Effects of maturity genes E2 and E3 on yield formation in soybean cultivar Enrei in warm region, Fukuyama in Japan

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**ABSTRACT**

Understanding how maturity genes affect soybean yield formation will provide important information for crop management decisions. This study aimed to reveal how maturity genes E2 and E3 in the soybean cultivar ‘Enrei’ affect yields and yield formation in warm regions of Japan. ‘Enrei’ (e2e3) and three near-isogenic lines of ‘Enrei’ (e2E3, E2e3, and E2E3) were cultivated in 2016 and 2017 in Fukuyama, Japan (34°30′N, 133°23′E). Two sowing dates were set in each year (June sowing and July sowing). E2 extended the period from emergence to R1 and also the period from R1 to R7, whereas E3 extended only the period from emergence to R1. Interaction between E2 and E3 did not affect duration of the period from emergence to R1, but did affect the period from R1 to R7. Although seed yield did not differ between genotypes in the June sowings, the effects of E2 and E3 on seed yield in July sowing were both significant and interaction between E2 and E3 also observed. The total number of nodes increased in E3 genotypes in both sowing dates, especially in E2E3. Pod-set ratio was lower in E2 and E3 genotypes than in e2 and e3 genotypes in the June sowings, but did not differ between genotypes in the July sowings. The high yield of E2E3 genotypes in the July sowings was attributed to increased number of nodes and flower production while maintaining pod-set ratio. Appropriate choice of sowing date is suggested to be essential when using E3 genotypes.

Abbreviations: HI: harvest index; NIL: near-isogenic line; RUE: radiation use efficiency; TDM: total above-ground dry matter; TRI: total solar radiation intercepted

1. Introduction

Soybean (*Glycine max* [L.] Merr.) is an important crop for food and protein and is widely cultivated throughout the world, from the tropics to temperate regions. The combination of maturity genes plays an important role in obtaining high yields, with the most effective combination varying according to the area where soybeans are cultivated (Jiang et al., 2014; Tsubokura et al., 2014). Several maturity genes have been identified so far (Watanabe, Harada, & Abe, 2012) and the effects of those genes on basic agronomic traits such as growth duration or seed yield have been studied (Cober & Morrison, 2010; Kumudini, Pallikonda, & Steele, 2007). However, those experiments were conducted in the United States and Canada using near-isogenic lines (NILs) of soybean cultivars ‘Clark’ and ‘Harosoy’, and information on the effects of maturity genes in Japanese cultivars is still limited, especially in warm regions of Japan.

However, NILs of the Japanese cultivar ‘Enrei’ have now been developed through marker assisted selection (Yamada et al., 2012). ‘Enrei’ is so far the only Japanese soybean cultivar to have had its whole genome sequenced (Shimomura et al., 2015). It is used as a parent for breeding due to its higher protein content which is suitable for tofu production (Takahashi et al., 2004). ‘Enrei’ is also used as a standard cultivar for crop development models in Japan (Nakano et al., 2015). It is widely cultivated in the Hokuriku region and in Yamagata prefecture and is the third most planted cultivar in Japan (Ministry of Agriculture, Forestry and Fisheries, 2017). In warm regions, ‘Enrei’ is classified as an early maturing cultivar (Okabe, Kikuchi, & Saruta, 2006).

‘Enrei’ possesses the maturity gene combination E1e2e3E4 (Tsubokura et al., 2014). E1, E2 (Bernard, 1971), E3 (Buzel, 1971), and E4 (Buzzell & Voldeng, 1980) are known as major genes (Watanabe et al., 2012). E1 is reported to be a repressor of *GmFT2a* and *GmFT5a*, orthologues of *FLOWERING LOCUS T* of *Arabidopsis* (Xia et al., 2012); E2 is reported to be an orthologue of the *GIANTEA* gene of *Arabidopsis*, related to a circadian clock (Watanabe et al., 2011); and E3 and E4 are reported to be phytochrome *A* (*phyA*) genes sensitive to the ratio of red light to far-red
light (R:FR ratio), named as GmphyA3 and GmphyA2, respectively (Liu et al., 2008; Watanabe et al., 2009). Yamada et al. (2012) developed NILs of maturity genes E2 and E3 of ‘Enrei’, and compared seed yield and basic agronomic traits at several locations in Japan. Although Yamada et al. observed a significant difference in growth duration and seed yield between ‘Enrei’ and the NILs, the key traits differentiating seed yield were not revealed.

The objective of our experiment was to elucidate how maturity genes E2 and E3 in the ‘Enrei’ genetic background affect yield from the aspect of crop production in a warm region. Our results will provide useful information for breeding high-yielding cultivars suitable for warm regions and information for selecting appropriate combinations of growing season and cultivar. To achieve this objective, field experiments were conducted at Fukuyama in 2016 and 2017. Two sowing dates, June sowing and July sowing, were set in each year.

### 2. Materials and methods

#### 2.1 Plant materials and growth conditions

‘Enrei’ (E1e2e3E4) and three NILs of ‘Enrei’ (e2E3, E2e3, and E2E3), developed by the Institute of Crop Science, NARO, were used in this study. The NILs for e2E3 and E2e3 were developed by backcrossing between ‘Enrei’ and ‘Fukuyutaka’ (E1E2E3E4) or ‘Sachiyutaka’ (E1E2e3E4), respectively. Each of these two lines was used in the field experiment after six times of backcrossing (BC6).

The NIL for E2E3 was developed by crossing the NIL for E2e3 (BC6) as a seed parent and the NIL for e2E3 (BC6) as a pollen parent. These three NILs were used in the field experiments at the F6 generation after backcrossing or crossing was complete.

Field experiments were conducted at the Western Region Agricultural Research Center, NARO, Fukuyama, Hiroshima, Japan (34°30’S, 133°23’E, 1 m above sea level) in 2016 and 2017. In 2016, seeds were sown into a lysimeter (alluvial soil) on June 17, and into an upland field converted from a paddy field (gray lowland soil) on July 19. In 2017, seeds were sown on June 19 into an upland field converted from a paddy field where a water table controlling system had been installed (FOEAS: farm-oriented enhancing aquatic system, Shimada et al., 2012; Wakasugi & Fujimori, 2009), and into a lysimeter on July 18. June sowing is a conventional sowing date in Hiroshima prefecture, and July sowing is now focused as a new alternative sowing date, which can avoid wet injury during rainy season from June to July. Inorganic fertilizers were applied as a basal dressing at 3 g m⁻² of N, 10 g m⁻² of P₂O₅, and 10 g m⁻² of K₂O before sowing except for the July sowings in 2017. In the July sowings in 2017, application of inorganic fertilizers was changed to 3 g m⁻² of N, 6 g m⁻² of P₂O₅, 6 g m⁻² of K₂O, and 12 g m⁻² of Mn in accordance with the soil test conducted after the experiment in 2016. Seeds were sown in rows by hands at 0.6 m between rows with 0.15 m intra-row spacing (11.1 plants m⁻²). The single-plot area of the upland field converted from the paddy field was 3.3 m × 3.6 m in 2016 and 2.6 m × 3.6 m in 2017. The single-plot area of the lysimeter was 3.7 m × 3.7 m in 2016 and 1.8 m × 1.8 m in 2017. Except for the July sowings in 2017, three replications were set in a randomized block design. Two replications were set in the July sowings in 2017. Furrow irrigation was conducted in the July sowings in 2016 one or two times a week from late July to late August to ensure adequate soil moisture. The water table level was maintained at ~0.3 m in the lysimeter and the FOEAS field during the growing season. Lodging was prevented by the installation of a net (0.3 m × 0.3 m mesh) at 0.3 m above the ground at flowering. Hand weeding was conducted until canopy closure. Insecticides and fungicides were applied periodically to avoid biotic stresses.

#### 2.2. Measurements

Daily solar radiation and temperature data were recorded at the Western Region Agricultural Research Center. The day length at experimental site was obtained from national astronomical observatory (National Astronomical Observatory of Japan, 2018). Dates of reproductive growth stages (R1, R5, R7; Fehr & Caviness, 1977) were recorded. Destructive measurements were conducted three times during the growth period, at R1, R5, and around R6 only in the June sowings. Total above-ground dry matter (TDM) was determined from eight plants harvested from each replication. Leaf area index (LAI) was determined from the leaf area of four representative plants measured by leaf area meter (LI-3100; LI-COR, Lincoln, NE, USA). TDM was determined by weighing after oven-drying for 72 h at 80°C. In the June sowings, canopy coverage was measured by digital image analysis twice a week from emergence (VE) until canopy closure according to the method of Purcell (2000). Cumulative solar radiation intercepted was calculated by interpolating the daily canopy coverage, and solar radiation use efficiency (RUE) from VE to R1 and from R1 to R5, and the mean RUE (by regression from VE to R1 and R1 to R5) was estimated. Total solar radiation was used to estimate RUE.

Seed yield was determined from 12 plants (occupying 1.08 m⁻²) per replication in the June sowings. In the July sowings, 18 randomly selected plants were
harvested from one replication and yield were estimated from six representative plants which showed intermediate weight in order to exclude plants whose emergence was delayed due to ununiform emergence in 2016 caused by severe drought after rainy season. Because ‘Enrei’ shows a shattering-susceptible trait (Funatsuki et al., 2008), plants were harvested after R7 and air-dried before measurement to avoid yield loss due to shattering. After separating seeds from pod walls, stem and pod walls were dried for 72 h at 80°C and weighed. Seed moisture content was adjusted to 15% in the calculation of seed yield. Leaves and petioles were completely excluded from the measurement of the TDM at maturity and the calculation of harvest index (HI). Yield components (total number of nodes, total number of flowers, total number of pods, pod-set ratio [total number of pods per total number of flowers], number of embryos [>1 mm diam.] per pod, seed set ratio [number of fertile seeds per embryo], and 100-seed weight) were determined from six representative plants per replication. Total number of flowers was estimated from the number of flower scars at R8. Pod-set ratio was calculated from the sum of pods and scars.

The effects of genotype and year were tested by two-way ANOVA. And the effects of E2 and E3 were also tested by two-way ANOVA. All analyses were conducted in BellCurve for Excel software v. 2.15 (Social Survey Research Information Co., Ltd., Tokyo).

3. Results

Table 1 shows the changes in mean air temperature, solar radiation and day length at the Western Region Agricultural Research Center in 2016 and 2017. The mean temperature over the whole growing season, from mid-June to late October, was 0.8°C higher in 2016 than in 2017. In particular, the temperature in the late growing season, from early September to late October, was 1.9°C higher in 2016 than in 2017. Although the mean solar radiation over the whole growing season was quite similar between the two years, the trends were different. In 2016, the solar radiation from early July to mid-August was 2.3 MJ m⁻² d⁻¹ higher than in 2017, but the solar radiation from late August to early October in 2016 was 1.6 MJ m⁻² d⁻¹ lower than in 2017. The longest day length in Fukuyama was 14.46 h in middle and late June and declined to 10.98 h in late October.

Emergence (VE) was observed on June 21 and July 24 in 2016 and on June 22 and July 23 in 2017. Table 2 shows the observed reproductive growth stages of the June and July sowings in 2016 and 2017. R1 of the June sowings ranged from 29.7 DAE (Days after Emergence) (e2e3) to 43.0 DAE (E2E3) in 2016 and from 29.7 DAE (e2e3) to 45.0 DAE (E2E3) in 2017. R1 of the July sowings ranged from 25.0 DAE (e2e3) to 35.0 DAE (E2E3) in 2016 and from 25.5 DAE (e2e3) to 34.0 DAE (E2E3) in 2017. R7 of the June sowings ranged from 101.7 DAE (e2e3) to 117.0 DAE (E2E3) in 2016 and from 97.3 DAE (e2e3) to 126.0 DAE (E2E3) in 2017. R7 of the July sowings ranged from 76.0 DAE (e2e3) to 90.0 DAE (E2E3) in 2016 and from 75.0 DAE (e2e3) to 95.0 DAE (E2E3) in 2017. Significant effect was observed in genotype, year and genotype × year interaction in VE-R1 and R1–R7 in both sowing dates.

Table 3 shows the analysis of variance of maturity genes E2 and E3 with respect to durations of growth periods in each of the sowings in the two years. E2 significantly extended the period from VE to R1. E3 extended the period from VE to R1 for both sowing dates. The interaction between E2 and E3 was significant in the period from R1 to R7 for both sowing dates. E3 lengthens the reproductive period in e2 background and shortens the reproductive periods in E2 background. E2 has a larger effect in e3 background than E3 background for the reproductive periods. Although the ANOVA results were similar in the June and July sowings, the durations of the growth periods from VE to R1 and from R1 to R7 were shorter in the July sowings.

Table 4 shows the effects of maturity genes E2 and E3 on LAI in the June sowings. The LAI at R1 ranged from 1.74 (e2e3 in 2017) to 7.76 (E2E3 in 2017). The LAI at R5 ranged from 5.66 (e2e3 in 2016) to 9.77 (E2E3 in 2017). Although the difference between genotypes was significant at R1, R5, and R6, the difference was smaller at the later growth stages.

Table 5 shows the TDM, total solar radiation intercepted (TRI), and RUE from VE to R5. TDM at R1 and R5 showed trends similar to that with LAI. E2E3 showed significantly higher dry matter accumulation than the
other genotypes. Although RUE from VE to R1 differed significantly between genotype and year, RUE from R1 to R5 did not differ significantly. The interaction between genotype and year was significant for both periods. Overall, the mean RUE ranged from 0.723 to 1.088 g MJ⁻¹ and did not differ significantly between genotypes.

Table 6 shows seed yield, TDM at maturity, and HI in the June sowings. Although seed yield ranged from 305 g m⁻² (e2E3 in 2016) to 603 g m⁻² (e2E3 in 2017), the difference between genotypes was not significant and the difference between years was significant. TDM at maturity ranged from 550 g m⁻² (e2E3 in 2016) to 867 g m⁻² (e2E3 in 2017) and the ANOVA showed the same trends as were observed with seed yield. On the other hand, HI ranged from 0.449 (E2E3 in 2016) to 0.623 (e2e3 in 2017) and the differences between years and genotypes were both significant. While HI in E2E3 genotype was the lowest in both years, the effect of E2 and E3 was not statistically significant. A significant interaction between genotype and year was not observed for seed yield, HI, or TDM at maturity.

Table 7 shows yield components in the June sowings. Number of main stem nodes ranged from 11.3 (e2e3 in 2016) to 19.1 (E2E3 in 2017). Both E2 and E3 gene
Table 4. Effect of maturity genes $E_2$ and $E_3$ on LAI at R1, R5, and R6 in June sowing.

|        | LAI (R1) | LAI (R5) | LAI (R6) | Remarks |
|--------|----------|----------|----------|---------|
| 2016   |          |          |          |         |
| $e_2e_3$ | 2.90     | 5.66     | 4.72     | Enrei   |
| $e_2E_3$ | 4.85     | 6.89     | 6.80     | $e_3$$\rightarrow$$E_3$ |
| $E_2e_3$ | 4.43     | 6.55     | 5.29     | $e_2$$\rightarrow$$E_2$ |
| $E_2E_3$ | 6.44     | 9.43     | 6.56     | $E_2$$+$$E_3$ |
| 2017   |          |          |          |         |
| $e_2e_3$ | 1.74     | 6.51     | 5.46     | Enrei   |
| $e_2E_3$ | 4.34     | 7.16     | 5.84     | $e_3$$\rightarrow$$E_3$ |
| $E_2e_3$ | 4.39     | 7.52     | 6.13     | $e_2$$\rightarrow$$E_2$ |
| $E_2E_3$ | 7.76     | 9.77     | 8.38     | $E_2$$+$$E_3$ |

Analysis of variance:

| Source         | $F$     | df     | $p$   |
|----------------|---------|--------|-------|
| Genotype       | 40.1*** | 1      | < 0.001 |
| Year           | 0.1 NS  | 1      | 0.45  |
| Genotype $\times$ Year | 2.9 NS  | 1      | 0.119 |

Table 5. Effects of maturing genes $E_2$ and $E_3$ on TDM, TRI, and RUE from VE to R5 in the June sowings.

|        | TDM (R1) (g m$^{-2}$) | TRI (VE-R1) (MJ) | RUE (VE-R1) (g MJ$^{-1}$) | TDM(R5) (g m$^{-2}$) | TRI(VE-R5) (MJ) | RUE (R1-R5) (g MJ$^{-1}$) | Mean RUE (VE-R5) (g MJ$^{-1}$) | Remarks |
|--------|-----------------------|-----------------|---------------------------|-----------------------|-----------------|---------------------------|--------------------------------|---------|
| 2016   |                       |                 |                           |                       |                 |                           |                                 |         |
| $e_2e_3$ | 127                  | 161             | 0.777                     | 457                   | 534             | 0.886                     | 0.847                          | Enrei   |
| $e_2E_3$ | 243                  | 294             | 0.822                     | 653                   | 735             | 0.929                     | 0.877                          | $e_3$$\rightarrow$$E_3$ |
| $E_2e_3$ | 213                  | 269             | 0.794                     | 677                   | 945             | 0.688                     | 0.723                          | $e_2$$\rightarrow$$E_2$ |
| $E_2E_3$ | 345                  | 430             | 0.800                     | 886                   | 1003            | 0.944                     | 0.871                          | $E_2$$+$$E_3$ |
| 2017   |                       |                 |                           |                       |                 |                           |                                 |         |
| $e_2e_3$ | 86                   | 120             | 0.732                     | 560                   | 507             | 1.224                     | 1.088                          | $e_3$$\rightarrow$$E_3$ |
| $e_2E_3$ | 216                  | 211             | 1.025                     | 590                   | 655             | 0.844                     | 0.913                          | $e_2$$\rightarrow$$E_2$ |
| $E_2e_3$ | 212                  | 210             | 1.014                     | 663                   | 710             | 0.901                     | 0.942                          | $E_2$$+$$E_3$ |
| $E_2E_3$ | 428                  | 362             | 1.178                     | 784                   | 845             | 0.738                     | 0.965                          | $E_2$$+$$E_3$ |

Analysis of variance:

| Source         | $F$     | df     | $p$   |
|----------------|---------|--------|-------|
| Genotype       | 38.1*** | 1      | < 0.001 |
| Year           | 0.0 NS  | 1      | 0.45  |
| Genotype $\times$ Year | 2.9 NS  | 1      | 0.119 |

Table 6. Effects of maturing genes $E_2$ and $E_3$ on seed yield, TDM at maturity, and HI in June sowing.

|        | Seed yield (g m$^{-2}$) | TDM at maturity (g m$^{-2}$) | Harvest index$^a$$^c$ | Remarks |
|--------|-------------------------|-------------------------------|------------------------|---------|
| 2016   |                         |                               |                        |         |
| $e_2e_3$ | 376                    | 675                           | 0.473                  | Enrei   |
| $e_2E_3$ | 305                    | 550                           | 0.473                  | $e_3$$\rightarrow$$E_3$ |
| $E_2e_3$ | 337                    | 553                           | 0.515                  | $e_2$$\rightarrow$$E_2$ |
| $E_2E_3$ | 320                    | 602                           | 0.449                  | $E_2$$+$$E_3$ |
| 2017   |                         |                               |                        |         |
| $e_2e_3$ | 547                    | 746                           | 0.623                  | Enrei   |
| $e_2E_3$ | 603                    | 867                           | 0.591                  | $e_3$$\rightarrow$$E_3$ |
| $E_2e_3$ | 515                    | 716                           | 0.612                  | $e_2$$\rightarrow$$E_2$ |
| $E_2E_3$ | 543                    | 852                           | 0.541                  | $E_2$$+$$E_3$ |

Analysis of variance:

| Source         | $F$     | df     | $p$   |
|----------------|---------|--------|-------|
| Genotype       | 0.2 NS  | 1      | 0.65  |
| Year           | 55.3*** | 1      | < 0.001 |
| Genotype $\times$ Year | 1.0 NS  | 1      | 0.32  |

| Source         | $F$     | df     | $p$   |
|----------------|---------|--------|-------|
| $E_2$          | 0.3 NS  | 1      | 0.51  |
| $E_3$          | 0.0 NS  | 1      | 0.97  |
| $E_2$$\times$$E_3$ | 0.0 NS  | 1      | 0.97  |

$^a$With 15% moisture content.

$^b$Leaves and petioles were excluded.

$^c$Arc sine transformation was done before the analysis of variance.

$^*, **, *** F$ values significance at $p < 0.05, p < 0.01, p < 0.001$, respectively. NS means non-significant at $p = 0.05$ level.
significantly increased the number of main stem nodes. Total number of nodes per plant ranged from 41.2 (e2E3 in 2016) to 74.4 (E2E3 in 2017). Although the range differed between the 2 years, E3 increased total number of nodes and the highest was E2E3. The total number of flowers per plant also showed the similar trend as the total number of nodes per plant. On the other hand, the difference in total number of pods per plant was not significant. Both E2 and E3 gene significantly decreased pod-set ratio. Pod-set ratio was significantly lower in late-maturing lines E2E3 and E2E3 than in 'Enrei'.

Table 8 shows seed yield, TDM at maturity, and HI in the July sowings. Seed yield ranged from 366 g m⁻² (e2E3 in 2017) to 660 g m⁻² (E2E3 in 2017), and the difference between genotypes was significant. The effects of E2 and E3 on seed yield were both significant and interaction between E2 and E3 also observed. E2 increased seed yield greater in E3 background. The difference between years was not significant. TDM at maturity ranged from 504 g m⁻² (e2E3 in 2017) to 954 g m⁻² (E2E3 in 2016) and the ANOVA showed the same trends as were seen with seed yield. HI ranged from 0.548 (e2e3 in 2016) to 0.618 (e2E3 in 2017) and the difference between years was significant but the difference between genotypes was not significant. A significant interaction between genotype and year was not observed in seed yield, HI, or TDM at maturity.

Table 9 shows yield components in the July sowings. Number of main stem nodes ranged from 11.4 (e2e3 in 2016 and 2017) to 15.1 (E2E3 in 2017). E3

**Table 7. Effects of maturing genes E2 and E3 on yield components in June sowing.**

| Year | No. of main stem nodes | Total no. of nodes (plant⁻¹) | Total no. of flowers (plant⁻¹) | No. of flowers/node | Total no. of pods (plant⁻¹) | Pod set ratio (%) | No. of embryos/pod | Seed set ratio (%) | 100-seed weight (g) | Remarks |
|------|------------------------|-------------------------------|--------------------------------|-------------------|-----------------------------|------------------|-------------------|------------------|------------------|---------|
| 2016 | e2e3                   | 11.3                          | 43.4                           | 153               | 3.52                        | 94.0             | 61.7              | 2.01             | 66.8             | 31.4    |
|      | e2E3                   | 13.4                          | 41.2                           | 116               | 2.81                        | 68.8             | 59.4              | 2.01             | 76.3             | 30.8    |
|      | E2e3                   | 13.5                          | 45.1                           | 149               | 3.27                        | 73.3             | 50.1              | 1.98             | 79.7             | 30.8    |
|      | E2E3                   | 17.3                          | 60.1                           | 212               | 3.52                        | 91.3             | 43.5              | 2.05             | 79.2             | 22.7    |
| 2017 | e2e3                   | 13.1                          | 51.0                           | 180               | 3.53                        | 104.9            | 58.7              | 2.08             | 83.1             | 31.9    |
|      | e2E3                   | 15.4                          | 62.8                           | 220               | 3.23                        | 111.8            | 50.8              | 2.03             | 81.4             | 34.5    |
|      | E2e3                   | 14.9                          | 57.6                           | 184               | 3.18                        | 88.6             | 48.5              | 2.09             | 85.0             | 34.8    |
|      | E2E3                   | 19.1                          | 74.4                           | 243               | 3.32                        | 97.7             | 40.5              | 2.06             | 79.4             | 36.1    |

Analysis of variance

| Genotype | Year | No. of main stem nodes | Total no. of nodes (plant⁻¹) | Total no. of flowers (plant⁻¹) | No. of flowers/node | Total no. of pods (plant⁻¹) | Pod set ratio (%) | No. of embryos/pod | Seed set ratio (%) | 100-seed weight (g) | Remarks |
|----------|------|------------------------|-------------------------------|--------------------------------|-------------------|-----------------------------|------------------|-------------------|------------------|------------------|---------|
| E2       |      | 45.9 **                | 3.1 NS                        | 2.8 NS                        | 0.1 NS            | 1.0 NS                      | 33.9 ***         | 0.1 NS            | 2.1 NS            | 0.3 NS            |         |
| E3       |      | 51.3 **                | 6.1 *                         | 3.2 NS                        | 1.0 NS            | 0.1 NS                      | 91.2 *           | 0.1 NS            | 0.0 NS            | 0.5 NS            |         |
| E2×E3    |      | 4.2 NS                 | 0.8 NS                        | 2.8 NS                        | 5.1 *             | 2.5 NS                      | 0.3 NS           | 1.9 NS            | 1.6 NS            |       |

With 15% moisture content.

* * *, **, *** F values significance at p < 0.05, p < 0.01, p < 0.001, respectively. NS means non-significant at p = 0.05 level.

**Table 8. Effects of maturing genes E2 and E3 on seed yield, TDM at maturity, and HI in July sowing.**

| Year | Seed yield (g m⁻²) | TDM at maturity (g m⁻²) | Harvest index | Remarks |
|------|-------------------|-------------------------|---------------|---------|
| 2016 | e2e3             | 403                     | 624           | 0.548   | Enrei   |
|      | e2E3             | 521                     | 798           | 0.557   | e2→E3   |
|      | E2e3             | 433                     | 652           | 0.567   | e2→E2   |
|      | E2E3             | 631                     | 954           | 0.563   | E2 + E3 |
| 2017 | e2e3             | 366                     | 504           | 0.618   | Enrei   |
|      | e2E3             | 481                     | 723           | 0.564   | e3→E3   |
|      | E2e3             | 395                     | 559           | 0.600   | e2→E2   |
|      | E2E3             | 660                     | 949           | 0.590   | E2 + E3 |

Analysis of variance

| Genotype | Year | Seed yield (g m⁻²) | TDM at maturity (g m⁻²) | Harvest index | Remarks |
|----------|------|-------------------|-------------------------|---------------|---------|
| E2       |      | 12.2 **            | 9.2 **                  | 0.4 NS        |         |
| E3       |      | 50.6 ***           | 51.6 ***                | 0.7 NS        |         |
| E2×E3    |      | 5.1 *              | 3.9 NS                  | 0.2 NS        |         |

With 15% moisture content

* Leaves and petals were excluded.

Arc sine transformation was done before the analysis of variance.

* * *, **, *** F values significance at p < 0.05, p < 0.01, p < 0.001, respectively. NS means non-significant at p = 0.05 level.
gene significantly increased the number of main stem nodes and interaction between E2 and E3 also observed. Total number of nodes per plant ranged from 35.0 (E2E3 in 2016) to 52.2 (E2E3 in 2017). The total number of nodes per plant was significantly larger in E2E3 than in ‘Enrei’, the same as was seen in the June sowings. Both E2 and E3 increased total number of flowers. In addition, E2E3 showed a significantly larger number of flowers per node. The total number of pods per plant was significantly increased in E2 and E3 genotypes and significant interaction between E2 and E3 was observed. Although a significant interaction between genotype and year was observed, the pod-set ratio ranged from 48.0 to 59.3 and the range was smaller than in the June sowings.

### 4. Discussion

The effect of maturity genes E2 and E3 on growth period was tested in this experiment. NILs with E2 and E3 showed extended duration of the vegetative growth period (VE to R1) (Table 3). In a previous study conducted in a growth chamber, E2 extended the duration of the growth period consistently, regardless of short day length or long day length treatment; in contrast E3 extended the duration of the growth period more as the day length increased to longer than 14 h through the experiment (McBlain, Hesketh, & Bernard, 1987). Yamada et al. (2014) conducted a QTL analysis of the numbers of days from sowing to flowering and from first flowering date to maturity in several different environments (two locations and 6 years), and reported that E3 was detected constantly in the period from sowing to flowering, but was not always detected in the period from first flowering to maturity. Because E3 regulates phyA, which responds to R:FR ratio (Watanabe et al., 2009), day length can be an important factor which explain the different results between locations. We conducted the field experiments at Fukuyama (34° 30’N), a relatively low latitude and short day length as compared to Yamada et al.’s (2014) experiments at 36° 00’N and 36°01’N where the effect of E3 was not always detected in the period from first flowering to maturity. The average day length in late June at 36°01’N (Yawara experimental field) was 14.63 h (National Astronomical Observatory of Japan, 2018) and 0.17 h longer than that of Fukuyama. Therefore, it is reasonable to understand that the effect of E3 was not detected in our experiments. The interaction between E2 and E3 was not significant from VE to R1, but was significant from R1 to R7. This result means that E2 and E3 extend the duration of the period from VE to R1 additively. On the other hand, E3 lengthens the reproductive period in e2 background and shortens the reproductive periods in E2 background. For all genotypes, the number of days from R1 to R7 in the July sowings was smaller than that of the June sowings, which suggests that other factors, for example, short day length or low temperature might promote maturity. Although vegetative growth was extended by E2 and E3, the change in the R7 date was relatively moderate as compared with the R1 date. This fact is remarkable for its utility in breeding, because it suggests that the ratio of vegetative growth period and reproductive growth period can be changed by modifying the combination of maturity genes. However, further study is needed to reveal the quantitative effects of the day length and temperature.

In the June sowings, LAI was measured on the dates at which R1, R5, and R6 were reached. Late-maturing
genotypes showed larger LAI at R1 and R5. At R6, however, LAI in late-maturing lines tended to decrease. There is a possibility that leaves and petioles in the lower nodes abscised owing to degradation of the canopy structure or to leaf senescence. The changes in total dry matter production (TDM) and TRI were analyzed only for the period from VE to R5 to minimize the fluctuation caused by leaf abscission during the period from R5 to R6. TDM at R1 and R5 showed trends similar to that of LAI, with late-maturing genotypes showing larger TDM. TRI from VE to R1 and from R1 to R5 differed between genotypes. Though the effect of genotype on RUE (VE-R1) was significant, the effects of E2 or E3 on RUE were not clearly detected. Overall, the mean RUE from VE to R5 was not significantly different between genotypes. The mean RUE from VE to R5 obtained in this experiment ranged from 0.723 to 1.088 g MJ\(^{-1}\). Bajgain et al. (2015) reported that the RUE in ‘Enrei’ ranged from 0.85 to 1.06 g MJ\(^{-1}\) when measured in the same way and for the same duration, and our results were quite similar to the results of that previous report. Consequently, the increase in TDM at R5 was attributed to the increase in TRI rather than to a difference in RUE. This finding suggests the possibility of estimating dry matter accumulation until R5 under a no-lodging environment after modifying maturity genes in an ‘Enrei’ background, if the date of R5 is predicted by a crop development model.

Although TDM at R5 differed between genotypes, the difference in seed yield between genotypes was not significant in the June sowings. The difference in TDM at maturity (attached leaves and petioles were completely removed) between genotypes was also not significant. On the other hand, HI differed between genotypes: E2E3 showed the lowest HI in both years. The differences between genotypes in TDM at R1 and R5 were presumed to be caused mainly by the differences in leaf DM and petiole DM. On the other hand, E3 genotypes showed greater seed yield than other genotypes in the July sowings. The difference in the HI in the July sowings was not significant, but TDM at maturity differed between genotypes. The difference in seed yield was attributed to TDM at maturity rather than HI. Yamada et al. (2012) reported that the trend in seed yield of different genotypes differed between northern sites (comparing ‘Enrei’ and E2E3) and southern sites (comparing ‘Enrei’, E2E3, and e2E3), and the difference in seed yield between genotypes was more obvious in the southern sites. Severe lodging and self-shading were mentioned in their discussion. In our experiment, the differences between genotypes were not clear in June sowings. However, the difference in seed yield was significant in the July sowings. In the context of day length, the day length of the July sowings was shorter than that of the June sowings, and the day length in the southern sites in the experiment of Yamada et al. (2012) also were shorter than in the northern sites, and the results in these experiments coincided. In addition, as lodging was prevented in our experiment by using nets in the June sowings, the existence of other factors which reduced the difference in seed yield is suggested.

To reveal factors which differentiate the seed yield, yield components were analyzed. The total number of nodes was larger in E3 genotypes in both the June and July sowings, and the total number of flowers was largest in E2E3. Although pod-set ratio was smallest in E2E3 in the June sowings, no significant difference in pod-set ratio between genotypes was observed in July sowing. The pod-set ratio obtained in this experiment ranged from 40.5 to 61.7. Mochizuki, Shiraiwa, Nakagawa, and Horie (2005) reported a difference in pod-set ratio between cultivars ranging from 45 to 60, which was similar to our values. Thus, genotype can be a candidate by which to differentiate pod-set ratio. However, the difference in temperature during the reproductive growth stage can also be a reason for the difference in pod-set ratio between genotypes.

The temperature and day length differed between sowing dates and were presumed to be factors which decreased the pod-set ratio in later maturing genotypes.

Table 10. Mean air temperature and solar radiation during reproductive growth duration.

|             | June sowing |                                        | July sowing |                                        |
|-------------|-------------|----------------------------------------|-------------|----------------------------------------|
|             | Mean air temperature (°C) | Solar radiation (MJ m\(^{-2}\) d\(^{-1}\)) | Mean air temperature (°C) | Solar radiation (MJ m\(^{-2}\) d\(^{-1}\)) |
|             | R1–R5      | R1–R7                                 | R1–R5      | R1–R7                                 |
| 2016 e2e3   | 28.5        | 26.8                                  | 20.4        | 16.0                                  |
| 2016 E2E3   | 29.2        | 26.3                                  | 20.8        | 15.3                                  |
| 2016 E2E3   | 29.1        | 25.8                                  | 20.1        | 15.2                                  |
| 2016 E2E3   | 28.5        | 25.4                                  | 19.7        | 14.6                                  |
| 2017 e2e3   | 29.0        | 26.4                                  | 17.9        | 16.0                                  |
| 2017 E2E3   | 28.7        | 25.1                                  | 17.7        | 15.3                                  |
| 2017 E2E3   | 28.8        | 23.9                                  | 17.9        | 13.9                                  |
| 2017 E2E3   | 27.9        | 23.0                                  | 17.9        | 13.4                                  |
| 2017 E2E3   | 27.6        | 25.4                                  | 17.6        | 13.0                                  |
| 2017 E2E3   | 26.7        | 23.8                                  | 16.0        | 12.2                                  |
| 2017 E2E3   | 26.7        | 23.6                                  | 15.6        | 12.2                                  |
| 2017 E2E3   | 25.8        | 23.0                                  | 15.0        | 11.5                                  |
in the June sowings. Previous studies have reported that high temperatures during the reproductive period decrease the pod-set ratio (Djanaguiraman, Prasad, & Schapaugh, 2013; Oh-E et al., 2007). The pod-set ratio in ‘Enrei’ observed by Oh-E et al. (2007) decreased from 69.5 to 39.8 as the temperature during reproductive growth stage increased. In the June sowings, the temperature in the pod-set period (estimated from R1 to R5) was higher than that in the July sowings owing to the high temperature in mid-August (Tables 1 and 10). Although pod-set ratio decreased in the late-maturing genotypes in the June sowings, ‘Enrei’ did not change between the two sowing dates. In contrast, there is also the possibility that long day length decreases the pod-set ratio. Han et al. (2006) reported the long day length treatment after flowering inhibited not only plant development but also pod set. E3 genotypes, which yielded higher in the July sowings, respond to the R:FR ratio and may be affected by day length. Xu et al. (2013) reported that with ‘Harosoy’ NILs, E3E4 genotypes develop fewer pods than do e3E4 genotypes under long day length treatment over 30 days after flowering. Even though the effect of E3 on growth duration was not significant from R1 to R7 (Table 3), there is the possibility that the multiple functions of phyA affected pod set in soybeans under the long day length in the June sowings.

While the extent of increase in seed yield in e2E3 genotype is larger than E2e3 genotype in July sowing in our experiment, Yamada et al. (2012) reported E2e3 genotype also significantly increased seed yield in southern experimental site. The difference in management method also can be an important factor. We conducted irrigation systematically to maintain optimum soil moisture condition at Fukuyama. In June sowing, the conventional sowing in warm region, ‘Enrei’ reached to flowering and pod setting stage in late July to middle August (Table 2). The drought is easy to occur during this period after rainy season due to lower precipitation if irrigation was not conducted. And there is a possibility that the other late-maturing genotypes reached to flowering and pod setting stage after this period. Though further research is needed, the irrigation also can be an important factor in our experiment.

Shimada, Hirokawa, and Miyagawa (1990) reported that the highest yield was achieved by early June sowing in Fukuyama using the cultivar ‘Tamahomare’, and pointed out that in order to achieve seed yield more than 600 g m⁻², the LAI needed to be around 9. In our experiment, E2E3 achieved a LAI of 9.77 at R5 but did not yield more than 600 g m⁻². On the other hand, e2E3 yielded 603 g m⁻² in 2017, even though LAI was 7.16 at R5. The response of seed yield to LAI may differ in NILs in which the duration of the vegetative growth period is extended by E3. Matsuo, Fukami, and Tsuchiya (2016) reported that an increase in seed yield was only observed in US cultivars and not in Japanese cultivars under early planting and longer growth duration. The difference in background genotype may play an important role. In addition, several studies in the United States showed that longer growth durations do not always result in higher yields (Egli, 1993; Kane & Grabau, 1992). The total number of nodes and total number of pods were reported to be important for achieving high yield in warm regions in Japan (Saitoh, Isobe, & Kuroda, 1998; Shimada et al., 1990). Our research suggests the importance of maintaining pod set by selecting an appropriate sowing date in addition to achieving larger node production or higher LAI by extending growth duration for high yield. More detailed information about factors causing fluctuations in sink formation is needed to provide appropriate selection of growth duration and cultivars. In addition, there is a possibility that seed appearance also can be improved by changing the growth duration. ‘Enrei’ is classified as an early maturing cultivar in warm region and severe occurrence of green stem disorder was pointed (Okabe et al., 2006; Saruta et al., 2012), which causing the degradation in seed appearance and quality. Though we did not evaluate the extent of green stem disorder and seed appearance in this experiment, the utilization of maturity genes can be a solution for improving the seed appearance. Yamada et al. (2012) reported that the effects of E2 and E3 gene on protein content and 100-seed weight were not statistically significant. On the other hand, in our experiment 100-seed weight of E2E3 in June sowing was relatively low and E2e3 in July sowing was relatively high in 2016. The amount of solar radiation in middle and late September in 2016 was quite low as compared with 2017 and this can be reason for smaller seed size in E2E3 in June sowing. As for July sowing, the effect of E2 on 100-seed weight was significant. The effect of E2e3 genotype on total number of pods was smaller than e2E3 genotype while E2e3 extending growth duration longer than e2E3. That may supply more assimilates to seeds and resulted in larger seed size. However, the difference between June and July sowing is still unclear. Further research is needed the effect of growth duration on seed quality and seed size.

The Chugoku region, where our Fukuyama experimental site is located, is one of the warm regions of Japan. The leading cultivar in the region is presently ‘Sachiyutaka’. ‘Sachiyutaka’ was developed by crossing ‘Enrei’ (E1e2e3E4) and ‘Fukuyutaka’ (E1E2E3E4) (Takahashi et al., 2004), and ‘Sachiyutaka’ possesses the combination of E1E2e3E4 (Tsubokura et al., 2014). Recently, cultivars suitable for late sowing are strongly required to avoid injury from excess water in the rainy season (Fatichin et al., 2013; Takeda & Sasaki, 2013). Although the only cultivar for warm regions that
presently possesses E3 is ‘Fukuyutaka’, ‘Fukuyutaka’ shows a longer growth duration and the risk of lodging is a concern (Matsuo et al., 2017). Our research suggests that genotypes including the E3 allele may be suitable for late sowing owing to increasing node production while maintaining pod-set ratio. Although a direct comparison between the yielding ability of NILs of ‘Enrei’ with E3 genotypes and the yielding ability of ‘Fukuyutaka’ or ‘Sachiyutaka’ is still needed, the utilization of E3 instead of E2 in breeding new cultivars for late sowing in warm regions, as an alternative to ‘Sachiyutaka’, is worth considering.

Our experiment provides important information about the effects of maturity genes E2 and E3 on yield formation over 2 years with two sowing dates. TDM at R5, LAI at R5, total number of nodes at maturity, and total number of flowers at maturity were all increased by introducing E2 and E3 in this warm region. However, the pod-set ratio differed between genotypes in the June sowings, although not in the July sowings. The most appropriate combination of genotype and growing season for this warm region was considered to be late-maturing genotypes with E3 and July sowing in ‘Enrei’ background, which can produce more nodes and flowers while maintaining pod-set ratio.

5. Conclusions

Maturity genes E2 and E3 extended the duration from emergence to beginning of flowering (R1) in both the June sowings and the July sowings in the cultivar ‘Enrei’ at Fukuyama. Although seed yield did not differ between lines in the June sowings, the effects of E2 and E3 on seed yield in July sowing were both significant and interaction between E2 and E3 also observed. E2 increased seed yield greater in E3 background. The total number of nodes increased in the late-maturing genotypes, especially in E2E3, in both sowing dates. Pod-set ratio differed between genotypes in the June sowings but not in the July sowings. The most appropriate combination of genotype and growing season for ‘Enrei’ in warm regions was considered to be late-maturing genotypes with E2E3 in July sowing, which can produce more nodes and flowers while maintaining pod-set ratio. Appropriate selection of sowing date is suggested to be essential when using E3 genotypes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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