Genetic Studies of Photoperiod Response Genes and Their Effect on Heading Time in Japanese Wheat Cultivars

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Abstract

Wheat (*Triticum aestivum* L.) should be harvested before the rainy season in Japan. 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. Heading time of wheat is a complex characteristic determined by three factors: narrow-sense earliness (also termed earliness *per se*), vernalization response, and photoperiod response. For fine tuning of heading time in wheat breeding, genetic factors controlling these characteristics should be combined properly.

In this study, genotypes of the photoperiod response genes, *Ppd-A1*, *Ppd-B1* and *Ppd-D1*, in 240 Japanese cultivars were determined using PCR-based methods, and the effect of *Ppd-1* genotype on heading time was discussed. In addition, the introduction of photoperiod-insensitive alleles into Japanese cultivars was discussed.

The distribution of photoperiod-insensitive alleles differed among *Ppd-1* genes, as well as among geographic areas. Most Tohoku-Kyushu cultivars (97.5%) carried *Ppd-D1a*, and 10 cultivars, including three commercial extra-early cultivars, carried both *Ppd-B1a* and *Ppd-D1a*, while no cultivars carried *Ppd-A1a*. Among Hokkaido winter wheat cultivars, 41.4% and 24.1% carried *Ppd-A1a* and *Ppd-D1a*, respectively, and none of the cultivars carried *Ppd-B1a*. In contrast, in Hokkaido spring wheat cultivars, only one experimental line carried *Ppd-D1a*, and the other cultivars did not carry photoperiod-insensitive alleles.

The effect of *Ppd-1* alleles on heading time also differed among areas. In the Tohoku-Kyushu region, wheat cultivars carrying the photoperiod-insensitive allele *Ppd-D1a* headed earlier by 10.3 days than did photoperiod-sensitive cultivars, and *Ppd-B1a*/*Ppd-D1a* genotype headed earlier by 6.7 days than did *Ppd-B1b*/*Ppd-D1a* genotype. In the Hokkaido region, photoperiod-insensitive cultivars with *Ppd-A1a* or *Ppd-D1a* headed earlier by 2.5 days than did photoperiod-sensitive cultivars with no photoperiod-insensitive alleles, although the effect of these alleles was less than that in the Kanto region. The geographical difference of distribution and the effect of photoperiod-insensitive alleles in this study may result from the difference of day-length of the wheat apical spike formation stage.

Pedigree analysis of extra-early wheat cultivars 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. In Japan, except in Hokkaido, the rainy season starts before the wheat harvest; thus, early cultivars with *Ppd-D1a* have been selected to avoid damages such as preharvest sprouting and Fusarium head blight. Furthermore, it is suggested that the introduction of the *Ppd-B1a* accelerated early-maturity wheat breeding in Japan. Three cultivars with *Ppd-B1a*/*Ppd-D1a* genotype are successfully established only in the Chugoku or Kyushu region. It was considered that such cultivars often suffered from late frost because of early stem elongation in eastern Japan, like the Kanto region, where the daily minimum temperature was below freezing in the winter. Besides, heading time of extra-early cultivars is variable depending on winter temperature, and causes instability of grain yield. However, heading time of extra-early cultivars can be stabilized by the introduction of adequate vernalization requirements.

Pedigree analysis of Hokkaido winter wheat cultivars showed that ‘Purple Straw’ and ‘Tohoku 118’ were one of the donor(s) 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.

The results in this study clearly indicated that heading time varied largely even among the Japanese spring type cultivars except those in the Hokkaido region, in spite of having the same set of photoperiod response and vernalization response genes. It was suggested that their heading time is affected by other photoperiod response genetic factor(s) and other photoperiod insensitive allele(s) of *Ppd-1*. In addition, variation in heading time could be partly explained by narrow-sense earliness, since it is also important for the control of heading time in Japanese wheat cultivars. Further advancement of molecular genetics of narrow-sense earliness should be expected, and would make it possible to discuss the significance of this characteristic in Japanese wheat breeding.

Key Words: wheat, photoperiod response gene, *Ppd-1*, *Ppd-A1*, *Ppd-B1*, *Ppd-D1*, heading time, Japanese wheat cultivars

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日本コムギ品種における日長反応性遺伝子とその出穂期への効果に関する遺伝学的研究

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抄　録

日本のコムギ作では、収穫期の雨害回避のために早生品種が求められる。しかし、早生品種は、生育期間が短いこと、茎立期が早く凍霜害に遭いやすいことなどにより、収量が低下しやすい。コムギの出穂早晩性は、日長反応性、低温要求性、純粋早晩性の複合形質であり、これらの形質を遺伝的にコントロールし組み合わせることで、生育や収量が安定したコムギ品種の育成が可能となると考えられる。

本研究では、日本品種240品種におけるコムギ日長反応性遺伝子*Ppd-A1*、*Ppd-B1*、*Ppd-D1*の遺伝子型をPCR法により判別するとともに、各*Ppd-1*遺伝子の出穂期への効果について検討した。さらに、日本のコムギ育種における各*Ppd-1*遺伝子の導入経緯および意義について議論した。

各*Ppd-1*の不感光性遺伝子の分布地域には差がみられた。東北-九州地域品種の97.5%が不感光性遺伝子*Ppd-D1a*を保有し、極早生コムギ品種を含む10品種が不感光性遺伝子*Ppd-B1a*と*Ppd-D1a*を併せ持ち、不感光性遺伝子*Ppd-A1a*を保有する品種はなかった。北海道の秋播コムギ品種では、*Ppd-A1a*、*Ppd-D1a*を保有する品種がそれぞれ41.4%、24.1%あり、*Ppd-B1a*を保有する品種はなかった。北海道の春播コムギ品種では、実験系統1系統を除いて、不感光性遺伝子を保有する品種はなかった。

*Ppd-1*の出穂期への効果については、東北-九州地域の品種では、*Ppd-D1a*が感光性型の*Ppd-D1b*に対して明確に早生化効果を示し、*Ppd-B1a*を併せて保有することで、さらなる早生化効果が示された。北海道では*Ppd-A1a*もしくは*Ppd-D1a*の保有により早生化効果がみられたが、関東地域での効果より小さかった。これは、北海道では幼穂形成期の日長が関東地域よりも長いことによると考えられた。

実用的な極早生コムギ3品種の*Ppd-B1a*は、白ボロ21号から早生の中国系統（中国農業試験場育成）を経て遺伝したことが示された。東北-九州品種では雨害回避のため、より早生となる*Ppd-D1a*を有する早生品種が選抜され、*Ppd-B1a*の導入によりさらに極早生品種が育成されたことが確認された。*Ppd-B1a*/*Ppd-D1a*型の実用品種は、中国地域と九州地域でのみ育成されてきた。冬期の最低気温が氷点下となる関東地域では、*Ppd-B1a*の導入は凍霜害の危険を高める恐れがあったため*Ppd-B1a*/*Ppd-D1a*型の実用品種が育成されてこなかったと推察されるが、適度な低温要求性を組み合わせることで、その懸念は回避されると考えられる。

北海道品種への*Ppd-A1a*および*Ppd-D1a*それぞれの供与親のひとつはPurple Strawおよび東北118号であることが確認された。近年、北海道で育成されたコムギ品種の多くが不感光性遺伝子を保有している。北海道のコムギ育種における*Ppd-1*の有用性について明らか

にするためには、*Ppd-1*の生育特性、収量特性への効果を調査する必要がある。

東北-九州品種では、同じ低温要求性遺伝子と日長反応性遺伝子を持つ品種間でも、出穂期に変異がある。日本品種の中に、*Ppd-1*の別の対立遺伝子や、*Ppd-1*とは異なる日長反応性遺伝子が存在する可能性がある。また、これまでに、日本でのコムギの早生化に純粋早晩性が重要な役割を果たしたことが示されている。今後、これらの遺伝子についても、日本のコムギ育種における意義について議論が可能となるよう、分子生物学的研究の発展が期待される。なお、本論文は岡山大学大学院自然科学研究科審査学位論文を加筆修正したものである。

キーワード： コムギ、日長反応性遺伝子、*Ppd-1*、*Ppd-A1*、*Ppd-B1*、*Ppd-D1*、出穂期、日本コムギ品種

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I Introduction

Wheat (Triticum aestivum L.) is one of the most important crops in the world, its production was 662 million tons in the year 2012. However, like other crops, wheat production is affected by the recent global climate change and has become unstable because of frequent drought in various areas around the world. Furthermore, the rapid growth of world population will lead to severe food shortage, and, thus, stable production of wheat is required in Japan as well as in the world. In such circumstances, the Japanese government is making efforts to increase food self-sufficiency and sets the target of increasing wheat production from 880 thousand tons in 2008 to 1.8 million tons in 2020.

In Japan, wheat should be harvested before the rainy season to avoid damage such as preharvest sprouting and Fusarium head blight, and before transplanting of rice for double cropping of wheat and rice. Therefore, early heading is one of the most important traits in wheat breeding. Since the 1970's, early heading cultivars such as 'Sakigakekomugi' (Yoshida *et al.* 1973), 'Goga tsukomugi' (Yoshida *et al.* 1975), 'Shiroganekomugi' (Yoshida *et al.* 1977), 'Asakazekomugi' (Nonaka *et al.* 1979) and 'Abukumawase' (Ujihara *et al.* 1994) have been bred in the Kyushu region, while 'Fukuwasekomugi' (Sumida *et al.* 1988) and 'Bandowase' (Seko *et al.* 1997) were bred in Chugoku and Kanto regions, respectively. 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 (Eguchi *et al* 1984, Hukumoto and Takahashi 1950, Taya 1993). Taya (1993) proposed the scheme for the breeding of early maturing wheat varieties with higher grain yield. Following his scheme, early cultivars with high yield like 'Nishikazekomugi' (Nonaka *et al.* 1987), 'Daichinominori' (Ujihara *et al.* 1991) and 'Chikugoizumi' (Ujihara *et al.* 1995) have been successfully bred. However, instability of grain yield is arising from the yearly or regional fluctuation of the length of each growth stage. Thus, heading characteristics related to the length of growth stages must be adjusted for the stable production of wheat in each area.

Heading time of wheat is a complex characteristic determined by three factors, narrow-sense earliness (also termed earliness *per se*), vernalization response and photoperiod response (Kato and Yamashita 1991, Yasuda and Shimoyama 1965). For fine tuning of heading time in wheat breeding, genetic factors controlling these characteristics should be combined properly.

Vernalization response was roughly estimated by the degree of spring growth habit, which was partly affected by narrow-sense earliness and photoperiod response in earlier studies (Kakizaki and Szuki 1937), and, thereafter, several methods to evaluate vernalization response have been proposed (Gotoh 1976, Kakizaki and Suzuki 1937, Kato and Yamagata 1988, Yasuda and Shimoyama 1965). Hashimoto and Hirano (1963) hypothesized that early cultivars with winter growth habit would be available to avoid frost injury, and this hypothesis was confirmed by Fujita *et al.* (1995) who compared heading characteristics among winter- and spring-type near-isogenic lines of the Japanese wheat cultivars. Based on these findings, the early heading cultivar with winter growth habit, 'Iwainodaichi', was successfully bred (Taya *et al.* 2003). It was reported that photoperiod response is the major determinant of earliness in autumn-sown wheat in central and south-western

Japan (Tanio *et al.* 2006, Yasuda and Shimoyama 1965, Yoshida *et al.* 1983), and it is closely related to the *Ppd* genotype but independent of the *Vrn* genotype, controlling vernalization response (Fujita *et al.* 1995, Kato and Yamashita 1991, Tanio *et al.* 2005). For narrow-sense earliness, Kato and Wada (1999) indicated that it was a heritable character, in spite of its quantitative nature, and could be efficiently optimized through artificial selection. It was also indicated that small response to photoperiod and short narrow-sense earliness should be combined for breeding of early heading varieties (Hashimoto and Hirano 1963, Yoshida *et al.* 1983).

Recently, *Vrn-1* and *Ppd-1* genes, the major genetic factors controlling vernalization response and photoperiod response in wheat, have been cloned (Beales *et al.* 2007, Fu *et al.* 2005, Nishida *et al.* 2013, Yan *et al.* 2003, 2004), and DNA markers for the distinction of variant or mutant alleles of each gene have been developed. These advances in molecular genetics facilitated genotyping of a large number of wheat cultivars and, hence, the elucidation of adaptive significance of *Vrn-1* and *Ppd-1* genes in various areas. *Vrn-1* genotype of the Japanese wheat cultivars has been analyzed by conventional segregation analysis, and it was revealed that most wheat cultivars in the central and south-western region carried *Vrn-D1*, and spring wheat cultivars in Hokkaido region carried *Vrn-A1* (Gotoh 1979, Iwaki *et al.* 2000). These results have been confirmed by analysis using DNA markers (Matsunaka *et al.* unpublished). On the contrary, little is known about the *Ppd-1* genotype of the Japanese wheat cultivars, except eight cultivars analyzed by the conventional segregation analysis (Tanio *et al.* 2005). Therefore, the comprehensive analysis of the *Ppd-1* genotype of Japanese wheat cultivars is required to know the effect of *Ppd-1* genes on heading time and the significance of *Ppd-1* genes for Japanese wheat breeding. Such an analysis would bring useful information for fine tuning of heading characteristics in breading program.

In this study, the *Ppd-1* genotype of Japanese cultivars was determined by PCR-based methods and the effect of *Ppd-1* genotype on heading time was discussed. First, in chapter II, *Ppd-B1* and *Ppd-D1* genotypes were determined and their important role in adjusting the heading time in Japan except the Hokkaido region was evaluated. Furthermore, the pedigree of extra-early wheat cultivars was analyzed. In chapter III, *Ppd-A1* genotype was determined and its effect on the heading time in Hokkaido region was evaluated. In addition, the introduction of *Ppd-A1a* and *Ppd-D1a* alleles into Hokkaido wheat cultivars was discussed. This report is the revision of the dissertation submitted to Graduate School of Natural Science and Technology, Okayama University.

II Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time

The photoperiod response is a very important physiological character in wheat, as it determines the earliness of heading with vernalization response and narrow-sense earliness (Yasuda and Shimoyama 1965). 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.* 2013), 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 'Akakomugi' (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.* (2013). 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 chapter, 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.

1 Materials and Methods

A total of 263 wheat cultivars, consisting of Japanese commercial cultivars (161 cultivars), Japanese breeding lines (47 cultivars), Japanese landraces (22 cultivars), and foreign cultivars introduced for breeding (33 cultivars), were used in the present study. Geographical origins are summarized in Table 2.

For DNA extraction, all wheat genotypes were grown in a growth chamber maintained at 20 ℃ 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.* 2013). 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 1.

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 Bio Inc., Otsu, Japan; 20 mM Tris-HCl at pH 8.0, 100 mM KCl, 20 mM Mg^{2+}), 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 ℃ for 30 sec; 35 PCR cycles at 98 ℃ for 10 sec, 54 ℃ for 1 min, and 72 ℃ for 30 sec; and a final extension step at 72 ℃ 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 Mg^{2+}), 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 ℃ for 30 sec; 35 PCR cycles at 98 ℃ for 10 sec, 64 ℃ for 1 min, and 72 ℃ for 30 sec; and a final extension step at 72 ℃ for 2 min. Amplification reactions were conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, CA, USA). PCR products were electrophoresed on acrylamide gel. Electrophoresis and polymorphism detection were based on the description by Hori *et al.* (2003).

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.

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

Table 1. Primers used to determined the *Ppd-D1* and *Ppd-B1* genotypes

Locus	Primer name	Sequence(5’→3’)
Ppd-B1	TaPpd-B1proF1	ACACTAGGGCTGGTCGAAGA
	TaPpd-B1int1R1	CCGAGCCAGTGCAAATTAAC
Ppd-D1	TaPpd-D1_F1	ACGCCTCCCACTACACTG
	TaPpd-D1_R1	TGTTGGTTCAAACAGAGAGC
	TaPpd-D1_R2	CACTGGTGGTAGCTGAGATT

2 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 2089bp, 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 263 cultivars, 221 cultivars (84.0%) proved to carry the photoperiod-insensitive allele *Ppd-D1a* (Table 2). 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 196 of 201 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 2, Fig. 1). On the other hand, only 11 cultivars (4.2%) carried *Ppd-B1a* allele (Table 2). 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, 'Fukuwasekomugi', and five breeding lines. Two cultivars from the Kyushu region, i.e., 'Sakigakekomugi' and 'Abukumawase', and one cultivar from Korea, i.e., 'Tapdongmil', also carried *Ppd-B1a* allele (Table 2, Fig. 2). All of the cultivars with *Ppd-B1a* allele carried *Ppd-D1a* allele as well; the *Ppd-B1a*/*Ppd-D1b* genotype was not found in this study.

Table 2. Distribution of photoperiod insensitive alleles *Ppd-B1a* and *Ppd-D1a* in Japanese and foreign wheat cultivars

Area of origin	Total number of cultivars	*Ppd-B1*		*Ppd-D1*	
		Ppd-B1a	*Ppd-B1b*	*Ppd-D1a*	*Ppd-D1b*
Hokkaido(Winter wheat)	19	0	19	7	12
Hokkaido(Spring wheat)	10	0	10	1[f]	9
Tohoku,Hokuriku	47	0	47	43	4[g]
Kanto,Tokai	63	2[b]	61	63	0
Kinki,Chugoku,Shikoku	43	6[c]	37	42	1[h]
Kyushu	48	2[d]	46	48	0
Foreign cultivars [a]					
High latitude area	11	0	11	4	7
Low latitude area	22	1[e]	21	13	9
Total	263	11	252	221	42

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

[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*; 'OS21-5'

[g] Carrier of *Ppd-D1b*; 'Fultz Daruma', 'Norin 6', 'Norin 24' and 'Norin 38'

[h] Carrier of *Ppd-D1b*; 'Eshima'

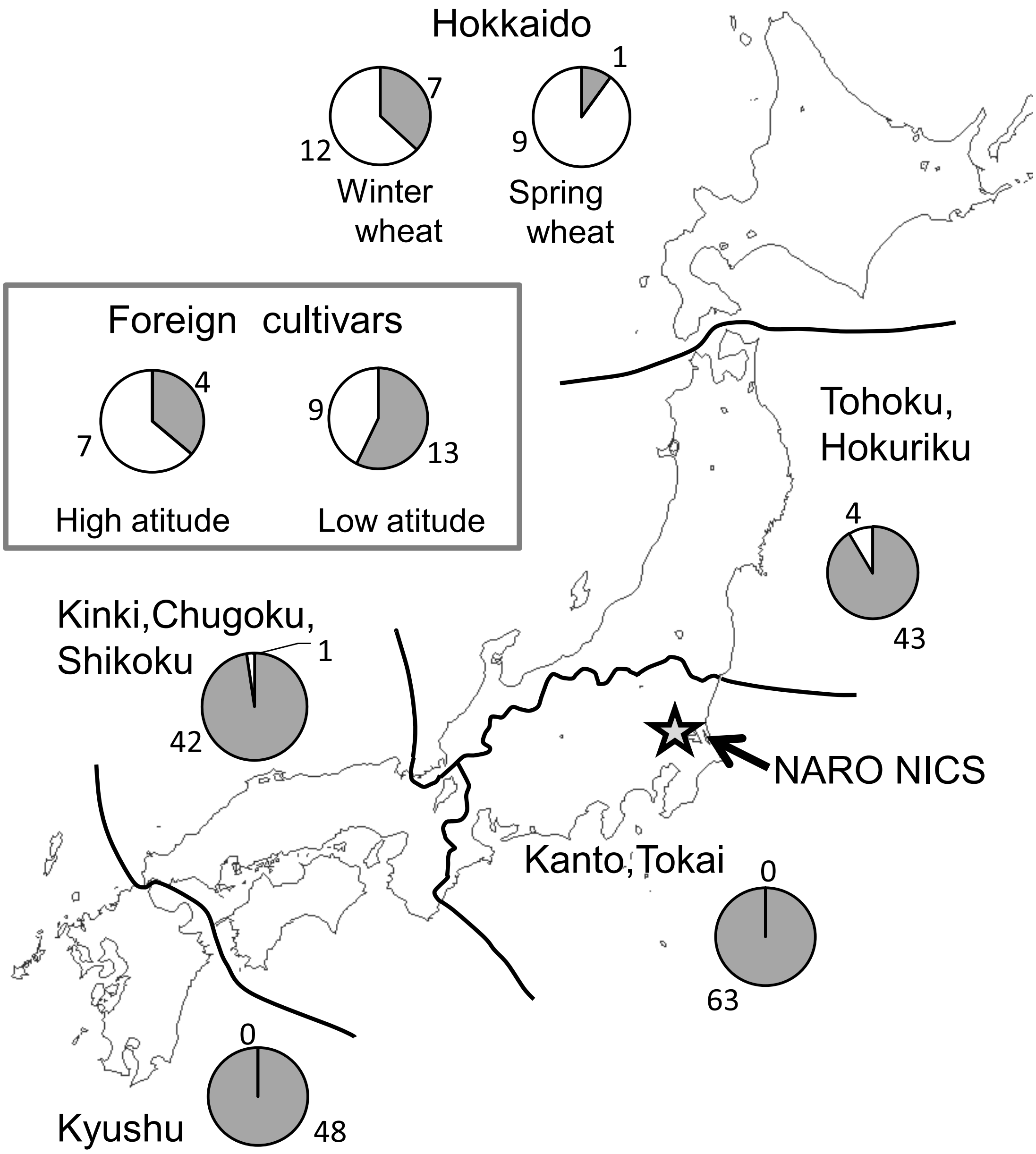

Fig. 1. Geographical distribution of *Ppd-D1a* and *Ppd-D1b*. Solid and open parts of the circular chart indicate the proportions of wheat cultivars carrying *Ppd-D1a* and *Ppd-D1b*, respectively. Number of cultivars was also indicated.

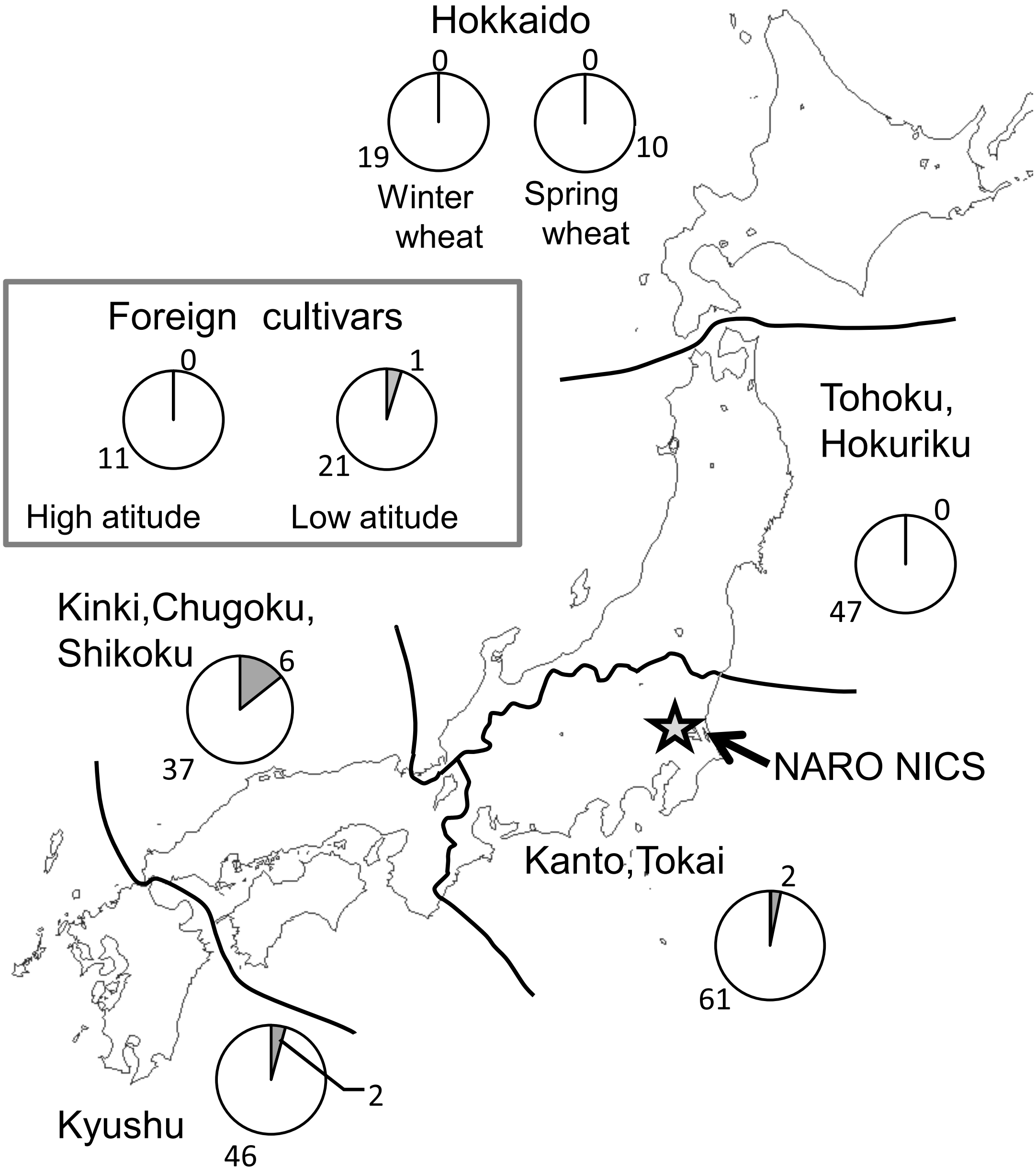

Fig. 2. Geographical distribution of *Ppd-B1a* and *Ppd-B1b*. Solid and open parts of the circular charts indicate the proportions of wheat cultivars carrying *Ppd-B1a* and *Ppd-B1b*, respectively. Number of cultivars was also indicated.

As summarized in Table 3, heading date of wheat cultivars differed significantly among the three seasons as well as among *Ppd-D1* genotypes (P $\leq$ 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.

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

		Total		Hokkaido				Tohoku-Kyushu		Foreign cultivars					
Growing season	*Ppd-D1* genotype			Total		Winter wheat				Total		High latitude area[b]		Low latitude area[b]	
		n	Heading date[a]	n	Heading date	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	4	32.0±1.3	12	26.9±1.4
	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	7	37.3±2.8	9	33.0±1.4
			***		ns		ns		***		***		ns		***
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	4	38.3±1.0	12	30.7±1.9
	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	7	43.3±2.7	9	40.7±1.7
			***		ns		ns		***		***		ns		***
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	4	24.0±1.4	12	18.4±1.9
	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	7	30.0±2.7	9	27.1±1.6
			***		ns		ns		***		***		ns		***
F-value of ANOVA [c]															
Growing seoson(A)			225.2 ***		34.30 ***		24.70 ***		30.27 ***		38.66 ***		13.33 ***		27.93 ***
Ppd-D1 genotype(E			410.1 ***		3.133 ns		1.906 ns		80.45 ***		44.84 ***		6.217 *		34.18 ***
(A)*(B)			4.775 *		0.850 ns		0.311 ns		1.300 ns		0.394 ns		0.018 ns		0.664 ns

[a] 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.

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

[c] * and *** indicate significance at 5% and 0.1% level, respectively, and 'ns' indicates no significance at 5% level.

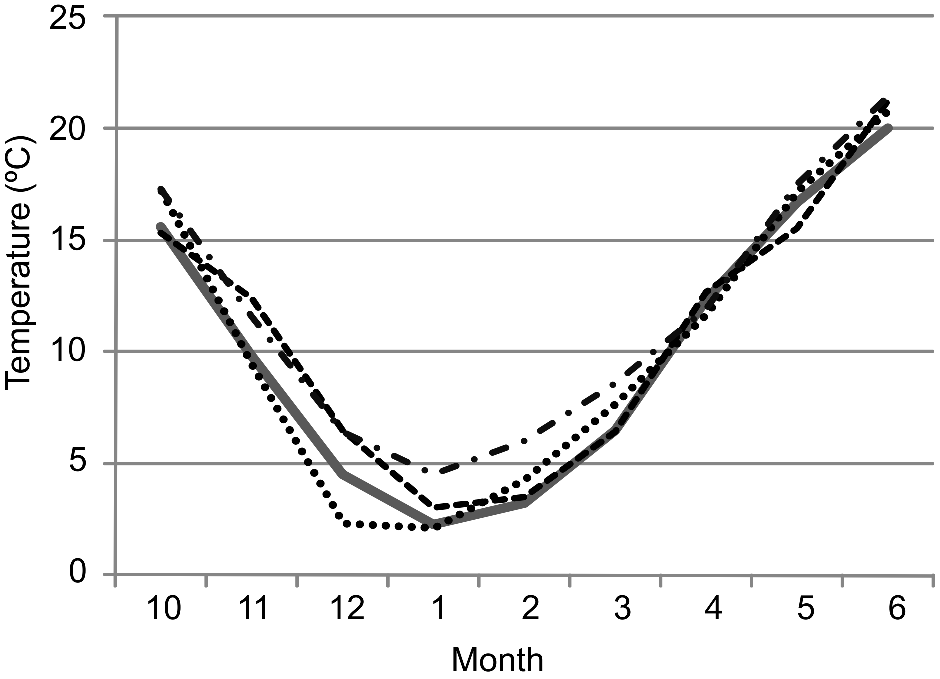

Fig. 3. Monthly mean temperature at Tateno, Tsukuba.

▬▬ ; Average of the last 30 years, ▬ ▬ ▬ ; 2004/2005 growing season,

••••• ; 2005/2006 growing season, ▬ • ▬ ; 2006/2007 growing season.

Data by Japan Meteorological Agency

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*.

Table 4. Additive effect of *Ppd-D1a* and *Ppd-B1a* for heading date

Growing season	*Ppd-1* genotype	Number of cultivars	Heading date [a]
2004/2005	*Ppd-B1a/Ppd-D1a*	10	22.1±0.8^a
	Ppd-B1b/Ppd-D1a	206	28.5±0.3^b
	Ppd-B1b/Ppd-D1b	42	35.7±0.8^c
2005/2006	*Ppd-B1a/Ppd-D1a*	10	27.5±0.7^a
	Ppd-B1b/Ppd-D1a	206	32.4±0.3^b
	Ppd-B1b/Ppd-D1b	42	43.0±0.9^c
2006/2007	*Ppd-B1a/Ppd-D1a*	10	11.6±0.8^a
	Ppd-B1b/Ppd-D1a	206	20.3±0.4^b
	Ppd-B1b/Ppd-D1b	42	30.5±0.8^c
F-value of ANOVA [b]			
	Growing seoson(A)		144.4 ***
	Ppd-1 genotype(B)		208.7 ***
	(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.

[b] ** and *** indicate significance at 1% and 0.1% level, respectively.

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

Ppd-1 genotype	Number of cultivars	Difference of heading date [a]
Ppd-B1a/Ppd-D1a	10	15.9 ± 0.5^a
Ppd-B1b/Ppd-D1a	206	12.0 ± 0.2^b
Ppd-B1b/Ppd-D1b	42	12.5 ± 0.4^b
F-value of ANOVA [b]		
Ppd-1 genotype		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.

[b] ** indicates significance at 1% level.

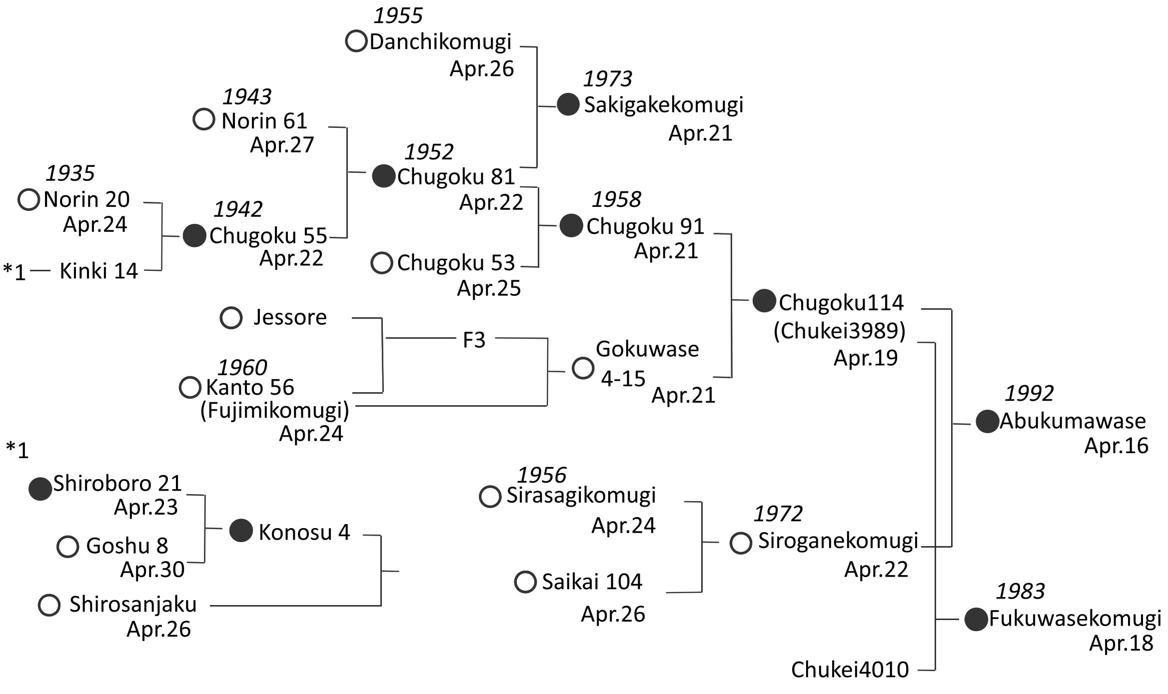

Fig. 4. *Ppd-B1* genotype and heading date of wheat cultivars on the pedigree of extra early wheat cultivars
● : Carrier of Ppd-B1a. ○ : Carrier of Ppd-B1b.

Ppd-1 genotype and heading date of ‘Kinki 14’ and ‘Chukei4010’ and heading date of ‘Jessore’ and ’Konosu4’ were not tested. The average of heading date for three growing seasons is indicated by calendar date.
The year ofregistration was shown in italics. *1 shows the pedigree of ‘Kinki14’.

3 Discussion

Examination of the *Ppd-D1* genotypes of 230 Japanese wheat cultivars by PCR-based analysis detected a deletion of 2089 bp in the 5′ upstream region (Beales *et al*. 2007), showing that 204 cultivars (88.7%) carried the insensitive allele *Ppd-D1a* (Table 2). In the Tohoku-Kyushu region, most of the cultivars (97.5%) carried *Ppd-D1a*. The predominance of the *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), *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: *Ppd-D1a* accelerates flowering by 4–8 days, so that only the early genotype carrying *Ppd-D1a* is able to fill grain before the hot, dry summer season in southern Europe. The result in this study also indicated that *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 *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 2, Fig. 1). *Ppd-D1a* also 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 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*. 2013). 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*. 2013, Tanio *et al*. 2005). 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*. Therefore, as summarized in Fig. 4, it was 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, ‘Tapdong mil’, also carried *Ppd-B1a.* Although ‘Tapdongmil’ 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 ‘Tapdongmil’ 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, 2014). 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 ‘Abuk umawase’ 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 varnalization requirement.

The previous genetic analysis indicated that spring type cultivars originated in the Tohoku-Kyushu region carry the vernalization response gene *Vrn-D1* (Gotoh 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.* 2013), 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*.

4 Summary

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-D1a*. 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.

Ⅲ Distribution of photoperiod-insensitive alleles *Ppd-A1a* and their effect on heading time

Heading time of wheat is a complex character controlled by narrow-sense earliness (also termed earliness *per se*) and is modified by vernalization response and photoperiod response (Kato and Yamashita 1991, Yasuda and Shimoyama 1965). Photoperiod response is controlled mainly by three major genes, namely, *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* (Scarth and Law 1983, 1984, Welsh *et al.* 1973). Recently, these genes have been cloned (Beales *et al.* 2007, Fu *et al.* 2005, Nishida *et al.* 2013, Yan *et al.* 2003, 2004,) and DNA markers for the distinction of variant or mutant alleles of each gene have been developed.

There are some investigations about geographical distribution of *Ppd-D1a* before. 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 the frequency of *Ppd-D1a* allele varies among areas. Guo *et al.* (2010) showed that most Italian wheat cultivars carry *Ppd-D1a*. As for *Ppd-A1* and *Ppd-B1*, little is known about the distribution of photoperiod-insensitive alleles because the critical sequence polymorphism among alleles has not been detected until recently by Nishida *et al.* (2013).

In Chapter II, the *Ppd-B1* and *Ppd-D1* genotypes of Japanese wheat cultivars were determined, and it was revealed that most of the cultivars in Tohoku-Kyushu region carried *Ppd-D1a* and extra-early cultivars in southwestern Japan carried *Ppd-B1a* and *Ppd-D1a*. *Ppd-B1a* showed a stronger effect on accelerating heading compared with *Ppd-D1a* (Chapter II, Tanio and Kato 2007). Therefore, it was suggested that introduction of the *Ppd-B1a* allele enabled breeding of early maturity wheat cultivars required for avoiding preharvest sprouting and Fusarium head blight during the rainy season (Chapter II, Tanio and Kato 2007). The photoperiod-insensitive allele *Ppd-D1a* has also been found in Hokkaido wheat cultivars, though its effect on heading time was insignificant under the genetic background of Hokkaido wheat cultivars. In addition to these two alleles, another photoperiod-insensitive allele *Ppd-A1a* was found in a Hokkaido cultivar ‘Chihokukomugi’ by Nishida *et al.* (2013). They compared the heading time among DH lines differing in *Ppd-1* genotype, and showed that the effect of *Ppd-A1a* was weaker than that of *Ppd-B1a* or *Ppd-D1a*. However, since an insensitive allele of *Ppd-A1* had not been previously reported, very little is known regarding the effect of *Ppd-A1a* and its distribution in Japanese wheat cultivars.

In this chapter, the *Ppd-A1* genotype of Japanese wheat cultivars was determined by PCR-based method to detect the deletion in the upstream region of this gene. Thereafter, the *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 *Ppd-A1a* allele and to discuss the effect of *Ppd-A*1*a* and *Ppd-D1a* on heading time in Hokkaido wheat cultivars.

1 Materials and Methods

A total of 280 wheat cultivars consisting of Japanese commercial cultivars (164 cultivars), Japanese breeding lines (54 cultivars), Japanese landraces (22 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 7.

For DNA extraction, all wheat genotypes were grown in a growth chamber maintained at 20 ℃ under a natural photoperiod. Genomic DNA was extracted from 2-week-old seedlings by using a modified CTAB method (Murray and Thompson 1980).

The *Ppd-1* genotypes were determined using PCR-based methods with the primer sets designed to identify the deletion of 1085 bp in the upstream region of *Ppd-A1a*, the insertion of 308 bp in the upstream region of *Ppd-B1a* (Nishida *et al.* 2013), or the deletion of 2089 bp in the upstream region of *Ppd-D1a* (Beales *et al.* 2007). Three primers, namely, TaPpd-A1prodelF1, TaPpd-A1prodelR3, and TaPpd-A1prodelR2 (developed by Nishida *et al.* 2013) were used for *Ppd-A1*, and two primers, namely, TaPpd-B1proF1 and TaPpd-B1int1R1 (developed in this study) were used for *Ppd-B1*. Three primers, namely, Ppd-D1_F1, Ppd-D1_R1, and Ppd-D1_R2 (developed by Beales *et al.* 2007) were used for *Ppd-D1*. The nucleotide sequence of each primer is shown in Table 6.

Table 6. Primers used to determined the *Ppd-1* genotypes

Locus	Primer name	Sequence(5’→3’)
Ppd-A1	TaPpd-A1prodelF1	CGTACTCCCTCCGTTTCTTT
	TaPpd-A1prodelR3	AATTTACGGGGACCAAATACC
	TaPpd-A1prodelR2	GTTGGGGTCGTTTGGTGGTG
Ppd-B1	TaPpd-B1proF1	ACACTAGGGCTGGTCGAAGA
	TaPpd-B1int1R1	CCGAGCCAGTGCAAATTAAC
Ppd-D1	TaPpd-D1_F1	ACGCCTCCCACTACACTG
	TaPpd-D1_R1	TGTTGGTTCAAACAGAGAGC
	TaPpd-D1_R2	CACTGGTGGTAGCTGAGATT

For the analysis of *Ppd-A1* and *Ppd-D1*, PCR amplification was performed in a 5-μl mixture containing 10 ng genomic DNA, 0.5 μl 10× *Ex Taq* buffer (TaKaRa; 20 mM Tris-HCl at pH 8.0, 100 mM KCl, 20 mM Mg^{2+}), 0.2 mM dNTP, 0.2 μM of each primer, and 0.125 U *Ex Taq* Hot Start Version (TaKaRa). The PCR cycle for *Ppd-A1* was as follows: an initial denaturing step at 98 ℃ for 30 sec; 35 PCR cycles at 98 ℃ for 10 sec, 57 ℃ for 30 sec, and 72 ℃ for 30 sec; and a final extension step at 72 ℃ for 2 min. The PCR cycle for *Ppd-D1* was as follows: an initial denaturing step at 98 ℃ for 30 sec; 35 PCR cycles at 98 ℃ for 10 sec, 54 ℃ for 1 min, and 72 ℃ for 30 sec; and a final extension step at 72 ℃ 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 Mg^{2+}), 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 ℃ for 30 sec; 35 PCR cycles at 98 ℃ for 10 sec, 64 ℃ for 1 min, and 72 ℃ for 30 sec; and a final extension step at 72 ℃ for 2 min. Amplification reactions were conducted using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems). PCR products were separated by electrophoresis on a 13% polyacrylamide gel. Electrophoresis and polymorphism detection were based on the description by Hori *et al.* (2003). For 263 cultivars, the *Ppd-B1* and *Ppd-D1* genotypes have been analyzed in chapter II, and their genotype data was used in this chapter.

Among 280 wheat cultivars, 23 cultivars and breeding lines appeared in the pedigree of ‘Kitahonami’, the latest registered winter wheat cultivar cultivated in Hokkaido, were tested in the 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 8.

At Tsukuba, they were sown on November 6 and November 5 in the 2008/2009 and 2009/2010 wheat-growing 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 one-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 at Tsukuba and Kunneppu was calculated according to methods presented by Gotoh (1977). According to Gotoh (1977), effective day-length should include predawn time with light intensity over 20 lux and twilight time with light intensity over 10 lux, which was 26 and 23 min at Fukuyama (34°30′N), respectively. The calculation formula was as follows:

(effective day-length in minutes at θ degrees latitude) = (astronomical day-length) + 49 (cos 34°30′/ cos θ).

2 Results

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. 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 7), i.e., 12 Hokkaido winter wheat cultivars and two foreign cultivars, ‘Purcam (U-11)’ and ‘Purple Straw’ (Table 7 and Fig. 5). The *Ppd-A1a* allele was not found in Hokkaido spring wheat cultivars or Tohoku-Kyushu cultivars. As reported in Chapter II, 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-D1a*. The other genotypes with two or three photoperiod-insensitive alleles, i.e., the *Ppd-A1a/Ppd-B1a/Ppd-D1b*, *Ppd-A1a/*P*pd-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-A*1*a and five carried *Ppd-D1a* as a photoperiod-insensitive allele, while the others did not carry insensitive alleles of *Ppd-1* (Table 8). The heading date examined at 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 9). Genotypic difference of heading date was significant at the 0.1% level at Tsukuba and 5% level at Kunneppu. At Tsukuba, the average heading date of the *Ppd-A1a/Ppd-B1b /Ppd-D1b*, *Ppd-A1b/Ppd-B1b/Ppd-D1a*, and *Ppd-A1b/Ppd-B1b/Ppd-D1b* genotypes was 28.4 April, 25.0 April, and 5.3 May, respectively, for the 2008/2009 season. A similar genotypic difference was observed for the 2009/2010 seasons, indicating that the *Ppd-A1b/Ppd-B1b/Ppd-D1b* genotype headed 6.9-9.8 days later than other genotypes with a photoperiod-insensitive allele. Among the genotypes with a photoperiod-insensitive allele, the *Ppd-A1a/Ppd-B1b/Ppd-D1b* genotype headed 2-3 days later than the *Ppd-A1b/Ppd-B1b/Ppd-D1a* genotype, though the difference was not significant. At Kunneppu, the *Ppd-A1b/Ppd-B1b/Ppd-D1b* genotype headed 2.5 days later than other genotypes with a photoperiod-insensitive allele, though the difference was less than at Tsukuba.

Table 7. Result of genotyping of *Ppd-1*

Area of origin	Total number of cultivars	*Ppd-A1*		*Ppd-B1*		*Ppd-D1*	
		Ppd-A1a	*Ppd-A1b*	*Ppd-B1a*	*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[f]	9
Tohoku,Hokuriku	47	0	47	0	47	43	4[g]
Kanto,Tokai	63	0	63	2[b]	61	63	0
Kinki,Chugoku,Shikoku	43	0	43	6[c]	37	42	1[h]
Kyushu	48	0	48	2[d]	46	48	0
Foreign cultivars	40	2[a]	38	1[e]	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*; 'OS21-5'

[g] Carrier of *Ppd-D1b*; 'Fultz Daruma', 'Norin 6', 'Norin 24' and 'Norin 38'

[h] Carrier of *Ppd-D1b*; 'Eshima'

Table 8. Cultivars tested in fields

	Ppd-1 genotype			Heading date						
	Ppd-A1[a]	*Ppd-B1*[b]	*Ppd-D1*[c]	Tsukuba[d]			Kunneppu[e]			
				2008/2009	2009/2010	Av.	2007/2008	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	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] "a" and "b" indicate *Ppd-A1a* and *Ppd-A1b*, respectively.

[b] "a" and "b" indicate *Ppd-B1a* and *Ppd-B1b*, respectively.

[c] "a" and "b" indicate *Ppd-D1a* and *Ppd-D1b*, respectively.

[d] Heading date at Tsukuba, 21 cultivars were tested; 'Hokuei' and 'Mukakomugi' were not tested.

[e] Heading date at Kunneppu, 18 cultivars were tested; 'Kitakei 221', 'Kitakei 497', 'Tohoku 118', 'Kitakei 320', and 'Kitami 18' were not tested.

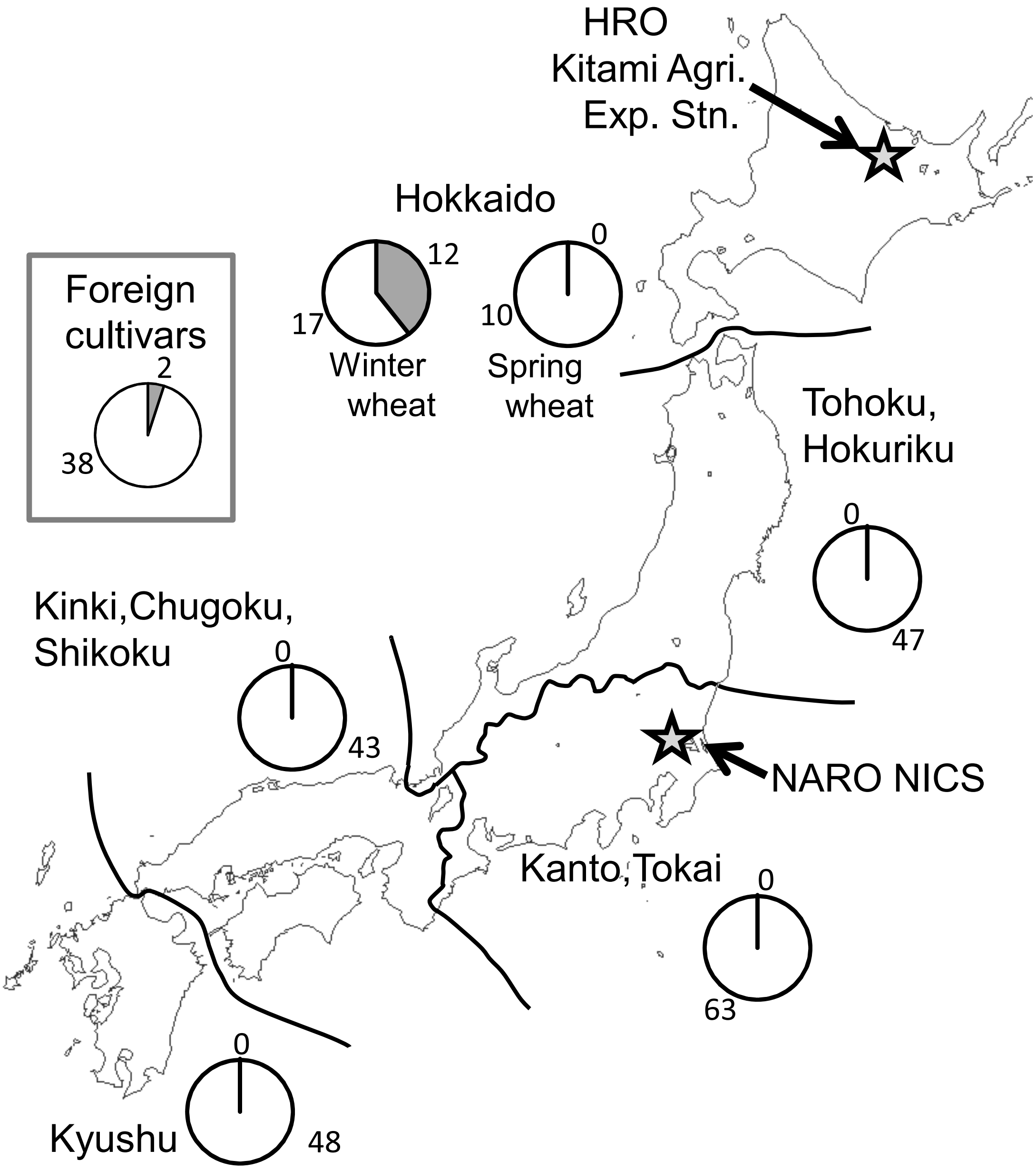

Fig. 5. Geographical distribution of *Ppd-A1a* and *Ppd-A1b*. Solid and open parts of the circular charts indicate the proportions of wheat cultivars carrying *Ppd-A1a* and *Ppd-A1b*, respectively. Number of cultivars was also indicated.

Table 9. Effect of *Ppd-1* genotype on heading date in cultivars on the pedigree of ‘Kitahonami'

		Tsukuba		Kunneppu	
Growing season	*Ppd* genotype	n	Heading date [a]	n	Heading date [a]
2007/2008	*Ppd-A1a/Ppd-B1b/Ppd-D1b*		–	8	11.4±0.9^{a}
	Ppd-A1b/Ppd-B1b/Ppd-D1a		–	4	10.5±1.7^{a}
	Ppd-A1b/Ppd-B1b/Ppd-D1b		–	6	14.2±1.2^{a}
2008/2009	*Ppd-A1a/Ppd-B1b/Ppd-D1b*	10	28.4±1.4^{ab}	8	7.9±0.9^{a}
	Ppd-A1b/Ppd-B1b/Ppd-D1a	5	25.0±2.9^{a}	4	8.0±2.0^{a}
	Ppd-A1b/Ppd-B1b/Ppd-D1b	6	35.3±1.7^{b}	6	10.5±0.9^{a}
2009/2010	*Ppd-A1a/Ppd-B1b/Ppd-D1b*	10	35.3±0.9^{a}	8	14.0±1.0^{a}
	Ppd-A1b/Ppd-B1b/Ppd-D1a	5	33.0±2.1^{a}	4	14.5±1.2^{a}
	Ppd-A1b/Ppd-B1b/Ppd-D1b	6	42.3±2.0^{b}	6	15.8±0.7^{a}
F-value of ANOVA [b]					
Growing seoson(A)			25.03 ***		17.57 ***
genotype(B)			14.47 ***		4.228 *
(A)*(B)			0.053 ns		0.201 ns

[a] Values showed mean ± standard error.
1=1st April in Tsukuba, and =1st June in Kunneppu.
Values followed by the different letters in same growing season are significantly different($P<0.05$) by Tukey HSD multiple range test.

[b] * and *** indicate significance at 5% and 0.1% level, respectively, and 'ns' indicates no significance at 5% level.

As shown in Fig. 6B, monthly average temperature differed among the three wheat-growing seasons. At 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 on the test field at Kunneppu, in the 2007/2008 season, snow melted very early after that, 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 significance 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. 7. 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.

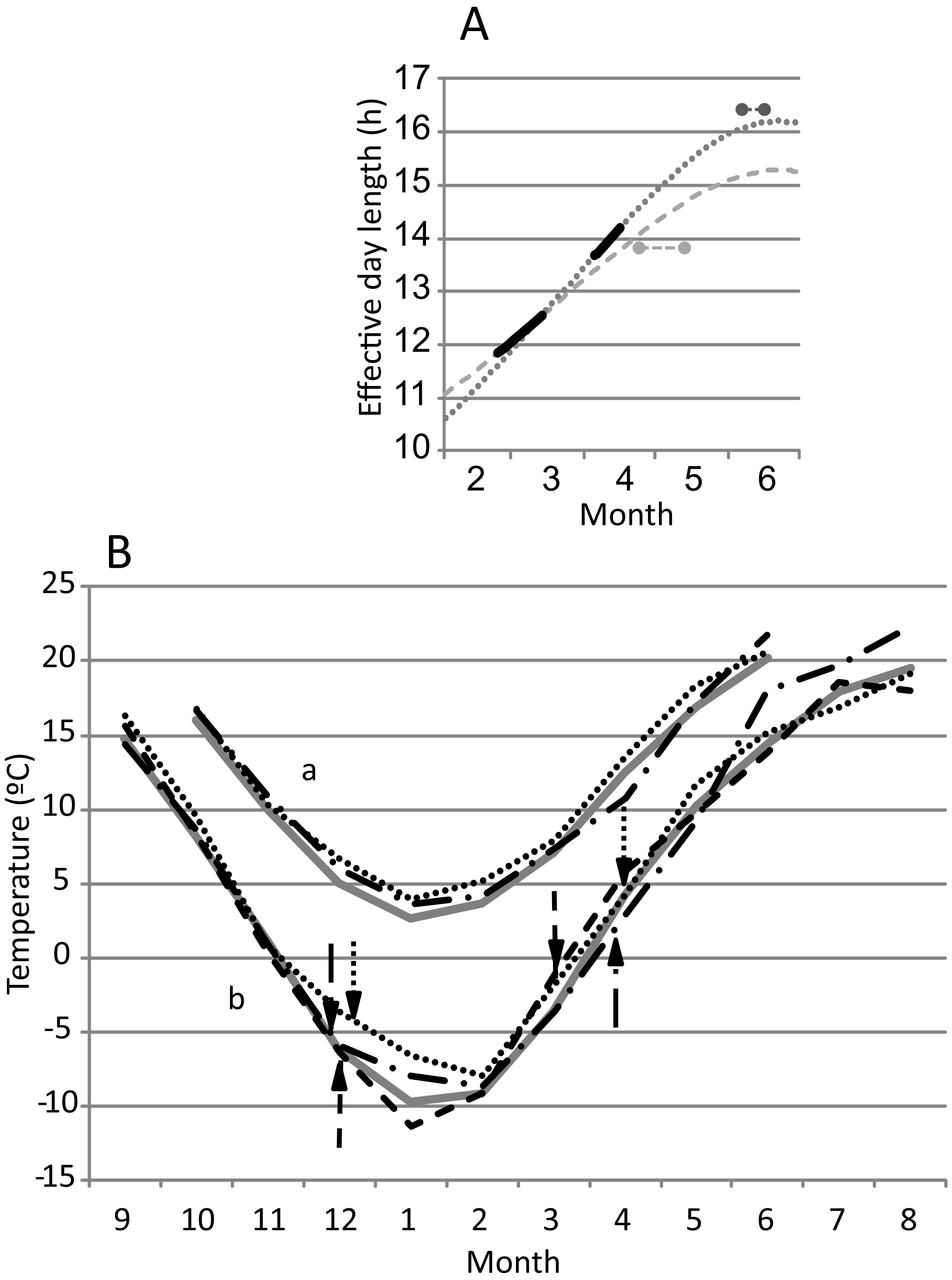

Fig. 6. A. Effective day-length at two sites.

– – – : 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 the end of continuous snow cover duration during each season at Kunneppu.

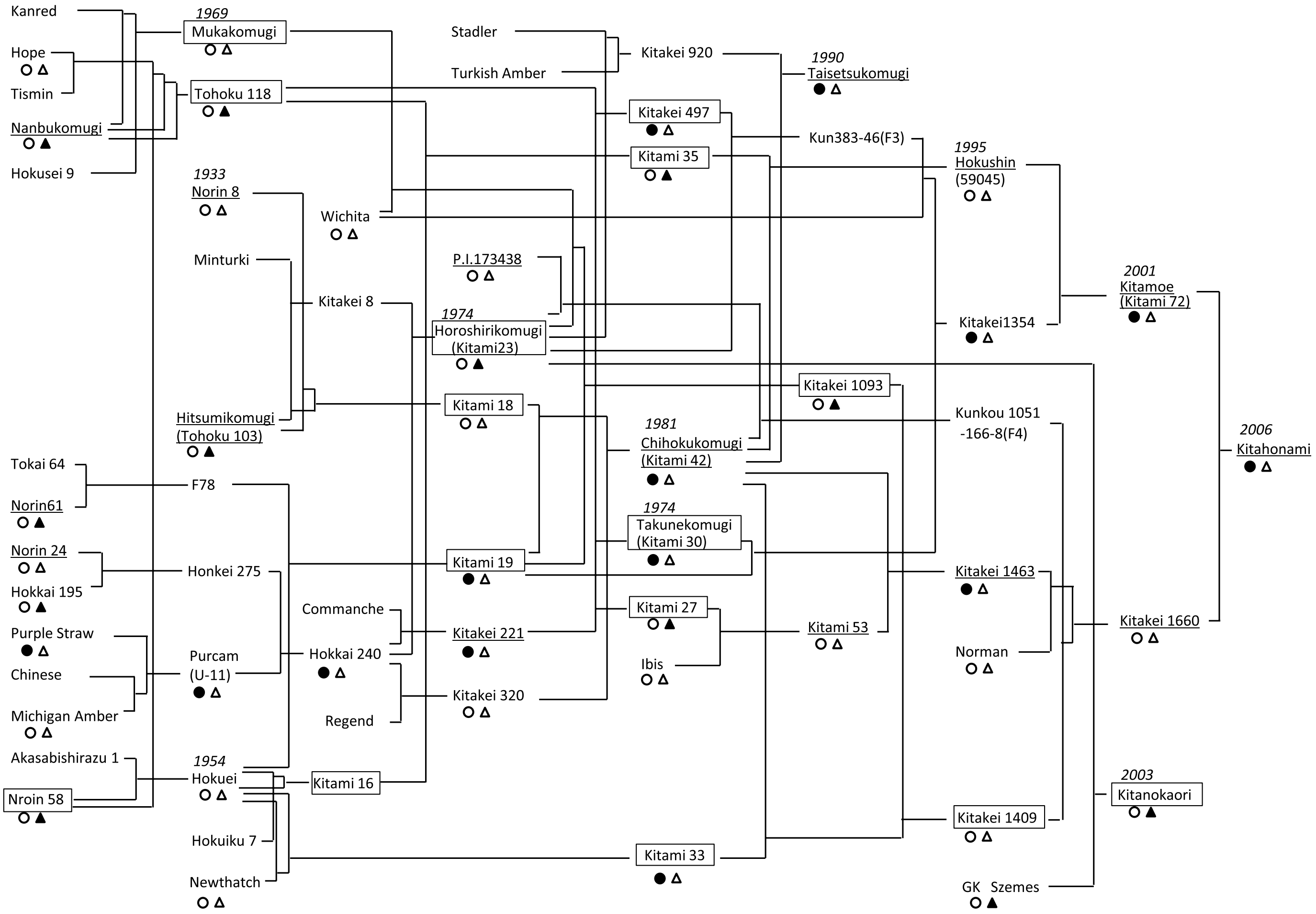

Fig. 7. *Ppd-1* genotypes of wheat cultivars in the pedigree of Hokkaido winter wheat

●:Carrier of *Ppd-A1a*.　○:Carrier of *Ppd-A1b*.
▲:Carrier of *Ppd-D1a*.　△:Carrier of *Ppd-D1b*.

Cultivars with no symbols were not tested for genotyping of *Ppd-1* .

Some cultivars were tested for genotyping of *Pina* and *Pinb* using the method of 'Marker assist selection in wheat' project (http://maswheat.ucdavis.edu/protocols/Hardness/index.htm).

In a previous report, Japanese hard wheat cultivars were classified as one of three genotypes, *Pina-D1b*/P*inb-D1a*, *Pina-D1a/Pinb-D1b* and *Pina-D1a/Pinb-D1c* (Ikeda et al. 2005). In this pedigree, cultivars enclosed rectangles were *Pina-D1a/Pinb-D1b* or *Pina-D1a/Pinb-D1c*, showing these cultivars hard wheat. Cultivars with underlines were not the three genotypes classified as Japanese hard wheat cultivars, suggested that these cultivars were soft wheat.

The year of registration is shown in italics.

3　Discussion

The combination of *Ppd-1* alleles is important for the control of 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 that enable stable wheat production in Japan. To investigate the *Ppd-1* genotypes of Japanese wheat cultivars using the Japanese cultivars, including 164 commercial cultivars, 22 landraces, and 54 breeding lines, the *Ppd-B1* and *Ppd-D1* genotypes were determined in Chapter II; the *Ppd-A1* genotype was analyzed in this chapter.

Most Tohoku-Kyushu cultivars (97.5%) carried *Ppd-D1a*; extra-early cultivars (5.0%) carried *Ppd-B1a* as reported in Chapter II. However, no cultivars carried *Ppd-A1a* (Table 7). In contrast, among Hokkaido cultivars, none of the cultivars carried *Ppd-B1a*, and the frequency of the *Ppd-A1a* and *Ppd-D1a* alleles generally differed between winter wheat and spring wheat. Among winter wheat cultivars, 41.4% and 24.1% carried *Ppd-A1a* and *Ppd-D1a*, respectively (Table 7). In contrast, in spring wheat cultivars, most (90%) did not carry photoperiod-insensitive alleles (Table 7).

As shown in a chapter II, in Tohoku-Kyushu cultivars, heading times differed depending on their *Ppd-1* genotypes, i.e., cultivars without photoperiod-insensitive alleles headed late, those with *Ppd-D1a* headed early to middle, while those with *Ppd-B1a* and *Ppd-D1a* headed very early. Consequently, the importance of photoperiod response in controlling heading time was 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 chapter, the heading date was investigated at Tsukuba in the Kanto region and at Kunneppu in the Hokkaido region using 23 Japanese cultivars and lines in the pedigree of ‘Kitahonami’. The heading date was significantly different between the *Ppd-1* genotypes (Table 9), indicating acceleration of heading due to the photoperiod-insensitive alleles *Ppd-A1a* or *Ppd-D1a*, and the effect of *Ppd-1* was lower at Kunneppu than at Tsukuba (Table 9). 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 that includes Kanto. Analysis of photoperiod-sensitive and -insensitive wheat cultivars clearly showed drastic heading time difference between 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 of ‘Kitahonami’ is approximately 14 h at Kunneppu and similar to that shown by Gotoh (1977) (Fig. 6A). 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. 7). In Hokkaido, artificial cross-breeding of wheat has been performed since 1919 to develop hard wheat cultivars. Tohoku cultivars were used as cross-parents as well as foreign cultivars, resulting in 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. 7). ‘Hokkai 240’, which is tolerance to leaf rust and lodging (Iriki et al. 1985), should have contributed to introduction of *Ppd-A1a* into Hokkaido cultivars. Since the 1980s, soft wheat cultivars suitable for the Japanese noodles have been developed in Hokkaido. Thereafter, no commercial cultivar with *Ppd-D1a* was developed except for ‘Kitanokaori’, a hard wheat cultivar for bread. ‘Chihokuko mugi’, a soft wheat cultivar with *Ppd-A1a*, was registered in 1981. This cultivar possessed high quality for Japanese noodle and was frequently crossed in wheat breeding. The shift of 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 a less effect on heading time under long day conditions. A possible explanation would be the pleiotropic effects of *Ppd-D1a* detected under the growing conditions in Europe, high latitude area such as Hokkaido (Börner *et al.* 1993, Worland *et al.* 1988, Worland 1996). 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 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 the previous reports, the *Ppd-D1a* allele reduced the plant height independently of the semi-dwarf gene Rht (Börner *et al.* 1993, Worland *et al.* 1988). The possibility cannot be excluded that the introduction of the lodging tolerance from ‘Hokkai 240’ resulted in the introduction of *Ppd-A1a* into Hokkaido cultivars. On the contrary, there is no previous works showing close linkage between *Ppd-1* and genes for disease tolerance or flour quality, while some QTLs were reported on homoeologous group 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 vernalizarion 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. A photoperiod-insensitive allele *Ppd-A1a* analyzed in this study was first found by Nishida *et al.* (2013), and thus the effect of this allele on heading time has not been extensively investigated. According to Nishida *et al.* (2013), 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, though 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*, though the difference was insignificant (Table 9). Although early heading is important to avoid various damages 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 heading time in the Tohoku-Kyushu region. Nishida *et al.* (2013) 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 allele(s) 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*.

4　Summary

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 compared to 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 one of the donor(s) 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 of 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*.

Ⅳ　Conclusion

Heading characteristics must be adjusted to the growing conditions in each area for the stable production of wheat. Heading time of wheat is a complex characteristic, determined by three factors: narrow-sense earliness (also termed earliness *per se*), vernalization response and photoperiod response (Kato and Yamashita 1991, Yasuda and Shimoyama 1965). Fine tuning of heading characteristics is achievable by combining these genetic factors in wheat breeding. Genotyping of wheat cultivars, established in each area, is one of the promising approaches for discerning the best combination of these genetic factors and would bring useful information for fine tuning of heading characteristics in breeding programs.

In this study, the *Ppd-1* genotypes of 240 Japanese wheat cultivars were examined and photoperiod-insensitive alleles of three *Ppd-1* genes were successfully detected. The distribution of photoperiod-insensitive alleles differed among *Ppd-1* genes, as well as among geographic areas (Table 2, Table 7, Fig. 1, Fig. 2, and Fig. 5). Almost all cultivars in the Tohoku-Kyushu region carried the photoperiod-insensitive allele *Ppd-D1a* (Table 2, Table 7, and Fig. 1), and 10 cultivars including three commercial extra-early cultivars carried both *Ppd-B1a* and *Ppd-D1a* (Table 2, Table 7, and Fig. 2), while no cultivars carried *Ppd-A1a* (Table 7 and Fig. 5). Of 29 winter wheat cultivars in the Hokkaido region, 12 cultivars carried *Ppd-A1a* (Table 7 and Fig. 5), and 7 cultivars carried *Ppd-D1a* (Table 2, Table 7, and Fig. 1), while no cultivars carried *Ppd-B1a* (Table 2, Table

7, and Fig. 2). Of 10 spring wheat cultivars in Hokkaido region, only one experimental line carried *Ppd-D1a* (Table 2, Table 7, and Fig. 1) and the other cultivars carried no photoperiod-insensitive alleles. The effect of Ppd-*1* alleles on heading time also differed between areas (Table 3, Table 4, and Table 9). In Tohoku-Kyushu region, wheat cultivars carrying the photoperiod-insensitive allele *Ppd-D1a* headed earlier by 10.3 days than did photoperiod-sensitive cultivars (Table 3), and *Ppd-B1a/Ppd-D1a* genotype headed earlier by 6.7 days than did *Ppd-B1b/Ppd-D1a* genotype (Table 4). These results support the hypothesis that early-maturity wheat breeding in Japan has been accelerated by the introduction of *Ppd-B1a* (Tanio and Kato 2005). In the Hokkaido region, among wheat cultivars on the pedigree of 'Kitahonami', photoperiod-insensitive cultivars with *Ppd-A1a* or *Ppd-D1a* headed earlier by 2.5 days than did photoperiod-sensitive cultivars with no photoperiod-insensitive alleles, although the effect of these alleles was less than that in the Kanto region (Table 9). Gotoh (1977) suggested that the importance of photoperiod sensitivity for the earliness of heading is less in the Hokkaido region than in the Kanto region based on the day-length of two months before the wheat heading time, which is equivalent to the wheat spike formation stage, and 2-3 hours longer in the Hokkaido region than in the Kanto region. The geographical difference of distribution and effect of photoperiod-insensitive alleles in this study may be result from the difference of day-length of wheat apical specializing time.

Fujita (1997) indicated that photoperiod-insensitivity of 'Abukumawase' was traced to 'Chugoku 114', 'Chugoku 91' and 'Chugoku 81' on the pedigree. Hashimoto and Hirano (1963) mentioned that 'Chugoku 81' was one of the best cross parents for breeding early maturing wheat cultivars. By the pedigree analysis of extra-early cultivars in central to south-western regions of Japan, it was clearly demonstrated that the *Ppd-B1a* allele of three extra-early cultivars was inherited from 'Shir oboro 21' by these Chugoku lines (Fig. 4). The pedigree analysis in this study confirmed the adequacy of the above two reports. Three cultivars with *Ppd-B1a/Ppd-D1a* genotype are successfully established only in the Chugoku or Kyushu region. It was considered that such cultivars often suffered from late frost because of early stem elongation in eastern Japan where the daily minimum temperature was below freezing in the winter. Furthermore, heading time of extra-early cultivars is variable depending on winter temperature (Table 5), causing instability of grain yield. However, according to Fujita *et al.* (1995) and Seki *et al.* (2007) who compared heading time among spring- and winter-type NILs, heading time of extra-early cultivars can be stabilized by the introduction of adequate vernalization requirements.

Fujita (1997) also mentioned that photoperiod-insensitivity of the early cultivar 'Asakazeko mugi' was traced to 'Hiyokukomugi', 'Junreikomugi' and 'Norin 26'. Yoshida *et al.* (1983) also suggested that photoperiod-insensitivity of extra-early breeding lines 'Gokuwase 2' and 'Gokuwase 4-15' was inherited from 'Jesssore'. However, none of these cultivars carried *Ppd-B1a* (Table 2, Fig. 4) and no cultivars in the Tohoku-Kyushu region carried *Ppd-A1a* (Table 7, Fig. 5). These results clearly indicated that heading time varied largely even among the Japanese wheat cultivars of *Ppd-A1b/Ppd-B1b/Ppd-D1a* genotype, suggesting that their heading time is affected by other photoperiod response genetic factor(s) such as the FT-like gene reported in barley (Kikuchi *et al.* 2009) and other photoperiod-insensitive allele(s) of *Ppd-1* like *Ppd-B1* allele of Chinese Spring (Law *et al.* 1978, Scarth and Law 1983).

A photoperiod-insensitive allele *Ppd-A1a* was first found in a Hokkaido cultivar 'Chihokukomu gi' by Nishida *et al.* (2013), and no cultivars carried this allele in the Tohoku-Kyushu region (Table 7 and Fig. 5). According to Nishida *et al.* (2013), the effect of *Ppd-A1a* on photoperiod insensitivity was smaller than those of *Ppd-B1a* and *Ppd-D1a*. Therefore, *Ppd-A1a* may be useful as a unique gene source for fine tuning of heading time in the Tohoku-Kyushu region.

The *Ppd-1* genes have a strong effect on heading time and, thus, also affect the growth and yield of wheat cultivars, as reported by Börner *et al.* (1993), Foulkes *et al.* (2004) and Worland *et al.* (1988, 1998). However, as to the Japanese wheat cultivars, the effect of *Ppd-1* genes on growth and yield has not been investigated, although the effect of *Vrn-1* vernalization response genes on growth and yield in the south-western region of Japan was recently clarified (Seki *et al.* 2007). For the identification of the *Ppd-1* alleles which can be utilized for fine tuning of heading time in the south-western region of Japan, the effect of *Ppd-1* genes and interactions of *Ppd-1* genes and *Vrn-1* genes on growth and yield of the Japanese wheat cultivars should be investigated.

The significance of *Ppd-1* in Hokkaido region was also discussed. It was clarified that 'Purple Straw' and 'Tohoku 118' are one of the donor(s) of *Ppd-A1a* and *Ppd-D1a* respectively of Hokkaido wheat cultivars (Fig. 7). As mentioned above, the effect of these alleles on heading time in the Hokkaido region was less than in the Kanto region. Additionally, studies examining the relationship between *Ppd-1* and improved tolerance to lodging and diseases or flour qualities in wheat breeding, will reveal details of *Ppd-A1a* introduction into Hokkaido cultivars. Although the frequency of wheat cultivars carrying a photoperiod-insensitive allele is increasing in Hokkaido (Appendix Table, Fig. 7), the reason is unknown, and, thus, the effect of *Ppd-1* on growth and yield in Hokkaido should be also studied.

Although most of the Japanese spring wheat cultivars except those in the Hokkaido region have the same set of *Ppd-1* and *Vrn-1* genes, heading time varies largely between these cultivars. The variation of heading time could be partly explained by narrow-sense earliness, since it is also important for the control of heading time in Japanese wheat cultivars (Fujita 1997, Hashimoto and Hirano 1963, Kato and Wada 1999). The narrow-sense earliness genes (*Eps*: earliness per se genes) have been identified on different wheat chromosomes, and some of them have been mapped as QTLs for heading time (Kato *et al.* 2002, Kucheul *et al.* 2006, Scarth and Law 1984, Sourdille *et al.* 2000, Worland and Law 1986). The *Eps-A*^{m}*1* was mapped on chromosome 1AmL of diploid wheat Triticum monococcum L. (Lewis *et al.* 2008). Further advancement of molecular genetics of narrow-sense earliness should be expected and would make it possible to discuss the significance of this characteristic in Japanese wheat breeding.

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Appendix Table. List of wheat cultivars tested.

A. Hokkaido cultivars

		Registraion year		*Ppd-1* genotype		
Cultivar			Reference	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*
Winter Wheat						
Akakawa-aka	Japanese landrace			*b*	*b*	*b*
Norin 8	commercial cultivar	1933	1	*b*	*b*	*b*
Norin 62	commercial cultivar	1944	1	*b*	*b*	*a*
Hokuei	commercial cultivar	1954	2	*b*	*b*	*b*
Mukakomugi	commercial cultivar	1969	1	*b*	*b*	*b*
Horoshirikomugi	commercial cultivar	1974	1	*b*	*b*	*a*
Takunekomugi	commercial cultivar	1974	1	*a*	*b*	*b*
Chihokukomugi	commercial cultivar	1981	1	*a*	*b*	*b*
Taisetsukomugi	commercial cultivar	1990	1	*a*	*b*	*b*
Hokushin	commercial cultivar	1995	1	*b*	*b*	*b*
Kitamoe	commercial cultivar	2001	1	*a*	*b*	*b*
Kitanokaori	commercial cultivar	2003	1	*b*	*b*	*a*
Kitahonami	commercial cultivar	2006	1	*a*	*b*	*b*
Hokkai 195	breeding line			*b*	*b*	*a*
Hokkai 240	breeding line			*a*	*b*	*b*
Kitakei 221	breeding line			*a*	*b*	*b*
Kitakei 320	breeding line			*b*	*b*	*b*
Kitakei 497	breeding line			*a*	*b*	*b*
Kitakei 1093	breeding line			*b*	*b*	*a*
Kitakei 1354	breeding line			*a*	*b*	*b*
Kitakei 1409	breeding line			*b*	*b*	*b*
Kitakei 1463	breeding line			*a*	*b*	*b*
Kitakei 1660	breeding line			*b*	*b*	*b*
Kitami 18	breeding line			*b*	*b*	*b*
Kitami 19	breeding line			*a*	*b*	*b*
Kitami 27	breeding line			*b*	*b*	*a*
Kitami 33	breeding line			*a*	*b*	*b*
Kitami 35	breeding line			*b*	*b*	*a*
Kitami 53	breeding line			*b*	*b*	*b*
Spring Wheat						
Norin 3	commercial cultivar	1930	1	*b*	*b*	*b*
Norin 29	commercial cultivar	1938	1	*b*	*b*	*b*
Norin 35	commercial cultivar	1938	1	*b*	*b*	*b*
Norin 75	commercial cultivar	1948	1	*b*	*b*	*b*
Haruhikari	commercial cultivar	1965	1	*b*	*b*	*b*
Haruminori	commercial cultivar	1969	1	*b*	*b*	*b*
Haruyutaka	commercial cultivar	1985	1	*b*	*b*	*b*
Harunoakebono	commercial cultivar	1994	1	*b*	*b*	*b*
Haruhinode	commercial cultivar	2001	1	*b*	*b*	*b*
OS21-5	breeding line			*b*	*b*	*a*

B. Tohoku cultivars

		Registraion year		*Ppd-1* genotype		
Cultivar			Reference	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*
Furutsudaruma	Japanese landrace			*b*	*b*	*b*
Norin 1	commercial cultivar	1929	1	*b*	*b*	*a*
Norin 2	commercial cultivar	1929	1	*b*	*b*	*a*
Norin 6	commercial cultivar	1932	1	*b*	*b*	*b*
Norin 10	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 14	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 15	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 17	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 18	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 22	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 24	commercial cultivar	1937	1	*b*	*b*	*b*
Norin 27	commercial cultivar	1937	1	*b*	*b*	*a*
Norin 31	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 33	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 38	commercial cultivar	1938	1	*b*	*b*	*b*
Norin 39	commercial cultivar	1939	1	*b*	*b*	*a*
Norin 40	commercial cultivar	1939	1	*b*	*b*	*a*
Norin 54	commercial cultivar	1943	1	*b*	*b*	*a*
Norin 55	commercial cultivar	1943	1	*b*	*b*	*a*
Norin 58	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 66	commercial cultivar	1944	1	*b*	*b*	*a*
Susonokomugi	commercial cultivar	1950	1	*b*	*b*	*a*
Mutsubenkei	commercial cultivar	1950	1	*b*	*b*	*a*
Aobakomugi	commercial cultivar	1951	1	*b*	*b*	*a*
Nanbukomugi	commercial cultivar	1951	1	*b*	*b*	*a*
Yukichabo	commercial cultivar	1952	1	*b*	*b*	*a*
Hikarikomugi	commercial cultivar	1952	1	*b*	*b*	*a*
Myokokomugi	commercial cultivar	1952	1	*b*	*b*	*a*
Hitsumikomugi	commercial cultivar	1953	1	*b*	*b*	*a*
Kokeshikomugi	commercial cultivar	1953	1	*b*	*b*	*a*
Okukomugi	commercial cultivar	1955	1	*b*	*b*	*a*
Sakyukomugi	commercial cultivar	1956	1	*b*	*b*	*a*
Furutsumasari	commercial cultivar	1956	1	*b*	*b*	*a*
Kitakamikomugi	commercial cultivar	1959	1	*b*	*b*	*a*
Shimofusakomugi	commercial cultivar	1963	1	*b*	*b*	*a*
Miyaginokomugi	commercial cultivar	1964	1	*b*	*b*	*a*
Hachimankomugi	commercial cultivar	1973	1	*b*	*b*	*a*
Hanagasakomugi	commercial cultivar	1974	1	*b*	*b*	*a*
Wakamatsukomugi	commercial cultivar	1982	1	*b*	*b*	*a*
Koyukikomugi	commercial cultivar	1988	1	*b*	*b*	*a*
Akitakko	commercial cultivar	1992	1	*b*	*b*	*a*
Nebarigoshi	commercial cultivar	2001	1	*b*	*b*	*a*
Haruibuki	commercial cultivar	2001	1	*b*	*b*	*a*
Yukichikara	commercial cultivar	2002	1	*b*	*b*	*a*

B. Tohoku cultivars (continue)

		Registraion year		*Ppd-1* genotype		
Cultivar			Reference	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*
Tohoku 71	breeding line			*b*	*b*	*a*
Tohoku 101	breeding line			*b*	*b*	*a*
Tohoku 118	breeding line			*b*	*b*	*a*

C. Kanto and Tokai cultivars

		Registraion year		*Ppd-1* genotype		
Cultivar			Reference	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*
Akabozu	Japanese landrace			*b*	*b*	*a*
Akadaruma	Japanese landrace			*b*	*b*	*a*
Akakomugi	Japanese landrace			*b*	*b*	*a*
Igachikugo Oregon	Japanese landrace			*b*	*b*	*a*
Shiroboro 21	Japanese landrace			*b*	*a*	*a*
Shirochabo	Japanese landrace			*b*	*b*	*a*
Shirodaruma	Japanese landrace			*b*	*b*	*a*
Shirosanjaku	Japanese landrace			*b*	*b*	*a*
Norin 7	commercial cultivar	1932	1	*b*	*b*	*a*
Norin 9	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 12	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 13	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 16	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 28	commercial cultivar	1937	1	*b*	*b*	*a*
Norin 30	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 41	commercial cultivar	1939	1	*b*	*b*	*a*
Norin 42	commercial cultivar	1939	1	*b*	*b*	*a*
Norin 44	commercial cultivar	1939	1	*b*	*b*	*a*
Norin 48	commercial cultivar	1942	1	*b*	*b*	*a*
Norin 50	commercial cultivar	1942	1	*b*	*b*	*a*
Norin 53	commercial cultivar	1943	1	*b*	*b*	*a*
Norin 57	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 64	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 67	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 68	commercial cultivar	1946	1	*b*	*b*	*a*
Norin 69	commercial cultivar	1946	1	*b*	*b*	*a*
Norin 70	commercial cultivar	1946	1	*b*	*b*	*a*
Yuyakekomugi	commercial cultivar	1950	1	*b*	*b*	*a*
Fujimikomugi	commercial cultivar	1960	1	*b*	*b*	*a*
Mikunikomugi	commercial cultivar	1962	1	*b*	*b*	*a*
Omasekomugi	commercial cultivar	1969	1	*b*	*b*	*a*
Zenkojikomugi	commercial cultivar	1969	1	*b*	*b*	*a*
Kobushikomugi	commercial cultivar	1969	1	*b*	*b*	*a*
Toyohokomugi	commercial cultivar	1975	1	*b*	*b*	*a*
Fukuhokomugi	commercial cultivar	1979	1	*b*	*b*	*a*
Shiranekomugi	commercial cultivar	1986	1	*b*	*b*	*a*
Airakomugi	commercial cultivar	1988	1	*b*	*b*	*a*
Bandowase	commercial cultivar	1990	1	*b*	*b*	*a*
Shunyo	commercial cultivar	1995	1	*b*	*b*	*a*
Ayahikari	commercial cultivar	2000	1	*b*	*b*	*a*
Kinuhime	commercial cultivar	2000	1	*b*	*b*	*a*
Kinunonami	commercial cultivar	2000	3	*b*	*b*	*a*
Kinuazuma	commercial cultivar	2001	1	*b*	*b*	*a*
Yumeseiki	commercial cultivar	2001	1	*b*	*b*	*a*
Tamaizumi	commercial cultivar	2002	1	*b*	*b*	*a*
Fusetsu	commercial cultivar	2004	1	*b*	*b*	*a*

C. Kanto and Tokai cultivars (continue)

	Registraion year		*Ppd-1* genotype		
Cultivar		Reference	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*
Kankei w361	breeding line		*b*	*b*	*a*
Kankei w364	breeding line		*b*	*b*	*a*
Kanto 101	breeding line		*b*	*b*	*a*
Kanto 107	breeding line		*b*	*b*	*a*
Kanto 66	breeding line		*b*	*b*	*a*
Kanto 79	breeding line		*b*	*b*	*a*
Kanto 82	breeding line		*b*	*b*	*a*
Kanto 87	breeding line		*b*	*b*	*a*
Kanto 95	breeding line		*b*	*b*	*a*
Kanto 99	breeding line		*b*	*b*	*a*
Kitakanto 44	breeding line		*b*	*b*	*a*
Kitakanto 48	breeding line		*b*	*b*	*a*
Konosu 4	breeding line		*b*	*a*	*a*
Saitama 29	breeding line		*b*	*b*	*a*
Tosan 18	breeding line		*b*	*b*	*a*
Tosan 38(Yumeasahi)	breeding line		*b*	*b*	*a*
Tosan 40(Hanamanten)	breeding line		*b*	*b*	*a*

D. Kinki, Chugoku and Shikoku cultivars

Cultivar		Registraion year	Reference	*Ppd-1* genotype		
				Ppd-A1	*Ppd-B1*	*Ppd-D1*
Eshima	Japanese landrace			*b*	*b*	*b*
Hirakikomugi	Japanese landrace			*b*	*b*	*a*
Hiroshimashipuree 3	Japanese landrace			*b*	*b*	*a*
Sanin 1	Japanese landrace			*b*	*b*	*a*
Shinchunaga	Japanese landrace			*b*	*b*	*a*
Shinrikikomugi	Japanese landrace			*b*	*b*	*a*
Yushoki 347	Japanese landrace			*b*	*b*	*a*
Norin 4	commercial cultivar	1931	1	*b*	*b*	*a*
Norin 11	commercial cultivar	1935	1	*b*	*b*	*a*
Norin 19	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 21	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 23	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 25	commercial cultivar	1937	1	*b*	*b*	*a*
Norin 26	commercial cultivar	1937	1	*b*	*b*	*a*
Norin 32	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 37	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 43	commercial cultivar	1939	1	*b*	*b*	*a*
Norin 46	commercial cultivar	1940	1	*b*	*b*	*a*
Norin 47	commercial cultivar	1940	1	*b*	*b*	*a*
Norin 51	commercial cultivar	1943	1	*b*	*b*	*a*
Norin 52	commercial cultivar	1943	1	*b*	*b*	*a*
Norin 56	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 59	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 63	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 65	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 71	commercial cultivar	1948	1	*b*	*b*	*a*
Norin 72	commercial cultivar	1948	1	*b*	*b*	*a*
Norin 73	commercial cultivar	1948	1	*b*	*b*	*a*
Norin 74	commercial cultivar	1948	1	*b*	*b*	*a*
Iyokomugi	commercial cultivar	1950	1	*b*	*b*	*a*
Akatsukikomugi	commercial cultivar	1951	1	*b*	*b*	*a*
Shirasagikomugi	commercial cultivar	1956	1	*b*	*b*	*a*
Junreikomugi	commercial cultivar	1957	1	*b*	*b*	*a*
Ushiokomugi	commercial cultivar	1967	1	*b*	*b*	*a*
Fukuwasekomugi	commercial cultivar	1983	1	*b*	*a*	*a*
Sanukinoyume 2000	commercial cultivar	2000	4	*b*	*b*	*a*
Fukusayaka	commercial cultivar	2002	1	*b*	*b*	*a*
Chugoku 53	breeding line			*b*	*b*	*a*
Chugoku 55	breeding line			*b*	*a*	*a*
Chugoku 81	breeding line			*b*	*a*	*a*
Chugoku 91	breeding line			*b*	*a*	*a*
Chugoku 98	breeding line			*b*	*a*	*a*
Chugoku 114	breeding line			*b*	*a*	*a*

E. Kyushu cultivars

		Registraion year		*Ppd-1* genotype		
Cultivar			Reference	*Ppd-A1*	*Ppd-B1*	*Ppd-D1*
Eshimashinriki	Japanese landrace			*b*	*b*	*a*
Hayakomugi	Japanese landrace			*b*	*b*	*a*
Igachikugo	Japanese landrace			*b*	*b*	*a*
Shirokomugi	Japanese landrace			*b*	*b*	*a*
Sojukuakage	Japanese landrace			*b*	*b*	*a*
Norin 5	commercial cultivar	1931	1	*b*	*b*	*a*
Norin 20	commercial cultivar	1936	1	*b*	*b*	*a*
Norin 34	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 36	commercial cultivar	1938	1	*b*	*b*	*a*
Norin 45	commercial cultivar	1940	1	*b*	*b*	*a*
Norin 49	commercial cultivar	1942	1	*b*	*b*	*a*
Norin 60	commercial cultivar	1944	1	*b*	*b*	*a*
Norin 61	commercial cultivar	1944	1	*b*	*b*	*a*
Hatamasari	commercial cultivar	1950	1	*b*	*b*	*a*
Ebisukomugi	commercial cultivar	1952	1	*b*	*b*	*a*
Yutakakomugi	commercial cultivar	1956	1	*b*	*b*	*a*
Danchikomugi	commercial cultivar	1956	1	*b*	*b*	*a*
Hayatokomugi	commercial cultivar	1961	1	*b*	*b*	*a*
Nichirinkomugi	commercial cultivar	1964	1	*b*	*b*	*a*
Hiyokukomugi	commercial cultivar	1969	1	*b*	*b*	*a*
Sakigakekomugi	commercial cultivar	1972	1	*b*	*a*	*a*
Shiroganekomugi	commercial cultivar	1974	1	*b*	*b*	*a*
Gogatsukomugi	commercial cultivar	1974	1	*b*	*b*	*a*
Setokomugi	commercial cultivar	1976	1	*b*	*b*	*a*
Chikushikomugi	commercial cultivar	1977	1	*b*	*b*	*a*
Shirowasekomugi	commercial cultivar	1977	1	*b*	*b*	*a*
Asakazekomugi	commercial cultivar	1978	1	*b*	*b*	*a*
Minaminokomugi	commercial cultivar	1979	1	*b*	*b*	*a*
Nishikazekomugi	commercial cultivar	1984	1	*b*	*b*	*a*
Daichinominori	commercial cultivar	1989	1	*b*	*b*	*a*
Abukumawase	commercial cultivar	1992	1	*b*	*a*	*a*
Kinuiroha	commercial cultivar	1994	1	*b*	*b*	*a*
Chikugoizumi	commercial cultivar	1994	1	*b*	*b*	*a*
Nishihonami	commercial cultivar	1995	1	*b*	*b*	*a*
Iwainodaichi	commercial cultivar	2000	1	*b*	*b*	*a*
Nishinokaori	commercial cultivar	2000	1	*b*	*b*	*a*
Minaminokaori	commercial cultivar	2004	1	*b*	*b*	*a*
Aki 9	breeding line			*b*	*b*	*a*
Chogokuwase	breeding line			*b*	*b*	*a*
Fukuokakomugi 18	breeding line			*b*	*b*	*a*
Gokuwase 2	breeding line			*b*	*b*	*a*
Gokuwase 4-15	breeding line			*b*	*b*	*a*
Saikai 77	breeding line			*b*	*b*	*a*
Saikai 87	breeding line			*b*	*b*	*a*
Saikai 95	breeding line			*b*	*b*	*a*
Saikai 98	breeding line			*b*	*b*	*a*
Saikai 104	breeding line			*b*	*b*	*a*
Saikai 113	breeding line			*b*	*b*	*a*

F. Foreign cultivars

Cultivar	Origin	*Ppd-1* genotype		
		Ppd-A1	*Ppd-B1*	*Ppd-D1*
Alsen	USA	*b*	*b*	*b*
Ardito	Italy	*b*	*b*	*a*
Aroona	Australia	*b*	*b*	*a*
Arrino	Australia	*b*	*b*	*b*
AUS1408	Australia	*b*	*b*	*b*
Combine	Italy	*b*	*b*	*a*
Corringin	Australia	*b*	*b*	*b*
Creopatra	Mexico	*b*	*b*	*a*
D6899	USA	*b*	*b*	*a*
Dawson 1	Canada	*b*	*b*	*b*
Eradu	Australia	*b*	*b*	*b*
Geurumil	Korea	*b*	*b*	*a*
GK Szemes	Hungary	*b*	*b*	*a*
Goshu 13	Australia	*b*	*b*	*b*
Goshu 8	Australia	*b*	*b*	*a*
Hope	GB	*b*	*b*	*b*
IA7873	Mexico	*b*	*b*	*a*
Ibis	Nederland	*b*	*b*	*b*
Jagger	USA	*b*	*b*	*b*
Jessore	Bangradesh	*b*	*b*	*a*
Kanred	USA	*b*	*b*	*b*
KS831957	USA	*b*	*b*	*a*
Martin	USA	*b*	*b*	*b*
Michigan Amber	USA	*b*	*b*	*b*
Newthatch	USA	*b*	*b*	*b*
Norman	GB	*b*	*b*	*b*
Olgrumil	Korea	*b*	*b*	*a*
Pampa INTA	Argentina	*b*	*b*	*a*
PI 173438	Turkey	*b*	*b*	*b*
Purcam(U-11)	USA	*a*	*b*	*b*
Purple Straw	USA	*a*	*b*	*b*
Reeder	USA	*b*	*b*	*b*
Rieti	Italy	*b*	*b*	*b*
Roblin	Canada	*b*	*b*	*a*
Rosella	Australia	*b*	*b*	*b*
Suweon235	Korea	*b*	*b*	*a*
Tapdongmil	Korea	*b*	*a*	*a*
Tordo	Mexico	*b*	*b*	*a*
Victoria INTA	Argentina	*b*	*b*	*a*
Wichita	USA	*b*	*b*	*b*

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