Growth and Yield Properties of Near-Isogenic Wheat Lines Carrying Different Photoperiodic Response Genes

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Abstract: Near-isogenic lines (NILs), carrying different combinations of Ppd-I genes in the genetic background of an early-maturity cultivar Abukumawase were grown at two sites for two years to elucidate the effects of photoperiodic response genes on the growth and yield of early-maturity wheat (Triticum aestivum L.) in central and southwestern Japan. Photoperiod-insensitive genes, Ppd-B1a and Ppd-D1a, accelerated young spike development, and this effect was predominant with Ppd-B1 with no additive effect among them. Ppd-B1a and Ppd-D1a also advanced the jointing stage, heading, and maturity, and the effect of Ppd-B1a on the jointing stage and heading was stronger than that of Ppd-D1a. An additive effect of two genes was detected for heading. Besides, Ppd-B1a and Ppd-D1a reduced culm length and grain weight, although the reduction effect on grain weight was not significant. Meanwhile, the mean temperature from double ridge formation stage to heading was lower in NILs with photoperiod-insensitive genes than in NILs with photoperiod-sensitive gene, and there was a significant correlation between mean temperature from double ridge formation stage to terminal spikelet formation stage and spikelet number per spike as well as between mean temperature from terminal spikelet formation stage to heading and grain number per spikelet. Therefore in a genetic background of extremely early-maturity line of spring type wheat, photoperiod-insensitive genes accelerated wheat growth and reduced spikelet numbers in central and southwestern Japan, and the effect of Ppd-B1a was stronger than that of Ppd-D1a.

Key words: Climate change, Near-isogenic line, Photoperiodic response gene, Ppd-I, Wheat, Yield components.

Wheat heading time is determined by a complex process controlled by vernalization response, photoperiodic response, and narrow-sense earliness (Yasuda and Shimoyama, 1965; Kato and Yamagata, 1988; Kato and Yamashita, 1991). Among these three traits, photoperiodic response is the major determinant of heading time and its control is stable even under changing climate. Three major genes responsible for the response to photoperiod, Ppd-A1, Ppd-B1, and Ppd-D1 are located on the short arm of chromosomes 2A, 2B, and 2D, respectively (Pirasteh and Welsh, 1975; Scarth and Law, 1983).

In Japan, especially in the central and southwestern regions, earliness of heading has been one of the most important targets of wheat breeding, in order to avoid the disease and pre-harvest sprouting caused by monsoon rain. Fujita et al. (1995) reported that the difference in heading time between the middle-maturity cultivar Norin 61 and the early-maturity cultivars Asakazekomugi and Abukumawase was caused by the difference in photoperiodic response. Tanio et al. (2005) reported that the late-maturity cultivar Haruhikari had no photoperiod-insensitive alleles, while the middle-maturity cultivars Norin 61 and Saitama 27 had a single insensitive allele Ppd-D1a, and the early-maturity cultivars Fukuwasekomugi and Abukumawase had two insensitive alleles, Ppd-B1a and Ppd-D1a. These studies indicated that the early-maturity
Photoperiodic response genes | NILs | Recurrent Parent | [Ppd-B1, Ppd-D1] | [Ppd-B1] | [Ppd-D1] | [ppd]
---|---|---|---|---|---|---
Ppd-A1 | insensitive | sensitive | sensitive | sensitive | sensitive
Ppd-B1 | insensitive | insensitive | sensitive | sensitive | sensitive
Ppd-D1 | insensitive | sensitive | insensitive | sensitive | sensitive

Table 1. Ppd-I genotype of near-isogenic lines (NILs).

characteristics of Japanese wheat cultivars had been conferred by introduction of insensitive allele Ppd-B1a into middle-maturity cultivars carrying another insensitive allele Ppd-D1a (Tanio and Kato, 2007; Seki et al., 2011). According to Seki et al. (2011), nearly all Japanese cultivars, except those in Hokkaido region, have insensitive allele Ppd-D1a and the sensitive allele Ppd-B1b, while only a few early-maturity cultivars have two insensitive alleles Ppd-B1a and Ppd-D1a. There is little variation in the combination of photoperiodic response genes among Japanese wheat cultivars, suggesting the possibility to modify the developmental pattern by modulating the photoperiodic response.

The effects of Ppd-1 genes on heading time and young spike development under the condition of controlled day length were reported by Tanio and Kato (2007). The effect of Ppd-1 genes on wheat yield in Europe has been reported by Foulkes et al. (2004), Worland and Law (1986), Börner et al. (1993), and Worland (1996). According to these reports, the effects on yield differ depending on the genetic background of cultivars tested and the growth conditions. Consequently, it is necessary to clarify the effects of Ppd-1 genes on the growth pattern and yield under Japanese field conditions to control wheat growth by modulating the photoperiodic response.

In this study, growth pattern and yield characteristics in central and southwestern Japan were studied using NILs, carrying different combinations of Ppd-1 genes with a common genetic background of an extremely early-maturity line, Abukumawase.

**Materials and Methods**

1. **Plant materials**

Four NILs carrying different combinations of Ppd-1 gene were used in this study. These lines were developed by backcrossing five times with Vrn-A1 type NIL of Abukumawase, “Ab (VrnA1)” after Seki et al. (2007), since “Ab (VrnA1)” was highly spring type with no requirement of vernalization and thus the effect of vernalization requirement could be neglected. “Ab (VrnA1)” carried photoperiodic-insensitive alleles Ppd-B1a and Ppd-D1a, and Haruhikari was used as a donor of photoperiod-sensitive alleles Ppd-B1b and Ppd-D1b. BC1 and BC3 plants were selfed to raise F2 generation in which segregants carrying both Ppd-B1b and Ppd-D1b were selected using the SSR markers linked to Ppd-B1 and Ppd-D1 (Nishida et al., 2013) based on heading time under short-day condition with 8 hr of daylight. Finally, BC5 plants were selfed, and BC5 F2 plants carrying different combinations of photoperiodic response genes were selected using a diagnostic DNA marker to detect the mutant alleles of Ppd-B1 and Ppd-D1 (Beales et al., 2007; Nishida et al., 2013). The NILs with insensitive allele Ppd-B1a and Ppd-D1a was designated as [Ppd-B1] and [Ppd-D1] respectively, while the NIL with Ppd-B1b and Ppd-D1b was denoted [ppd], Vrn-A1 type Abukumawase with Ppd-B1a and Ppd-D1a, the recurrent parent “Ab (VrnA1)”, was termed [Ppd-B1, Ppd-D1] (Table 1).

2. **Field management and analysis**

Field experiments were conducted at two sites in 2009 and 2010 crop seasons. One site was NARO Agricultural Research Center located in central Japan (Ibaraki, 36°0’ N latitude, 140°0’ E longitude, 24 m altitude), and the other site was NARO Kyushu-Okinawa Agricultural Research Center located in southwestern Japan (Fukuoka, 33°2’ N latitude, 130°5’ E longitude, 11 m altitude). These four field experiments are termed as Ibaraki 2009, Ibaraki 2010, Fukuoka 2009, and Fukuoka 2010. The seeds of four NILs were sown on 6 November 2009 and on 5 November 2010 in Ibaraki, and on 20 November 2009 and on 19 November 2010 in Fukuoka, with four seeds in each spot at 8.5 cm intervals (planting density was 67 seeds m⁻²). In Ibaraki, a chemical fertilizer (N:PO:KO= 10:14:13) was applied at a rate of 36 g m⁻², and magnesium multiphosphate was applied at a rate of 12.5 g m⁻² prior to sowing. In Fukuoka, a chemical fertilizer (N:PO:KO= 14:12:12) was applied at a rate of 30 g m⁻² prior to sowing, and ammonium sulfate was applied at a rate of 3 g m⁻² in early February and early March (total of 6 g m⁻²). The other field managements were done following the local practices in each region. The experiments were performed with two replications in Ibaraki 2009 and Fukuoka 2009, with three replications in Ibaraki 2010 and Fukuoka 2010. Around tillering stage to stem elongation stage, three to five plants with moderate growth in each plot were selected to measure the length of main stem and to estimate the frost injury on young spike of the main stem. The developmental stage of young spike was also noted. These
observations were performed three to six times depending on the growth in each field. The jointing stage was defined as the stage at which the length of the main stem reached 20 mm, estimated by regression of exponential equation \( y = a^x \cdot b^x \), where \( x \) is the number of days after sowing and \( y \) is the length of the main stem (Fujita, 1997). The developmental stage of young spike was defined as follows after Inamura et al. (1956): V (initiation of spikelet primordia is not observed), VI (spikelet primordia initiate at the middle part of spike), VIIe (spikelet primordia initiate at the basal part of spike), VIIl (double ridges become less distinct), VIII (spikelet primordia begin to differentiate empty glumes, and terminal spikelet initiates), IXe (spikelet primordia differentiate into florets), IXm (first florets differentiate lemma, pelea, stamen and pistil), IXl (differentiation of lemma, pelea, stamen and pistil cover the florets) and X (empty glume and awn begin to elongate). In addition to determination of the young spike development stages, the double ridge formation stage (initiation of reproductive period) and terminal spikelet formation stage were determined in Ibaraki 2010 and Fukuoka 2010 according to the method of Porter et al. (1987). At the maturity stage, grain yield, spike number, grain weight and test weight were determined, by unit area sampling in Ibaraki and by whole sampling in Fukuoka. Spikelet number per spike was determined by observation.
of 10 spikes in each plot in all cultivation except Fukuoka 2009. Grain number per spike was calculated from the spike number, grain weight, and grain yield, and grain number per spikelet was calculated from the grain number per spike and spikelet number per spike. Culm length was determined as the average length of the longest culms of 10 plants in each plot in Ibaraki and as the average by unit area in Fukuoka. Daily maximum and minimum temperatures during wheat growing season were recorded at each field. The 30-year-average daily maximum and minimum temperature in Ibaraki was calculated by using data from 1971 – 2001 recorded in Tateno, Tsukuba, and Ibaraki with the automated Meteorological Data Acquisition System (AMeDAS), while that in Fukuoka was calculated using data from 1971 – 2001 in Chikugo, Fukuoka acquired by the NARO Kyushu-Okinawa Agricultural Research Center. The day length was calculated by using data of daily sunrise and sunset time at each field estimated from latitude, longitude and altitude.

Results

1. Temperature during wheat growing season

In Ibaraki 2009 and Ibaraki 2010, the daily minimum temperature from sowing to middle January was higher than the normal value (1971 – 2001) (Fig. 1a). It was warmer in Ibaraki 2009 than in Ibaraki 2010 (Fig. 1a). In Fukuoka 2009 and Fukuoka 2010, the daily minimum temperature from sowing to late December was equal to the normal value (1971 – 2001). On the contrary, the daily minimum temperature from late January to early March in Fukuoka 2009 was higher than the normal value (1971 – 2001), while in Fukuoka 2010 the daily minimum temperature in January was lower than the normal value (1971 – 2001) (Fig. 1b). The daily minimum temperature was often below freezing point from middle December to early February commonly in Ibaraki 2009 and Ibaraki 2010, and it dropped below 0°C for six days even in middle March 2010 with the lowest at –5.6°C on 18 March (Fig. 2).

2. Growth properties

The timing of young spike development differed significantly among four NILs, being earliest in [Ppd-B1, Ppd-D1] and [Ppd-B1] followed by [Ppd-D1] and [ppd] with [ppd] the latest (Table 2). In Ibaraki 2009, some tillers of [Ppd-B1, Ppd-D1] and [Ppd-B1] were withered and died by frost injury on young whole spikes on 8 February. In Ibaraki 2010, frost injuries on whole spike were also observed in [Ppd-B1, Ppd-D1] on 27 January and 17 February, and in three NILs, [Ppd-B1, Ppd-D1], [Ppd-B1], and [Ppd-D1] on 23 March (Table 2, Fig. 2). In Fukuoka...
Table 2. Young spike development and frost injury on young whole spikes of NILs.

| Cultivation   | Date              | NILs       | [Ppd-B1,Ppd-D1] | [Ppd-B1] | [Ppd-D1] | [ppd] |
|---------------|-------------------|------------|----------------|----------|----------|-------|
|               |                   | Stage      | Frost injury   | Stage    | Frost injury | Stage | Frost injury |
| Ibaraki 2009  | 27 January 2010   | IX e       | IX m           | VII      | VI        |       |
|               | 8 February 2010    | IX m       | IX m           | VII      | VI        |       |
|               | 8 March 2010       | X          | X              | X        | IX e      |       |
| Ibaraki 2010  | 16 December 2010   | VII e      | VII e          | VI       | < V       |       |
|               | 6 January 2011     | VII        | VII            | VII l    | VI        |       |
|               | 27 January 2011    | IX e       | IX e           | VII l    | VI        |       |
|               | 17 February 2011   | IX l       | IX m           | IX e     | VII l     |       |
|               | 8 March 2011       | X <        | X <            | X        | IX e      |       |
|               | 23 March 2011      | X <        | X <            | X <      | X         |       |
| Fukuoka 2009  | 27 January 2010   | IX e       | IX e           | VII l    | VI        |       |
|               | 10 February 2010   | IX m       | IX m           | IX e     | VII l     |       |
|               | 26 February 2010   | X          | X              | IX l     | IX e      |       |
| Fukuoka 2010  | 8 January 2011     | VI         | VII e          | VI       | < V       |       |
|               | 3 February 2011    | IX e       | IX e           | VII l    | < V       |       |
|               | 24 February 2011   | X          | IX l           | IX m     | VII       |       |
|               | 9 March 2011       | X          | X              | X        | IX l      |       |

General linear mixed model

NILs ***
Date ***
Cultivation ns
NILs×Date ns
Tukey HSD test a a b c

“+” indicate the occurrence of frost injury on young whole spikes.
Statistical analysis was performed with numerical values converted from the growth stage of young spikes, V (initiation of spikelet primordia is not observed) = 5, VI (spikelet primordia initiate at the middle part of spike) = 6, VIIe (spikelet primordia initiate at the basal part of spike) = 7, VIIl (double ridges become less distinct) = 8, VIII (spikelet primordia begin to differentiate empty glumes, and terminal spikelet initiates) = 9, IXe (spikelet primordia differentiate into florets) = 10, IXm (first florets differentiate lemma, pelea, stamen and pistil) = 11, IXl (differentiation of lemma, pelea, stamen and pistil cover the florets) = 12, X (empty glume and awn begin to elongate) = 13, X<= 14), using general linear mixed model (Random effect : cultivation Fixed effects : NILs, date and interaction) in statistical analysis software JMP 8.0.2 (SAS Institute Inc.).

“***” indicates significance at 0.1% level, and “ns” indicates insignificant difference, and the different alphabetic characters indicate significant difference between NILs at 5% level, based on the Tukey honestly significant difference (HSD) test.

2009 and Fukuoka 2010, no frost injury on whole spike was observed (Table 2). Double ridge formation stage and terminal spikelet formation stage occurred earlier in the order of [Ppd-B1]> [Ppd-B1, Ppd-D1]> [Ppd-D1]> [ppd] (Table 3). The days from sowing to double ridge formation was shorter in Ibaraki where the sowing date was earlier, while the days from double ridge formation to terminal spikelet formation was shorter in Fukuoka where the mean temperature of that period was higher (Table 3, Table 4). The timing of jointing stage was earlier in the order of [Ppd-B1]> [Ppd-B1, Ppd-D1]> [Ppd-D1] = [ppd] while the order of heading was earlier in the order of [Ppd-B1, Ppd-D1]> [Ppd-B1]> [Ppd-D1]> [ppd] (Table 5). Maturity date was the same among three NILs, [Ppd-B1, Ppd-D1], [Ppd-B1], and [Ppd-D1], and the latest was [ppd] (Table 5).

3. Culm length and yield properties

Culm length in early heading NILs was shortest in [Ppd-B1, Ppd-D1] followed by [Ppd-D1] [Ppd-B1] and [ppd] in this order, though the order of [Ppd-B1] and [Ppd-D1] was inconsistent (Table 6). The difference between the shortest NIL [Ppd-B1, Ppd-D1] and the longest NIL [ppd] was 24cm on the average (Table 6). The average culm length of four NILs was shorter in Ibaraki 2010 than that in the other experiments (Table 6). Grain weight was lighter in early heading NILs (Table 6). The grain weight of [Ppd-B1, Ppd-D1] was lighter by 120 g m$^{-2}$
### Table 3. Date of double ridge formation stage and terminal spikelet formation stage and days of each growth period of NILs.

| Cultivation  | NILs                | Days Sowing - Double ridge formation stage | Days Double ridge formation stage - Terminal spikelet formation stage | Date Double ridge formation stage | Date Terminal spikelet formation stage |
|--------------|---------------------|--------------------------------------------|---------------------------------------------------------------------|----------------------------------|---------------------------------------|
| Ibaraki 2010 | [Ppd-B1,Ppd-D1]     | 43 x                                       | 54                                                                  | 16 December 2010                | 10 February 2011                      |
|              | [Ppd-B1]            | 43 x                                       | 53                                                                  | 16 December 2010                | 8 February 2011                       |
|              | [Ppd-D1]            | 53                                          | 60                                                                  | 28 December 2010                | 26 February 2011                      |
|              | [ppd]               | 94                                          | 37                                                                  | 6 February 2011                 | 15 March 2011                         |
| Fukuoka 2010 | [Ppd-B1,Ppd-D1]     | 63                                          | 24                                                                  | 21 January 2011                 | 13 February 2011                      |
|              | [Ppd-B1]            | 50 x                                       | 37                                                                  | 8 January 2011                  | 13 February 2011                      |
|              | [Ppd-D1]            | 63                                          | 41                                                                  | 21 January 2011                 | 2 March 2011                          |
|              | [ppd]               | 87                                          | 17                                                                  | 13 February 2011                | 2 March 2011                          |
| Average Ibaraki |                  | 58                                          | 51                                                                  |                                   |                                       |
| Average Fukuoka |                | 66                                          | 29                                                                  |                                   |                                       |
| [Ppd-B1,Ppd-D1] | 53 bc             | 39 b                                       | 16                                                                  |                                   |                                       |
| [Ppd-B1]     | 46 c               | 45 b                                       | 18                                                                  |                                   |                                       |
| [Ppd-D1]     | 58 b               | 51 a                                       | 19                                                                  |                                   |                                       |
| [ppd]        | 90 a               | 27 a                                       | 14                                                                  |                                   |                                       |
| ANOVA Cultivation |                   | ***                                        | ***                                                                 | ***                              | ***                                   |
| NILs         | ***                | ***                                        | ***                                                                 | ***                              | ***                                   |
| NILs×Cultivation |                 | ***                                        | *                                                                   |                                   |                                       |

"x": The double ridge has been already formed on the first observation date, and thus this date is indicated.

"***" and "*" indicate significance at 0.1% and 5% level respectively, and the different alphabetic characters indicate significant difference between NILs at 5% level, based on the Tukey HSD test.

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### Table 4. Mean temperature during each growth period of NILs.

| Cultivation  | NILs                | Sowing - Double ridge formation stage | Double ridge formation stage - Terminal spikelet formation stage | Terminal spikelet formation stage - Heading | Heading - Maturity |
|--------------|---------------------|--------------------------------------|-----------------------------------------------------------------|---------------------------------------------|---------------------|
| Ibaraki 2010 | [Ppd-B1,Ppd-D1]     | 9.6                                  | 3.4                                                             | 7.3                                         | 17.0                |
|              | [Ppd-B1]            | 9.6                                  | 3.4                                                             | 7.3                                         | 17.1                |
|              | [Ppd-D1]            | 8.9                                  | 3.5                                                             | 8.1                                         | 17.2                |
|              | [ppd]               | 6.3                                  | 5.2                                                             | 9.8                                         | 18.0                |
| Fukuoka 2010 | [Ppd-B1,Ppd-D1]     | 6.3                                  | 3.6                                                             | 8.7                                         | 17.2                |
|              | [Ppd-B1]            | 7.4                                  | 3.1                                                             | 8.8                                         | 17.2                |
|              | [Ppd-D1]            | 6.3                                  | 6.0                                                             | 9.0                                         | 17.3                |
|              | [ppd]               | 5.6                                  | 9.0                                                             | 9.7                                         | 18.5                |
| Average Ibaraki 2010 |                | 8.6                                  | 3.9                                                             | 8.1                                         | 17.3                |
| Average Fukuoka 2010 |             | 6.4                                  | 5.4                                                             | 9.0                                         | 17.6                |
| [Ppd-B1,Ppd-D1] | 8.0 ab             | 3.5 c                                | 8.0 c                                                            | 17.1 d                                      |
| [Ppd-B1]     | 8.5 a               | 3.2 d                                | 8.1 bc                                                           | 17.2 c                                      |
| [Ppd-D1]     | 7.6 b               | 4.7 b                                | 8.6 b                                                            | 17.3 b                                      |
| [ppd]        | 5.9 c               | 7.1 a                                | 9.7 a                                                            | 18.3 a                                      |
| ANOVA NILs   | ***                 | ***                                  | ***                                                             | ***                                         |
| Cultivation  | ***                 | ***                                  | ***                                                             | ***                                         |
| NILs×Cultivation |                  | ***                                  | *                                                               | ***                                         |

"***", "**", and "*" indicates significance at 0.1%, 1%, and 5% level respectively.

Different alphabetic characters indicate significant difference between NILs at 5% level, based on the Tukey HSD test.
in Ibaraki 2009, by 193 g m\(^{-2}\) in Fukuoka 2009, and by 89 g m\(^{-2}\) in Fukuoka 2010, compared with the highest-yielding NIL [ppd] (Table 6). Grain number per m\(^{2}\) was smaller in early heading NILs significantly, whereas 1,000-grain weight and spike number per m\(^{2}\) was not affected (Table 6). Grain number per spike was also lower in early heading NILs, though the difference was insignificant. Grain weight correlated significantly with grain number per m\(^{2}\), and grain number per m\(^{2}\) was correlated significantly with grain number per spike (Fig. 3). Meanwhile, there was no significant correlation between grain weight and 1000-grain weight, and also between grain number per m\(^{2}\) and spike number per m\(^{2}\). Grain number per spike was correlated significantly both with spikelet number per spike and grain number per spikelet (Fig. 3). Late maturity NIL [ppd] was severely damaged by heavy rain during grain filling stage in Ibaraki 2010, and yield and yield properties could not be evaluated for NIL [ppd].

**Discussion**

Tanio and Kato (2007) detected that photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* accelerate double ridge formation and subsequent growth of young spikes, the *Ppd-B1a* allele has a larger effect than *Ppd-D1a*, and *Ppd-B1a* and *Ppd-D1a* have additive effect, under controlled 8 hr of daylight and 20ºC, using NILs in a genetic background of the late-maturity cultivar Haruhikari. In this study, the acceleration effect of *Ppd-B1a* and *Ppd-D1a* was confirmed under field condition (Table 2), and NILs with photoperiod-insensitive alleles had shorter periods of

| Cultivation | NILs | Days | Date |
|-------------|------|------|------|
|             |      | Sowing-Jointing | Jointing-Heading | Heading-Maturity | Jointing | Heading | Maturity |
| Ibaraki     | [Ppd-B1,Ppd-D1] | 116 | 37 | 64 | 2 March 2010 | 8 April 2010 | 11 June 2010 |
| 2009        | [Ppd-B1] | 111 | 44 | 63 | 25 February 2010 | 9 April 2010 | 11 June 2010 |
|             | [Ppd-D1] | 123 | 41 | 54 | 9 March 2010 | 18 April 2010 | 11 June 2010 |
|             | [ppd] | – | – | 49 | – | 28 April 2010 | 16 June 2010 |
| Ibaraki     | [Ppd-B1,Ppd-D1] | 129 | 33 | 59 | 14 March 2011 | 16 April 2011 | 14 June 2011 |
| 2010        | [Ppd-B1] | 130 | 34 | 57 | 15 March 2011 | 18 April 2011 | 14 June 2011 |
|             | [Ppd-D1] | 138 | 27 | 56 | 23 March 2011 | 19 April 2011 | 14 June 2011 |
|             | [ppd] | 137 | 34 | 56 | 22 March 2011 | 24 April 2011 | 20 June 2011 |
| Fukuoka     | [Ppd-B1,Ppd-D1] | 92 | 30 | 64 | 20 February 2010 | 22 March 2010 | 25 May 2010 |
| 2009        | [Ppd-B1] | 93 | 33 | 60 | 21 February 2010 | 25 March 2010 | 25 May 2010 |
|             | [Ppd-D1] | 100 | 31 | 56 | 28 February 2010 | 31 March 2010 | 26 May 2010 |
|             | [ppd] | – | – | 50 | – | 12 April 2010 | 1 June 2010 |
| Fukuoka     | [Ppd-B1,Ppd-D1] | 101 | 38 | 51 | 28 February 2011 | 6 April 2011 | 28 May 2011 |
| 2010        | [Ppd-B1] | 100 | 39 | 52 | 27 February 2011 | 7 April 2011 | 29 May 2011 |
|             | [Ppd-D1] | 108 | 34 | 50 | 7 March 2011 | 10 April 2011 | 30 May 2011 |
|             | [ppd] | 108 | 41 | 50 | 7 March 2011 | 17 April 2011 | 6 June 2011 |
| Average     | Ibaraki 2009 | – | – | 56 | |
|             | Ibaraki 2010 | 133 | 33 | 57 | |
|             | Fukuoka 2009 | – | – | 58 | |
|             | Fukuoka 2010 | 104 | 38 | 51 | |
|             | [Ppd-B1,Ppd-D1] | 110 b | 34 c | 60 b | |
|             | [Ppd-B1] | 108 b | 38 b | 58 b | |
|             | [Ppd-D1] | 117 a | 33 a | 54 b | |
|             | [ppd] | – | – | 51 a | |
| ANOVA       | NILs | *** | *** | *** |
|             | Cultivation | *** | *** | *** |
|             | NILs×Cultivation | ns | *** | *** |

“-”; Not recorded.

“***” indicates significance at 0.1% level, and different alphabetic characters indicate significant difference between NILs at 5% level, based on the Tukey HSD test.
vegetative growth before the double ridge formation stage (Table 3). In contrast, additive effect of Ppd-B1a and Ppd-D1a on young spike development was not detected in this study (Table 2). These results suggest that their additive effect on young spike development is not detectable under the field condition of central and southwestern Japan, where the day length around young spike development stage is longer than 8 hrs (Fig. 1, Table 3). It was reported that frost injury of young spikes was caused by low temperature during the glumaceous flower developing stage (Uchijima, 1978). In this study, frost injury on young whole spikes was observed in [Ppd-B1, Ppd-D1] and [Ppd-B1] in Ibaraki 2009, and in [Ppd-B1, Ppd-D1], [Ppd-B1], and [Ppd-D1] in Ibaraki 2010 (Table 2). These results indicated that young spike development was strongly accelerated in NILs carrying Ppd-B1a and Vrn-A1, and this increased the risk of frost injury in colder regions such as Ibaraki. Jointing stage was accelerated by Ppd-B1a, whereas it was not affected by Ppd-D1a (Table 5). The effect of each gene on heading time and their additive effect were confirmed in this study, as also reported under controlled short-day condition (Tanio and Kato, 2007). Furthermore, Ppd-B1a and Ppd-D1a individually accelerated the maturity with similar magnitude and had no additive effect (Table 5).

In the previous studies using European wheat cultivars, early-maturity wheat with photoperiod-insensitive alleles of Ppd-1 had shorter culms (Worland et al., 1998), fewer spikes (Worland et al., 1998; Foulkes et al., 2004), fewer spikelets (Worland et al., 1994, 1998), and lighter grain

**Table 6. Culm length, yield and yield properties of NILs.**

| Cultivation | NILs | Culm length (cm) | Grain weight (g m⁻²) | Grain number per m² | 1000-grain weight (g) | Spike number per m² | Spikelet number per spike | Grain number per spikelet | Test weight (g L⁻¹) |
|-------------|------|------------------|----------------------|------------------|---------------------|-------------------|-------------------------|-------------------------|-------------------|
| Ibaraki 2009 | [Ppd-B1, Ppd-D1] | 71 c | 309 b | 12935 c | 30.7 b | 365 b | 35.4 a | 15.5 b | 2.3 a | 806 b |
| | [Ppd-B1] | 88 b | 369 ab | 13866 b | 34.1 a | 376 b | 36.9 a | 15.8 b | 2.3 a | 826 a |
| | [Ppd-D1] | 86 b | 385 ab | 15612 b | 31.8 b | 403 b | 38.7 a | 16.8 a | 2.5 a | 814 ab |
| | [ppd] | 92 a | 429 a | 17767 a | 30.7 b | 451 b | 39.2 a | 17.7 a | 2.2 a | 825 a |
| Ibaraki 2010 | [Ppd-B1, Ppd-D1] | 66 c | 367 a | 10269 a | 35.7 b | 375 a | 27.4 a | 13.7 b | 2.0 a | 821 ab |
| | [Ppd-B1] | 81 b | 368 a | 10118 a | 37.1 a | 374 a | 27.1 a | 13.6 b | 2.0 a | 823 a |
| | [Ppd-D1] | 81 b | 429 a | 11967 a | 36.0 b | 366 a | 32.7 a | 15.5 a | 2.1 a | 811 b |
| | [ppd] | 87 a | - | - | - | - | - | - | - | - |
| Fukuoka 2009 | [Ppd-B1, Ppd-D1] | 70 c | 270 b | 7921 b | 33.9 b | 403 a | 19.7 c | - | - | 814 a |
| | [Ppd-B1] | 84 b | 336 ab | 9320 b | 36.0 a | 439 a | 21.3 bc | - | - | 817 a |
| | [Ppd-D1] | 83 b | 377 ab | 10872 ab | 34.7 b | 402 a | 27.1 ab | - | - | 817 a |
| | [ppd] | 104 a | 463 a | 13348 a | 34.7 b | 412 a | 32.4 a | - | - | 794 b |
| Fukuoka 2010 | [Ppd-B1, Ppd-D1] | 72 c | 407 b | 12906 b | 31.6 b | 370 a | 34.9 a | 15.5 c | 2.3 a | 805 a |
| | [Ppd-B1] | 90 ab | 442 ab | 13114 b | 33.9 a | 368 a | 35.7 a | 15.7 c | 2.3 a | 805 a |
| | [Ppd-D1] | 87 b | 467 a | 14866 ab | 32.2 b | 351 a | 42.4 a | 17.2 b | 2.5 a | 801 ab |
| | [ppd] | 92 a | 496 a | 15878 a | 31.3 b | 380 a | 41.8 a | 18.7 a | 2.2 a | 790 b |
| Average | [Ppd-B1, Ppd-D1] | 70 c | 338 a | 11008 b | 33.0 b | 379 a | 29.4 a | 14.9 b | 2.2 a | 811 a |
| | [Ppd-B1] | 86 b | 370 a | 11604 ab | 35.3 a | 389 a | 30.2 a | 14.5 b | 2.2 a | 818 a |
| | [Ppd-D1] | 84 b | 415 a | 13329 a | 33.7 ab | 380 a | 35.2 a | 16.5 a | 2.3 a | 811 a |
| | [ppd] | 94 a | - | - | - | - | - | - | - | - |
| Ibaraki 2009 | 84 a | 373 a | 15022 a | 31.8 b | 399 ab | 37.6 a | 16.4 a | 2.3 a | 818 a |
| Ibaraki 2010 | 79 b | - | - | - | - | - | - | - | - |
| Fukuoka 2009 | 85 a | 361 a | 10365 b | 34.8 a | 414 a | 25.1 b | - | - | 811 a |
| Fukuoka 2010 | 85 a | 453 a | 14191 a | 32.2 b | 368 b | 38.6 a | 16.8 a | 2.3 a | 801 b |

**ANOVA**

| NILs | Cultivation | NILs × Cultivation |
|------|-------------|-------------------|
| *** | ns | * | * | * | * | ns | ns | ** | ns | ns |
| ** | ns | * | * | * | * | ns | ns | * | ns | ns |
| * | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

- **; Not recorded.
- ****, ***, and * indicate significance at 0.1%, 1% and 5% level respectively.
- Different alphabetic characters indicate significant difference between NILs at 5% level, based on the Tukey HSD test.
weight (Foulkes et al., 2004), but an increased percentage of ripening and grain number (Worland et al., 1998; Foulkes et al., 2004). Foulkes et al. (2004) concluded that photoperiodic response genes have a neutral effect on grain yield. However, Worland and Law (1986) reported photoperiod-insensitive alleles decreased yield 5 – 10%; in contrast, Börner et al. (1993) and Worland (1996) reported a 15 – 35% increase. The effect of photoperiodic response genes on yield is likely different depending on the fields or cultivation method. In this study, using NILs with a genetic background of Japanese early-maturity wheat, Ppd-B1a and Ppd-D1a shortened the culm length and decreased grain weight, though the reduction of grain weight was insignificant (Table 6). There was a significant correlation between grain weight and grain number per m$^2$, which was correlated significantly with grain number per spike (Fig. 3). These results are consistent with those of Taya (1993) in that early-maturity breeding caused a decrease of grain number and yield. Furthermore, grain number per spike was correlated significantly both with

**Fig. 3.** Correlation among grain yield components.

- ◆ shows the data of [Ppd-B1, Ppd-D1], △ shows [Ppd-B1], □ shows [Ppd-D1], and ● shows [ppd].
- (a), (b), (c) and (d): All data except [ppd] in Ibaraki 2010 was plotted.
- (c) and (f): All data except four NILs in Fukuoka 2009 and [ppd] in Ibaraki 2010 was plotted.
- **“***”, and “**” indicate significance at 0.1%, and 1% level respectively, and “ns” indicates no significance.
spikelet number per spike and grain number per spikelet (Fig. 3).

The spikelet number per spike of standard sown wheat increased under warm temperatures conditions during the flag leaf initiation stage to the terminal spikelet formation stage (Zhang et al., 2007). The floret number per spike is likely related to the temperature during the terminal spikelet formation stage to the flowering stage (Fukushima et al., 2006). The number of spikelet per spike ranges from 10 to 25 in wheat (Rawson, 1970; Lucas, 1972; Kirby, 1974; Rahman and Wilson, 1977). In each spikelet, around 8 floret primordia are formed. Some of these will be aborted, and floret number per spike is determined (one to four florets per spikelet). Fertile florets ripe sufficiently, and grain number per spike is determined (Langer and Hanif, 1973; Whingwiri and Stern, 1982; Sibony and Pinthus, 1988). In this study, the mean temperature during the double ridge formation stage to the terminal spikelet formation stage of [Ppd-B1, Ppd-D1] was lower by 3.6°C than that of [ppd], and the mean temperature during the terminal spikelet formation stage to the flowering stage of [Ppd-B1, Ppd-D1] was lower by 1.7°C than that of [ppd] (Table 4). Furthermore, there was a significant correlation between the mean temperature during the double ridge formation stage to terminal spikelet formation stage and spikelet number per spike, and also between mean temperature during the terminal spikelet formation stage to the heading stage and grain number per spikelet (Fig. 4). These results presumed that the temperature around the young spike development stage and stem elongation stage affected the grain number per spike.

In conclusion, in the genetic background of extremely early-maturity wheat cultivars with spring growth habit, wheat photoperiod-insensitive alleles of Ppd-1 genes accelerated young spike formation and subsequent growth, which caused the decrease in the grain number per spike. Meanwhile, Foulkes et al. (2004) indicated that the decrease of yield in early-maturity wheat was caused by the reduction in green area index at the flowering stage and reduced accumulation of above-ground dry matter during the stem elongation stage to the flowering stage. In this study, biomass yields of above ground should vary with the...
NILs, because the culm lengths of the early heading NILs were significantly short, though the spike numbers were not different (Table 6). Accordingly, the difference in biomass yield between the NILs presumably influenced the difference in grain yield. Additionally, frost injury on young whole spike decreased grain yield in some cases (Batten and Khan, 1987; Sato et al., 1993). In this study, early heading NILs were damaged by frost injury on whole spikes in Ibaraki (Table 2). There was a possibility that frost injury caused the decrease in grain weight of these NILs in Ibaraki, though it was not clearly indicated in this study.

Lastly, although [Ppd-D1] was an early-maturity line, this NIL showed little frost injury and relatively high yield. These results support the fact that wheat cultivars with photoperiod-insensitive allele Ppd-D1a and sensitive allele Ppd-B1b have been selected in central regions of Japan where spring type wheat cultivars have been commonly grown (Seki et al., 2011). Recently, some winter type cultivars with vernalization requirement have been bred in these regions such as Iwainodaichi and Satonosora. It is necessary to elucidate the interaction between vernalization response and photoperiodic response, for the stable production of wheat under changing environments.

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