Genetic Analysis of Photoperiod Response in Wheat and Its Relation with the Earliness of Heading in the Southwestern Part of Japan

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Genetic analysis of vernalization and photoperiod responses was carried out using eight wheat cultivars, in order to investigate the relationship between their genotype and the earliness of heading in the southwestern part of Japan. Allelism test of the genes for the vernalization response showed that three wheat cultivars, ‘Fukuwasekomugi’, ‘Zenkoujikomugi’ and ‘Schomburgk’, harboured *Vrn-D1*, *Vrn-D1* and *Vrn-A1*, respectively. Among the eight cultivars tested, ‘Haruhikari’ was sensitive to short photoperiod, while the others were insensitive. Segregation analysis of the photoperiod response in the F_2 and B_1F_1 populations showed that the very late heading cultivar ‘Haruhikari’ did not harbour major genes for insensitivity to photoperiod. Six cultivars characterized by medium to late heading, ‘Norin 61’, ‘Zenkoujikomugi’, ‘Saitama 27’, ‘Schomburgk’, ‘Norin 59’ and ‘Norin 67’, carried a single gene for insensitivity, *Ppd-S*, in common. The extremely early heading cultivar ‘Fukuwasekomugi’ was found to carry two genes for insensitivity, *Ppd-S* and *Ppd-F*, the latter displaying a stronger effect for insensitivity to photoperiod. The present study revealed that the earliness of heading in the southwestern part of Japan was closely related to the *Ppd* genotype of wheat cultivars, whereas it was independent of the *Vrn* genotype.

Key Words: wheat, vernalization response, photoperiod response, genetic analysis, earliness of heading.

Introduction

Earliness of heading is one of the most important traits for wheat breeding, and cultivars with early heading have been bred in the southwestern part of Japan to be harvested before monsoonal rain. According to Yasuda and Shimoyama (1965), three physiological characters, vernalization response, photoperiod response and earliness *per se* (originally designated as narrow-sense earliness), are responsible for the earliness of heading, and the photoperiod response is most important in the southwestern part of Japan. Kato and Yamashita (1991) and Fujita *et al.* (1995) reported that heading time of wheat in the southwestern part of Japan was independent of the genotype for vernalization response. Therefore, early heading cultivars with a winter growth habit, such as ‘Iwainodaichi’ (Taya *et al.* 2003), had been successfully bred to avoid frost injury in the southwestern part of Japan. However, the relationship between the genotype for the photoperiod response and heading time of wheat in the southwestern part of Japan remains to be investigated.

The major genes for vernalization insensitivity that

convert winter wheat into spring wheat are *Vrn-A1* (formerly *Vrn1*), *Vrn-B1* (*Vrn2*), *Vrn-D1* (*Vrn3*), *Vrn-D5* (*Vrn4*) and *Vrn-B4* (*Vrn5*) (Pugsley 1972, Law *et al.* 1976, Kato *et al.* 1993, Snape *et al.* 2001, Iwaki *et al.* 2002, Kato *et al.* 2003). The photoperiod response is controlled by three major genes, *Ppd-A1* (formerly *Ppd3*), *Ppd-B1* (*Ppd2*) and *Ppd-D1* (*Ppd1*), which induce wheat insensitivity to daylength (Welsh *et al.* 1973, Keim *et al.* 1973, Pirasteh and Welsh 1975, Scarth and Law 1983, Scarth and Law 1984, Snape *et al.* 2001). QTL analysis revealed that additional genetic factors for the photoperiod response are located on chromosome 4B (Sourdille *et al.* 2000), on chromosomes 4B, 5A, 5B, 7A, and 7B (Shindo *et al.* 2003) and on chromosomes 5A and 7D (Hanocq *et al.* 2004). Genetic analysis of the Japanese wheat cultivars has been carried out for the vernalization response, and most of the Japanese cultivars tested were found to carry *Vrn-D1* (Gotoh 1979, Iwaki *et al.* 2000). On the other hand, while the Japanese wheat cultivars with extremely early heading are essentially highly insensitive to short photoperiod (Yoshida *et al.* 1983), the *Ppd* genotype has not been identified in any Japanese wheat cultivars or lines.

In the present study, we analyzed the genotype for the response to vernalization and photoperiod in seven Japanese and one Australian wheat cultivars, and investigated the

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relationship between the genotype for vernalization and photoperiod responses and the earliness of heading in the southwestern part of Japan.

Materials and Methods

Plant materials

Eight wheat cultivars analyzed in this study were selected, based mainly on their heading traits (Table 1). The seeds of eight cultivars were supplied by the National Institute of Agrobiological Sciences. For the genetic analysis of the vernalization and photoperiod responses, seeds were pre-germinated and sown in containers filled with a mix of sterilized soil containing adequate amounts of chemical fertilizer and vermiculite (1 : 1) at 3 cm × 3 cm intervals (130 seeds per container).

Genetic analysis of vernalization response

Vrn genotype of three cultivars, 'Norin 61', 'Saitama 27' and 'Haruhikari', has been identified by Gotoh (1979) (Table 1). Therefore, the *Vrn* genotype of the other three cultivars with spring growth habit, 'Fukuwasekomugi', 'Zenkoujikomugi' and 'Schomburgk', was analyzed. F₁ and F₂ populations of crosses between these three cultivars and five tester lines, 'Saitama 27' (*Vrn-A1*), 'Triple Dirk (B)' ('TD(B)', *Vrn-B1*), 'TD(E)' (*Vrn-D1*), 'TD(F)' (*Vrn-D5*) and 'Norin 59' (winter type), were grown in a glasshouse, with parental lines, at 20°C under a 24 hour photoperiod in which natural daylength was extended by the use of incandescent light. The same populations of equal size were vernalized at 5°C under continuous illumination of fluorescent light for 70 days and then grown at 20°C under a 24 hour photoperiod. Vernalization treatment was started when the first leaf emerged from the coleoptile. The number of days from sowing or after vernalization treatment to heading of

the main culm was recorded on a single plant basis. The seeds of three near-isogenic lines, 'TD(B)', 'TD(E)' and 'TD(F)' developed by Pugsley (1972), were provided by the National Agricultural Research Center for the Tohoku Region.

Genetic analysis of photoperiod response

The F₁, F₂ and B₁F₁ populations of the crosses between a photoperiod-sensitive cultivar 'Haruhikari' and seven insensitive cultivars were vernalized, with their parental lines, at 5°C under 8 hour illumination of fluorescent light for 70 days, and then grown in a glasshouse at 20°C under 8 hour of natural daylight or a 24 hour photoperiod in which natural daylength was extended by the use of incandescent light. The number of days to heading of the main culm after vernalization treatment was recorded on a single plant basis for up to 100 days at the end of the experiment. Allelism test between an insensitive cultivar 'Saitama 27' and the other insensitive cultivars was also carried out in the same way.

Earliness of heading in the southwestern part of Japan

Twenty seeds of each cultivar were sown in a field at the National Agricultural Research Center for the Western Region, located at Fukuyama (34°30'N latitude, 133°21'E longitude) in the southwestern part of Japan, at 12 cm intervals with two replications, each of which being sown on November 16 in 2001 and November 18 in 2002, respectively. The date when the tip of the spikes appeared from about 50 percent of tillers was recorded as heading date. The average heading date for the two years was calculated and is presented in Table 1. Based on these data, the eight cultivars were classified into four types of earliness of heading, namely extremely early, medium, late and very late, as shown in Table 1.

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

Cultivar	Country	Earliness of heading		Growth habit	Vernalization response			Photoperiod response	
		Earliness type	HD ¹⁾		V ²⁾	NV ²⁾	<i>Vrn</i> gene ³⁾	Sensitivity ⁴⁾	<i>Ppd</i> gene ⁵⁾
Fukuwasekomugi	Japan	extremely early	Apr 12	spring	9.3	38.5	<i>Vrn-D1</i>	insensitive	<i>Ppd-S Ppd-F</i>
Norin 61	Japan	medium	Apr 20	spring	10.9	40.3	<i>Vrn-D1</i>	insensitive	<i>Ppd-S</i>
Zenkoujikomugi	Japan	medium	Apr 21	spring	11.4	39.5	<i>Vrn-D1</i>	insensitive	<i>Ppd-S</i>
Saitama 27	Japan	medium	Apr 22	spring	13.7	34.5	<i>Vrn-A1</i>	insensitive	<i>Ppd-S</i>
Schomburgk	Australia	medium	Apr 22	spring	10.7	32.1	<i>Vrn-A1</i>	insensitive	<i>Ppd-S</i>
Norin 59	Japan	medium	Apr 22	winter	14.0	93.0	—	insensitive	<i>Ppd-S</i>
Norin 67	Japan	late	Apr 26	winter	17.9	84.7	—	insensitive	<i>Ppd-S</i>
Haruhikari	Japan	very late	May 1	spring	12.8	34.2	<i>Vrn-A1 Vrn-B1</i>	sensitive	—

¹⁾ HD: Heading date in the field in Fukuyama, Japan.

²⁾ V, NV: Number of days to heading after vernalization treatment (V) and days from sowing to heading without vernalization treatment (NV), respectively. Twenty plants were grown as described in "Materials and Methods", with two replications.

³⁾ *Vrn* genotype of 'Fukuwasekomugi', 'Zenkoujikomugi' and 'Schomburgk' was identified in the present study, while that of 'Norin 61', 'Saitama 27' and 'Haruhikari' was determined by Gotoh (1979).

⁴⁾ Sensitivity to short photoperiod was determined based on the results listed in Table 3.

⁵⁾ *Ppd-S* and *Ppd-F* are genes for photoperiod insensitivity tentatively designated in the present study.

—: no major *Vrn* or *Ppd* genes.

Results

Genotype for vernalization response

Frequency distribution of the number of days to heading was unimodal with small variations in the F_2 populations vernalized for 70 days, whereas it was discontinued and bimodal without vernalization treatment except for critical crosses. Therefore, the variations observed under the latter condition were ascribed to the difference in the vernalization response of each plant. Based on the values of F_1 and the parental populations and distinct discontinuity around 65 days, F_2 plants that headed within 65 days were classified into the spring type and those that headed after 66 days into the winter type. It was reconfirmed that the four tester lines, 'Saitama 27', 'TD(B)', 'TD(E)', and 'TD(F)', harboured a different single *Vrn* gene (Table 2). Segregation of spring and winter types in the F_2 populations of the crosses between a winter cultivar 'Norin 59' and three cultivars, 'Fukuwasekomugi', 'Zenkoujikomugi' and 'Schomburgk', fitted to the expected ratio of 3 : 1, showing that these three cultivars harboured a single *Vrn* gene (Table 2). F_2 populations of 'Fukuwasekomugi' $\times$ 'TD(E)', 'Zenkoujikomugi' $\times$

'TD(E)' and 'Schomburgk' $\times$ 'Saitama 27' failed to segregate into the winter type, while segregation into spring and winter types fitted to the expected ratio of 15 : 1 in other cross combinations (Table 2). Therefore, three cultivars, 'Fukuwasekomugi', 'Zenkoujikomugi' and 'Schomburgk', were found to carry *Vrn-D1*, *Vrn-D1* and *Vrn-A1*, respectively.

Characteristics of the *Vrn* genotype of the eight cultivars are presented in Table 1. Since the genotype of five wheat cultivars with medium heading was classified into *Vrn-A1* type, *Vrn-D1* type and winter genotype, it appeared that earliness of heading was independent of the *Vrn* genotype in the southwestern part of Japan.

Photoperiod response

The variations in the number of days to heading under a 24 hour photoperiod after vernalization treatment were small (18.0–27.0 days) among the eight wheat cultivars, whereas those under a 8 hour photoperiod were very large (25.6–80.4 days), as shown in Table 3. Therefore, the number of days to heading under the 8 hour photoperiod indicated the degree of sensitivity to short photoperiod. The cultivar 'Haruhikari' was highly sensitive to short photoperiod

Table 2. Segregation of spring and winter types in F_2 population of the crosses between tester lines and between three cultivars and tester lines

Cross combination	No. of plants			χ^2 for the expected ratio		
	Spring type	Winter type	Total	3 : 1	15 : 1	63 : 1
Norin 59 $\times$ Saitama 27	99	31	130	0.09	68.70 **	419.70 **
Norin 59 $\times$ TD(B)	99	31	130	0.09	68.70 **	419.70 **
Norin 59 $\times$ TD(E)	96	34	130	0.09	87.90 **	511.13 **
Norin 59 $\times$ TD(F)	98	32	130	0.01	74.83 **	449.17 **
Saitama 27 $\times$ TD(B)	119	11	130	18.96 **	1.09	40.23 **
Saitama 27 $\times$ TD(E)	121	9	130	22.66 **	0.10	24.29 **
Saitama 27 $\times$ TD(F)	123	7	130	26.68 **	0.17	12.35 **
TD(B) $\times$ TD(E)	122	8	130	24.63 **	0.00	17.82 **
TD(B) $\times$ TD(F)	123	7	130	26.68 **	0.17	12.35 **
TD(E) $\times$ TD(F)	125	5	130	31.03 **	1.28	4.41 *
Fukuwasekomugi $\times$ Saitama 27	124	6	130	28.81 **	0.59	7.88 **
Fukuwasekomugi $\times$ TD(B)	117	13	130	15.60 **	3.12	60.17 **
Fukuwasekomugi $\times$ TD(E)	130	0	130	43.33 **	8.67 **	2.06
Fukuwasekomugi $\times$ TD(F)	123	7	130	26.68 **	0.17	12.35 **
Fukuwasekomugi $\times$ Norin 59	95	35	130	0.26	94.82 **	543.60 **
Zenkoujikomugi $\times$ Saitama 27	120	10	130	20.77 **	0.46	31.76 **
Zenkoujikomugi $\times$ TD(B)	122	8	130	24.63 **	0.00	17.82 **
Zenkoujikomugi $\times$ TD(E)	130	0	130	43.33 **	8.67 **	2.06
Zenkoujikomugi $\times$ TD(F)	123	7	130	26.68 **	0.17	12.35 **
Zenkoujikomugi $\times$ Norin 59	103	27	130	1.24	46.77 **	311.80 **
Schomburgk $\times$ Saitama 27	130	0	130	43.33 **	8.67 **	2.06
Schomburgk $\times$ TD(B)	121	9	130	22.66 **	0.10	24.29 **
Schomburgk $\times$ TD(E)	123	7	130	26.68 **	0.17	12.35 **
Schomburgk $\times$ TD(F)	122	8	130	24.63 **	0.00	17.82 **
Schomburgk $\times$ Norin 59	99	31	130	0.09	68.70 **	419.70 **

*, ** Significant at the 5% and 1% levels, respectively.

and therefore did not carry any gene for photoperiod insensitivity, while the other cultivars were slightly sensitive or insensitive, 'Fukuwasekomugi' being most insensitive. We therefore assumed that seven cultivars other than 'Haruhikari' must harbour major gene(s) for photoperiod insensitivity. The F_1 hybrids of the crosses between 'Haruhikari' and the insensitive cultivars headed earlier, under the 8 hour photoperiod, by 8.0 to 17.8 days than their respective midparent value (Table 3), indicating that insensitivity was incompletely dominant over sensitivity.

Genotype for photoperiod response

As shown in Fig. 1A-B, Fig. 2A-E and Fig. 3A,C, the frequency distribution of the number of days to heading in the F_2 and B_1F_1 populations of crosses between a sensitive cultivar 'Haruhikari' and insensitive cultivars was unimodal with small variations under a 24 hour photoperiod (open bar), while those observed under a 8 hour photoperiod were discontinued and bimodal (solid bar). The variations observed under the 8 hour photoperiod were therefore due to the difference in sensitivity to short photoperiod. Based on the number of days to heading of the F_1 and the parental populations (Table 3) and the distinct discontinuity around 65 days (Fig. 1A-B, Fig. 2A-E and Fig. 3A,C), the F_2 and B_1F_1 plants that headed within 65 days were classified into photoperiod-insensitive and those that did not head by 66 days were classified into sensitive. Six cultivars insensitive to short photoperiod, 'Saitama 27', 'Norin 61', 'Zenkoujikomugi', 'Schomburgk', 'Norin 59' and 'Norin 67', were found to harbour a single dominant gene, since the segregation of the insensitive and sensitive types in the F_2 and B_1F_1 populations fitted to the expected ratios of 3 : 1 and 1 : 1, respectively (Table 4, Fig. 1A-B and Fig. 2A-E). In addition, allelism test between six insensitive cultivars (Table 4 and Fig. 2F-J) showed that the gene for photoperiod insensitivity

of these cultivars was allelic to each other. We tentatively designated this gene as *Ppd-S*. Table 4 and Fig. 3A,C also showed that the photoperiod insensitivity of the most insensitive cultivar 'Fukuwasekomugi' was controlled by two dominant genes. Although the size of the F_2 population of 'Saitama 27' $\times$ 'Fukuwasekomugi' was not large enough (Table 4), no transgressive segregants headed later than 'Saitama 27' (Fig. 3B). Therefore, it was suggested that 'Fukuwasekomugi' carried *Ppd-S* in common with 'Saitama 27', and the other gene carried by 'Fukuwasekomugi' was tentatively designated as *Ppd-F*.

The *Ppd* genes carried by the eight cultivars are listed in Table 1. The *Ppd* genotypes of the extremely early heading type, the medium to late heading types, and the very late heading type were *Ppd-S Ppd-F*, *Ppd-S ppd-F*, and *ppd-S ppd-F*, respectively, indicating that earliness of heading was closely related to the *Ppd* genotype of wheat cultivars in the southwestern part of Japan.

Discussion

Gotoh (1979) and Iwaki *et al.* (2000) reported that wheat cultivars indigenous to Japan carried *Vrn-D1* as a single *Vrn* gene, and suggested that cultivars with *Vrn-D1* were more adaptable to the growing conditions in the southern part of Japan than those with *Vrn-A1*, which are susceptible to frost damage. The present study also showed that the two Japanese wheat cultivars, 'Fukuwasekomugi' and 'Zenkoujikomugi', developed in the southern part of Japan, carried *Vrn-D1* (Table 2). According to Kato and Yamagata (1988), wheat cultivars with *Vrn-A1* are completely insensitive to vernalization and those with *Vrn-D1* are slightly sensitive. These facts strongly support the concept of Gotoh (1979) and Iwaki *et al.* (2000), according to which partial requirement of vernalization by *Vrn-D1* is important for

Table 3. Number of days to heading of parental and F_1 populations under 24 hour or 8 hour photoperiod after vernalization treatment

Parent and cross combination	Generation	Days to heading under 24 hour photoperiod			Days to heading under 8 hour photoperiod			Difference (B)–(A)
		Plant	Range	Mean (A)	Plant	Range	Mean (B)	
Haruhikari	P	5	25–28	27.0	5	71–87	80.4	53.4
Saitama 27	P	5	24–25	24.6	5	36–41	38.4	13.8
Norin 61	P	5	22–24	22.6	5	35–39	37.6	15.0
Zenkoujikomugi	P	5	19–21	20.2	5	33–36	33.8	13.6
Schomburgk	P	5	24–25	24.2	5	36–40	38.6	14.4
Norin 59	P	5	20–22	21.0	5	41–42	41.6	20.6
Norin 67	P	5	24–25	24.8	5	39–43	40.0	15.3
Fukuwasekomugi	P	5	18	18.0	5	25–26	25.6	7.6
Haruhikari $\times$ Saitama 27	F_1	5	27–28	27.6	5	48–52	49.8	22.2
Haruhikari $\times$ Norin 61	F_1	5	24–26	25.2	5	50–53	51.0	25.8
Haruhikari $\times$ Zenkoujikomugi	F_1	5	24–25	24.4	5	45–53	48.0	23.6
Haruhikari $\times$ Schomburgk	F_1	5	27–28	27.2	5	50–52	50.6	23.4
Haruhikari $\times$ Norin 59	F_1	5	25–26	25.2	5	47–50	48.0	22.8
Haruhikari $\times$ Norin 67	F_1	5	25–27	26.0	5	50–52	51.0	25.0
Haruhikari $\times$ Fukuwasekomugi	F_1	5	22–23	22.6	5	33–37	35.2	12.6

Table 4. Segregation of photoperiod response in F₂ and B₁F₁ populations

Cross combination (F ₂ populations)	Segregation of sensitivity to short photoperiod		Total	χ^2 for the expected ratio		
	Insensitive	Sensitive		3 : 1	15 : 1	63 : 1
Haruhikari × Saitama 27	104	26	130	1.73	41.95 **	287.32 **
Haruhikari × Norin 61	96	34	130	0.09	87.90 **	511.13 **
Haruhikari × Zenkoujikomugi	96	34	130	0.09	87.90 **	511.13 **
Haruhikari × Schomburgk	94	36	130	0.50	102.01 **	577.08 **
Haruhikari × Norin 59	107	23	130	3.70	29.05 **	219.90 **
Haruhikari × Norin 67	96	34	130	0.09	87.90 **	511.13 **
Haruhikari × Fukuwasekomugi	126	4	130	33.32 **	2.23	1.94
Saitama 27 × Norin 61	130	0	130	43.33 **	8.67 **	2.06
Saitama 27 × Zenkoujikomugi	130	0	130	43.33 **	8.67 **	2.06
Saitama 27 × Schomburgk	130	0	130	43.33 **	8.67 **	2.06
Saitama 27 × Norin 59	130	0	130	43.33 **	8.67 **	2.06
Saitama 27 × Norin 67	130	0	130	43.33 **	8.67 **	2.06
Saitama 27 × Fukuwasekomugi	130	0	130	43.33 **	8.67 **	2.06

Cross combination (B ₁ F ₁ populations)	Segregation of sensitivity to short photoperiod		Total	χ^2 for the expected ratio		
	Insensitive	Sensitive		1 : 1	3 : 1	7 : 1
Haruhikari × (Haruhikari × Saitama 27)	26	29	55	0.16	22.55 **	81.37 **
Haruhikari × (Haruhikari × Fukuwasekomugi)	87	37	124	20.16 **	1.55	34.08 **

** Significant at the 1% level.

adaptation in the southern part of Japan.

It was suggested that ‘Fukuwasekomugi’ carried *Ppd-F* in addition to *Ppd-S* commonly carried by the other insensitive cultivars. In the backcross population of ‘Haruhikari’ × (‘Haruhikari’ × ‘Fukuwasekomugi’), B₁F₁ segregants insensitive to short photoperiod showed an almost trimodal distribution when classified at two-day intervals (Fig. 3C). This segregation pattern appeared to fit to the 1 : 1 : 1 ratio expected, if ‘Fukuwasekomugi’ had two genes which showed different degrees of insensitivity and additive interaction. The number of days to heading of the F₁ plants of crosses between ‘Haruhikari’ and six cultivars carrying *Ppd-S* (Table 3) was relatively uniform (48.0–51.0 days), while ‘Haruhikari’ × ‘Fukuwasekomugi’ headed earlier (35.2 days) (Table 3). From these results, it is reasonable to assume that

the above three modes from early to late heading corresponded to the *Ppd-S ppd-S Ppd-F ppd-F*, *ppd-S ppd-S Ppd-F ppd-F* and *Ppd-S ppd-S ppd-F ppd-F* genotypes, respectively. Therefore it is likely that the effect of *Ppd-F* is probably stronger than that of *Ppd-S*. It was considered that *Ppd-S* and *Ppd-F* display an additive effect to increase insensitivity and consequently, that ‘Fukuwasekomugi’ is highly insensitive to short photoperiod, as shown in Table 3.

Photoperiod response is an important physiological factor controlling the earliness of heading in the southwestern part of Japan (Hashimoto and Hirano 1963, Yasuda and Shimoyama 1965, Gotoh 1977, Yoshida *et al.* 1983). Nevertheless, the effect of the genes for the photoperiod response on the earliness of heading in the southwestern part of Japan has not been investigated. The present study showed that the very

Fig. 1. Frequency distribution of the number of days to heading of F₂ and B₁F₁ populations in the cross between ‘Haruhikari’ and ‘Saitama 27’ under 24 hour (open bar) or 8 hour (solid bar) photoperiod after vernalization treatment. nh: Plants did not head within 100 days. ▼: Number of days to heading of parents and F₁ under 8 hour photoperiod. Parents were indicated by their initial letter.

Fig. 2. Frequency distribution of the number of days to heading of F₂ populations in crosses between two cultivars, 'Haruhikari' and 'Saitama 27', and five cultivars, 'Norin 61', 'Zenkoujikomugi', 'Schomburgk', 'Norin 59' and 'Norin 67', under 24 hour (open bar) or 8 hour (solid bar) photoperiod after vernalization treatment. nh: Plants did not head within 100 days. ▼: Number of days to heading of parents and F₁ under 8 hour photoperiod. Parents were indicated by their initial letter.

late heading cultivar 'Haruhikari' did not carry major genes for insensitivity to photoperiod. On the other hand, six medium to late heading cultivars, 'Norin 61', 'Zenkoujikomugi', 'Saitama 27', 'Schomburgk', 'Norin 59' and 'Norin 67', carried a single and common gene *Ppd-S* for insensitivity,

while the extremely early heading cultivar 'Fukuwasekomugi' carried two genes for insensitivity (Table 4). These results clearly indicated that the earliness of wheat cultivars in the southwestern part of Japan is strongly related to the *Ppd* genotype, but is independent of the *Vrn* genotype.

Fig. 3. Frequency distribution of the number of days to heading of F₂ and B₁F₁ populations in crosses between two cultivars, 'Haruhikari' and 'Saitama 27', and a cultivar 'Fukuwasekomugi' under 24 hour (open bar) or 8 hour (solid bar) photoperiod after vernalization treatment. nh: Plants did not head within 100 days. ▼: Number of days to heading of parents and F₁ under 8 hour photoperiod. Parents were indicated by their initial letter.

Therefore, the *Ppd* genes are undoubtedly very important for regulating the heading time of wheat in the southwestern part of Japan.

Since the photoperiod response is a quantitative trait, the genotype can not be identified without segregation analysis under short daylength. Therefore, marker-assisted selection is indispensable for introducing *Ppd* genes in a breeding programme. In barley, STS markers closely linked to *Ppd-H1* (0.6–0.8 cM) have been developed by AFLP analysis (Decousset *et al.* 2000), and can be utilized as selection markers, while in wheat, *Ppd* genes have been mapped by using RFLP markers (Gale *et al.* 1995, Shindo *et al.* 2003) which are usually less polymorphic among wheat cultivars. Recently, Hanocq *et al.* (2005) have succeeded in locating *Ppd-B1* and *Ppd-D1* on the SSR map. However, the linkage between the *Ppd* genes and SSR markers was not close enough to utilize them as selection markers. Therefore, further studies should be conducted to develop closely linked markers. By using these markers, it will be possible to determine which genes among *Ppd-A1*, *Ppd-B1* and *Ppd-D1* corresponded to the two genes, *Ppd-S* and *Ppd-F*, tentatively designated in the present study. Near-isogenic lines for the photoperiod response can be established also by using these markers, and may enable to clarify the effects of the *Ppd* genes on the earliness of heading in the southwestern part of Japan as well as their pleiotropic effects on culm length and other agronomic traits.

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