Early-Maturing and Chilling-Tolerant Soybean Lines Derived from Crosses between Japanese and Polish Cultivars

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Abstract: Early maturity is an important trait for soybean \textit{Glycine max} (L.) Merr. growing in Hokkaido where the growing period is restricted because of the short fall season and early snowfall. Development of an early-maturing line without decreased seed yield is difficult because of the positive correlation between days to maturity and seed yield. In this study, we developed two breeding lines, Tokei 1067 (T1067) and Toiku 251 (T251), that were derived from crosses between Japanese and Polish cultivars. T1067 and T251 had a significantly earlier maturing time than Yukihomare (YH), the standard cultivar in Hokkaido. The seed yield of T251 was similar to that of YH. Moreover, the chilling tolerance levels of the T1067 and T251 lines at the flowering stage were greater than the tolerance level of YH.

Key words: Chilling tolerance, Early maturity, Polish cultivar, Soybean, Yield.

In Hokkaido, situated in northern Japan, the growing period of soybean \textit{Glycine max} (L.) Merr. is restricted because of a short fall season and early snowfall. Late-maturing soybeans are damaged frequently by frost, and consequently the external appearance of the seed is poor. In years when the weather is cold, soybeans frequently reach maturity later than usual. For example, Hagihara et al. (2003) reported that in 2005 the maturing days were 19 to 22 d later than in years when it was less cold. When soybeans do not mature in time, combine harvesting is impossible because immature plant easily become entwined in the combine reels. Yukihomare (YH), a leading soybean variety in Hokkaido, is early maturing and tolerant to cold weather (Tanaka et al., 2003). However, in cold weather, maturing of YH is often late and as a result the seeds become frost damaged. Therefore, early maturity is an important trait for soybean grown in Hokkaido.

The chilling tolerance is also an important trait in Hokkaido. The decreased seed yields caused by chilling temperatures have been attributed to three main factors: poor growth during the early growth stage, abscission of flowers and pods at the flowering stage, and insufficient grain filling at the pod filling stage (Yamamoto and Narikawa, 1966). The abscission of flowers and pods was found to be the most important factor that contributed to reduced yield (Matsukawa, 1994). Previous studies have revealed differences in chilling tolerance among cultivars at the flowering stage (Kurosaki and Yumoto, 2003; Kurosaki et al., 2003, 2004; Funatsuki et al., 2004). The chilling tolerance level at the flowering stage of YH is lower than that of Toyoharuka, the most chilling-tolerant variety in Hokkaido (Tanaka et al., 2003, 2009). Therefore, new cultivars that mature earlier and are more tolerant to cold weather than YH need to be developed.

Poland lies near the northern limit for soybean cultivation and as a result Polish cultivars exhibit various climate-specific characteristics; for example, tolerance to a long daylight period and tolerance to cold temperatures (Koniecny and Shimamoto, 1989). The Polish cultivars were reported to flower and mature earlier than the Japanese cultivars in the Hokkaido fields (Koniecny and Shimamoto, 1989).

In soybean, several maturity loci have been reported to control the time to flowering and maturity. These loci have...
been designated as $E$ loci (Cober et al., 1996); namely, $E_1$ and $E_2$ (Bernard, 1971), $E_3$ (Buzzell, 1971), $E_4$ (Buzzell and Voldeng, 1980), $E_5$ (McBlain and Bernard, 1987), $E_6$ (Bonato and Vello, 1999), $E_7$ (Cober and Voldeng, 2001), and $E_8$ (Cober et al., 2010).

In this study, we developed two early-maturing and chilling-tolerant lines derived from crosses between Japanese and Polish cultivars. We also investigated genotypes in the $E$ loci using molecular markers to identify candidate early-maturing $E$-genes in the Polish cultivars. Our results suggested that Tokei 1067 (T1067) and Toiku 251 (T251) are useful breeding lines to develop early-maturing and chilling-tolerant cultivars.

Materials and Methods

1. Plant material

All the soybean breeding lines used in our study were developed at the Tokachi Agricultural Experiment Station (TAES, Hokkaido, Japan). Two early-maturing cultivars, Polan and Progres, were developed in Poland (Konieczny and Shimamoto, 1989). The growth habits of all the cultivars and breeding lines used in this study were determinate. The T1067 and T251 lines were developed from the BC$_1$ population derived from a YH (a recurrent parent) × Polan (a donor parent) cross (cross number: Tc1347) and a YH (a recurrent parent) × Progres (a donor parent) cross (cross number: Tc1348), respectively (Fig. 1A). The seed coat color and hilum of the T1067 and T251 seeds were similar to those of the YH seeds (Fig. 1B).

The breeding procedures of T1067 and T251 were as described below. The crosses were conducted at TAES in 2001. The F$_1$ plants were backcrossed with YH in 2002. The BC$_1$F$_1$ plants were cultivated at TAES in 2003. The bulked BC$_1$F$_2$ progeny were cultivated at Kamishihoro in 2005. The bulked BC$_1$F$_4$ progeny were cultivated at TAES in 2006. The individual selections for early maturity were carried out in both 2005 and 2006. The 31 and 42 plants were selected from the BC$_1$F$_4$ population derived from Tc1347 and Tc1348, respectively. The line selection was carried out at TAES in 2007 to 2010 according to the pedigree method. In 2007, the four and five early-maturing lines were selected from the BC$_1$F$_5$ lines derived from Tc1347 and Tc1348, respectively. In 2008, the selected lines that matured earlier than YH and yielded equal to YH were designated as T1067 and Tokei 1068. In 2009, Tokei 1068 was designated as T251. The generations of both T1067 and T251 were BC$_1$F$_6$ in 2008, BC$_1$F$_7$ in 2009, and BC$_1$F$_8$ in 2010.

Toyoharuka (Tanaka et al., 2009) and Hayahikari (Yumoto et al., 2000) were used as the reference cultivars for the marker analysis. Toyoharuka and Toyomusume (Sasaki et al., 1988) were used as the standard cultivars for the chilling tolerance test.

2. Field test procedure

The field tests were performed in Memuro (experimental fields of TAES) and Kamishihoro, Hokkaido, Japan. Memuro lies at a north latitude of 42°89′, is 93 m above sea level, and has relatively warm weather. Kamishihoro is a cool region that lies at a north latitude of 43°23′, and is 313 m above sea level. The average temperatures in the two locations are shown in Table 1. Both fields had dry Andosol soils.

In Memuro, the breeding lines were planted on 23 May 2008, 21 May 2009, and 19 May 2010 (Table 1). Each plot consisted of three (in 2008) or four rows (in 2009 and 2010) with lengths of 3 m (in 2008) or 3.5 m (in 2009 and 2010) that were spaced 60 cm apart with 20 cm inter-hill and two plants per hill, giving a plant population density of
16.7 plants m\(^{-2}\). Fertilizer was applied according to Hokkaido fertilization standards (0.2 kg a\(^{-1}\) of N, 1.8 kg a\(^{-1}\) of P\(_2\)O\(_5\), 0.9 kg a\(^{-1}\) of K\(_2\)O and 0.4 kg a\(^{-1}\) of MgO). In Kamishihoro, the breeding lines were planted on 30 May 2008, 28 May 2009, and 6 June 2010 (Table 1). Each plot consisted of two (in 2008) or three rows (in 2009 and 2010) with lengths of 3 m that were spaced 66 cm apart with 18 cm inter-hill and two plants per hill, giving a plant population density of 16.8 plants m\(^{-2}\). Fertilizer was applied according to Hokkaido fertilization standards (0.1 kg a\(^{-1}\) of N, 2.2 kg a\(^{-1}\) of P\(_2\)O\(_5\), and 0.5 kg a\(^{-1}\) of K\(_2\)O). In each cultivar with the use of a BioSprint 96 DNA Plant Kit (Qiagen, Hilden, Germany). Molecular markers for the E1 locus were designed based on the results of previous studies (Yamanaka et al., 2001, 2005; Liu et al., 2008; Watanabe et al., 2009, 2011; Xia et al., 2012). The four markers, E2at_U46k, E2at, E2at_D82k and GMES4019, were used for estimating E2 according to the previous study (Yamada et al., 2012). The four markers, E3at_U113k, FT3SSR4, FT3SSR1dom and FT3SSR3, were used for estimating E3 according to the previous study (Yamada et al., 2012). Toyoharuka and Hayahikari were used as the references for e1e1 e2e2 E3E3 E4E4 and E1E1 e2e2 e3e3 e4e4, respectively.

4. Chilling tolerance test at the flowering stage

The chilling tolerance tests were conducted at TAES in 2010 as described previously (Kurosaki et al., 2003). Toyoharuka and Toyomusume were used as the standard cultivars; the chilling tolerance levels of Toyoharuka and Toyomusume are strong and medium, respectively. Seeds (10 per pot) were planted in pots (1/2000 a) on 17 May 2010. The pots were filled with low-humic Andosol supplemented with a chemical fertilizer (N, 0.12 g; P\(_2\)O\(_5\), 1.00 g; K\(_2\)O, 0.52 g; MgO, 0.20 g per pot). Three (breeding lines) or five (YH and standard cultivars) pots were prepared for each soybean cultivar, and 2 weeks after seedling emergence, two of the plants from each pot were selected. The plants were grown under a plastic roof in an experimental facility without walls. At the beginning of flowering, the pots were transferred to a phytotron and grown for 28 d under the following chilling temperature conditions: 18°C days (0800 – 1800) and 13°C nights (1800 – 0800), with 55% shading to avoid excessive heating from sunlight. After this treatment, the pots were returned to the experimental facility and the plants were grown to maturity. The Tukey-Kramer multiple comparison test was used to determine the significance of differences in seed yield among the cultivars. The chilling tolerance index (CTI) for seed yield was calculated to evaluate the rate of

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**Table 1. Sowing dates and average temperatures in the two field test locations.**

| Field      | Year | Sowing date | Average temperature (°C) | Accumulated temperature from June to September (°C) |
|------------|------|-------------|---------------------------|---------------------------------|
|            |      |             | June | July | August | September |                             |
| Memuro     | 2008 | 23 May      | 14.5 | 18.5 | 18.7   | 16.7      | 2089                          |
|            | 2009 | 21 May      | 14.4 | 17.6 | 19.2   | 15.1      | 2026                          |
|            | 2010 | 19 May      | 17.2 | 20.4 | 22.8   | 17.4      | 2377                          |
| Kamishihoro| 2008 | 30 May      | 13.7 | 17.5 | 17.5   | 15.7      | 1967                          |
|            | 2009 | 28 May      | 13.9 | 16.9 | 18.8   | 14.4      | 1956                          |
|            | 2010 | 6 June      | 16.8 | 19.7 | 22.2   | 16.3      | 2292                          |
yield reduction by the chilling treatment according to previous reports (Sanbuichi, 1979; Kurosaki et al., 2004) as: \[ CTI = \frac{\text{seed yield in the chilling treatment}}{\text{seed yield in the control}} \times 100. \]

**Results and Discussion**

1. **Maturity and seed yield**
   
The field tests of T1067 and T251 were conducted in Memuro and Kamishihoro in 2008 to 2010. The accumulated temperatures in Kamishihoro were lower than in Memuro in each year (Table 1). The field tests revealed significant differences among the cultivars in all the agronomic traits we measured (Table 2). No significant differences were found among fields, and no interactions between cultivars and fields were detected (data not shown). T1067 and T251 had significantly earlier flowering time than YH in Memuro, and significantly earlier maturing time in both locations (Table 2). YH and T1067 had 4 and 3 days longer maturing time, respectively, in Kamishihoro than in Memuro while the maturing time of T251 in Memuro was similar to that in Kamishihoro (Table 2). These results indicated that the maturing time of T251 was relatively stable.

   In Kamishihoro, the seed yield was about 80\% of that in Memuro (Table 2). The seed yield of T1067 was significantly lower than that of YH in both locations, while the seed yield of T251 was similar to that of YH in both locations (Table 2). The main stem length of T251 was similar to that of YH and that of T1067 was significantly greater than that of YH in Memuro (Table 2). T1067 and T251 had significantly lighter 100-seeds weights than YH in both locations, but T251 had significantly heavier 100-seeds weight than T1067 (Table 2). These results indicated that the agronomic traits of T251 resembled the YH traits more than the T1067 traits.

   In general, late-maturing soybeans yield more seeds than early-maturing ones because their vegetative growth and reproductive periods are longer. A positive correlation was found between days to maturity and seed yield in the breeding lines developed in Hokkaido (Ohnishi et al., 2012; Kobayashi et al., 2013). Cober et al. (2010) reported that early-maturing lines yielded about 60\% of the yield of late-maturing lines using two near-isogenic lines with different backgrounds. Therefore, it has been difficult to develop an early-maturing line without decreasing seed yield. The present study showed that T1067 and T251 had significantly earlier maturing time than YH (Table 2), and T251 had seed yield similar to that of YH (Table 2). Therefore, we have successfully bred an early-maturing line without decreasing yield.

   Exotic germplasms can be a source of new alleles that can improve yield (Li et al., 2008; Palomeque et al., 2009a, 2009b; Kim et al., 2012). Previous reports have shown that Japanese and Polish cultivars did not grouped together in a cluster analysis using simple sequence repeat markers (Hudcovicová and Kraic 2003). Therefore, the Polish cultivars can be regarded as useful exotic germplasms in breeding programs in Japan. Pyramiding of the positive yield alleles in Progres and YH might have contributed to the success of the breeding process of T251.

2. **Genototyping the maturity genes**
   
   Based on the marker genotypes, the \( E \)-gene genotypes of YH, Polan, Progres, T1067, and T251 were all estimated as \( e1e1 e2e2 E3E3 \) (Table 3). The \( E4/e4 \) genotype of Polan could not be estimated because the lengths of the PCR products were different from those of the reference cultivars Toyoharuka and Hayahikari; therefore, the allele at the \( E4/e4 \) locus of Polan was named as \( e^f \) (Table 3). The \( E4 \) genotype of T1067 was estimated as \( e^f e^f \), while the \( E4 \) genotypes of YH, Progres, and T251 were all estimated as \( e^*e^* \) (Table 3). Tsukuba et al. (2013) reported loss-of-

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**Table 2.** Field tests of the early-maturing soybean lines in the two field test locations.

| Field          | Cultivar or line | Flowering time (days) | Maturing time (days) | Main stem length (cm) | Seed yield (Kg 10\( \text{a}^-1 \)) | 100 seeds weight (g) |
|----------------|------------------|-----------------------|----------------------|-----------------------|-----------------------------------|----------------------|
| Memuro         | Yukihomare       | 58.7 ± 3.2 a\(^1\)   | 123.3 ± 3.8 a        | 61.9 ± 5.5 b          | 316 ± 28 a                        | 34.8 ± 1.2 a         |
|                | Tokei 1067       | 55.7 ± 3.5 b          | 115.7 ± 2.9 c        | 78.4 ± 8.0 a          | 288 ± 19 b                        | 27.9 ± 1.1 c         |
|                | Toiku 251        | 56.3 ± 3.5 b          | 118.7 ± 4.6 b        | 61.6 ± 3.2 b          | 315 ± 8 a                         | 31.9 ± 1.4 b         |
| Kamishihoro    | Yukihomare       | ND\(^2\)              | 127.3 ± 6.2 a        | ND                    | 260 ± 51 a                        | 32.3 ± 1.6 a         |
| (cool region)  | Tokei 1067       | ND                    | 118.7 ± 6.8 b        | ND                    | 220 ± 40 b                        | 26.5 ± 0.5 c         |
|                | Toiku 251        | ND                    | 119.7 ± 6.3 b        | ND                    | 253 ± 48 a                        | 29.9 ± 1.9 b         |

ANOVA (Cultivars)\(^3\)

\( * \) ** *** ** ***

All the numbers are the averages ± standard errors of the values obtained from 2008 to 2010.

\(^1\) The Tukey–Kramer multiple comparison test was used to evaluate the significance of differences among the cultivars in each field. Values within a trait with the same letters were not significantly different at the 5\% level.

\(^2\) ND: no data.

\(^3\) Two-way analysis of variance with repeated measures was used to test differences among the cultivars. * and **: significantly different at \( P < 0.05 \) and 0.001.
function alleles at the E4 locus in soybean. These results indicated that the genetic variation of e4 might be one of the factors for early maturity in T1067. Investigations into the effects of various e4 genotypes on maturity may make their role clearer in the future. The E-gene genotypes of YH and T251 were both estimated as e1e1 e2e2 E3E3 e4e4 (Table 3), which indicated the presence of other genetic factors for early maturity in T251, rather than the E1, E2, E3, and E4 loci.

3. Chilling tolerance at the flowering stage

Chilling tolerance tests at the flowering stage were performed using three cultivars and two breeding lines (Table 4). In the controls, no significant differences in seed yield were detected among the three cultivars; however, among the treated plants, the seed yield of Toyoharuka was significantly greater than that of YH (Table 4). The CTIs of Toyoharuka and Toyomusume were 106 and 61, respectively, which confirmed the strong chilling tolerance levels of both lines as strong and medium, respectively (Table 4). The tolerance level of YH was confirmed as somewhat strong; the CTI of YH was 71 (Table 4). The results obtained for these cultivars are similar to previously reported results (Tanaka et al., 2003, 2009), indicating that the chilling tolerance tests were performed accurately. In both the control and treated plants, the maturing times of T1067 and T251 were earlier than that of YH (Table 4). The CTIs of T1067 and T251 were 90 and 91, respectively, which confirmed the strong chilling tolerance levels of both lines (Table 4). These results indicated that the chilling tolerance levels of both breeding lines were greater than that of YH.
estimated as $e_1 e_2 E_3$ (Table 3), although the chilling tolerance levels of T1067 and T251 were greater than that of YH (Table 4). These results indicated that the chilling tolerance of T1067 and T251 was not associated with the $E_1$, $E_2$, and $E_3$ loci.

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