Distribution of photoperiod-insensitive allele $Ppd-A1a$ and its effect on heading time in Japanese wheat cultivars

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The $Ppd-A1$ genotype of 240 Japanese wheat cultivars and 40 foreign cultivars was determined using a PCR-based method. Among Japanese cultivars, only 12 cultivars, all of which were Hokkaido winter wheat, carried the $Ppd-A1a$ allele, while this allele was not found in Hokkaido spring wheat cultivars or Tohoku-Kyushu cultivars. Cultivars with a photoperiod-insensitive allele headed 6.9–9.8 days earlier in Kanto and 2.5 days earlier in Hokkaido than photoperiod-sensitive cultivars. The lower effect of photoperiod-insensitive alleles observed in Hokkaido could be due to the longer day-length at the spike formation stage compared with that in Kanto. Pedigree analysis showed that ‘Purple Straw’ and ‘Tohoku 118’ were donors of $Ppd-A1a$ and $Ppd-D1a$ in Hokkaido wheat cultivars, respectively. Wheat cultivars recently developed in Hokkaido carry photoperiod-insensitive alleles at a high frequency. For efficient utilization of $Ppd-1$ alleles in the Hokkaido wheat-breeding program, the effect of $Ppd-1$ on growth pattern and grain yield should be investigated. $Ppd-A1a$ may be useful as a unique gene source for fine tuning the heading time in the Tohoku-Kyushu region since the effect of $Ppd-A1a$ on photoperiod insensitivity appears to differ from the effect of $Ppd-B1a$ and $Ppd-D1a$.

Key Words: wheat, photoperiod response gene, $Ppd-A1$, heading time, Japanese wheat cultivars.

Introduction

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

The photoperiod response is mainly controlled by three major genes, $Ppd-D1$ (previously designated $Ppd1$), $Ppd-B1$ ($Ppd2$) and $Ppd-A1$ ($Ppd3$), which are located in a homoeologous group of 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 bacterial artificial chromosome (BAC) libraries and sequence analyses revealed that the photoperiod-insensitive $Ppd-D1a$ allele is associated with a 2089-bp deletion in the 5′ upstream region (Beales et al. 2007). Recent data showed that the photoperiod-insensitive $Ppd-A1a$ and $Ppd-B1a$ alleles are associated with a 1085-bp deletion and 308-bp insertion, respectively, both in the 5′ upstream region (Nishida et al. 2012). Deletion of $Ppd-A1a$ shares a common region with the deletion of $Ppd-D1a$, while insertion of $Ppd-B1a$ interrupts the common region.

Previous studies have investigated the geographical
distribution of \( \text{Ppd-D1a} \). Based on the sequence polymorphism among \( \text{Ppd-D1} \) alleles reported by Beales et al. (2007), Yang et al. (2009) determined the \( \text{Ppd-D1} \) genotype of Chinese wheat landraces and found that the frequency of the \( \text{Ppd-D1a} \) allele varies among regions, even within China. The \( \text{Ppd-D1a} \) allele was not found in northern China, but was frequently observed in southeastern China. Most of the European wheat cultivars with photoperiod insensitivity likely carry \( \text{Ppd-D1a} \) derived from ‘Akkomugi’ (Worland 1996). This assumption is supported by Guo et al. (2010), who showed that most Italian wheat cultivars carry \( \text{Ppd-D1a} \). However, little is known regarding the distribution of the photoperiod-insensitive alleles \( \text{Ppd-A1} \) and \( \text{Ppd-B1} \) since the critical sequence polymorphism among alleles was not detected until recently by Nishida et al. (2012).

For Japanese wheat cultivars, most cultivars in the Tohoku-Kyushu region carried \( \text{Ppd-D1a} \) and extra-early cultivars in southwestern Japan carried \( \text{Ppd-B1a} \) and \( \text{Ppd-D1a} \) (Seki et al. 2011). \( \text{Ppd-B1a} \) showed a stronger effect on accelerating heading than \( \text{Ppd-D1a} \) (Seki et al. 2011, Tanio et al. 2007). Therefore, it was suggested that introduction of the \( \text{Ppd-B1a} \) allele enabled the breeding of early maturity wheat cultivars required to avoid preharvest sprouting and Fusarium head blight during the rainy season. The photoperiod-insensitive allele \( \text{Ppd-D1a} \) has also been found in Hokkaido wheat cultivars, although its effect on heading time was insignificant against the genetic background of Hokkaido wheat cultivars (Seki et al. 2011). In addition to these two alleles, another photoperiod-insensitive allele \( \text{Ppd-A1a} \) was found in a Hokkaido cultivar ‘Chihokukomugi’ by Nishida et al. (2012). They compared the heading time among DH lines differing in the \( \text{Ppd-D1} \) genotype and showed that the effect of \( \text{Ppd-A1a} \) was weaker than that of \( \text{Ppd-B1a} \) or \( \text{Ppd-D1a} \). However, since an insensitive allele of \( \text{Ppd-A1} \) had not been previously reported, very little is known regarding the effect of \( \text{Ppd-A1a} \) and its distribution in Japanese wheat cultivars.

In this study, the \( \text{Ppd-A1} \) genotype of Japanese wheat cultivars was determined using a PCR-based method to detect the deletion in the upstream region of this gene. Thereafter, the \( \text{Ppd-A1} \) genotype of wheat cultivars and breeding lines present in the pedigree of Hokkaido winter wheat cultivars was determined to reveal the origin of the \( \text{Ppd-A1a} \) allele and to discuss the effect of \( \text{Ppd-A1a} \) and \( \text{Ppd-D1a} \) alleles on heading time in Hokkaido wheat cultivars.

Materials and Methods

A total of 280 wheat cultivars, consisting of Japanese commercial cultivars (164 cultivars), Japanese breeding lines (52 cultivars), Japanese landraces (24 cultivars) and foreign cultivars introduced for breeding (40 cultivars), were used in the present study. The geographical origin of these cultivars is summarized in Table 1.

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).

The \( \text{Ppd-D1} \) genotypes were determined using PCR-based methods with primer sets designed to identify the deletion of 1085 bp in the upstream region of \( \text{Ppd-D1a} \), the insertion of 308 bp in the upstream region of \( \text{Ppd-B1a} \) (Nishida et al. 2012), or the deletion of 2089 bp in the upstream region of \( \text{Ppd-D1a} \) (Beales et al. 2007). Three primers, namely TaPpd-A1prodelF1, TaPpd-A1prodelR3 and TaPpd-A1prodelR2 (developed by Nishida et al. 2012), were used for \( \text{Ppd-A1} \) and two primers, namely TaPpd-B1prof1 and TaPpd-B1int1R1 (developed by Nishida et al. 2012), were used for \( \text{Ppd-B1} \). Three primers, namely Ppd-D1_F1, Ppd-D1_R1 and Ppd-D1_R2 (developed by Beales et al. 2007), were used for \( \text{Ppd-D1} \). The nucleotide sequence of each primer is shown in Table 2.

For the analysis of \( \text{Ppd-A1} \) and \( \text{Ppd-D1} \), PCR amplification was performed in a 5 μl mixture containing 10 ng genomic DNA, 0.5 μl 10 × Ex Taq buffer (TaKaRa, Shiga, Japan; 20 mM Tris-HCl at pH 8.0, 100 mM KCl, 20 mM Mg²⁺), 0.2 mM dNTP, 0.2 μM of each primer and 0.125 U Ex Taq Hot Start Version (TaKaRa). The PCR cycle for \( \text{Ppd-A1} \) was as follows: an initial denaturing step at 98°C for 30 sec; 35 PCR cycles at 98°C for 10 sec, 57°C for 30 sec and 72°C for 30 sec and a final extension step at 72°C for 2 min. The PCR cycle for \( \text{Ppd-D1} \) 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 \( \text{Ppd-B1} \), 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 Mg²⁺), 0.2 mM 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, Foster City, CA, USA). PCR products were separated by electrophoresis on a 13% polyacrylamide gel. Electrophoresis and polymorphism detection were based on the description by Horī et al. (2003). For 258 cultivars, the \( \text{Ppd-B1} \) and \( \text{Ppd-D1} \) genotypes were analyzed in our previous study (Seki et al. 2011) and their genotype data were used in this study.

Among them, 23 wheat cultivars and breeding lines appeared in the pedigree of ‘Kitahonami’, the latest registered winter wheat cultivar cultivated in Hokkaido and were grown in a field at the NARO Institute of Crop Science (Tsukuba, Ibaraki, 36°01′N, 140°06′E) in the Kanto region and the HRO Kitami Agricultural Experiment Station (Kunneppu, Hokkaido, 43°47′N, 143°42′E) in the Hokkaido region of Japan. The details are summarized in Table 3.

In Tsukuba, they were sown on November 6 and November 5 in the 2008/2009 and 2009/2010 wheat-growing
Table 1. Geographical origin of 280 wheat cultivars and geographical distribution of Ppd-1 alleles

| Area of origin                  | Total number of cultivars | Ppd-A1 | Ppd-A1b | Ppd-B1 | Ppd-B1b | Ppd-D1a | Ppd-D1b |
|---------------------------------|---------------------------|--------|---------|--------|---------|---------|---------|
| Hokkaido (Winter wheat)         | 29                        | 12     | 17      | 0      | 29      | 7       | 22      |
| Hokkaido (Spring wheat)         | 10                        | 0      | 10      | 0      | 10      | 1       | 9       |
| Tohoku, Hokuriku                | 47                        | 0      | 47      | 0      | 47      | 43      | 4       |
| Kanto, Tokai                    | 63                        | 0      | 63      | 2°     | 61      | 63      | 0       |
| Kinki, Chugoku, Shikoku         | 43                        | 0      | 43      | 6°     | 37      | 42      | 1°      |
| Kyushu                          | 48                        | 0      | 48      | 2°     | 46      | 48      | 0       |
| Foreign cultivars               | 40                        | 2°     | 38      | 1°     | 39      | 18      | 22      |
| Total                           | 280                       | 14     | 266     | 11     | 269     | 222     | 58      |

a Carrier of Ppd-A1a; ‘Purcam (U-11)’ and ‘Purple Straw’.
b Carrier of Ppd-B1a; ‘Konosu 4’ and ‘Shiroboro 21’.
c Carrier of Ppd-B1a; ‘Chugoku 55’, ‘Chugoku 81’, ‘Chugoku 91’, ‘Chugoku 98’, ‘Chugoku 114’ and ‘Fukuwasekomugi’.
d Carrier of Ppd-B1a; ‘Sakigakekomugi’ 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 2. Diagnostic PCR primers used to determine the Ppd-1 genotypes

| Locus | Primer name | Sequence (5'→3') |
|-------|-------------|------------------|
| Ppd-A1 | TaPpd-A1prodelF1 | GTGACCTCCCTCGGTTCTTT |
|       | TaPpd-A1prodelR3 | ATTATCGGGGACCAAATACCC |
|       | TaPpd-A1prodelR2 | GTTGTTGGTGCTGGGTTG |
| Ppd-B1 | TaPpd-B1proF1 | ACTAGGGCTGGTCGAG |
|       | TaPpd-B1intR1 | CGGACCACTGGAATAAC |
| Ppd-D1 | TaPpd-D1_F1 | CCGCTCCCACACTACGT |
|       | TaPpd-D1_R1 | TTGTTGTCAACAGAGAGC |
|       | TaPpd-D1_R2 | ACTGTTGTAAGCTGAGATT |

Expected PCR product sizes, i.e., 338 bp from Ppd-A1a or 299 bp from Ppd-A1b, a photoperiod-sensitive allele without deletion of 1085 bp, were successfully amplified by multiplex PCR in all of the cultivars tested (Supplemental Fig. 1). For Ppd-B1, 1600 bp from Ppd-B1a or 1292 bp from Ppd-B1b, a photoperiod-sensitive allele without an insertion of 308 bp and for Ppd-D1, 288 bp from Ppd-D1a or 415 bp from Ppd-D1b, a photoperiod-sensitive allele without deletion of 2089 bp, were successfully amplified in all of the cultivars tested. Among 280 cultivars, only 14 cultivars (5.0%) carried the Ppd-A1a allele (Table 1), i.e., 12 Hokkaido winter wheat cultivars and two foreign cultivars, ‘Purcam (U-11)’ and ‘Purple Straw’ (Table 1 and Supplemental Fig. 2). The Ppd-A1a allele was not found in Hokkaido spring wheat cultivars or Tohoku-Kyushu cultivars. Most of the cultivars in the Tohoku-Kyushu region and eight cultivars in the Hokkaido region carried the photoperiod-insensitive allele Ppd-D1a and 11 cultivars, including three extra-early commercial cultivars, carried the two photoperiod-insensitive alleles Ppd-B1a and Ppd-B1d, as previously reported (Seki et al. 2011). The other genotypes with two or three photoperiod-insensitive alleles, i.e., the Ppd-A1a/Ppd-B1a/Ppd-D1b, Ppd-A1a/Ppd-B1b/Ppd-D1a and Ppd-A1a/Ppd-B1a/Ppd-D1a genotypes, were not found in this study.

Among the 23 genotypes in the pedigree of ‘Kitahonami’, 10 carried Ppd-A1a and five carried Ppd-D1a as a photoperiod-insensitive allele, while the others did not carry insensitive alleles of Ppd-1 (Table 3). The heading date examined in Tsukuba and Kunneppu was compared among three Ppd-1 genotypes, namely Ppd-A1a/Ppd-B1b/Ppd-D1b, Ppd-A1b/Ppd-B1b/Ppd-D1a and Ppd-A1b/Ppd-B1b/Ppd-D1b (Table 4). The genotypic difference of heading date was significant at the 0.1% level in Tsukuba and 5% level in Kunneppu. In Tsukuba, the average heading date of

"seasons, respectively. Each experimental plot consisted of a single 1.0-m-long row; the planting distance was 70 cm between rows and 8.5 cm between plants. At Kunneppu, they were sown on September 21, September 20 and September 19 for the 2007/2008, 2008/2009 and 2009/2010 wheat-growing seasons, respectively. Each experimental plot consisted of six 4.5-m-long rows; the planting distance was 20 cm between rows and 255 seeds per square meter. Heading time was recorded when the tip of the first ear emerged from the flag leaf sheath in half of the plants for each cultivar.

Heading date data were analyzed using statistical software (SPSS Ver. 18.0 J for Windows; SPSS Japan Inc.). The effective day-length in Tsukuba and Kunneppu was calculated according to methods presented by Gotoh (1977), who stated that the effective day-length should include predawn with light intensity over 20 lux and twilight with light intensity over 10 lux, which were 26 and 23 min in Fukuyama (34°30'N), respectively. The calculation formula was as follows:

\[
\text{(effective day-length in minutes at 0 degrees latitude)} = (\text{astronomical day-length}) + 49 (\cos 34^\circ 30'/\cos 0).
\]
Heading date at Kunneppu, 18 cultivars were tested; 'Kitakei 221', 'Kitakei 497', 'Tohoku 118', 'Kitakei 320' and 'Kitami 18' were not tested.

At Tsukuba, 21 cultivars were tested; 'Hokuei' and 'Mukakomugi' were not tested.

"a" and "b" indicate differences in heading time between test years. A similar genotypic difference was observed among the three wheat-growing seasons. In Kunneppu, the difference was not significant. In Tsukuba, the temperature after snow melted was warmer until heading time.

As shown in Fig. 1B, monthly average temperature differed among the three wheat-growing seasons. In Tsukuba, compared with the average temperatures over the last 30 years, temperatures in winter were slightly higher during the 2008/2009 and 2009/2010 seasons. In the 2009/2010 season, temperatures were lower in April, which is just before heading. According to meteorological data from Sakaino, near Kunneppu, and the end of continuous snow cover of the test field in Kunneppu, in the 2007/2008 season, snow melted very early and then temperatures were warmer during March and April. In the 2008/2009 season, the temperature after snow melted was warmer until heading time. In contrast, the temperature after snow melted was lower until 2 or 4 weeks before heading time in the 2009/2010 season. These differences in temperature conditions resulted in a significant difference in heading time between test years.

The Ppd-1 genotypes of wheat cultivars and breeding lines in the pedigree of Hokkaido winter wheat cultivars are summarized in Fig. 2. Pedigree analysis showed that Ppd-A1a in the three cultivars, ‘Takunekomugi’, ‘Kitamoe’ and ‘Kitahonami’ was inherited from ‘Purple Straw’ through ‘Purcam (U-11)’ and ‘Hokkai 240’. Ppd-D1a was inherited from ‘Tohoku 118’ to ‘Kitami 27’ and ‘Kitami 35.’ However, the source of Ppd-A1a of ‘Kitami 19’ could not be identified.

### Discussion

The combination of Ppd-1 alleles is important for control of the photoperiod response and hence, for the fine tuning of heading time. The introduction of photoperiod-insensitive alleles of Ppd-1 is indispensable for breeding early-heading wheat cultivars.

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### Table 3. Wheat cultivars tested at Tsukuba and Kunneppu and their Ppd-1 genotype and heading date

| Ppd-1 genotype | Tsukuba | Kunneppu |
|----------------|---------|----------|
| Ppd-A1a | Ppd-B1b | Ppd-D1a | Heading date | Ppd-A1a | Ppd-B1b | Ppd-D1a | Heading date |
| 2008/2009 | 2009/2010 | Av. | 2007/2009 | 2008/2009 | 2009/2010 | Av. |
| Kitakei 221 | a | b | b | May 2 | May 7 | May 4 | – | – | – | – |
| Kitakei 497 | a | b | b | Apr. 22 | May 1 | Apr. 26 | – | – | – | – |
| Kitakei 1354 | a | b | b | May 1 | May 6 | May 3 | June 14 | June 10 | June 15 | June 13 |
| Kitakei 1463 | a | b | b | May 3 | May 10 | May 6 | June 13 | June 11 | June 16 | June 13 |
| Kitami 19 | a | b | b | May 2 | May 7 | May 4 | June 13 | June 9 | June 15 | June 12 |
| Kitami 33 | a | b | b | Apr. 22 | May 1 | Apr. 26 | June 9 | June 4 | June 12 | June 8 |
| Takunekomugi | a | b | b | Apr. 22 | May 2 | Apr. 27 | June 6 | June 1 | June 8 | June 5 |
| Chihokukomugi | a | b | b | May 1 | May 8 | May 4 | June 13 | June 10 | June 17 | June 13 |
| Kitamoe | a | b | b | Apr. 30 | May 6 | May 3 | June 12 | June 10 | June 15 | June 12 |
| Kitahonami | a | b | b | Apr. 29 | May 5 | May 2 | June 11 | June 8 | June 14 | June 11 |
| Kitakei 1093 | b | b | a | May 2 | May 8 | May 5 | June 12 | June 12 | June 17 | June 13 |
| Kitami 27 | b | b | a | Apr. 22 | May 2 | Apr. 27 | June 10 | June 5 | June 13 | June 9 |
| Kitami 35 | b | b | a | Apr. 20 | Apr. 29 | Apr. 24 | June 6 | June 4 | June 12 | June 7 |
| Tohoku 118 | b | b | a | Apr. 19 | Apr. 28 | Apr. 23 | – | – | – | – |
| Horoshirikomugi | b | b | a | May 2 | May 8 | May 5 | June 14 | June 11 | June 16 | June 13 |
| Kitakei 320 | b | b | b | May 2 | May 8 | May 5 | – | – | – | – |
| Kitakei 1409 | b | b | b | May 7 | May 12 | May 9 | June 14 | June 11 | June 15 | June 13 |
| Kitakei 1660 | b | b | b | May 8 | May 15 | May 11 | June 17 | June 13 | June 18 | June 16 |
| Kitami 18 | b | b | b | May 6 | May 16 | May 11 | – | – | – | – |
| Kitami 53 | b | b | b | May 10 | May 18 | May 14 | June 17 | June 12 | June 17 | June 15 |
| Hokuei | b | b | b | – | – | – | June 16 | June 11 | June 17 | June 14 |
| Mukakomugi | b | b | – | – | – | June 11 | June 9 | June 15 | June 11 |
| Hokushin | b | b | b | Apr. 29 | May 5 | May 2 | June 10 | June 7 | June 13 | June 10 |

* "a" and "b" indicate Ppd-A1a and Ppd-A1b, respectively.
* "a" and "b" indicate Ppd-B1a and Ppd-B1b, respectively.
* "a" and "b" indicate Ppd-D1a and Ppd-D1b, respectively.
* Heading date at Tsukuba, 21 cultivars were tested; ‘Kitakei 221’, ‘Kitakei 497’, ‘Tohoku 118’, ‘Kitakei 320’ and ‘Kitami 18’ were not tested.
* Heading date at Kunneppu, 18 cultivars were tested; ‘Kitakei 221’, ‘Kitakei 497’, ‘Tohoku 118’, ‘Kitakei 320’ and ‘Kitami 18’ were not tested.
wheat cultivars that enable stable wheat production in Japan. To investigate the Ppd-1 genotypes of Japanese wheat cultivars using Japanese cultivars, including 164 commercial wheat cultivars that enable stable wheat production in Japan.

Table 4. Effect of the Ppd-1 genotype on the heading time in cultivars on the pedigree of ‘Kitahonami’

| Growing season | Ppd genotype | Tsukuba | Kuneppu |
|----------------|--------------|---------|---------|
|                | n | Heading date | n | Heading date |
| 2007/2008      |   |             | 8 | 11.4 ± 0.9 |
| Ppd-A1a/Ppd-B1b/Ppd-D1b | – | 8 | 11.4 ± 0.9 |
| Ppd-A1b/Ppd-B1b/Ppd-D1a | – | 4 | 10.5 ± 1.7 |
| Ppd-A1b/Ppd-B1b/Ppd-D1b | – | 6 | 14.2 ± 1.2 |
| 2008/2009      | 10 | 28.4 ± 1.4 | 8 | 7.9 ± 1.2 |
| Ppd-A1a/Ppd-B1b/Ppd-D1b | 5 | 25.0 ± 2.9 | 4 | 8.0 ± 2.0 |
| Ppd-A1b/Ppd-B1b/Ppd-D1a | 6 | 35.3 ± 1.7 | 6 | 10.5 ± 0.9 |
| 2009/2010      | 10 | 35.3 ± 0.9 | 8 | 14.0 ± 1.0 |
| Ppd-A1a/Ppd-B1b/Ppd-D1b | 5 | 33.0 ± 2.1 | 4 | 14.5 ± 1.2 |
| Ppd-A1b/Ppd-B1b/Ppd-D1a | 6 | 42.3 ± 2.0 | 6 | 15.8 ± 0.7 |
| Average       | 2007/2008 | – | 12.0b |
| 2008/2009      | 29.6 | 8.8a |
| 2009/2010      | 36.9 | 14.8a |
| Ppd genotype  | Ppd-A1a/Ppd-B1b/Ppd-D1b | 31.9b |
| Ppd-A1b/Ppd-B1b/Ppd-D1a | 29.0a |
| Ppd-A1b/Ppd-B1b/Ppd-D1b | 38.8b |

F-value of ANOVA

- Growth season(A)
  - Growing season(B)
  - Ppd genotype
  - (A)*(B)

|                      | n Heading date | n Heading date |
|----------------------|---------------|---------------|
|                      |              |               |
|                      | 2007/2008 | 12.0b |
|                      | 2008/2009 | 8.8a |
|                      | 2009/2010 | 14.8a |
|                      | Ppd-A1a/Ppd-B1b/Ppd-D1b | 31.9b |
|                      | Ppd-A1b/Ppd-B1b/Ppd-D1a | 29.0a |
|                      | Ppd-A1b/Ppd-B1b/Ppd-D1b | 38.8b |

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- Heading date shows average duration (days ± standard error).
- In Tsukuba, days from March 31 to heading date.
- In Kuneppu, days from May 31 to heading date.
- Average values of each growing season and Ppd genotype followed by different letters are significantly different (P < 0.05) according to the LSD tests.
- * and *** indicate significance at the 5% and 0.1% level, respectively; ‘ns’ indicates no significance at the 5% level.

confirmed, indicating the possibility of adjusting the heading time by altering Ppd-1 genotypes in the Tohoku-Kyushu region. In contrast, in Hokkaido cultivars, an effect of Ppd-D1a on heading time was not observed, indicating the involvement of other factors in controlling heading time. In this study, the heading date was investigated in Tsukuba in the Kanto region and in Kuneppu in the Hokkaido region using 23 Japanese cultivars and lines in the pedigree ‘Kitahonami’. The heading date was significantly different between the Ppd-1 genotypes (Table 4), indicating that acceleration of heading due to the photoperiod-insensitive alleles Ppd-A1a or Ppd-D1a and the effect of Ppd-1 was lower in Kuneppu than in Tsukuba (Table 4). According to Gotoh (1977), the effective day-length at the spike formation stage, two months before heading, is approximately 14 h in Hokkaido winter wheat and 15 h in Hokkaido spring wheat, while it is shorter (approximately 12 h) in the southwestern region, which includes Kanto. Based on these data, Gotoh (1977) suggested that photoperiod sensitivity is less important for the earliness of heading in the Hokkaido region compared to in the southwestern region. Analysis of photoperiod-sensitive and -insensitive wheat cultivars clearly showed marked heading time differences between the two types under 8–12 h day-length, while the difference was small or negligible under 14–24 h day-length (Evans 1987, Klaimi,...
and Qualset 1973, Ormrod 1963, Slafer and Rawson 1996). These results also support the conclusion of Gotoh (1977).

In this study, effective day-length two months before heading of cultivars of the pedigree ‘Kitahonami’ was approximately 14 h in Kunneppu, similar to that shown by Gotoh (1977) (Fig. 1A). Therefore, the regional difference in the day-length could explain the geographical difference of the effect of Ppd-1 on the heading time.

Pedigree analysis of Hokkaido winter wheat cultivars suggested that Ppd-D1a in Hokkaido cultivars has been introduced from several Tohoku cultivars, among which ‘Tohoku 118’ is thought to be one of the donors (Fig. 2). In Hokkaido, artificial cross-breeding of wheat has been performed since 1919 to develop hard wheat cultivars. Tohoku-Kitahonami cultivars were used as cross-parents as well as foreign cultivars, resulting in the introduction of the Ppd-D1a allele. For Ppd-A1a, pedigree analysis showed that ‘Hokkai 240’ inherited the allele from a US wheat cultivar ‘Purcam (U-11)’, for which Ppd-A1a could be traced back to an old US cultivar ‘Purple Straw’ (Fig. 2), ‘Hokkai 240’, which is tolerant to leaf rust and lodging (Iriki et al. 1985), should have contributed to the introduction of Ppd-A1a into Hokkaido cultivars. Since the 1980s, soft wheat cultivars suitable for Japanese noodles have been developed in Hokkaido. Thereafter, no commercial cultivar with Ppd-D1a has been developed except for ‘Kitanokaoiri’, a hard wheat cultivar for bread. ‘Chihokukomugi’, a soft wheat cultivar with Ppd-A1a, was registered in 1981. This cultivar possesses high quality for Japanese noodles and is frequently crossed in wheat breeding. The shift of the breeding target from hard wheat to soft wheat was considered the turning point and then the frequency of Ppd-A1a increased in Hokkaido cultivars.

Hokkaido cultivars carried insensitive alleles of Ppd-1, although the photoperiod-insensitive alleles have less of an effect on heading time under long day conditions. A possible explanation is the pleiotropic effects of Ppd-D1a detected under the growing conditions in Europe and high latitude areas such as Hokkaido (Börner et al. 1993, Worland 1996, Worland et al. 1988). According to these reports, the Ppd-D1a allele reduced the number of spikelets per ear, resulting in increased spikelet fertility. These studies demonstrated that the photoperiod-insensitive allele Ppd-D1a is advantageous for increasing grain yield. The effect of Ppd-1 on the growth pattern and grain yield in the Hokkaido region should be investigated to clarify the reason for the higher frequency of photoperiod-insensitive alleles in recently developed Hokkaido cultivars. Another possibility could be the genetic linkage between Ppd-1 and other genes. Tolerance to lodging and diseases, particularly leaf rust, has been one of the main targets since the beginning of wheat breeding in Hokkaido. According to previous reports, the Ppd-D1a allele reduced plant height independently of the semi-dwarf gene Rht (Börner et al. 1993, Worland et al. 1988). We cannot exclude the possibility that the introduction of the lodging tolerance from ‘Hokkai 240’ resulted in the introduction of Ppd-A1a into Hokkaido cultivars. In contrast, no previous research has shown a close linkage between Ppd-1 and genes for disease tolerance or flour quality, while some QTLs were reported in a homoeologous group of two chromosomes on which Ppd-1 loci are located (Chhuneja et al. 2006, Roncallo et al. 2012, Watanabe et al. 2006). Additionally, studies examining the relationship between Ppd-1 and improvement of tolerance to lodging and diseases or flour qualities in wheat breeding will reveal details of Ppd-A1a introduction into Hokkaido cultivars.

In this study, no Tohoku-Kyushu cultivar carried Ppd-A1a. Cultivars carrying Ppd-A1a, such as ‘Chihokukomugi’ and ‘Kitamoe’, have been used as cross parents in Tohoku-Kyushu wheat breeding; however, these cultivars carried genetic factors of late heading such as vernalization genes, so that only one cultivar, ‘Nebarigoshi’, was developed successfully in Tohoku. Introduction of Ppd-A1a into Tohoku-Kyushu cultivars has not been carried out so far. The photoperiod-insensitive allele Ppd-A1a analyzed in this study

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**Fig. 1.** Meteorological data in test seasons. A. Effective day-length at two sites. : Tsukuba, : Kunneppu. Lines flanked by circles indicate the range of heading date at each site. Solid and thick lines indicate the effective day-length two months before heading. B. Monthly mean temperature. : Average temperature over the last 30 years, : 2007/2008 growing season, : 2008/2009 growing season, : 2009/2010 growing season. a: Tateno (in Tsukuba), b: Sakaino (near Kunneppu). Data were taken from the Japan Meteorological Agency. Arrows indicating the start and end of continuous snow cover duration during each season in Kunneppu.
was first found by Nishida et al. (2012), and thus the effect of this allele on heading time has not been extensively investigated. According to Nishida et al. (2012), who analyzed the heading time of a DH population segregating for three Ppd-1 genes, the Ppd-A1a/Ppd-B1b/Ppd-D1b genotype headed two days later in Okayama, Chugoku region, compared to the Ppd-A1b/Ppd-B1a/Ppd-D1b or the Ppd-A1b/Ppd-B1b/Ppd-D1a genotypes, although the difference was not significant. Consistent with these data, our results demonstrate that cultivars carrying Ppd-A1a headed 2–3 days later in the Kanto region than those carrying Ppd-D1a, although the difference was insignificant (Table 4). Although early heading is important to avoid various damage in the rainy season, shorter growth periods generally result in lower grain yields; early-heading 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. Ppd-A1a may be useful as a unique gene source for fine tuning the heading time in the Tohoku-Kyushu region. Nishida et al. (2012) developed DNA markers to determine Ppd-A1 genotypes, making it possible to introduce Ppd-A1a into Tohoku-Kyushu cultivars by MAS. However, the interaction between Ppd-A1a and other photoperiod-insensitive alleles is not clear. Furthermore, Eagles et al. (2010) indicated that the effect of Ppd-D1a depended on the genotype of vernalization genes. The additive effects and the interactions between Ppd-1 genes and those between the Ppd-1 and Vrn-1 genes should be investigated to elucidate the usefulness of Ppd-A1a.

Acknowledgments
The authors are grateful to the National Institute of Agro-biological Sciences and wheat-breeding sites in Japan, including Gunma Agricultural Technology Center, the Nagano Agricultural Experimental Station, Aichi Agricultural Research Center and the three sites of NARO, HARC, TARC.
and WARC for providing the seeds of the wheat cultivars examined in this study. We are also grateful to the members of the Research Support Center of NARO Agricultural Research Center for their management of the field. Thanks are also due to members of the NARO Institute of Crop Science for their technical assistance. This work was partly supported by the Ministry of Agriculture, Forestry and Fisheries, for their technical assistance. This work was partly supported by the Ministry of Agriculture, Forestry and Fisheries, for their technical assistance. This work was partly supported by the Ministry of Agriculture, Forestry and Fisheries, for their technical assistance.

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