Genetic and molecular genetic characterization of the Japanese early heading cultivar of wheat

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ABSTRACT

Genetic analysis of photoperiodic response and molecular genetic analysis of vernalization were conducted using Japanese wheat cultivars. Six cultivars ranging from early to late heading proved to have a single insensitive gene, *Ppd-D1*. The extremely early heading cultivars carried two insensitive genes, *Ppd-B1* and *Ppd-D1*. The days to heading under a 24 h photoperiod did not differ among the NILs of 'Haruhikari', while days to heading under 8 h photoperiod differed significantly. 'Haruhikari' did not reach heading within 160 days after sowing, while 'Haruhikari (*Ppd-B1*)', 'Haruhikari (*Ppd-D1*)', and 'Haruhikari (*Ppd-B1*, *Ppd-D1*)' headed 62.5, 76.5 and 55.0 days after sowing, respectively. This indicates that *Ppd-B1* had a stronger effect on insensitivity than *Ppd-D1*. The results indicated an interaction between *Ppd-B1* and *Ppd-D1*. Both *Ppd-B1* and *Ppd-D1* had significant effects on accelerating double-ridge initiation and on the development of the following spike under short photoperiod. These results suggest the important contribution of *Ppd-B1* for the breeding of extremely early heading cultivars in Japan. Spring-type cultivars carrying *Vrn-D1* have a large deletion in the first intron, as expected. However, the identical deletion was also found in winter-type cultivars with early heading.

INTRODUCTION

Wheat cultivars with early heading have been bred in the central to southwestern part of Japan to be harvested before monsoonal rain. Heading time is a complex character controlled by earliness *per se*, and modified by vernalization requirement and photoperiodic response. Correlation analysis indicated that photoperiodic response and earliness *per se* are equally important for the control of heading time of Japanese winter wheat [1, 2], and both factors are small in early heading cultivars [3]. Photoperiodic response is mainly determined by *Ppd-1* genes on the short arms of homoeologous group 2, that is, *Ppd-A1* (formerly *Ppd3*), *Ppd-B1* (*Ppd2*) and *Ppd-D1* (*Ppd1*). The dominant alleles of these genes confer the insensitive response. Therefore, the Japanese early heading cultivars would be expected to have the dominant allele of *Ppd-1*, and their *Ppd-1* genotype should be determined to facilitate the systematic breeding of early heading cultivars and to enable the MAS of *Ppd-1* genes.

In addition to early heading, late stem elongation is also highly desirable, because frost injury of young spikes in early spring is closely related to the earliness of stem elongation during which young spikes come out above the ground. Stem elongation is related to apical development, commencing at the double-ridge stage which is the sign of transition from vegetative to reproductive growth [4]. Therefore, the effect of photoperiodic response genes on apical development should be analyzed.

Spring-type cultivars are grown as winter cultivars in this area, since winter temperature is not low enough to vernalize winter-type cultivars. By conventional genetic analysis, most of the Japanese cultivars of the spring-type proved to carry *Vrn-D1* [5, 6] among the major genes for vernalization-insensitivity, *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Vrn-B3*, and *Vrn-D4* (formerly *Vrn4*) [7, 8, 9]. However, because of the instability of winter temperature, spring-type cultivars can be damaged by frost in winter and early spring. To overcome such a problem, the winter allele of *Vrn-D1* was introduced to the spring-type cultivars, since heading time proved to be independent of vernalization requirement in this region [2, 10]. The development of winter-type cultivars with early heading was successful, and several cultivars, such as 'Iwainodaichi', have been released. However, it is generally believed that heading time of winter-type wheat is later than that of spring-type wheat. Molecular genetic analysis of *Vrn-D1* allele is necessary to understand the earliness of these cultivars with winter growth habit.

In this study, we determined the *Ppd-1* genotype of ten Japanese cultivars, and phenotypic effect of *Ppd-1* genes was evaluated using the NILs for photoperiodic response. We also compared genomic sequence of *Vrn-D1* among early heading cultivars with spring or winter growth habit. Based on these results, the strategy for the breeding of early heading cultivars will be discussed.

MATERIALS AND METHODS

Genetic analysis of photoperiodic response: F_1, F_2, and B_1F_1 populations of crosses between a photoperiod-sensitive cultivar 'Haruhikari' and nine insensitive cultivars were vernalized, with their parental lines, at 5°C under 8 h illumination for 70 days, and then grown in a glasshouse at 20°C under 8 h or 24 h photoperiod. The number of days to heading after vernalization treatment was recorded. An allelism test between the insensitive cultivar 'Saitama 27' and the other insensitive cultivars was also carried out in a similar manner. Using the above F_2 populations, chromosomal location of photoperiod-insensitive genes detected was determined based on the linkage with three SSR markers, *Xwmc177* (2A), *Xgwm148* (2B), and *Xgwm484* (2D), since *Ppd-A1* and *Ppd-B1* have not been cloned [11].

Evaluation of photoperiodic response and apical development: Based on the genotype data obtained in the above analysis, 'Saitama 27' (*Ppd-D1*) and 'Fukuwasekomugi' (*Ppd-B1* and *Ppd-D1*) were crossed with 'Haruhikari' as a recurrent parent. In the BC_5F_2 generation, segregants homozygous for photoperiod insensitivity were selected and the following three NILs were established; 'Haruhikari (*Ppd-B1*)', 'Haruhikari (*Ppd-D1*)', and 'Haruhikari (*Ppd-B1*, *Ppd-D1*)'.

These NILs were grown in an incubator at 20°C under 8 h or 24 h photoperiod without vernalization treatment, since 'Haruhikari' carries vernalization-insensitive genes, *Vrn-A1* and *Vrn-B1*. Three plants of each line were sampled every 5 days (8 h photoperiod) or 2 days (24 h photoperiod) during the period from a few days prior to double-ridge initiation to elongation of empty glume. Stem length and stage of apical development were recorded. Apical development stage was scored, based on the criteria listed in the notes under Table 3.

Molecular genetic analysis of *Vrn-D1*: A total of 23 accessions (eight winter-type and 15 spring-type) of Japanese wheat cultivars were used for the analysis of *Vrn-D1*. Two primer sets (Intr1/D/F+Intr1/D/R3, Intr1/D/F+Intr1/D/R4) [12] were used for the detection of a large deletion (4235 bp) in the first intron.

RESULTS

***Ppd-1* genotype:** Varietal variation in days-to-heading under a 24 h photoperiod was small (18.0-27.0 d). On the contrary, it was large (25.6-80.4 d) under a 8 h photoperiod, which was mainly reflected by the degree of sensitivity to short photoperiod. The cultivar 'Haruhikari' is highly sensitive to short photoperiod. The other cultivars are slightly sensitive or insensitive, with 'Fukuwasekomugi' being the most insensitive.

The frequency distribution of days to heading in F_2 and B_1F_1 populations of crosses between 'Haruhikari' and insensitive cultivars was unimodal with small variation under 24 h photoperiod, while that observed under 8 h photoperiod was characterised as discontinued bimodal. From segregation analysis, six cultivars insensitive to short photoperiod proved to have a single dominant gene (Table 1). In addition, the photoperiod-insensitive gene of these cultivars was allelic with each other. Genetic linkage between this photoperiod-insensitive gene and *Xgwm484* was detected, thus these cultivars proved to carry *Ppd-D1*. Extremely early heading cultivars also carried *Ppd-D1*. Another gene carried by these cultivars was *Ppd-B1*, as shown by genetic linkage with *Xgwm148*.

Table 1. Heading traits of ten wheat cultivars examined in the present study.

Cultivar	Earliness of heading	Vernalization response: Growth habit	Vernalization response: *Vrn* genotype	*Vrn-D1* 1st intron	*Ppd-1* genotype
Fukuwasekomugi	extremely early	spring	*Vrn-D1*	deletion	*Ppd-B1 Ppd-D1*
Abukumawase	extremely early	spring	*Vrn-D1*	deletion	*Ppd-B1 Ppd-D1*
Abukumawase(W)	extremely early	winter	-	deletion	*Ppd-B1 Ppd-D1*
Hayakomugi	early	winter	-	deletion	*Ppd-D1*
Norin 61	medium	spring	*Vrn-D1*	deletion	*Ppd-D1*
Zenkoujikomugi	medium	spring	*Vrn-D1*	deletion	*Ppd-D1*
Saitama 27	medium	spring	*Vrn-A1*	-	*Ppd-D1*
Norin 59	medium	winter	-	-	*Ppd-D1*
Norin 67	late	winter	-	-	*Ppd-D1*
Haruhikari	very late	spring	*Vrn-A1 Vrn-B1*	-	-

Effect of *Ppd-1* genes on photoperiodic response: The days-to-heading did not differ among the NILs under the 24 h photoperiod, while it differed significantly under 8 h photoperiod (Table 2). Under the 8 h photoperiod, 'Haruhikari (*Ppd-B1*)' headed significantly earlier than 'Haruhikari (*Ppd-D1*)' by 14.0 days, indicating that *Ppd-B1* had a stronger effect on insensitivity than *Ppd-D1*. The interaction of *Ppd-B1* and *Ppd-D1* was also indicated by the fact that that 'Haruhikari (*Ppd-B1*, *Ppd-D1*)' headed significantly earlier by 7.5 and 21.5 days compared with 'Haruhikari (*Ppd-B1*)' and 'Haruhikari (*Ppd-D1*)', respectively.

Table 2. Variation in photoperiodic response among NILs (Haruhikari)

Line	Days to heading: 24 h photoperiod	Days to heading: 8 h photoperiod	Photoperiodic response (8h) - (24h)
Haruhikari	34.2	160<	125.8<
Haruhikari (*Ppd-B1*)	34.6	62.5	27.9
Haruhikari (*Ppd-D1*)	33.1	76.5	43.4
Haruhikari (*Ppd-B1*, *Ppd-D1*)	33.7	55.0	21.3

Effect of *Ppd-1* genes on apical development: Under the 24 h photoperiod, there was no difference in apical development among the NILs. Conversely, under the 8 h photoperiod, there was a large genotypic difference in the number of days to stage VI, or the double-ridge stage (Table 3). It was very long (55 days) in 'Haruhikari', while it was short in the NILs (20-30 days). The number of days from stage VI to stage X also differed among 'Haruhikari' and the NILs. Shoot apex of 'Haruhikari' remained at stage VIIe for more than 30 days, while the number of days from stage VI to stage X was small in the NILs (15–25 days). These results indicate that the number of days to stage VI and that from stage VI to stage X were both closely related to the *Ppd-1* genotype.

In all genotypes except 'Haruhikari', stem started to elongate at nearly stage VI, subsequently elongated exponentially, and rapid stem elongation occurred at stage IXm (Table 3). This pattern of stem elongation was not affected by the genotype or photoperiod.

Table 3. Effect of *Ppd-1* genes on apical development under 8h photoperiod

Days	Haruhikari Length	Haruhikari Stage	Haruhikari *Ppd-B1* Length	Haruhikari *Ppd-B1* Stage	Haruhikari *Ppd-D1* Length	Haruhikari *Ppd-D1* Stage	Haruhikari *Ppd-B1, Ppd-D1* Length	Haruhikari *Ppd-B1, Ppd-D1* Stage
15	-	-	1.0	<	-	-	1.0	<
20	-	-	1.0	<	1.0	<	1.0	<
25	-	-	1.0	VI	1.0	<	1.7	VIIe
30	1.0	<	2.7	VII1	1.4	VIIe	3.2	VIII
35	1.0	<	4.0	IXe	2.2	VII1	19.0	IXm
40	1.0	<	22.7	IXm	5.2	IXe	56.0	X
45	1.0	<	60.0	X	11.2	IXm	-	-
50	1.0	<	-	-	33.0	X	-	-
55	2.0	VI	-	-	-	-	-	-
60	2.0	VIIe	-	-	-	-	-	-

-: Not examined, Length: Stem length (mm), Stage: Score of apical development
<: Vegetative stage, VI: Double ridges at the middle part of spike, VIIe: Double ridges at the basal part of spike, VII1: Double ridges become less distinct, VIII: Terminal spikelet initiation, IXe: Spikelet primordia differentiate into florets, IXm: First florets differentiate floral organs, IX1: Differentiation of floral organs in all florets, X: Elongation of empty glume

Molecular genetic analysis of *Vrn-D1*: A large deletion (4235 bp) in the first intron was detected in all of the 15 spring-type cultivars, as expected. On the contrary, in eight winter-type cultivars, four cultivars with (extremely) early heading have this deletion, while the other four cultivars with medium to late heading lack this deletion (partly shown in Table 1).

DISCUSSION

As summarized in Table 1, six cultivars ranging from early to late heading carried a single and common gene *Ppd-D1* for insensitivity, while three cultivars of extremely early heading carried two genes (*Ppd-B1*, *Ppd-D1*). In contrast, 'Abukumawase(W)', a winter-type NIL of 'Abukumawase', is also an extremely early heading type, indicating that heading time was independent of *Vrn* genotype. Therefore, it was clearly indicated that the earliness of wheat cultivars from the central to southwestern part of Japan is strongly related with *Ppd-1* genotype. The breeding of extremely early heading cultivars could be achieved by the introduction of *Ppd-B1*.

Analysis of NILs showed that *Ppd-B1* had stronger effect on insensitivity to photoperiod than *Ppd-D1*, and the magnitude of insensitivity was *Ppd-D1* < *Ppd-B1* < *Ppd-B1 Ppd-D1* in increasing order (Table 2). On the contrary, *Ppd-D1* of 'Ciano 67' had stronger effect than *Ppd-B1* of 'Chinese Spring' [13, 14]. Such a discrepancy could be explained by multiple allelism at the *Ppd-1* loci. As suggested by Scarth and Law [13], the *Ppd-B1* allele of the Australian cultivar 'Timstein' had stronger effect than the *Ppd-B1* allele of 'Chinese Spring'.

In order to breed wheat and barley cultivars with late stem elongation and early heading, it has been proposed that the combination of adequate vernalization requirement and small photoperiodic response is important [15]. The *Vrn-D1* genotype with quantitative requirement for vernalization is more resistant to frost damage and appears more suitable to the central to southwestern regions of Japan than the *Vrn-A1* type with no requirement [5, 6]. Fujita *et al*. [10] developed a winter-type NIL of 'Abukumawase' (Table 1) and demonstrated that late stem elongation and early heading could be combined by the introduction of winter growth habit into extremely early heading cultivars. These results showed the advantage of the *Vrn-D1* allele and winter growth habit. Taking these facts and the results obtained in the present study into account, it is apparent that apical development may be controlled complementarily by *Vrn* and *Ppd* genes, and the following genotypic scheme could be proposed: a successful cultivar must have the appropriate combination of the *Vrn* genotype (*Vrn-D1* or winter type) and the *Ppd* genotype (*Ppd-B1* or *Ppd-B1 Ppd-D1*) depending upon the growth conditions. For the establishment of a MAS system, sequence polymorphisms among *Ppd-B1* and *Vrn-D1* alleles will need to be found among the Japanese wheat cultivars.

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