT-C1</td>
<td>Gm04</td>
<td>ID04101–ID04106</td>
<td>29.2</td>
<td>16.29</td>
<td>52.10</td>
<td>8.62</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>PGO</td>
<td>qFT-C2</td>
<td>Gm06</td>
<td>E1-ID06106</td>
<td>62.9</td>
<td>7.13</td>
<td>18.68</td>
<td>3.71</td>
<td></td>
</tr>
</tbody>
</table>

† Identification of QTLs by multiple QTL mapping (MQM), interval mapping (IM), and composite interval mapping (CIM), implemented by MapQTL 5.0, QTL IciMapping, and WinQTLcart, respectively.
‡ PGH, Paranagoiana × Harosoy; PGO, Paranagoiana × OT94-47; PGI, PI 159925 × Paranagoiana.
§ Marker or interval: Markers or support intervals on the linkage map in which the LOD is the largest.
¶ Position: The LOD peak for candidate QTL on the genetic linkage map.
# LOD, logarithm of odds.
†† Percentage of phenotypic variance explained by the QTL.
‡‡ The additive effects contributed by QTL. A positive value (+) of the additive effect indicates that the allele originated from the female; a negative value (−) of the additive effect indicates that the allele originated from the male.
§§ Significance at 0.05 probability by 1000 permutation tests.
flowering time corresponding to E6 and E1 loci under SD were consistently identified from two crosses in PGH and PGO (Table 4). To understand the genetic effects between QTLs of E6 and E1, we classified the allelic combinations using the tagging marker HRM101 of E6 and E1 functional markers in the two F2 populations PGH and PGO (Fig. 3). In the population PGH, in homozygous dominant E6E6 lines, there were no flowering time differences whether the E1 allele was dominant or recessive, which suggested that E6 had a suppressive effect on E1 (Fig. 3a). In homozygous recessive e6e6 lines, the partial loss of function allele e1 as reduced the suppressive effect of E6 on E1, which suggested that E6 is dependent on E1 (Fig. 3a). The suppressive effect of E6 on E1 on flowering

**Fig. 2.** Sequence alignment of E1 in Paranagoiana, Harosoy, and OT94-47. Rectangular windows indicate different E1 alleles, the e1ns allele (G44C) and e1fs allele (A46-).

**Fig. 3.** Allelic effects on flowering time of quantitative trait loci (QTLs) of E6 and E1 in two F2 populations, (a) PGH (Paranagoiana × Harosoy) and (b) PGO (Paranagoiana × OT94-47). Allelic combinations of E6 and E1 loci are indicated in each column. The numbers indicate the plants tested for each allelic combination of E6 and E1. The genotypes of the E6 allele were analyzed by tagging marker HRM101 (Supplemental Table 1). The E1 allele was genotyped by its functional markers, E1-dCAPs. DAE, days after emergence.
time was further confirmed in the F_2 population PGO (Fig. 3b). In the homozygous loss of function eFeF lines, E6 completely lost an effect on flowering time control, suggesting that E6 is fully dependent on E1 function (Fig. 3b). Molecular cloning of the E6 gene will further facilitate understanding the regulatory relationships between E6 and E1 and their molecular mechanisms controlling flowering time and the LJ trait.

In summary, the maturity gene E6 was molecular mapped on Gm04 adjacent to marker HRM101. The mapping results indicate that E6 and J genes might be the two genes that are tightly linked. The E1 gene has a role in control of flowering time under SD conditions. We found that E1 has an epistatic effect on E6 and that E6 has a suppressive effect on E1. The two F_2 populations of PGH and PGI will be selfed to advanced generations, and appropriate populations such as heterogeneous inbred family will be selected from residual heterozygous lines using the closest markers of E6 for positional cloning of E6. Molecular identification and functional characterization of the E6 gene will greatly facilitate understanding of the genetic and molecular mechanisms underlying the LJ trait. The markers for E6 are very useful for molecular breeding for wide adaptation and stable productivity of soybean under low-latitude environments.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

We thank Randall L. Nelson (Pathology and Genetics Research Unit and Department of Crop Sciences, Soybean/Maize Germplasm, USDA-ARS, University of Illinois, Urbana, IL) for providing some soybean germplasm. This work was funded by the National Natural Science Foundation of China (31430065, 31071445, 31501330, 31371643, and 31571686); the National Key Research and Development Program (no. 2016YFD0100401); the Open Foundation of the Key Laboratory of Soybean Molecular Design Breeding, Chinese Academy of Sciences; the “Hundred Talents” Program of the Chinese Academy of Sciences; and the Strategic Action Plan for Science and Technology Innovation of the Chinese Academy of Sciences (XDA08030108).

References


