Allelic Variation of *Rht-1*, *Vrn-1* and *Ppd-1* in Korean Wheats and Its Effect on Agronomic Traits

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ABSTRACT The allelic variations at the *Rht-1*, *Vrn-1* and *Ppd-1* of 410 Korean wheat cultivars, including 111 Korean experimental lines, 238 Korean landraces and 61 North Korean collections, were investigated to provide the information of plant height and heading date and to elucidate the relationship between those traits and allelic variation of these genes because earliness is major consideration in Korean wheat production. All Korean wheats displayed *vrn-A1* and *Ppd-A1b* alleles, while *Rht-B1a*, *Rht-D1a*, *vrn-B1*, *Vrn-D1*, *Ppd-B1b* and *Ppd-D1a* alleles were also predominantly found. Most Korean wheats carried both *Rht-B1a* and *Rht-D1a* alleles, both *vrn-B1* and *Vrn-D1* alleles, or both *Ppd-B1b* and *Ppd-D1a* alleles. The *Rht-B1a*, *vrn-D1*, *Ppd-B1b* and *Ppd-D1b* alleles were found to exhibit longer culm and spike length than their counterpart alleles. The *Rht-B1a* allele also showed longer spike length than *Rht-B1b*. *Vrn-B1b* and *vrn-D1* alleles exhibited longer days to heading date than their counterpart alleles at the *Vrn-B1* and *Vrn-D1* loci. Lines carrying both *Rht-B1b* and *Rht-D1b* alleles displayed shorter culm and longer spike length and days to heading date than any other combination of alleles at the *Rht-B1* and *Rht-D1* loci. In contrast, lines carrying both *Ppd-B1b* and *Ppd-D1b* alleles exhibited longer culm and spike length than any other combination of alleles at the *Ppd-B1* and *Ppd-D1* loci.

Keywords Korean wheat, Allele, *Rht-1*, *Vrn-1*, *Ppd-1*

INTRODUCTION

Semi-dwarfing genes, especially *Rht-B1b* and *Rht-D1b*, have been used as the main components of the green revolution, as these genes are insensitive to exogenous gibberellic acid (GA3, Tosovic-Maric *et al.* 2008). The *Rht-B1* and *Rht-D1* loci located on chromosome 4B and 4D, respectively, encode DELLA proteins, for which polymorphisms result in different alleles which have different effects on plant height (Börner *et al.* 1996). These semi-dwarfing genes have influenced the reduction of plant height and increased grain yield of wheat grown in favorable climatic conditions, albeit the grain yield was found to be reduced under high temperature and drought conditions (Flintham *et al.* 1997).

The flowering time in wheat, though complex in character, is mainly controlled by vernalization (*Vrn*) and

photoperiod (*Ppd*) response (Kamran *et al.* 2014). These response genes hasten or delay flowering in response to environmental stimuli, by which floral initiation is determined through interactions with growth temperatures (Kamran *et al.* 2014). The *Vrn-1* and *Ppd-1* alleles include three orthologous genes at each locus, and are located on the homoeologous group 5 and 2 chromosomes, respectively (Kamran *et al.* 2014). The *Vrn-1* alleles are known to have differential effects on flowering time, plant height and various yield components (Stelmakh 1998). Spring habit *Vrn-A1a* allele is predominant in wheat cultivars grown at high latitudes, while the *Vrn-D1* allele is predominantly found in cultivars from Asia and in Japanese and Chinese landraces, with particular prevalence in sub-tropical regions where the growing season is longer (Kamran *et al.* 2014). Photoperiod insensitive alleles, *Ppd-A1a*, *Ppd-B1a* and *Ppd-D1a*, were observed to positively affect the yield

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when wheat was grown in summer under hot and dry conditions, because these alleles displayed earlier flowering date and larger grain than the photoperiod sensitive alleles (Snape *et al.* 2001).

The wheat consumed in Korean has mostly been imported from Australia, Canada and the United States, though the per capita wheat consumption increased to 32.3 kg in 2013 (MAF 2014). The Korean government has recently been pushed to increase wheat production up to 200,000 MT by 2020, which was targeted to increase the country's self-sufficiency rate from its current level of 1.2% to 10%. Korean wheat has typically been cultivated by double-cropping with rice, as the time of the wheat harvest is overlapped with the rainy seasons and the time for rice transplanting in Korea. Therefore, earliness, as well as grain yield, have been the most important criteria considered in Korean wheat breeding programs. Korean wheat breeders have been focusing on achieving improved grain yield through the selection of wheat lines with reduced plant height and shortened days to flowering since the 1970s. Consequently, the Korean wheat cultivars have narrow genetic variations in semi-dwarf genes, growth habits and photoperiod response (Cho *et al.* 2015a, b). Therefore, the present study was conducted to investigate the distribution of the *Rht-1*, *Vrn-1* and *Ppd-1* alleles in Korean experimental lines and landraces using molecular markers, and to elucidate the relationship between agronomic traits and allelic variation of these genes.

MATERIALS AND METHODS

Plant materials

A total of 410 Korean experimental lines and landraces, including 111 Korean experimental lines, 238 Korean landraces and 61 North Korean collections, were kindly provided by the National Agrobiodiversity Center, National Academy of Agricultural Science of the Rural Development Administration (Suwon, Korea). Korean experimental lines contained wheat lines developed from 1950s to 2010s in National Institute of Crop Science (NICS), Rural Development Administration (RDA). Korean landraces and North Korean collections mainly collected in the past

1950s. These lines were sown in the Upland Crop Experimental Farm of the NICS, RDA in 2013 on 50% clay loam soil. The seeds were sown on October 25 with each plot consisting of three 2-m rows spaced 25 cm apart which were performed in randomized complete blocks with 3 replicates, and the plots were combine-harvested on June 20. Fertilizer was applied at a ratio of 5:7:5kg/10a (N:P:K) before sowing, and the plots were stringently controlled for weeds, insects and disease. No supplemental irrigation was applied.

Agronomic traits

Agronomic traits were recorded as follows: days to heading date as the number of days from last October 25 to exertion of the spike from the stem in 50 % of a plot; culm length as the length from ground level to the base of the spike; and spike length as the length of the spike excluding the awn. Culm length and spike length were determined from a sample of 10 main tillers randomly selected from each plot at maturity.

PCR conditions

Five seeds of each line were grown in a temperature-controlled greenhouse to analyze the allelic variations of *Rht-B1*, *Rht-D1*, *Vrn-A1*, *Vrn-B1*, *Ppd-A1*, *Ppd-B1* and *Ppd-D1*. Two weeks after germination, single leaves taken from five plants of each cultivar were collected, bulked and snap-frozen in liquid nitrogen, and then stored at -80°C until needed. All plants were kept in the greenhouse for analysis of allelic variations. Genomic DNA was extracted from young leaf tissue (100 mg) using a genomic DNA prep kit (Solgent Co., Korea) according to the manufacturer's instructions. PCR for *Rht-B1* and *Rht-D1* was carried out using allele-specific markers according to the methods of Ellis *et al.* (2002) and Zhang *et al.* (2006). The primers for *Vrn-1* allele-specific markers and PCR followed the methods of Yan *et al.* (2004), Fu *et al.* (2005), Santra *et al.* (2009), Zhang *et al.* (2012), and Chen *et al.* (2013). For PCR of *Ppd-1*, the allele-specific markers and methods from Beales *et al.* (2007) and Bentley *et al.* (2011) were employed. Amplified PCR fragments were separated on 1.5% agarose gels, stained with ethidium bromide, and visualized using UV light.

Statistical analysis

Statistical analysis of the data was performed by SAS software (SAS Institute, NC, USA) using Fisher's least significant difference test (LSD), analysis of variance (ANOVA) and pair-wise t-test. Analysis of variance was conducted using the general linear model (GLM) procedure. The GLM procedure was used for the randomized complete block (RCB) analysis. Sources of variation in the model were considered to be fixed effects. PROC GLM was used to estimate the relative contribution of the tested loci for the measured traits of Korean wheats. Pearson correlations

between pairs of traits were determined from the least square means of wheat cultivars for each year separately, and over all environments, using the CORR procedure.

RESULTS

Genetic variations at *Rht-1*, *Vrn-1* and *Ppd-1* loci

Genetic variations at the *Rht-1*, *Vrn-1* and *Ppd-1* loci in the Korean wheats are shown in Fig. 1 and 2, and summarized in Table 1. Thirteen types of allelic composition

Table 1. Differences in culm length, spike length and days to heading date in Korean wheats according to different alleles at the *Rht-1*, *Vrn-1* and *Ppd-1* loci.

	No. line	Culm length (Cm)	Spike length (Cm)	Days to heading date
<i>Rht-B1a</i>	318	101.4a ²⁾	9.2a	182.1a
<i>Rht-B1b</i>	92	90.0b	8.7b	185.1a
<i>Rht-D1a</i>	322	102.3a	9.1a	183.3a
<i>Rht-D1b</i>	88	86.3b	9.0a	180.7a
<i>Rht-B1a Rht-D1a</i>	233	107.3a	9.2ab	182.7b
<i>Rht-B1a Rht-D1b</i>	85	87.5ab	8.9ab	180.4b
<i>Rht-B1b Rht-D1a</i>	90	90.2ab	8.7b	184.9b
<i>Rht-B1b Rht-D1b</i>	2	82.0b	10.5a	194.5a
<i>vrn-B1</i>	385	99.4a	9.1a	182.7b
<i>Vrn-B1a</i>	23	87.4a	8.3a	183.1b
<i>Vrn-B1b</i>	2	89.0a	9.0a	193.5a
<i>vrn-D1</i>	271	100.8a	9.3a	183.9a
<i>Vrn-D1a</i>	139	95.0b	8.7b	180.5b
<i>vrn-B1 vrn-D1</i>	250	102.0a	9.3a	184.0a
<i>vrn-B1 Vrn-D1a</i>	135	95.1a	8.7a	180.2a
<i>Vrn-B1a vrn-D1</i>	20	87.9a	8.3a	182.2a
<i>Vrn-B1a Vrn-D1a</i>	3	84.7a	8.7a	189.7a
<i>Vrn-B1b vrn-D1</i>	1	74.0a	8.0a	194.0a
<i>Vrn-B1b Vrn-D1a</i>	1	104.2a	10.0a	193.0a
<i>Ppd-B1a</i>	25	81.2b	8.2b	183.6a
<i>Ppd-B1b</i>	385	100.0a	9.1a	182.7a
<i>Ppd-D1a</i>	308	93.5b	8.8b	182.6a
<i>Ppd-D1b</i>	102	114.8a	9.7a	183.5a
<i>Ppd-B1a Ppd-D1a</i>	25	81.2c	8.3c	183.6a
<i>Ppd-B1b Ppd-D1a</i>	283	94.6b	8.9b	182.5a
<i>Ppd-B1b Ppd-D1b</i>	102	114.8a	9.7a	183.5a

²⁾Values followed by the same letters within the same characteristics are not significantly different at $P < 0.05$.

were identified among the 410 Korean wheats. Two types of alleles were found at the *Rht-B1*, *Rht-D1*, *Vrn-D1*, *Ppd-B1* and *Ppd-D1* loci, and three types of alleles found at the *Vrn-B1* locus, while all of the Korean wheats tested displayed *vrn-A1* and *Ppd-A1b* alleles. The Korean wheats

showed higher frequency of *Rht-B1a*, *Rht-D1a*, *vrn-B1*, *Vrn-D1*, *Ppd-B1b* and *Ppd-D1a* alleles (77.6, 78.5, 93.9, 66.1, 93.9 and 75.1%, respectively) than their counterpart alleles at the same loci. Among the 111 Korean experimental lines tested, 85 (76.6%) carried *Rht-B1a* and 77 (69.4%)

Fig. 1. PCR patterns of *Rht-B1* (A), *Rht-D1* (B), *Vrn-A1* (C), *Vrn-B1* (D), *Vrn-D1* (E), *Ppd-A1* (F), *Ppd-B1* (G) and *Ppd-D1* (H) alleles in Korean wheats. M, molecular size marker.

Fig. 2. Frequency of *Rht-I* (A), *Vrn-I* (B) and *Ppd-I* (C) alleles in 410 Korean wheats, and frequency of *Rht-I* (D), *Vrn-I* (E) and *Ppd-I* (F) alleles in 111 Korean experimental lines according to the year released.

displayed *Rht-D1a* at the *Rht-B1* and *Rht-D1* loci, respectively. The *vrn-B1*, *Ppd-B1b* and *Ppd-D1a* alleles were predominantly found at the *Vrn-B1* and *Ppd-1* loci (91.9, 83.8 and 94.6%, respectively). The Korean experimental lines showed similar frequency of *Vrn-D1* and *Vrn-D1a* alleles (56.8 and 43.2%, respectively). The *Rht-D1a*, *vrn-B1*, *Vrn-D1* and *Ppd-B1b* alleles (83.6, 93.4, 82.0 and 98.4%, respectively) were found to be dominant in the 61 North Korean collections. In contrast, the North Korean collections showed similar frequencies of the *Rht-B1a* and *Rht-B1b* alleles (59.0 and 41.0%, respectively), while the frequencies of the *Ppd-D1a* and *Ppd-D1b* alleles (70.5 and 29.5%, respectively) were similar to those of Korean landraces.

Korean experimental lines released after 2005 exhibited lower frequency of the *Rht-B1a* allele (57.1%) and higher frequency of the *Rht-D1a* allele (92.9%) than the experimental lines released before 2004 (> 77.8% and < 69.4%, respectively). However, the experimental lines mainly possessed the *vrn-B1* allele (> 85.7%) regardless of the year released. The frequency of the *Vrn-B1a* allele increased in lines released after 2005, while only one line, Suwon229, had the *Vrn-B1b* allele. The Korean experimental lines released between 1991 and 2004 displayed higher frequency of the *Vrn-D1a* (58.3%) and *Ppd-B1a* (83.3%) alleles than the experimental lines released before 1990 or after 2005 (< 39.3% and < 21.3%, respectively). Only two lines released after 2005, Iksan311 and Iksan313, contained the *Ppd-B1a* allele. All Korean experimental lines were found to possess the *Ppd-D1a* allele, except six lines released before 1990.

The frequency of lines carrying both the *Rht-B1a* and *Rht-D1a* alleles was higher (56.8%) than that of any other combination (< 22.0%). In particular, those alleles were dominantly found in Korean landraces (64.7%). In contrast, the experimental lines showed higher frequency of both *Rht-B1a* and *Rht-D1b* alleles (30.6%) than the other lines, while North Korean collections exhibited a higher frequency of both *Rht-B1b* and *Rht-D1a* alleles (37.7%). Only two North Korean collections, Seuyuk28 and Seuyuk44, carried both the *Rht-B1b* and *Rht-D1b* alleles. Most of the Korean wheats carried either both the *vrn-B1* and *Vrn-D1* alleles (61.0%), or both the *vrn-B1* and

Vrn-D1a alleles (32.9%). Only 20 lines were found to carry both the *Vrn-B1a* and *Vrn-D1* alleles, while three carried both the *Vrn-B1a* and *Vrn-D1a* alleles. One North Korean collection, IT215959, displayed both the *Vrn-B1b* and *vrn-D1* alleles, while one Korean experimental line, Suwon229, carried both the *Vrn-B1b* and *Vrn-D1a* alleles. Though about 70% of Korean wheats carried both the *Ppd-B1b* and *Ppd-D1a* alleles, only 6.1% carried both the *Ppd-B1a* and *Ppd-D1a* alleles. Only one of the North Korean collections, IT246394, and 6 Korean landraces carried both the *Ppd-B1a* and *Ppd-D1a* alleles. Six of the Korean experimental lines displayed both the *Ppd-B1b* and *Ppd-D1b* alleles.

Relationship between allelic variations and agronomic traits

The ranges of the culm and spike length, and days to heading date of 410 Korean wheats are shown in Fig. 3. The mean values of the agronomic traits were 98.8 cm for culm length, 9.1 cm for spike length and 182.8 days for days to heading date. The culm and spike length, and days to heading date of the Korean wheats ranged from 48.8 – 161.7 cm, 4.9 – 13.3 cm and 162.0 – 207.0 days, respectively. The Korean experimental lines showed shorter culm and spike length (83.7 and 8.3 cm, respectively) than the Korean landraces (104.2 and 9.4 cm, respectively) and North Korean collections (105.5 and 9.1 cm, respectively). Regarding days to heading date, the experimental lines showed intermediate values (187.6) compared to the Korean landraces (177.1) and North Korean collections (196.0). Of the 111 experimental lines, those released before 1990 displayed longer culm and spike length, as well as days to heading date (92.0 cm, 8.7 cm and 189.7 days, respectively) than those released after 1991 (< 78.8 cm, < 8.0 cm and < 186.4 days, respectively). The culm lengths of Korean experimental lines released after 2005 were shorter than those of lines released between 1991 and 2004, though no significant differences were observed in spike length and days to heading date between those lines. Culm length positively correlated with spike length in the 410 Korean wheats and 111 Korean experimental lines ($r = 0.444^{***}$ and $r = 0.363^{***}$, respectively), while culm length also displayed a positive correlation with days to

Fig. 3. Frequency of culm length (A), spike length (B) and days to heading date (C) alleles in 410 Korean wheats, and frequency of culm length (D), spike length (E) and days to heading date (F) alleles in 111 Korean experimental lines according to the year released. Bars indicates ranges of Korean wheats.

heading date in the 111 experimental lines ($r = 0.258^{**}$).

Korean wheats with allelic variation at the *Rht-1*, *Vrn-1* and *Ppd-1* loci exhibited significant differences in culm and spike length, as well as days to heading date (Table 1). Those carrying the *Rht-B1a* allele exhibited longer culm and spike length (101.4 and 9.2 cm, respectively) than those with *Rht-B1b* (90.0 and 8.7 cm, respectively). Those carrying the *Rht-D1a* allele also displayed longer culm length (102.3 cm) than those with the *Rht-D1b* allele (86.3 cm), but no significant difference was observed in spike length. There were also no significant differences of days to heading date in the Korean wheats according to allelic variations at the *Rht-B1* and *Rht-D1* loci. Two Korean experimental lines released before 1990, Seuyuk28 and Seuyuk28, which carried the *Rht-B1b* and *Rht-D1b* alleles displayed shorter culm length (82.0 cm) but longer spike length and days to heading date (10.5 cm and 194.5 days, respectively) than those with the combined alleles of both *Rht-B1* and *Rht-D1* (> 87.5 cm, > 9.2 cm and < 184.9 days, respectively). One Korean experimental line and one North Korean collection, Suwon 229 and IT215959, which

carried the *Vrn-B1b* allele showed longer days to heading date (193.5) than the other Korean wheats carrying the *vrn-B1* and *Vrn-B1a* allele (182.7 and 183.1, respectively). However, significant differences of culm and spike length among Korean wheats were not observed according to allelic variations at the *Vrn-B1* locus. Korean wheats carrying the *Vrn-D1a* allele showed shorter culm and spike length (95.0 and 8.7 cm, respectively), as well as shorter days to heading date (180.5) than those carrying the *vrn-D1* allele (100.8 cm, 9.3 cm and 183.9 days, respectively). However, no significant differences of culm and spike length, as well as days to heading date, were found among the Korean wheats with a combination of both *Vrn-B1* and *Vrn-D1* alleles. Korean wheats carrying the *Ppd-B1b* or *Ppd-D1b* allele displayed longer culm and spike length than those with the *Ppd-B1a* or *Ppd-D1a* allele, though no significant differences in days to heading date were observed. Korean wheats carrying both the *Ppd-B1b* and *Ppd-D1b* alleles also showed longer culm and spike length than those with both the *Ppd-B1a* and *Ppd-D1a* or *Ppd-B1b* and *Ppd-D1a* alleles.

DISCUSSION

Korean wheat cultivars, as well as Chinese and Japan wheats, were found to display low frequency of the *Rht-B1b* allele and high frequency of the *Rht-D1b* allele (Yamada 1990; Zhang *et al.* 2006; Cho *et al.* 2015a). Korean experimental lines also exhibited similar frequency of *Rht-B1* and *Rht-D1* alleles as Korean wheat cultivars (Cho *et al.* 2015a). Only two North Korean collections showed both *Rht-B1b* and *Rht-D1b* alleles, which was similar frequency as previous reports (Yamada 1990; Zhang *et al.* 2006; Tosovic-Maric *et al.* 2008). The frequencies of *Rht-B1b* and *Rht-D1b* alleles increased when compared with the landraces and leading cultivars in China (Zhang *et al.* 2006). Korean experimental lines showed higher frequency of *Rht-B1b* and *Rht-D1b* alleles than Korean landraces and North Korean collections. Therefore, the Korean experimental lines displayed shorter culm length than the others. The *Rht-B1b* and *Rht-D1b* alleles were reported to reduce plant height by 15% and increase yield by 24% (Flintham *et al.* 1997). The experimental lines released after 2005 showed higher frequency of the *Rht-B1b* allele than those released before 2004, but the frequency of the *Rht-D1b* allele was decreased from 44.7% to 7.1%. In German wheat cultivars, those carrying the *Rht-D1b* were reported to be more susceptible to Fusarium head blight (Knopf *et al.* 2008). However, no significant difference in the culm and spike length were found, although significant difference in the frequency of the *Rht-D1b* allele was observed. The effects of the *Rht-B1b* and *Rht-D1b* alleles on plant height and grain yield are known to be influenced by the growth conditions (Flintham *et al.* 1997; Korzun *et al.* 1998). However, the *Rht8* dwarfing gene, which also reduced plant height by around 10%, has higher resistance to environments (Worland and Law 1986; Börner *et al.* 1993; Worland *et al.* 1998). Therefore, allelic variations of Korean wheats at the *Rht8* locus and other dwarfing genes should be evaluated to elucidate adaptations for improving the grain yield, including reducing the height for lodging resistance and increasing the disease resistance to factors such as Fusarium head blight.

Allelic variations at the *Vrn-1* loci have differential

effects on the flowering time, plant height and grain yield (Stelmakh 1998). Seven different *Vrn-1* alleles were reported in spring wheats with facultative growth habits: *Vrn-A1a*, *Vrn-A1b* and *Vrn-A1c* at the *Vrn-A1* locus; *Vrn-B1a* and *Vrn-B1b* at the *Vrn-B1* locus; and *Vrn-D1a* and *Vrn-D1b* at the *Vrn-D1* locus (Kamran *et al.* 2014). Herein, six different alleles were found in the 410 Korean wheats, all of which possessed the *vrn-A1* allele, while three types of alleles were found at the *Vrn-B1* locus and two types at the *Vrn-D1* locus. About 60% of the Korean wheats possessed the *vrn-A1*, *vrn-B1* and *vrn-D1* alleles, which was a higher frequency than in Korean wheat cultivars (Cho *et al.* 2015b). The *Vrn-B1a* and *Vrn-B1b* alleles were rarely found in the Korean lines, and no significant differences were observed in the frequency of *Vrn-B1* alleles among the 410 wheats tested. However, the North Korean wheat collections showed higher frequency of the *vrn-D1* allele and lower frequency of the *Vrn-D1a* allele than the Korean experimental lines and landraces. Conversely, the frequency of the *Vrn-D1a* allele increased in the Korean experimental lines compared to Korean landraces and North Korean collection. The *vrn-A1* allele was found to have moderate occurrence in Chinese wheats, while the *Vrn-A1a* and *Vrn-B1a* alleles appeared frequently in Canadian spring wheats, the *Vrn-A1b* or *Vrn-A1c* alleles have rarely been observed, and the *Vrn-B1c* allele was found to be highly prevalent in Russian spring wheats (Iqbal *et al.* 2007; Zhang *et al.* 2008; Shcherban *et al.* 2012; Kamran *et al.* 2014). While the *Vrn-D1a* allele was found to be highly prevalent in Indian spring wheats, none of the Canadian spring wheats displayed the *vrn-D1* allele, and only one sample among Russian spring wheats had the *vrn-D1* allele (Iqbal *et al.* 2007; Shcherban *et al.* 2012; Singh *et al.* 2013). The Korean experimental lines released after 2005 showed different frequencies of the *vrn-B1* and *Vrn-B1a* alleles, but exhibited similar frequency of the *Vrn-D1* alleles compared to wheats released before 1990. The *Vrn-A1* allele has the strongest effect on inhibiting the vernalization requirement, followed by the *Vrn-D1* and *Vrn-B1* alleles (Goncharov 2004). Facultative growth habits or the partial vernalization requirement of *Vrn-D1* plays a key role in determining the wider adaptability of wheat cultivars (Kamran *et al.* 2014). Recently, more

attention has been called to the development of facultative or spring wheat in Korea due to water logging during the sowing season in late October, frequently resulting in reduced growing area. Therefore, wheat lines which could be harvested in late summer after planting in the early spring, and introduction of the *Vrn-A1a* or *Vrn-A1b* alleles should be considered for the development of facultative or spring wheat. The *Vrn-B1b* and *vrn-D1* alleles had to be involved longer days to heading date in the 410 Korean wheats. The heading date of wheat is known to be determined 70 - 75 % by the growing habits and 20 - 25% by the photoperiod response (Stelmakh 1998). The rainy season has generally been overlapped with the harvest time of wheat in the southern part of Korea, which is closely related to the occurrence of pre-harvest sprouting at the maturation stage. Therefore, earliness has been a major consideration for wheat growing of Korea, and increase of the genetic diversity at the *Vrn-1* alleles, as well as investigation of other *Vrn* alleles should be considered in Korean wheat breeding programs.

Photoperiod response plays a key role in accelerating or delaying the heading time under field conditions in vernalized wheats, for which the *Ppd-1* genes are located on the homoeologous group 2 chromosomes (Kamran *et al.* 2014). The *Ppd-1a* alleles are known to confer insensitivity in response to the photoperiod, while the *Ppd-1b* alleles are related to sensitive response (Snape *et al.* 2001; Kamran *et al.* 2014). Photoperiod insensitive cultivars were also preferred in the southern regions of Europe and North America, whereas cultivars in the northern regions were generally photo sensitive (Worland *et al.* 1994; Dyck *et al.* 2004). Wheats carrying *Ppd-1a* alleles displayed earlier flowering date and larger grain yield than those carrying the *Ppd-1b* alleles in Southern and Eastern Europe, wherein the summer conditions were hot and dry (Snape *et al.* 2001). *Ppd-D1* is the most photoperiod insensitive locus, followed by *Ppd-B1* (Worland 1996). All of the 410 Korean wheats tested were found to have the *Ppd-A1b* allele, while higher frequency of the *Ppd-B1b* allele than the *Ppd-B1a* allele was observed, in spite of the high frequency of the *Ppd-D1a* allele. In addition, the Korean experimental lines showed higher frequency of the *Ppd-B1b* allele than the Korean landraces and North

Korean collections. Furthermore, the experimental lines released between 1991 and 2004 showed higher frequency of the *Ppd-B1b* allele than the others. The *Ppd-D1a* allele is also wide spread in most of the European, Canadian and Chinese insensitive cultivars (Kamran *et al.* 2014). All of the Korean wheat cultivars also possessed the *Ppd-A1b* and *Ppd-D1a* alleles, and about 90% of cultivars exhibited the *Ppd-B1b* allele (Cho *et al.* 2015b). Almost all of the improved cultivars released in China after 1970 possessed the *Ppd-D1a* allele, whereas below 50% of Chinese landraces had this allele (Yang *et al.* 2009). The pleiotropic influence of the *Ppd-D1* gene on the yield potential of the major wheat growing regions of Europe could be involved in reducing the flowering time, plant height and number of spikelets per spike (Snape *et al.* 2001; Foulkes *et al.* 2004; Kamran *et al.* 2014). Almost all high-yield improved cultivars are insensitive to photoperiod, while all landraces in North Africa, Mediterranean regions and Asia are sensitive, because selection of the photoperiod insensitive trait has enhanced the adaptability of the improved cultivars to a wide range of environments in comparison with the photoperiod-sensitive landraces in these regions (Kamran *et al.* 2014). Herein, no significant difference in days to heading date according to the allelic variation at *Ppd-1* loci was observed, although wheats with the *Ppd-B1b* or *Ppd-D1b* allele showed longer culm and spike length than those with the *Ppd-B1a* or *Ppd-D1a* allele. The *Ppd-B1b* allele, when combined with *Vrn-D1b* alleles, could be involved in shortening the days to maturation and increasing the grain yield in Korean wheat cultivars (Cho *et al.* 2015b). The introduction of the *Ppd-A1a* allele and increase of the frequency of the *Ppd-B1a* allele should be considered for reducing the flowering time of Korean wheats, and further investigation is also needed to elucidate the relationship between specific alleles combined with *Vrn-1* and *Ppd-1* alleles and the major agronomic traits in Korean wheat breeding programs.

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