Distribution of photoperiod-insensitive alleles Ppd-B1a and Ppd-D1a and their effect on heading time in Japanese wheat cultivars

Masako Seki*1,2), Makiko Chono1), Hitoshi Matsunaka1), Masaya Fujita3), Shunsuke Oda1), Katashi Kubo3), Chikako Kiribuchi-Otobe1), Hisayo Kojima1), Hidetaka Nishida2) and Kenji Kato2)

1) NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan
2) Okayama University, 3-1-1 Tsushima-naka, Kita, Okayama 700-8530, Japan
3) NARO Kyushu Okinawa Agricultural Research Center, 496 Izumi, Chikugo, Fukuoka 833-0041, Japan

doi:10.1270/jsbbs.61.405
Breeding Science 61: 405–412 (2011)
*Corresponding author (e-mail: msk@affrc.go.jp)

The genotypes of photoperiod response genes Ppd-B1 and Ppd-D1 in Japanese wheat cultivars were determined by a PCR-based method, and heading times were compared among genotypes. Most of the Japanese wheat cultivars, except those from the Hokkaido region, carried the photoperiod-insensitive allele Ppd-D1a, and heading was accelerated 10.3 days compared with the Ppd-D1b genotype. Early cultivars with Ppd-D1a may have been selected to avoid damage from preharvest rain. In the Hokkaido region, Ppd-D1a frequency was lower and heading date was late regardless of Ppd-D1 genotype, suggesting another genetic mechanism for late heading in Hokkaido cultivars. In this study, only 11 cultivars proved to carry Ppd-B1a, and all of them carried another photoperiod-insensitive allele, Ppd-B1a. The Ppd-B1a/Ppd-D1a genotype headed 6.7 days earlier than the Ppd-B1b/Ppd-D1a genotype, indicating a significant effect of Ppd-B1a in the genetic background with Ppd-D1a. Early-maturity breeding in Japan is believed to be accelerated by the introduction of the Ppd-B1a allele into medium-heading cultivars carrying Ppd-D1a. Pedigree analysis showed that Ppd-B1a in three extra-early commercial cultivars was inherited from ‘Shiroboro 21’ by early-heading Chugoku lines bred at the Chugoku Agriculture Experimental Station.

Key Words: wheat, photoperiod response gene, Ppd-B1, Ppd-D1, heading date, Japanese wheat cultivars.

Introduction

Wheat (Triticum aestivum L.) should be harvested before the rainy season in Japan, except in the Hokkaido area, to avoid damage such as preharvest sprouting and Fusarium head blight. Therefore, early heading is one of the most important traits in wheat breeding. However, shorter growth periods generally result in lower grain yields, and early-heading wheat cultivars with early apical development and stem elongation are prone to frost injury (Hukumoto and Takahashi 1950, Taya 1993). Thus, heading characteristics must be adjusted for the stable production of wheat in each area.

Heading time of wheat is a complex character controlled by narrow-sense earliness (also termed earliness per se) and is modified by vernalization responses and photoperiod responses (Kato and Yamashita 1991, Yasuda and Shimoyama 1965). It was also reported that photoperiod response is the major determinant of earliness in autumn-sown wheat in central and southwestern Japan (Tanio et al. 2006, Yasuda and Shimoyama 1965, Yoshida et al. 1983), and that heading time is independent of the Vrn genotype controlling vernalization response (Fujita et al. 1995, Kato and Yamashita 1991, Tanio et al. 2005).

Photoperiod response is controlled mainly by three major genes, namely, Ppd-D1 (previously designated Ppd1), Ppd-B1 (Ppd2) and Ppd-A1 (Ppd3), located on homoeologous group two chromosomes (Scarth and Law 1983, 1984, Welsh et al. 1973). The barley homoeologue Ppd-H1 was identified as a member of the pseudo-response regulator (PRR) gene family (Turner et al. 2005). The orthologous PRR genes of the A, B and D genomes have been isolated from wheat BAC (bacterial artificial chromosome) libraries, and sequence analyses revealed that the photoperiod-insensitive Ppd-D1a allele is associated with a 2,089-bp deletion upstream of the coding region (Beales et al. 2007). Recent data show that the photoperiod-insensitive Ppd-A1a and Ppd-B1a alleles are associated with a 1085-bp deletion and a 308-bp insertion, respectively (Nishida et al. submitted, GenBank sequence accessions are AB646973 and AB646974), both of which share the common region with a deletion of Ppd-D1a.

Based on the sequence polymorphism among Ppd-D1 alleles reported by Beales et al. (2007), Yang et al. (2009) determined the Ppd-D1 genotype of Chinese wheat landraces and indicated that Ppd-D1a allele frequency varies among different areas, even within China. The Ppd-D1a allele was...
not found in northern China but was found frequently in southeastern China. Most of the European wheat cultivars with photoperiod insensitivity probably carry *Ppd-D1a* derived from ‘Akakomugī’ (Worland 1996). This assumption is supported by Guo *et al.* (2010), who showed that most Italian wheat cultivars carry *Ppd-D1a*. Tanio *et al.* (2005) analyzed the *Ppd-1* genotype of Japanese wheat cultivars by conventional segregation analysis and reported the following results. The very late-heading cultivar ‘Haruhikari’ does not carry any major photoperiod-insensitive alleles. Medium- to late-heading cultivars such as ‘Norin 61’ and ‘Saitama 27’ carry a single allele for photoperiod insensitivity. Extremely early-heading cultivars carry two alleles for photoperiod insensitivity. Lately, it was revealed that the former single allele is *Ppd-D1a* and the latter two alleles are *Ppd-B1a* and *Ppd-D1a* by Nishida *et al.* (submitted). However, only eight cultivars were analyzed because the conventional segregation procedure is labor intensive. On the contrary, genotyping based on the detection of sequence differences allows for the analysis of many wheat cultivars and a comprehensive analysis of the relationship between the *Ppd-1* genotype and the heading time of Japanese wheat cultivars.

In this study, the *Ppd-B1* and *Ppd-D1* genotypes of Japanese wheat cultivars were determined by a PCR-based method to detect large deletions or insertions in the upstream region of the respective gene. Heading date in the field was compared among wheat cultivars carrying a sensitive or insensitive allele of each gene, and the effect of *Ppd-1* genotypes on heading time was successfully evaluated. In addition, the pedigree of extremely early-heading cultivars was discussed based on *Ppd-1* genotype.

### Materials and Methods

A total of 260 wheat cultivars, consisting of Japanese commercial cultivars (157 cultivars), Japanese landraces (25 cultivars) and foreign cultivars introduced for breeding (33 cultivars), were used in the present study. Geographical origins are summarized in Table 1. The cultivars were grown in a field at the NARO Institute of Crop Science (36°01′N, 140°06′E) in the Kanto region of Japan and were sown on November 4, November 10 and October 31 for the 2004/2005, 2005/2006 and 2006/2007 wheat growing seasons, respectively. Each experimental plot consisted of a single 1.0-m-long row, and the planting distance was 70 cm between rows and 8.5 cm between plants. Heading date was recorded when the tip of the first ear emerged from the flag leaf sheath in one-half of the plants for each cultivar.

For DNA extraction, all wheat genotypes were grown in a growth chamber maintained at 20°C under a natural photoperiod. Genomic DNA was extracted from 2-week-old seedlings using a modified CTAB method (Murray and Thompson 1980). *Ppd-B1* and *Ppd-D1* genotypes were determined using PCR-based methods with the primer sets designed to identify the deletion of 2089 bp in the upstream region of *Ppd-D1a* (Beales *et al.* 2007) or the insertion of 308 bp in the upstream region of *Ppd-B1a* (Nishida *et al.* submitted). Three primers, namely, *Ppd-D1* F1, *Ppd-D1* R1 and *Ppd-D1* R2 (developed by Beales *et al.* 2007), were used for *Ppd-D1*, and two primers, i.e., TaPpd-B1proF1 and TaPpd-B1int1R1 (developed in this study), were used for *Ppd-B1*. The nucleotide sequence of each primer is shown in Table 2.

For the analysis of *Ppd-D1*, PCR amplification was performed in a 5-μl mixture containing 10 ng genomic DNA, 0.5 μl 10× *Ex Taq* buffer (TaKaRa, Japan; 20 mM Tris-HCl at pH 8.0, 100 mM KCl, 20 mM MgCl₂), 0.2 mM dNTP, 0.2 μM of each primer and 0.125 U *Ex Taq* Hot Start Version (TaKaRa). The PCR cycle was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 54°C for 1 min and 72°C for 30 sec; and a final extension step at 72°C for 2 min. For the analysis of *Ppd-B1*, PCR amplification was performed in a 5-μl mixture containing 10 ng genomic DNA, 0.5 μl 10× *Pyrobest* buffer (TaKaRa; 50 mM Tris-HCl at pH 8.2, 10 mM MgCl₂), 0.2 mM

| Area of origin          | Total number of cultivars | *Ppd-B1a* | *Ppd-B1b* | *Ppd-D1a* | *Ppd-D1b* |
|------------------------|---------------------------|-----------|-----------|-----------|-----------|
| Hokkaido (Winter wheat) | 19                        | 0         | 19        | 7         | 12        |
| Hokkaido (Spring wheat)| 10                        | 10        | 10        | 1^f       | 9         |
| Tohoku, Hokuriku       | 47                        | 0         | 47        | 43        | 4^f       |
| Kanto, Tokai           | 62                        | 2^g       | 60        | 62        | 0         |
| Kinki, Chugoku, Shikoku| 42                        | 6^h       | 36        | 41        | 1^h       |
| Kyushu                 | 47                        | 2^i       | 45        | 47        | 0         |
| **Total**              | **260**                   | **11**    | **249**   | **218**   | **42**    |

^a^ High and low latitude areas are tentatively separated by 40 degrees.

^b^ Carrier of *Ppd-B1a*, ‘Konosu 4’ and ‘Shirohoro 21’

^c^ Carrier of *Ppd-B1a*, ‘Chugoku 55’, ‘Chugoku 81’, ‘Chugoku 91’, ‘Chugoku 98’, ‘Chugoku 114’ and ‘Fukuwasekomugi’

^d^ Carrier of *Ppd-B1a*, ‘Sagakakekomugi’ and ‘Abukumawase’

^e^ Carrier of *Ppd-B1a*, ‘Tapdongmil’

^f^ Carrier of *Ppd-D1a*, ‘OS-21’

^g^ Carrier of *Ppd-D1b*, ‘Fultz Daruma’, ‘Norin 6’, ‘Norin 24’ and ‘Norin 38’

^h^ Carrier of *Ppd-D1b*, ‘Eshima’

### Table 1. Distribution of photoperiod insensitive alleles *Ppd-B1a* and *Ppd-D1a* in Japanese and foreign wheat cultivars

### Table 2. Primers used to determine the *Ppd-D1* and *Ppd-B1* genotypes

| Locus       | Primer name      | Sequence (5′→3′)          |
|-------------|------------------|----------------------------|
| *Ppd-B1*    | TaPpd-B1proF1    | AACATAGGGCTGTCGAGGAGA      |
|             | TaPpd-B1int1R1   | CCGAGGCGTGCCAAATTAC       |
| *Ppd-D1*    | TaPpd-D1_F1      | ACGGCTCCCACACTACG         |
|             | TaPpd-D1_R1      | TGTGGTGTCAAAACAGAGC       |
|             | TaPpd-D1_R2      | CACTGGTGCGTCTGAGGATT      |
dNTP, 0.2 μM of each primer and 0.125 U Pyrobest DNA polymerase (TaKaRa). The PCR cycle was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 64°C for 1 min, and 72°C for 30 sec and a final extension step at 72°C for 2 min. Amplification reactions were conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, USA). PCR products were electrophoresed on acrylamide gel. Electrophoresis and polymorphism detection were based on the description by Hori et al. (2003).

Heading date data were analyzed using statistical software (SPSS Ver. 18.0 J for Windows, SPSS Japan Inc.).

Results

Expected PCR product sizes, i.e., 288 bp from Ppd-D1a or 415 bp from Ppd-D1b, photoperiod-sensitive allele without a deletion of 2089 bp, were successfully amplified by multiplex PCR in all of the cultivars tested. For Ppd-B1, 1600 bp from Ppd-B1a or 1292 bp from Ppd-B1b, photoperiod-sensitive allele without an insertion of 308 bp, were successfully amplified in all of the cultivars tested. Among 260 cultivars, 218 cultivars (83.8%) proved to carry the photoperiod-insensitive allele Ppd-D1a (Table 1). Frequency of the Ppd-D1a genotype was different among geographical groups. Only seven cultivars of winter wheat carried the Ppd-D1a allele in Hokkaido, whereas 193 of 198 Japanese wheat cultivars (97.5%), except those from the Hokkaido region, and half of the foreign cultivars (51.5%) carried the Ppd-D1a allele (Table 1 and Fig. 1). On the other hand, only 11 cultivars (4.2%) carried Ppd-B1a allele (Table 1). Among them, two accessions from the Kanto and Tokai regions and six accessions from the Kinki, Chugoku and Shikoku regions included an extra-early cultivar, ‘Fukunakako’ and ‘Kumawase’ and five breeding lines. Two cultivars from the Koyama region, i.e., ‘Sakigakekomugi’ and ‘Abukumawase’ and one cultivar from Korea, i.e., ‘Tapdongmil’, also carried Ppd-B1a allele (Table 1 and Fig. 2). All of the cultivars with Ppd-B1a allele carried Ppd-D1a allele as well; the Ppd-B1a/Ppd-D1a genotype was not found in this study.

As summarized in Table 3, heading date of wheat cultivars differed significantly among the three seasons as well as among Ppd-D1 genotypes (P < 0.001). As shown in Fig. 3, monthly average temperatures differed among the three wheat growing seasons. Compared with average temperatures for the last 30 years, temperatures during the early growing stage of wheat were higher in the 2004/2005 season and lower in the 2005/2006 season. In contrast, a warm winter in 2006/2007 resulted in higher temperatures for the entire growing season.

Average heading date of the Ppd-D1a and Ppd-D1b genotypes were 28.2 April and 5.7 May, respectively, for the 2004/2005 season. A similar genotypic difference was observed in the other two seasons, indicating that the Ppd-D1a genotype headed approximately 10 days earlier than the Ppd-D1b genotype. The genotypic difference was not significant in wheat cultivars from the Hokkaido region, where winter wheat and spring wheat are grown in different areas. For winter wheat cultivars in this region, average heading date of the Ppd-D1a and Ppd-D1b genotypes were 7.1 May and 7.8 May, respectively, for the 2004/2005 season. In foreign cultivars from high-latitude areas, the Ppd-D1a genotype headed 5–6 days earlier than the Ppd-D1b genotype. The genotypic differences in each season were not significant. However, ANOVA results showed that the Ppd-D1 genotype was significant for heading at the 5% level.

Heading date of the three genotypes detected in the present study, namely, Ppd-B1a/Ppd-D1a, Ppd-B1b/Ppd-D1a and Ppd-B1b/Ppd-D1b, are shown in Table 4. Because wheat cultivars of the Ppd-B1a/Ppd-D1b genotype were not detected, the effect of the Ppd-B1 gene under the Ppd-D1b genetic background could not be analyzed. Therefore, heading date was compared between two genotypes, Ppd-B1a/Ppd-D1a and Ppd-B1b/Ppd-D1a, to determine the interaction between the Ppd-B1a and Ppd-D1a alleles. Heading date of Ppd-B1a/Ppd-D1a and Ppd-B1b/Ppd-D1a genotypes were 22.1 April and 28.5 April for the 2004/2005 season, respectively; similar results were obtained for the other two seasons. Heading date of photoperiod-insensitive wheat cultivars carrying Ppd-D1a was accelerated by 4.9–8.7 days when combined with Ppd-B1a. Heading time stability among the three seasons also differed depending on the Ppd-1 genotype. Heading time differences between the 2005/2006 season...
with a cold winter and the 2006/2007 season with a warm winter was 15.9 days in the Ppd-B1a/Ppd-D1a genotype and 12.0–12.5 days in the other Ppd-1 genotypes (Table 5).

The Ppd-B1 genotypes of wheat lines in the pedigree of extremely early wheat cultivars carrying Ppd-B1a allele (‘Sakigakekomugi’, ‘Fukuwasekomugi’ and ‘Abukumawase’) are summarized in Fig. 4. Four early-heading breeding lines, ‘Chugoku 55’, ‘Chugoku 81’, ‘Chugoku 91’ and ‘Chugoku 114’, bred at the Chugoku Agriculture Experimental Station proved to carry Ppd-B1a. The old cultivars ‘Konosu 4’ and ‘Shiroboro 21’ also carried Ppd-B1a, whereas the other cultivars carried Ppd-B1b (Fig. 4). This result indicated that Ppd-B1a of extremely early wheat cultivars was inherited from ‘Shiroboro 21’ by early-heading Chugoku lines, although the Ppd-1 genotype was not determined for ‘Kinki 14’ because seed was not available.

### Table 3. Average of heading date of each Ppd-D1 genotype

| Growing season | Ppd-D1 genotype | Total | Hokkaido | Tohoku-Kyushu | Foreign cultivars |
|----------------|-----------------|-------|----------|---------------|------------------|
|                | n | Heading date | n | Heading date | n | Heading date | n | Heading date | n | Heading date |
| 2004/2005 Ppd-D1a | 216 | 28.2 ± 0.3 | 8 | 36.1 ± 1.5 | 7 | 37.1 ± 1.3 | 192 | 27.8 ± 0.3 | 16 | 28.2 ± 1.2 |
| Ppd-D1b | 42 | 35.7 ± 0.8 | 21 | 36.3 ± 0.9 | 12 | 37.8 ± 1.1 | 5 | 36.0 ± 3.3 | 16 | 34.9 ± 1.5 |
| 2005/2006 Ppd-D1a | 216 | 32.1 ± 0.3 | 8 | 40.3 ± 1.9 | 7 | 40.7 ± 2.1 | 192 | 31.8 ± 0.2 | 16 | 32.6 ± 1.7 |
| Ppd-D1b | 42 | 43.0 ± 0.9 | 21 | 44.1 ± 1.2 | 12 | 43.8 ± 1.7 | 5 | 41.8 ± 3.5 | 16 | 41.8 ± 1.5 |
| 2006/2007 Ppd-D1a | 216 | 19.9 ± 0.4 | 8 | 29.6 ± 1.7 | 7 | 30.5 ± 1.6 | 192 | 19.5 ± 0.4 | 16 | 19.8 ± 1.6 |
| Ppd-D1b | 42 | 30.5 ± 0.8 | 21 | 31.6 ± 1.0 | 12 | 32.2 ± 1.2 | 5 | 32.2 ± 3.1 | 16 | 28.4 ± 1.5 |

F-value of ANOVA:

| Growing season (A) | 225.2 *** | 34.30 *** | 24.70 *** | 27.90 *** | 38.66 *** | 13.33 *** |
| Ppd-D1 genotype (B) | 410.1 *** | 3.133 ns | 1.906 ns | 80.45 *** | 44.84 *** | 6.217 * |
| (A)*(B) | 4.775 * | 0.850 ns | 0.311 ns | 0.018 ns | 0.018 ns | 0.664 ns |

Values showed mean ± standard error. 1 = 1st April. *** and ‘ns’ indicate significance at 0.1% levels and no significance at 5% level, respectively, by T-test.

High and low latitude areas are tentatively separated by 40 degrees.

* and *** indicate significance at 5% and 0.1% level, respectively, and ‘ns’ not significant.
Examination of the \textit{Ppd-D1} genotypes of 227 Japanese wheat cultivars by PCR-based analysis detected a deletion of 2089 bp in the 5′ upstream region (Beales et al. 2007), showing that 201 cultivars (88.4%) carried the insensitive allele \textit{Ppd-D1a} (Table 1). In the Tohoku-Kyushu region, most of the cultivars (97.5%) carried \textit{Ppd-D1a}. The predominance of \textit{Ppd-D1a} allele was also found in Chinese improved wheat cultivars (90.6%, Yang et al. 2009) and Pakistani spring bread wheat cultivars (98.3%, Iqbal et al. 2011). According to Guo et al. (2010), \textit{Ppd-D1a} was carried in 11 of 12 Italian cultivars and in all 29 Mexican cultivars but was uncommon in Europe and North America except for Italy and Mexico. For such a geographical difference, Worland et al. (1996) explained as follows: \textit{Ppd-D1a} accelerates flowering by 4–8 days, so that only the early genotype carrying \textit{Ppd-D1a} is able to fill grain before the hot, dry summer season in southern Europe. Our result also indicated that \textit{Ppd-D1a} accelerates heading by 8.2–12.7 days in the Tohoku-Kyushu cultivars (Table 3). In Japan, except in Hokkaido, the rainy season starts before the wheat harvest; thus, early cultivars with \textit{Ppd-D1a} have been selected to avoid damage.

**Table 4.** Additive effect of \textit{Ppd-D1a} and \textit{Ppd-B1a} for heading date

| Growing season | \textit{Ppd-I} genotype | Number of cultivars | Heading date $^a$ |
|----------------|--------------------------|---------------------|-------------------|
| 2004/2005      | \textit{Ppd-B1a/Ppd-D1a} | 10                  | 22.1 ± 0.8$^a$    |
|                | \textit{Ppd-B1b/Ppd-D1a} | 206                 | 28.5 ± 0.3$^a$    |
|                | \textit{Ppd-B1b/Ppd-D1b} | 42                  | 35.7 ± 0.8$^a$    |
| 2005/2006      | \textit{Ppd-B1a/Ppd-D1a} | 10                  | 27.5 ± 0.7$^a$    |
|                | \textit{Ppd-B1b/Ppd-D1a} | 206                 | 32.4 ± 0.3$^a$    |
|                | \textit{Ppd-B1b/Ppd-D1b} | 42                  | 43.0 ± 0.9$^a$    |
| 2006/2007      | \textit{Ppd-B1a/Ppd-D1a} | 10                  | 11.6 ± 0.8$^a$    |
|                | \textit{Ppd-B1b/Ppd-D1a} | 206                 | 20.3 ± 0.4$^a$    |
|                | \textit{Ppd-B1b/Ppd-D1b} | 42                  | 30.5 ± 0.8$^a$    |

F-value of ANOVA

\[ F_{(A)} = 144.4^{***} \]
\[ F_{(B)} = 208.7^{***} \]

\[ F_{(A*B)} = 3.380^{**} \]

$^a$ Values showed mean ± standard error. 1 = 1st April. Values with the different letter indicate significant difference (P < 0.001) by Tukey HSD multiple range test, for each growing season.

$^{**}$ and $^{***}$ indicate significance at 1% and 0.1% level, respectively.

**Table 5.** Difference of heading time between 2005/2006 (cold winter) and 2006/2007 (warm winter)

| \textit{Ppd-I} genotype | Number of cultivars | Difference of heading date $^a$ |
|-------------------------|---------------------|-------------------------------|
| \textit{Ppd-B1a/Ppd-D1a} | 10                  | 15.9 ± 0.5$^a$               |
| \textit{Ppd-B1b/Ppd-D1a} | 206                 | 12.0 ± 0.2$^b$               |
| \textit{Ppd-B1b/Ppd-D1b} | 42                  | 12.5 ± 0.4$^b$               |

F-value of ANOVA

\[ F_{(Ppd-I)} = 7.173^{**} \]

$^a$ Values showed mean ± standard error. Values with the different letter indicate significant difference (P < 0.01) by Tukey HSD multiple range test.

$^{**}$ indicates significance at 1% level.

**Discussion**

Examination of the \textit{Ppd-D1} genotypes of 227 Japanese wheat cultivars by PCR-based analysis detected a deletion of 2089 bp in the 5′ upstream region (Beales et al. 2007), showing that 201 cultivars (88.4%) carried the insensitive allele \textit{Ppd-D1a} (Table 1). In the Tohoku-Kyushu region, most of the cultivars (97.5%) carried \textit{Ppd-D1a}. The predominance of the \textit{Ppd-D1a} allele was also found in Chinese improved wheat cultivars (90.6%, Yang et al. 2009) and Pakistani spring bread wheat cultivars (98.3%, Iqbal et al. 2011). According to Guo et al. (2010), \textit{Ppd-D1a} was carried in 11 of 12 Italian cultivars and in all 29 Mexican cultivars but was uncommon in Europe and North America except for Italy and Mexico. For such a geographical difference, Worland et al. (1996) explained as follows: \textit{Ppd-D1a} accelerates flowering by 4–8 days, so that only the early genotype carrying \textit{Ppd-D1a} is able to fill grain before the hot, dry summer season in southern Europe. Our result also indicated that \textit{Ppd-D1a} accelerates heading by 8.2–12.7 days in the Tohoku-Kyushu cultivars (Table 3). In Japan, except in Hokkaido, the rainy season starts before the wheat harvest; thus, early cultivars with \textit{Ppd-D1a} have been selected to avoid damage.
such as preharvest sprouting and Fusarium head blight.

Ppd-D1a frequency in the Hokkaido cultivars was 27.6%, which was lower than Ppd-D1a frequency in the Tohoku-Kyushu region (Table 1 and Fig. 1). Ppd-D1a was less frequent in wheat landraces from northern China than in wheat landraces from southern China (Yang et al. 2009). In addition, heading dates of the Ppd-D1a genotype was not significantly different from that of the Ppd-D1b genotype in Hokkaido cultivars, and was similar to that of Ppd-D1b genotype in the Tohoku-Kyushu cultivars. The lateness of the Hokkaido cultivars is an important trait for adaptation to longer winters. However, in foreign cultivars from high-latitude areas, heading date differed significantly among the Ppd-D1 genotype (Table 3, P < 0.05). According to Foulkes et al. (2004) who analyzed the NILs of United Kingdom cultivars ‘Merica’ and ‘Cappela-Desprez’, differing in Ppd-D1 genotypes, flag leaf unfolding was 12.5 days earlier in Ppd-D1a NILs than in Ppd-D1b NILs. These findings suggest that the Ppd-D1a genotype of the Hokkaido cultivar has another genetic mechanism for late heading. Although this mechanism is unknown, foreign wheat cultivars have been introduced from Europe and the United States for breeding with Hokkaido cultivars (Fukunaga and Inagaki 1985, Hoshino et al. 2001); thus, the genetic background of Hokkaido cultivars is considered different from that of wheat cultivars in other areas of Japan. Another possibility is the functional difference among the Ppd-D1a alleles, though Ppd-D1a allele of the Hokkaido cultivars has not been sequenced. Further study is required to uncover the genetic factors involved in the control of heading time in Hokkaido cultivars.

Although ‘Chinese Spring’ carries the photoperiod-insensitive allele Ppd-B1 (Law et al. 1978, Scarth and Law 1983), a 308-bp insertion was not detected in the 5’ upstream region, and this result shows that ‘Chinese Spring’ carried an allele that was different from Ppd-B1a (Nishida et al. submitted). In the present study, 11 cultivars were proved to carry Ppd-B1a with the 308-bp insertion, as well as Ppd-D1a. Among them, ‘Fukuwasekomugi’ and ‘Abukumawase’ were already confirmed to have Ppd-B1a by conventional segregation or molecular genetics analysis (Nishida et al. submitted, Tanio and Kato 2007). The results of this study were consistent with those of previous studies. However, the effect of Ppd-B1a could not be determined, because cultivars with the Ppd-B1a/Ppd-D1b genotype were not found in the Japanese improved cultivars. Tanio and Kato (2007) analyzed ‘Haruhikari’ NILs with different Ppd-1 genotypes and revealed that photoperiodic response was smallest in the Ppd-B1a/Ppd-D1a genotype followed by the Ppd-B1a/Ppd-D1b, Ppd-B1b/Ppd-D1a, and Ppd-B1b/Ppd-D1b genotypes. The result, summarized in Table 4, also showed that the Ppd-B1a/Ppd-D1a genotype headed 4.9–8.7 days earlier than the Ppd-B1b/Ppd-D1a genotype, indicating a significant effect of Ppd-B1a in the genetic background with Ppd-D1a and suggesting that early-maturity wheat breeding in Japan has been accelerated by the introduction of the Ppd-B1a allele.

Although the Bangladeshi cultivar ‘Jessore’ was considered the donor of the Ppd-B1a allele in extremely early cultivars in Japan (Tanio and Kato 2007, Yoshida et al. 1983), the Ppd-1 genotype proved to be Ppd-B1b/Ppd-D1a (Fig. 4). Therefore, as summarized in Fig. 4, we concluded that the old Japanese cultivar ‘Shiroboro 21’ was the Ppd-B1a donor. Ppd-B1a probably had been introduced from ‘Kinki 14’ for the breeding of early-heading wheat at the Chugoku Agriculture Experimental Station during the 1940s–1950s. As shown in Table 1, one of the introduced cultivars, ‘Tadongmii’, also carried Ppd-B1a. Although ‘Tadongmii’ is a Korean cultivar, ‘Chugoku 81’, bred at the Chugoku Agriculture Experimental Station, was used as one of the cross parents (Sung et al. 1987). Therefore, Ppd-B1a in ‘Tadongmii’ must be inherited from ‘Chugoku 81’.

Using near-isogenic lines of ‘Haruhikari’ with different Ppd-1 genotypes, Tanio and Kato (2007) showed that NILs carrying the photoperiod-insensitive allele Ppd-B1a started floral development and stem elongation earlier than the other NILs. Three cultivars confirmed to carry Ppd-B1a are successfully grown in the Chugoku or Kyushu regions of Japan, where the average daily minimum temperature from 1971 to 2000 was above freezing all year (Japan Meteorological Agency 2010). In contrast, in the north Kanto region, where the average daily minimum temperature was below freezing for 2–3 months, the cultivars suffered from late frost because of early stem elongation and poor adaptability (Inamura et al. 1958). Reflecting such a climatic difference, no cultivars carried Ppd-B1a in eastern Japan.

Taya (1993) reported that yield decreased with the advancement of heading time because of the decrease in spikelet number. The photoperiod-insensitive allele Ppd-1 genes also reportedly shortened the duration of spikelet initiation (Gonzalez et al. 2005, Scarth et al. 1985, Tanio and Kato 2007). In the present study, heading date of cultivars carrying two genes, namely, Ppd-B1a/Ppd-D1a, were earlier than those of the other Ppd-1 genotypes. In addition, heading date differences between two crop years, 2005/2006 (cold winter) and 2006/2007 (mild winter), were larger in the Ppd-B1a/Ppd-D1a genotype than in the other genotypes (Table 5). These results suggest that grain yield of early-heading cultivars carrying the two photoperiod-insensitive genes Ppd-B1a/Ppd-D1a will be lower, especially in warm-winter seasons. However, Fujita et al. (1995) and Seki et al. (2007) reported that the yearly fluctuation of heading time is smaller in the winter-type NILs of ‘Abukumawase’ carrying Ppd-B1a/Ppd-D1a. The fluctuation of heading time due to early sowing is also estimated to be smaller in winter type wheat (Fujita et al. 1995). Therefore, to breed early-heading cultivars adaptable to the Kanto region, Ppd-B1a and Ppd-D1a genes should be introduced with adequate vernalization requirement.

The previous genetic analysis indicated that spring type cultivars originated in the Tohoku-Kyushu region carry the vernalization response gene Trn-D1 (Gotho 1979, Iwaki et
al. 2000), and the present study showed that most of the cultivars in the Tohoku-Kyushu region carried the photoperiod-insensitive gene Ppd-D1a. These results indicate that most of the Japanese spring type cultivars, except those in the Hokkaido region, have the same set of genes for both the vernalization response and the photoperiod response. However, heading time varies between cultivars. Therefore, to refine heading time, further study is required to determine the genetic factors involved, including Ppd-A1 (Nishida et al. submitted), the FT-like gene known as the photoperiod response gene in barley (Kikuchi et al. 2009), and other QTLs for photoperiod response and earliness per se.

Acknowledgements

The authors are grateful to the National Institute of Agro-biological Sciences and the wheat breeding sites in Japan: the Kitami Agricultural Experimental Station of HRO, the Gunma Agricultural Technology Center, the Nagano Agricultural Experimental Station, the Aichi Agricultural Research Center, and four sites of NARO, NARCH, NARCT, WeNARC and KONARC for providing the seeds of wheat cultivars examined in this study. We are also grateful to members of the Research Support Center of National Agricultural Research Center for their management of the field. Thanks are also due to members of NICS for their technical assistance.

Literature Cited

Beales, J., A. Turner, S. Griffiths, J.W. Snape and D.A. Laurie (2007) A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). Theor. Appl. Genet. 115: 721–733.

Foulkes, M.J., R. Sylvester-Bradley, A.J. Worland and J.W. Snape (2004) Effects of a photoperiod-response gene Ppd-D1 on yield potential and drought resistance in UK winter wheat. Euphytica 135: 63–73.

Fujita, M., Y. Taniguchi, K. Uijihara and A. Sasaki (1995) Ear primordia development and stem elongation of near-isogenic lines for vernalization requirement in extremely-early maturing wheat cultivars (Triticum aestivum L.). Breed. Sci. 45: 97–104.

Fukunaga, K. and M. Inagaki (1985) Genealogical pedigrees of Japanese wheat cultivars. Jpn. J. Breed. 35: 89–92.

Gonzalez, F.G., G.A. Slafier and D.J. Miralles (2005) Pre-anthesis development and number of fertile florets in wheat as affected by photoperiod sensitivity genes Ppd-D1 and Ppd-B1. Euphytica 146: 253–269.

Gotoh, T. (1979) Genetic studies on growth habit of some important spring wheat cultivars in Japan, with special reference to the identification of the spring genes involved. Jpn. J. Breed. 29: 133–145.

Guo, Z., Y. Song, R. Zhou, Z. Ren and J. Jia (2010) Discovery, evaluation and distribution of haplotypes of the wheat Ppd-D1 gene. New Phytol. 185: 841–851.

Hori, K., T. Kobayashi, A. Shimizu, K. Sato, K. Takeda and S. Kawasaki (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theor. Appl. Genet. 107: 806–813.

Hoshino, T., K. Kato and K. Ueno (2001) Japanese wheat pool. In: Bonjean, A.P. and W.J. Angus (eds.) The World Wheat Book: A History of Wheat Breeding, Lavoisier Publishing, Paris, France, pp. 703–726.

Hukumoto, T. and M. Takashashi (1950) About unusual warmth in winter and cold injury of wheat and barley in 1949. Bulletin of the Nagano Prefectural Agricultural experiment Station 10: 1–81.

Inamura, H., I. Yamaga, K. Suzuki and M. Gokan (1958) Study on the breeding of early varieties cultivars of wheat and barley. (I.) Varietal differences of the early wheat and barley in the intermediate elongation in relation on the death of young ear by frost damage in early spring. Bull. Kanto-Tozan Natl. Agric. Exp. Stn. 11: 20–28.

Iqbal, M., A. Shahzad and I. Ahmed (2011) Allelic variation at the Vrn-A1, Vrn-B1, Vrn-D1, Vrn-B3 and Ppd-D1a loci of Pakistani spring wheat cultivars. Electronic Journal of Biotechnology 14 (issue): fulltext-6.

Iwaki, K., K. Nakagawa, H. Kuno and K. Kato (2000) Ecogeographical differentiation in east Asian wheat, revealed from the geographical variation of growth habit and Vrn genotype. Euphytica 111: 137–143.

Japan Meteorological Agency (2010) Climate statistic. http://www.data.jma.go.jp/obd/stats/etrn/index.php

Kato, K. and S. Yamashita (1991) Varietal variation in photoperiodic response, chilling requirement and narrow-sense earliness and their relation to heading time in wheat (Triticum aestivum L.). Jpn. J. Breed. 41: 475–484.

Kikuchi, R., H. Kawahigashi, T. Ando, T. Tonooka and H. Handa (2009) Molecular and functional characterization of PEBP genes in barley reveal the diversification of their roles in flowering. Plant Physiol. 149: 1341–1353.

Law, C.N., J. Sutka and A.J. Worland (1978) Genetic study of day-length response in wheat. Heredity 41: 185–191.

Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4325.

Scarth, R. and C.N. Law (1983) The location of the photoperiod gene, Ppd2 and an additional genetic factor for ear-emergence time on chromosome 2B of wheat. Heredity 51: 607–619.

Scarth, R. and C.N. Law (1984) The control of the day-length response in wheat by the group 2 chromosomes. Z. Pflanzenzüchtg 92: 140–150.

Scarth, R., E.J.M. Kirby and C.N. Law (1985) Effects of the photoperiod genes Ppd1 and Ppd2 on growth and development of the shoot apex in wheat. Ann. Bot. 55: 351–359.

Seki, M., S. Oda, H. Matsuaka, K. Hatta, M. Fujita, T. Hatano, C. Kiribuchi-Otobe, N. Kawada and K. Kato (2007) Growth and yield of near-isogenic wheat lines carrying different vernalization response genes. Breed. Res. 9: 125–133.

Sung, B.R., K.B. Youn, J.J. Hwang, H.S. Song, C.S. Park, W.S. Ahn, M.W. Park and S.Y. Lee (1987) A new early maturing, semi-dwarf, lodging resistance and good quality bread wheat variety “Tapdongmil”. Res. Rept. (Crops). 29: 154–158.

Tanio, M., K. Kato, N. Ishikawa, Y. Tamura, M. Sato, H. Takagi and M. Matsuoka (2005) Genetic analysis of photoperiod response in wheat and its relation with the earliness of heading in the southwestern part of Japan. Breed. Sci. 55: 327–334.

Tanio, M., K. Kato, N. Ishikawa, T. Tabiki, Z. Nishio, K. Nakamichi, Y. Tamura, M. Sato, H. Takagi and M. Matsuoka (2006) Effect of shuttle breeding with rapid generation advancement on heading traits of Japanese Wheat. Breed. Sci. 56: 311–320.

Tanio, M. and K. Kato (2007) Development of near-isogenic lines for photoperiod-insensitive gene, Ppd-B1 and Ppd-D1, carried by
Japanese wheat cultivars and their effect on apical development. Breed. Sci. 57: 65–72.

Taya, S. (1993) Breeding of early maturing wheat varieties with higher grain yield in southwestern regions of Japan. Bull. Kyushu Natl. Agric Exp. Stn. 27: 333–398.

Turner, A., J. Beales, S. Faure, R. P. Dunford and D. A. Laurie (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310: 1031–1034.

Welsh, J. R., D. L. Keim, B. Pirasteh and R. D. Richards (1973) Genetic control of photoperiod response in wheat. Proc. 4th Int. Wheat Genet. Symp., Columbia, USA. pp. 879–884.

Worland, A. J. (1996) The influence of flowering time gene on environmental adaptability in European wheats. Euphytica 89: 49–57.

Yang, F. P., X. K. Zhang, X. C. Xia, D. A. Laurie, W. X. Yang and Z. H. He (2009) Distribution of the photoperiod insensitive Ppd-D1a allele in Chinese wheat cultivars. Euphytica 165: 445–442.

Yasuda, S. and H. Shimoyama (1965) Analysis of internal factors influencing the heading time of wheat varieties. Ber. Ohara Inst. landw. Biol. Okayama U. 13: 23–38.

Yoshida, H., M. Kamio and K. Kawaguchi (1983) Evaluation of cultivars for early matureness in the Japanese wheat breeding. Proc. 6th Int. Wheat Genet. Symp., Kyoto, Japan. pp. 601–612.