AFLP Mapping of Soybean Maturity

Gene E4

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Days to flowering and maturity are controlled by genes E1–E7 and J in soybean. Previous studies revealed that E1–E5 and E7 influence tolerances to low-temperature-induced seed coat browning in different directions at various intensities. The E4 locus is useful for the development of early maturing cultivars with chilling tolerance because the recessive allele conditions both the early-maturing habit and chilling tolerance. This study was conducted to obtain a fine map of E4 by amplified fragment length polymorphism (AFLP) analysis using a F₈:9 family segregating for E4 that was developed from a cross between photoperiod-insensitive Japanese landraces, Saka-motowase (E4) and Miharudaizu (e4). AFLP analysis using a total of 4096 primer pairs detected 20 polymorphic markers between near-isogenic lines for E4. Linkage mapping incorporated 16 AFLP markers into a previously constructed genetic map around E4 in linkage group I. Eight AFLP markers were localized to unfilled areas between E4 and the closest markers identified previously. Two AFLP markers flanking E4, e48m41-8 and e18m38-8, were mapped at positions 0.6 and 5.4 cM apart from E4, respectively. They were dominant and in cis arrangement with the recessive allele (e4) conditioning the photoperiod insensitivity and chilling tolerance. These markers can be used in developing more precise markers for fine mapping and marker-assisted selection and in isolating the underlying gene via genome walking approaches.

Most of soybean (Glycine max [L.] Merr.) cultivars are photoperiod sensitive, and flower only when the daylength is shorter than a critical daylength. Soybean cultivars are generally adapted within a narrow north–south band due primarily to photoperiodic response; southern cultivars remain vegetative under long days and are too late maturing in the north, whereas northern cultivars flower in response to shorter days and mature too early in the south (Scott and Aldrich 1970). Thus, at certain latitudes, specific cultivars are grown because they have the appropriate time to flowering and maturity necessary to attain optimal commercial production.

Eight genes have been reported to control time to flowering and maturity in soybean: E1 and E2 (Bernard 1971), E3 (Buzzell 1971), E4 (Buzzell and Voldeng 1980), E5 (McBlain and Bernard 1987), E7 (Cober and Voldeng 2001), and E6 (Bonato and Vello 1999) for long juvenility. Of these loci, E1, E3, E4, and E7 are involved in the response of flowering to artificially induced long daylength (Buzzell 1971; Buzzell and Voldeng 1980; Cober et al. 1996; Cober and Voldeng 2001). The e3 locus controls the insensitivity to fluorescent long daylength obtained by extending natural daylength to 20 h using cool white fluorescent lamps with a high red to far-red quantum (R:FR) ratio (Buzzell 1971). On the other hand, e4 combines with e3 to control the insensitivity to incandescent long daylength (ILD) obtained by extending natural daylength to 20 h using incandescent lamps with a low R:FR ratio (Buzzell and Voldeng 1980). E7 also was reported to be involved in ILD insensitivity (Cober and Voldeng 2001). Further, E1 markedly retards flowering under ILD relative to e1, when combined with e3 and e4 (Cober et al. 1996).

Chilling temperatures (about 15 °C) during flowering induce browning and cracking of the seed coats (Sunada and Ito 1982). Genetic analysis using F₁ hybrids between chilling temperature sensitive and tolerant cultivars and their F₂ population revealed that susceptibility was partially dominant to tolerance as a whole and a few major genes were involved in tolerance (Takahashi and Abe 1994). We also found that one of the genes for chilling tolerance was closely associated with a dominant gene for late maturity, the recessive allele of which was involved in floral induction under ILD. Quantitative trait locus (QTL) analysis using DNA markers identified at QTL having a large effect on pigmentation at a position similar to E1 (Githiri et al. 2007).

To evaluate the separate effects of 5 soybean maturity genes (E1–E5) on the intensity of seed coat pigmentation and cracking, Takahashi and Abe (1999) treated cultivar Harosoy (e1e2E3E4e5E7) and its near-isogenic lines (NILs)
for $E1$–$E5$ with chilling temperatures. When compared with Harosoy, intensity of pigmentation was not different in Harosoy-$e3$, slightly lower in Harosoy-$E2$ and Harosoy-$e4$ and profoundly lower in Harosoy-$E1$ and Harosoy-$E5$. Relative to Harosoy, degree of cracking was slightly higher in Harosoy-$e3$ and drastically lower in Harosoy-$e4$, Harosoy-$E1$, and Harosoy-$E5$. Benitez et al. (2004) found that the dominant allele of the $E7$ locus also had inhibitory effects on both pigmentation and cracking. Thus, the $E4$ is particularly useful for the development of early-maturing cultivars with chilling tolerance because the recessive allele conditions both early maturity and chilling tolerance.

Benitez et al. (2004) investigated the combination effects of $E3$ and $E4$ by exposing a double-recessive NIL to 15 °C. Harosoy-$e3e4$ had an intermediate degree of pigmentation and cracking between Harosoy-$e3$ and Harosoy-$e4$, suggesting that the effects of $E3$ and $E4$ were additive. Takahashi et al. (2005) exposed Harosoy, Harosoy-$E1$, Harosoy-$e3e4$, and Harosoy-$E1e3e4$ to 15 °C to evaluate the combination effects of $E1$ and $E3E4$. Harosoy-$E1e3e4$ had an intermediate degree of pigmentation and cracking relative to Harosoy-$E1$ and Harosoy-$e3e4$, suggesting that the effects of $E1$ and $E3E4$ may be additive. The additive effects among $E1$, $E3$, and $E4$ alleles suggest that the combination effect of maturity genes on low-temperature–induced seed coat deterioration may roughly be estimated by the individual gene actions.

These results suggest that maturity genes control low-temperature–induced seed coat pigmentation at various intensities and directions specific to each maturity gene. Common pathways may be shared in the control of both transition from vegetative to reproductive growth and low-temperature–induced seed coat pigmentation. Molecular cloning and characterization of maturity genes may provide further insight into the mechanism of low-temperature response in soybean.

Insensitive of flowering to long daylength is an essential trait in adaptation of soybean to high latitudes with short growing seasons and long daylengths. The double-recessive genotype $e3e4$ is insensitive to ILD and flowers under long daylength conditions (Saindon et al. 1989). Takahashi et al. (2005) evaluated the effects of the maturity alleles on seed yield reduction under chilling treatments. Weight of seed per plant in NILs with $E1e3e4$ was higher than NILs with $E1E3E4$ under chilling treatments. The above results suggest that the allelic combination of $E1e3e4$ is preferable to $E1E3E4$ to enhance quality and yield of seeds under chilling conditions. Selection for the appropriate combination of maturity genes may be effective in improving location-specific adaptation and producing high-quality seeds at high-latitude regions. The $e4$ allele may be useful for the development of chilling-tolerant soybean cultivars adapted to high-latitude regions.

$E4$ has been mapped in the molecular linkage group I using simple sequence repeat (SSR) and isozyme markers (Abe et al. 2003; Molnar et al. 2003). Tasma and Shoemaker (2003) analyzed the association of $E1$–$E5$ with soybean orthologs of 18 *Arabidopsis* genes involved in time to flowering and photoperiod recognition. Mapping and genomic southern analysis using NILs for $E1$–$E5$ revealed an association between *FCA* ortholog and $E3$. However, they could not associate any of the orthologs with $E4$. The present study was conducted to more precisely determine the genomic location of $E4$ by amplified fragment length polymorphism (AFLP) markers using a family segregating for $E4$ that was developed by repetitive heterozygote selection.

### Materials and Methods

**Plant Materials**

A F$_{8:9}$ family segregating for $E4$ was used for linkage mapping. This family was developed by repetitive heterozygote selection from a F$_2$ plant (#130) derived from a cross between Miharudaizu ($e4$) and Sakamotowase ($E4$) (Abe et al. 2003). The family had a heterozygous segment that was segregating for 3 SSR markers, Satt239, Satt496, and Satt354 and an isozyme marker (*Enpi* endopeptidase isozyme). This segment encompassed a region of 37.2 cM in soybean linkage group I (Abe et al. 2003). The genotype at the $E4$ locus was determined based on the results of a progeny test. Progeny of 2 F$_3$ plants, #130-66 (designated #130I) and #130-48 (#130S), was selected as a pair of NILs for $E4$ and used for screening of polymorphisms. #130I was a ILD-insensitive plant homozygous for the $e4$ allele, and #130S was a ILD-sensitive plant homozygous for the $E4$ allele. These NILs possessed different alleles for the 4 SSR and isozyme markers.

**DNA Extraction and AFLP Analysis**

Total DNA of each NIL was extracted from trifoliolate leaves by the cetetyl trimethyl ammonium bromide method (Murray and Thompson 1980). AFLP analysis was performed using a total of 4096 primer pairs according to the method of Kawaguchi et al. (2001). Polymerase chain reactions (PCRs) were performed with *Eco*I- and *Mol*-digested DNA ligated to 2 sets of primers using ExTaq enzyme (Takara Bio, Otsu, Japan). PCR products were separated on 13% non-denaturing acrylamide gels (38:2). The fragments were visualized by the vistra green according to the manufacturer’s instructions (GE Healthcare Bio-Science, Piscataway, NJ). Designation of AFLP markers followed the report of Mano et al. (2001).

**Segregation Analysis and Linkage Mapping**

DNA samples for 116 plants in the family were subjected to AFLP analysis using the primer pairs that amplified polymorphic fragments between the NILs. Unstable or weak markers were excluded from the linkage mapping. Data from AFLP analysis and those for $E4$ and the 4 SSR and isozyme markers (Abe et al. 2003) were combined and then a new linkage map was reconstructed using MapMaker/EXP version 3.0 (Lander et al. 1987) with the threshold logarithm of odds score of 3.0.
Results and Discussion

AFLP analysis using a total of 4096 primer pairs revealed that 20 primer pairs produced polymorphic amplified fragments between the NILs. Linkage analysis using the 116 F8 plants incorporated 16 AFLP markers into the previously constructed genetic map flanking E4 (Abe et al. 2003) (Figure 1). The newly constructed map spanned 86.2 cM from e47m32-6 to Satt354. On the other hand, 3 AFLP markers formed a different linkage group spanning 3.5 cM and 1 marker remained unlinked (data not shown). The 3 linked and the unlinked AFLP markers may locate in regions with residual heterogeneity other than the region around E4. Presence/absence and fragment size of the AFLP bands assigned to the linkage group are summarized in Table 1. e58m32-8 was a co-dominant marker, whereas the others were dominant markers.

E4 was surrounded by Satt496 at a distance of 5.1 cM and by Enp at a distance of 8.0 cM (Abe et al. 2003). Eight AFLP markers were assigned to unfilled areas around E4. Six AFLP markers (e3m57-9, e29m62-9, e21m18-9, e18m64-6, e22m26-9, and e48m41-8) were mapped between Satt496 and E4. Among them, e48m41-8 was the nearest marker to and only 0.6 cM away from E4. Two AFLP markers (e18m38-8 and e19m20-9) were mapped between E4 and Enp. e18m38-8 was the closest marker to and 5.4 cM apart from E4. More exact positioning of the AFLP markers requires a larger segregating population for fine mapping. The 2 AFLP markers flanking E4, e48m41-8 and e18m38-8, were dominant but in cis arrangement with the recessive allele (e4) conditioning the photoperiod.
insensitivity and chilling tolerance (Table 1). The next step to be taken is to clone and analyze the AFLP bands and convert them to sequence characterized amplified region (SCAR) markers. The SCAR markers may be useful in fine mapping and marker-assisted selection and in isolating the underlying gene via genome walking approaches. Psf and McBlain digested AFLP markers may be useful to add more markers in the region.

Early-maturing cultivars native to the shores of the Sea of Okhotsk (Sakhalin and Kuril Islands) and eastern Hokkaido (northern Japan) have been used in breeding for chilling tolerance in Japan and Sweden (Holmberg 1973). These cultivars have a strong tendency to produce cleistogamous flowers throughout their blooming period (Takahashi et al. 2001). A gene controlling cleistogamy was also mapped at a similar position with $E_4$ (Khan NA, Githiri SM, Abe J, Kawasaki S, Hayashi T, Takahashi R unpublished data). Fine mapping of the region and positional cloning of $E_4$ may thus be useful to elucidate mechanisms for adaptation of soybean to northern regions with low temperatures, short growing seasons, and long daylengths.

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**Table 1.** AFLP markers flanking $E_4$, their specific sequences of selective primers and approximate size of amplified bands in NILs for $E_4$ (#130S and #130I)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Selective nucleotide for EcoRI primer</th>
<th>Selective nucleotide for Msel primer</th>
<th>Size of amplified bands (bp)</th>
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<tr>
<td>e47m32-6</td>
<td>GTG</td>
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<tr>
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<td>AAC</td>
<td>TCG</td>
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<td>TGA</td>
<td>TGG</td>
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<td>e15m10-8</td>
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<td>AGC</td>
<td>250</td>
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<tr>
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<td>TAC</td>
<td>TCA</td>
<td>200</td>
</tr>
<tr>
<td>e55m10-9</td>
<td>TCG</td>
<td>AGC</td>
<td>215</td>
</tr>
<tr>
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<td>e19m20-9</td>
<td>CAG</td>
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**References**


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