Effects on flowering and seed yield of dominant alleles at maturity loci \textit{E2} and \textit{E3} in a Japanese cultivar, \textit{Enrei}

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\textit{Enrei} is the second leading variety of soybean (\textit{Glycine max} (L.) Merr.) in Japan. Its cultivation area is mainly restricted to the Hokuriku region. In order to expand the adaptability of \textit{Enrei}, we developed two near-isogenic lines (NILs) of \textit{Enrei} for the dominant alleles controlling late flowering at the maturity loci, \textit{E2} and \textit{E3}, by backcrossing with marker-assisted selection. The resultant NILs and the original variety were evaluated for flowering, maturity, seed productivity and other agronomic traits in five different locations. Expectedly, NILs with \textit{E2} or \textit{E3} alleles flowered later than the original variety in most locations. These NILs produced comparatively larger plants in all locations. Seed yields were improved by \textit{E2} and \textit{E3} in the southern location or in late-sowing conditions, whereas the NIL for \textit{E2} exhibited almost the same or lower productivity in the northern locations due to higher degrees of lodging. Seed quality-related traits, such as 100-seed weight and protein content, were not significantly different between the original variety and its NILs. These results suggest that the modification of genotypes at maturity loci provides new varieties that are adaptive to environments of different latitudes while retaining almost the same seed quality as that of the original.

\textbf{Key Words:} soybean, \textit{Glycine max} (L.) Merr., maturity gene, backcrossing, marker-assisted selection, near-isogenic lines, seed productivity.

\textbf{Introduction}

\textit{Enrei} is the second leading variety of soybean (\textit{Glycine max} L. Merr.) in Japan; its production area accounts for 12.3\% of the total soybean production area [Ministry of Agriculture, Forestry and Fisheries (2011)]. This variety was registered in 1971 and has been used preferably for tofu production because of its high protein content; however, its production area is limited to the Hokuriku region around Niigata Prefecture because of its narrow-ranged adaptability to latitudes. Most of the total production area of \textit{Enrei} (99.3\%) is occupied by three prefectures in the Hokuriku region (Niigata, Toyama and Ishikawa) and Yamagata Prefecture [Ministry of Agriculture, Forestry and Fisheries (2011)]. The modification of flowering and maturing habits is thus very important to extend the adaptability of \textit{Enrei}, toward the southern regions of lower latitudes and/or the northern regions of higher latitudes.

Cober and Morrison (2010) exemplified the usefulness of modified maturity genotypes of soybean varieties to adapt to
a wide range of latitudes. They evaluated flowering and maturing habits and seed yield for 20 near-isogenic lines (NILs) of cv. Harosoy for the five maturity loci (E1, E2, E3, E4 and E7) and growth habit loci (Dit) in Ottawa, Canada, and observed that the seed yield increased linearly with maturity until about 112 days, and then reached a plateau. They concluded that variations in the photoperiod sensitivity and growth habit alleles give rise to a range of maturities, with pleiotropic effects on seed yield and agronomic characteristics, and that the variations play an important role in providing adaptation across latitudes (Cober and Morrison 2010).

Time to flowering and maturity in soybeans is controlled by E1, E2 (Bernard 1971), E3 (Buzzell 1971), E4 (Buzzell and Voldeng 1980), E5 (McBlain et al. 1987), E6 (Bonato and Vello 1999), E7 (Cober and Voldeng 2001), E8 (Cober et al. 2010) and J (Ray et al. 1995). In addition to these major genes, a number of quantitative trait loci (QTL) have been known to control the time to flowering (review by Watanabe et al. 2012). Of the major E genes, E1 to E4 were mapped on a fine scale on linkage groups (LGs) C2 for E1 (Yamanaka et al. 2001, Watanabe et al. 2004), O for E2 (Watanabe et al. 2011), L for E3 (Watanabe et al. 2009) and I for E4 (Matsumura et al. 2008, Liu et al. 2008). Furthermore, recent molecular assays have identified the genes responsible for some of the E genes; E2 is a GIGANTEA ortholog, GmGia (Watanabe et al. 2011), E3 and E4 are genes encoding phytochrome A (phyA), GmphyA3 (Watanabe et al. 2009) and GmphyA2 (Liu et al. 2008), respectively. The information on the exact position in linkage maps and physical maps and the molecular bases may facilitate the use of DNA markers tagging agronomically important genes in the tailor-made breeding of soybean varieties. In this paper, we report the development of NILs for maturity genes, E2 and E3, of ‘Enrei’, and the results of field evaluations on agronomic traits, such as flowering and maturing times, seed productivity and plant morphology, under various environmental conditions.

**Materials and Methods**

**Plant materials and development of NILs**

Two NILs for E2 and E3, and their parental varieties, ‘Enrei’, ‘Sachiyutaka’ and ‘Fukuyutaka’, and three leading varieties, ‘Suzuyutaka’, ‘Tachinagaha’ and ‘Tamahomare’, were used in this study. ‘Sachiyutaka’ had been bred from BC2 progeny between ‘Fukuyutaka’ and ‘Enrei’, in which the latter was used as a recurrent parent (Takahashi et al. 2004). The NILs for E2 and E3 used in this study were developed by backcrossing between ‘Enrei’ and ‘Sachiyutaka’ or ‘Fukuyutaka’ (Table 1). The maturity genotypes at E2 and E3 were estimated as e2e2e3e3 for ‘Enrei’, E2E2e3e3 for ‘Sachiyutaka’ and E2E2E3E3 for ‘Fukuyutaka’, based on the functional DNA markers developed for E2 (Watanabe et al. 2011) and E3 (Watanabe et al. 2009) (personal communication from Dr. Yasutaka Tsubokura). F1 hybrids between ‘Enrei’ and ‘Sachiyutaka’ and between ‘Enrei’ and ‘Fukuyutaka’ were backcrossed with ‘Enrei’, and in each backcrossing, plants heterozygous for the maturity locus were selected based on the marker genotypes (Table 2). In the BC2F1 generation, polymorphic SSR markers covering the whole genome were surveyed. In the subsequent generations, plants heterozygous at the maturity locus but homogeneous for the allele from ‘Enrei’ at as many SSR loci as possible were selected as a pollen parent in backcrossing.

**Table 1. Summary of back-crossing for E2 and E3 in ‘Enrei’**

| Parent | BC2 | BC1 | BC2 | BC3 | BC4 |
|--------|-----|-----|-----|-----|-----|
| Sachiyutaka | 20 | 19 | 2 | 80 |  
| Number of plants genotyped at F1 | 20 | 9 | 2 | 1 |  
| Number of plants developing next generation after DNA marker-assisted selection |  
| Sowing year and month | 2007.2 | 2007.7 | 2007.11 | 2008.2 | 2008.7 |  
| Number of plants genotyped at F2 | 73 |  
| Number of samples carrying donor allele in homozygote at E2 | 19 |  
| Sowing year and month | 2008.11 |  
|  
| Fukuyutaka | 10 | 35 | 4 | 30 | 98 |  
| Number of plants genotyped at F1 | 10 | 21 | 1 | 1 | 1 |  
| Number of plants developing next generation after DNA marker-assisted selection |  
| Sowing year and month | 2007.2 | 2007.7 | 2007.11 | 2008.2 | 2008.7 | 2008.11 |  
| Number of plants genotyped at F2 | 111 |  
| Number of samples carrying donor allele in homozygote at E3 | 23 |  
| Sowing year and month | 2009.2 |  

* ‘Enrei’ was crossed as recurrent parent in every generation.  
* BC2 indicates single-cross.
Marker analysis

Total DNA was extracted from young leaves using Biorobot EZ1 (Qiagen, Valencia, CA, USA) or Biosprint 96 (Qiagen). To estimate the $E_2$ or $E_3$ genotype, we developed several DNA markers based on sequence information on the $E_2$ or $E_3$ gene and their flanking regions (Table 2). These markers were labeled by different fluorescence dyes and multiplex polymerase chain reactions (PCRs) were performed in a 5.5 µl reaction mixture [containing 50 nM of each fluorescent primer pair, 5 ng total genomic DNA, and 2.5 µl of 2X Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany)] using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The amplification protocol and detection of the resulting amplicons using a fluorescence-based DNA sequencer followed the method described by Sayama et al. (2011). We screened the polymorphism between ‘Enrei’ and ‘Fukuyutaka’ for 245 simple sequence repeat (SSR) markers with high polymorphism information content values, which were selected at approximately every 12 cM (Hwang et al. 2009). The method of detection for SSR genotypes followed the method described by Sayama et al. (2011).

Growth conditions

Field experiments were conducted in 2009 and 2010 at the five agricultural experimental stations shown in Fig. 1 and Table 3. In each experiment, soybeans were grown using common cultivation methods at the stations (Table 3). ‘Enrei’ and ‘Enrei-E2’ were examined in all of the experiments, whereas ‘Enrei-E3’ was examined in three experiments conducted in a field of the National Agriculture and Food Research Organization Institute of Crop Science (Tsukubamirai, Ibaraki) in 2009 and 2010 and in a field of the National Agriculture and Food Research Organization Western Region Agricultural Research Center (Zentsuji, Kagawa). Each line was planted in two replications in accordance with the breeding methods for the primary yield test.

Evaluation of agronomic traits

Flowering time was defined as the number of days from sowing to flowering of 50% of the plants in a plot. Maturity was defined as the number of days from sowing to matura!

| Table 2. The marker panels for estimating $E_2$ and $E_3$ genotypes used in the multiplex PCRs |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Marker name | Dye | Forward sequence (5' to 3') | Reverse sequence (5' to 3') | Location in Phytozome database | Amplicon size (bp) |
|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| E2at_U46k | 6-FAM | GGATAATTTTCTGCAGCCATG | TCGAACCCTTGTAGTGCATTTC | Enrei Fuku-yutaka |
| E2at | PET | GTGCCCTTCCTGGCCTTTTCA | TCGGCCATTTTTAATTGTTG | 34 kb upstream away from $E_2$ region |
| E2at_D82k | NED | CGTCATTTCTATGGTTGCTT | GAAATGGACATTTTGTTG | 70 kb downstream away from $E_2$ region |
| GMES4019 | PET | TCAATTGGTTAAAATCCTGTTG | ATGGTAGTGTTGTTG | Inside of $E_2$ region |
| E3at-U113k | NED | CAACCTAATCGTGACAC | CACAAAGCCTTATCCTT | 189 kb downstream away from $E_2$ region |
| FT3SSR4 | VIC | GCCTATTTAGAAAAACATCCA | CCGCTAGCAATTTACTG | 113 kb upstream away from $E_3$ region |
| FT3SSR1dom | VIC | ATTAATCGTGACTCGTGACTCC | GGACTTAGATGAGGGCCATAA | 1 kb upstream away from $E_3$ region |
| FT3SSR3 | PET | CATTCCATTGTGCCTTTCC | TTCTACCTTCCTCACC | 19 kb downstream away from $E_3$ region |

$^a$ Fluorescent material referred to Applied Biosystems was attached together with the tail of several nucleotides before 5' end of forward primer.  
$^b$ http://www.phytozome.net/soybean (Schmutz et al. 2010).  
$^c$ ‘Sachiyutaka’ showed the same pattern as ‘Fukuyutaka’ around $E_2$ and ‘Enrei’ around $E_3$.  
$^d$ No amplicons.
agronomic traits. Coefficients of correlations were calculated between seed productivity and other agronomical traits. One-way ANOVA was conducted according to Tukey’s test for seed productivity among lines and cultivars.

Results and Discussion

Development of NILs for the E2 and E3 alleles

‘Enrei’ and ‘Fukuyutaka’ showed polymorphisms at 206 of 245 SSR markers tested. BC2F1 plants were polymorphic for 12 and 51 of the 206 markers tested in the cross between ‘Enrei’ and ‘Sachiyutaka’ and between ‘Enrei’ and ‘Fukuyutaka’, respectively. Those polymorphic SSR markers were distributed over 8 and 17 linkage groups. In the BC3F1 generation, single plants were selected from 80 plants for ‘Enrei-E2’ and from 30 plants for ‘Enrei-E3’, respectively, based on the genotypes for maturity locus and SSRs (Table 1). No genomic region of the donor parent (‘Sachiyutaka’) was left in the selected BC3F1 plant for E2 for the markers tested, so BC3F2 plants homozygous for E2 were used as NIL ‘Enrei-E2’. Thirty-five of the 51 markers tested were homozygous for the allele from ‘Enrei’ in the selected BC3F1 plant for E3, which was used for further backcrossing to generate BC4F1 plants. Of 98 BC4F1 plants obtained, only one plant was homozygous at 15 of the 16 markers for the alleles from ‘Enrei’. The BC4F1 plant was used to develop BC4F2 progeny. Among them, BC4F2 plants homozygous for E3 were used as NIL ‘Enrei-E3’. The developed NILs for E2 and E3 were registered as ‘Sakukei 74 (Enrei-E2)’ and ‘Sakukei 78 (Enrei-E3)’. The NILs had the same morphological phenotypes as ‘Enrei’: determinantal growth, round leaflets, purple flowers, gray pubescence, yellow hilum, yellow seed coats, and spherical seeds.

As shown in Table 1, in this study, backcrossing combined with marker-assisted selection was repeated three times a year. This method could provide sufficient seeds for BC3 or BC4 lines for a primary yield test in three years and could shorten the period of the breeding program by several years. In particular, DNA marker-assisted selection had the advantages that we could identify the degree to which the genome had been restored for the recurrent parent, where a backcrossed line had residual regions from the donor parent including the target region, and when it would reach the anticipated degree of similarity to the recurrent parent.

Effects of maturity genes on flowering, maturity and seed productivity

Both NILs flowered significantly later than ‘Enrei’ in all of the experiments, as expected (Tables 4–6). Accordingly, the substitution of early-maturity by late alleles was effective in modifying the flowering time of ‘Enrei’. Similarly, the seed-filling period and maturity showed significant differences between ‘Enrei’ and ‘Enrei-E2’, although the interaction between lines and experimental sites was also significant. The E2 allele delayed the seed-filling period and maturity compared to the e2 allele, particularly in three locations, Niigata, Nagano and Mito/Ibaraki, in 2009, whereas there was no clear difference in Kagawa and late-sowing experiments in Tsukubamirai/Ibaraki (Table 4), in which maturation proceeded under a relatively shorter daylength. The effect of E3 on the seed-filling period and maturity were also not clear; the order of the seed-filling period varied with the locations tested (Table 4). The absence of an effect of E3 over e3 on the seed-filling period and maturity may be due to a shorter daylength in the environmental conditions tested. McBlain et al. (1987) indicated that post-flowering development (R1 to R8) was slowed by E2 and E3 relative to their respective recessive alleles. The effect of E3 on maturity should thus be evaluated under a longer photoperiod condition as in Niigata and Nagano. MSN and MSL, plant size-related traits, showed higher values in NILs than ‘Enrei’ in all of the experiments, indicating that NILs produced larger plants than ‘Enrei’ (Table 4). Seed productivity also showed significant differences between ‘Enrei’ and NILs (Tables 5, 6); however, in the comparison of the E2 locus, the interaction between lines and experimental sites was significant at 1% probability; seed productivity was increased in ‘Enrei-E2’ relative to ‘Enrei’ in three experiments, Kagawa and two in Tsukubamirai/Ibaraki, whereas ‘Enrei-E2’ showed almost the same or lower seed productivity than ‘Enrei’ in three locations, Niigata, Nagano and Mito/Ibaraki (Table 4). Particularly in Kagawa and Tsukubamirai/Ibaraki in 2009, the seed productivity of ‘Enrei-E2’ was comparable to that of other high-yielding varieties, ‘Sachiyutaka’ or ‘Tamahomare’ (Fig. 2A, 2B), although later-maturing varieties, such as ‘Tamahomare’ and ‘Fukuyutaka’, showed greater productivity than ‘Enrei-E2’ under delayed sowing conditions (July, 15) of Tsukubamirai/Ibaraki in 2010 (Fig. 2C).

The relationships between seed productivity and other agronomical traits are shown in Fig. 3 and Table 7. Generally, later maturing varieties are expected to have an advantage in seed productivity because the total amount of insolation received from the canopy is greater than for early maturing varieties; however, the correlation was not significant for any agronomical traits in all lines combined (Table 7). As mentioned above, seed productivity for ‘Enrei-E2’ and ‘Enrei’ showed opposite trends among the location/experiments tested; ‘Enrei-E2’ produced higher seed productivity relative to ‘Enrei’ under environmental conditions where the daylength was relatively short, whereas seed productivity was lower in ‘Enrei-E2’ than ‘Enrei’ when daylength was relatively long (Table 4). We classified the five experiments into two groups: southern environment with shorter daylength and northern environment with longer daylength, and reevaluated relationships between flowering and maturity times and seed productivity. As a result, the correlation between flowering time and seed productivity was significantly positive only in the southern environment; a similar but non-significant correlation was observed between maturity and seed productivity (Fig. 3 and Table 7). On the other hand, correlations were negative for these traits
### Table 3. Experimental sites and growth conditions

| Experimental sites                                      | GPS Year       | Day of sowing | Inter-low (cm) | Intra-low (cm) | Number of plants per hill | Measurement area | Soil type | Amount of fertilizer applied |
|----------------------------------------------------------|----------------|---------------|----------------|----------------|--------------------------|----------------|-----------|-----------------------------|
| Niigata Agricultural Research Institute (Niigata, Japan) | 37°20′14N, 138°52′26E | 2009          | 21-May         | 75             | 15                       | 3.0            | 3         | 60                          | 11.25     | 2 Andosol                  | 16.40 : 60 : 60 | —         | —                           |
| Nagano Vegetable and Ornamental Crops Experiment Station (Shiojiri, Nagano) | 36°6′11N, 137°56′0E | 2010          | 1-Jun          | 75             | 16.7                     | 2.0            | 2         | 24                          | 9.00      | 2 Andosol                  | 36 : 144 : 72 | 600       | 10                          |
| Plant Biotechnology Institute, Ibaraki Agricultural Center (Mito, Ibaraki) | 36°26′24N, 140°26′57E | 2010          | 22-Jun         | 60             | 15                       | 2.7            | 5         | 30                          | 9.00      | 2 Andosol                  | 30 : 100 : 100 | —         | 10                          |
| National Agriculture and Food Research Organization Institute of Crop Science (Tsukuba, Ibaraki) | 36°0′26N, 140°1′19E | 2009          | 26-Jun         | 70             | 13                       | 3.0            | 4         | 92                          | 8.40      | 2 gray lowland soil         | 30 : 100 : 100 | 1000      | —                           |
| National Agriculture and Food Research Organization Institute of Crop Science (Tsukuba, Ibaraki) | 36°0′26N, 140°1′19E | 2010          | 15-Jul         | 70             | 13                       | 3.0            | 4         | 92                          | 8.40      | 2 gray lowland soil         | 30 : 100 : 100 | 1000      | —                           |
| National Agriculture and Food Research Organization Western Region Agricultural Research Center (Zentsuji, Kagawa) | 34°13′47N, 133°46′36E | 2010          | 11-Jun         | 70             | 13                       | 2.5            | 3         | 90                          | 5.25      | 2 gray lowland soil         | 30 : 100 : 100 | 1000      | 10                          |

### Table 4. Averages and standard deviations of agronomic traits of ‘Enrei’ and its NIL for maturity genes, E2 and E3

| Experimental sites | Lines | Flowering time (day) | Seed-filling period (day) | Maturity (day) | MSN<sup>a</sup> (plant<sup>1</sup>) | MSL<sup>b</sup> (cm) | Seed productivity (kg/a) | 100-seed weight (g) | Protein (%) |
|--------------------|-------|-----------------------|--------------------------|----------------|-------------------------------------|---------------------|------------------------|---------------------|-------------|
| Niigata, Niigata   | Enrei-E2 | 67.0 ± 1.4            | 82.0 ± 2.8               | 149.0 ± 4.2    | 76.7 ± 5.6                          | 31.1 ± 4.0          | 35.7 ± 2.4             | 45.9 ± 1.8          | 44.6 ± 0.5   |
| Shiojiri, Nagano   | Enrei-E2 | 59.0 ± 0.0            | 73.5 ± 0.7               | 132.5 ± 0.7    | 66.0 ± 3.6                          | 32.3 ± 2.4          | 34.7 ± 2.5             | 44.6 ± 0.5          | 46.1 ± 0.4   |
| Mito, Ibaraki      | Enrei-E2 | 44.5 ± 0.7            | 78.0 ± 4.2               | 122.5 ± 3.5    | 71.1 ± 4.5                          | 32.9 ± 0.6          | 28.6 ± 2.6             | 44.2 ± 1.8          | 43.9 ± 1.3   |
| Tsukubamirai, Ibaraki in 2009 | Enrei-E2 | 45.5 ± 0.7            | 69.0 ± 0.0               | 114.5 ± 0.7    | 76.5 ± 3.3                          | 44.0 ± 5.1          | 33.0 ± 0.6             | 46.9 ± 1.5          | 43.9 ± 1.3   |
| Zentsuji, Kagawa   | Enrei-E2 | 43.5 ± 0.7            | 69.0 ± 1.4               | 112.5 ± 2.1    | 71.7 ± 0.2                          | 35.6 ± 1.7          | 31.2 ± 2.9             | 46.6 ± 1.2          | 42.5 ± 1.5   |
| Tsukubamirai, Ibaraki in 2010 | Enrei-E2 | 39.0 ± 0.0            | 65.0 ± 2.8               | 104.0 ± 2.8    | 53.8 ± 0.8                          | 31.5 ± 6.8          | 29.6 ± 2.8             | 46.2 ± 1.5          | 46.2 ± 1.5   |

<sup>a</sup> The number of nodes on the main stem (MSN) was measured as the number of nodes on main stem from the cotyledonary node to the top node without the top peduncle for ten normally grown plants.

<sup>b</sup> Main stem length (MSL) was measured as the length from the cotyledonary node to the top node without the top peduncle for ten normally grown plants.
Fig. 1. Locations of experimental sites and cultivation areas of Enrei, Sachiyutaka and Fukuyutaka

Table 5. Mean squares of agronomic traits of ‘Enrei’ and its NIL for maturity gene, $E_2$

| Mean square     | Flowering time (day) | Seed-filling period (day) | Maturity (day) | MSN (plant$^{-1}$) | MSL (cm) | Seed productivity (kg/a) | 100-seed weight (g) | Protein (%) |
|-----------------|----------------------|---------------------------|----------------|---------------------|----------|--------------------------|---------------------|-------------|
| Line            | 280.2 ***            | 240.7 **                  | 1040.2 ***     | 36.7 ***            | 1127.5 ***| 151.5 *                  | 0.1                 | 1.7         |
| Experimental site | 458.7 ***         | 320.8 ***                 | 1049.6 ***     | 7.5 ***             | 221.4 **  | 225.6 **                 | 22.4 *              | 3.6         |
| Line * Experimental site | 1.9               | 46.7 **                  | 62.2 ***       | 0.7 **              | 39.2     | 72.4 **                  | 3.9                 | 3.1         |
| Error           | 0.9                  | 6.0                      | 5.8            | 0.1                 | 14.1     | 10.7                     | 3.0                 | 1.2         |

*F-test using mixed model. *, ** and *** indicate significant difference at 5%, 1% and 0.1% levels, respectively.

Table 6. Mean squares of agronomic traits of ‘Enrei’ and its NIL for maturity gene, $E_3$

| Mean square     | Flowering time (day) | Seed-filling period (day) | Maturity (day) | MSN (plant$^{-1}$) | MSL (cm) | Seed productivity (kg/a) | 100-seed weight (g) | Protein (%) |
|-----------------|----------------------|---------------------------|----------------|---------------------|----------|--------------------------|---------------------|-------------|
| Line            | 75.0 ***             | 0.3                       | 65.3           | 11.6 *              | 387.6    | 115.3 *                  | 0.4                 | 1.0         |
| Experimental site | 46.1 *              | 553.0 *                   | 717.6 *        | 1.0                 | 121.8    | 213.8 *                  | 4.2                 | 4.0         |
| Line * Experimental site | 0.8            | 26.3 *                    | 18.6           | 0.6                 | 43.2 *   | 5.6                      | 3.0                 | 0.7         |
| Error           | 0.2                  | 3.2                       | 3.7            | 0.3                 | 5.5      | 12.5                     | 3.1                 | 1.0         |

*F-test using mixed model. *, ** and *** indicate significant difference at 5%, 1% and 0.1% levels, respectively.
Development of near-isogenic late maturity soybean varieties in the northern environment (Fig. 3 and Table 7).

As indicated, some agronomic traits related to plant shape, such as MSN and MSL, of ‘Enrei-E2’ were greater than those of ‘Enrei’ (Tables 4, 5). The differences in plant shape appear to lead to differences in lodging and self-shading because severe lodging was more frequently observed for ‘Enrei-E2’ than for ‘Enrei’ in the northern environment. The adverse effect of the $E2$ allele on seed productivity is most likely due to severe lodging and self-shading.

**Seed quality of NILs**

The differences in 100-seed weights and protein contents were not significant between ‘Enrei’ and NILs (Tables 5, 6), although a significant effect of the experimental locations

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### Table 7. Coefficient of correlation between seed productivity and other agronomical traits

| Lines/sites     | Flowering time (day) | Seed-filling period (day) | Maturity (day) | MSN (plant$^{-1}$) | MSL (cm) | 100-seed weight (g) | Protein (%) |
|-----------------|----------------------|---------------------------|----------------|--------------------|----------|---------------------|-------------|
| Total           | 0.03                 | –0.14                     | –0.07          | 0.13               | 0.27     | 0.29                | 0.02        |
| Northern sites  | –0.44                | –0.72 **                  | –0.64 *        | –0.73 *            | –0.41    | 0.20                | –0.55       |
| Southern sites  | 0.91 ***             | 0.13                      | 0.43           | 0.70 **            | 0.71 **  | 0.51                | 0.26        |

* *, ** and *** indicate significant difference at 5%, 1% and 0.1% levels, respectively.
was detected in 100-seed weight in the comparison of the E2 locus. In addition, a significant difference was not observed in the processing suitability for tofu, which was evaluated by breaking stress and other seed quality-related traits (data not shown). These results suggest that the NILs had a similar seed quality to ‘Enrei’.

**Concluding remarks**

The present study revealed that modification of maturity genes in a Japanese variety ‘Enrei’ improved seed productivity to different degrees, depending on the environment evaluated. The later flowering and longer seed-filling period resulted in higher seed productivity only in relatively southern locations or under late-sowing conditions, whereas the E2 allele provided no clear or adverse effect on seed productivity in relatively northern locations. As suggested by Cober and Morisson (2010), there may be flowering and maturing habits that are most suitable for each environment. Furthermore, the earlier maturation of ‘Enrei’ might be rather advantageous for rotation cropping in non-snow-covered areas, such as Nagano and Mito/Ibaraki, and for escaping the risk of being covered with snow during harvesting in Niigata; thus, different genotypic combinations at maturity loci may be preferred in each location. In order to identify the most adaptive genotype in each region, it is very important to develop NILs for various maturity genes and to evaluate their seed productivity in diverse environments. In addition to E2 and E3 loci, DNA markers tagging E1 (Yamanaka et al. 2005) and E4 (Liu et al. 2008) are available to modify genotypes at these loci. Furthermore, such NILs would be useful to supply the same lot of seeds on a larger scale by cultivating more adaptive NILs in each region, if seed quality such as for tofu processing is retained at the same level as the original, as suggested in our present study. Further studies are required for our better understanding of the effects of alternating the maturity genotype on all types of agronomic traits.

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**Literature Cited**

Bernard, R.L. (1971) Two major genes for time of flowering and maturity in soybeans. Crop Sci. 11: 242-244.

Bonato, E.R. and N.A. Vello (1999) *E6*, a dominant gene conditioning early flowering and maturity in soybeans. Genet. Mol. Biol. 22: 229–232.

Buzzell, R.I. (1971) Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can. J. Genet. Cytol. 13: 703–707.

Buzzell, R.I. and H.D. Voldeng (1980) Inheritance of insensitivity to long daylength. Soybean Genet. Newsl. 7: 26–29.

Cober, E.R. and H.D. Voldeng (2001) A new soybean maturity and photoperiod-sensitivity locus linked to E1 and T. Crop Sci. 41: 698–701.

Cober, E.R., S.J. Molnar, M. Charrette and H.D. Voldeng (2010) A new locus for early maturity in soybean. Crop Sci. 50: 524–527.

Cober, E.R. and M.J. Morrison (2010) Regulation of seed yield and agronomic characters by photoperiod sensitivity and growth habit genes in soybean. Theor. Appl. Genet. 120: 1005–1012.

Hwang, T.Y., T. Sayama, M. Takahashi, Y. Takada, Y. Nakamoto, H. Funatsuki, H. Hisano, S. Sasamoto, S. Sato, S. Tabata et al. (2009) High-density integrated linkage map based on SSR markers in soybean. DNA Res. 16: 213–225.

Liu, B., A. Kanazawa, H. Matsumura, R. Takahashi, K. Harada and J. Abe (2008) Genetic redundancy in soybean photoreponses associated with duplication of the photochrome A gene. Genetics 180: 995–1007.

Matsumura, H., B. Liu, J. Abe and R. Takahashi (2008) AFLP mapping of soybean maturity gene E4. J. Hered. 99: 193–197.

McBlain, B.A., J.D. Hesketh and R.L. Bernard (1987) Genetic effects on reproductive phenotype in soybean isolines differing in maturity genes. Can. J. Plant Sci. 67: 105–116.

Ministry of Agriculture, Forestry and Fisheries (2011) Home page of soybean. http://www.maff.go.jp/j/seisan/ryuuto/daiyu/index.html

Ray, J.D., K. Hinson, E.B. Mankono and F.M. Malo (1995) Genetic control of a long juvenile trait in soybean. Crop Sci. 35: 1001–1006.

Sayama, T., T.Y. Hwang, K. Komatsu, Y. Takada, M. Takahashi, S. Kato, H. Sasama, A. Higashi, Y. Nakamoto, H. Funatsuki et al. (2011) Development and application of a whole-genome simple sequence repeat panel for high-throughput genotyping in soybean. DNA Res. 18: 107–115.

Schnutz, J., S.B. Cannon, J. Schluetter, J.X. Ma, T. Mitros, W. Nelson, D.L. Hyten, Q.J. Song, J.J. Thelen, J.J. Cheng et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature 463: 178–183.

Takahashi, M., R. Matsunaga, K. Komatsu, Y. Nakazawa, M. Hajika, S. Sakai and K. Igita (2004) New soybean cultivar “Sachiyutaka.” Bull. Natl. Agric. Res. Cent. Kyushu Okinawa Reg. 45: 15–39.

Watanabe, S., T. Tajuddin, M. Hayashi and K. Harada (2004) Analysis of QTLs for reproductive development and seed quality traits in soybean using recombinant inbred lines. Breed. Sci. 54: 399–407.

Watanabe, S., R. Hideshima, Z. Xia, Y. Tsubokura, S. Sato, Y. Nakamoto, N. Yamanaka, R. Takahashi, M. Ishimoto, T. Anai et al. (2009) Map-based cloning of the gene associated with the soybean maturity locus E3. Genetics 182: 1251–1262.

Watanabe, S., Z. Xia, R. Hideshima, Y. Tsubokura, S. Sato, N. Yamanaka, R. Takahashi, T. Anai, S. Tabata, K. Kitamura et al. (2011) A map-based cloning strategy employing a residual heterozygous line reveals that the GIGANTEA4 gene is involved in soybean maturity and flowering. Genetics 188: 395–407.

Watanabe, S., K. Harada and J. Abe (2012) Genetic and molecular bases of photoperiod responses of flowering in soybean. Breed. Sci. 61: 531–543.

Yamanaka, N., S. Ninomiya, M. Hoshi, Y. Tsubokura, M. Yano, Y. Nagamura, T. Sasaki and K. Harada (2001) An informative linkage map of soybean reveals QTLs for flowering time, leaflet morpholm and regions of segregation distortion. DNA Res. 8: 61–72.

Yamanaka, N., S. Watanabe, K. Toda, M. Hayashi, H. Fujigami, R. Takahashi and K. Harada (2005) Fine mapping of the FT1 locus for soybean flowering time using a residual heterozygous line derived from a recombinant inbred line. Theor. Appl. Genet. 110: 634–639.