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The effects of *Rht* semi-dwarfing alleles on agronomic traits in Korean wheat cultivars

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Abstract

The allelic variation at *Rht-B1*, *Rht-D1* and *Rht8* loci of Korean wheat cultivars was determined to evaluate the relationship between agronomic traits and different *Rht* alleles. Korean wheat cultivars had high frequency of *Rht-B1a* (70.4%) and *Rht-D1a* (51.9%) alleles. Thirteen cultivars carried both *Rht-B1a* and *Rht-D1b* alleles but no cultivar carried both *Rht-B1b* and *Rht-D1b* alleles. Only two cultivars, Namhae and Ol, showed 206bp fragment and whereas remaining genotypes showed 196bp at *Rht8* loci. Cultivars carrying *Rht-D1a* and $Rht8_{196bp}$ alleles exhibited higher thousand kernel weight and test weight (40.2g and 809.9g, respectively) as compared to their counterpart alleles (36.6g and 788.1g, respectively). Cultivars carrying *Rht-B1a* and *Rht-D1a* alleles showed lower number of kernels per spike than those with other alleles at *Rht-B1* and *Rht-D1* loci. Cultivars, Namhae and Ol, carrying $Rht8_{206bp}$ allele showed lower test weight regardless the allelic compositions at *Rht-B1* and *Rht-D1* loci. Significant differences were recorded in culm and spike length regardless of allelic variation of these semi-dwarf genes.

Key words: Wheat, semi-dwarf, allelic variation, agronomic traits.

Introduction

Reduced height trait (*Rht*) genes are an important component of the 'green revolution' (Hedden, 2003). Over 90% of the semi-dwarf wheat cultivars grown worldwide carry three major *Rht* genes, *Rht-B1, Rht-D1* (formerly known as *Rht1* and *Rht2*, respectively), and *Rht-8* (Tosovic-Maric et al. 2008). The *Rht-B1b* and *Rht-D1b* genes originated from Korean old landrace, Anzunbaengimil, which was being grown as early as the third and fourth centuries (Cho et al. 1980). This was introduced to Japan cultivars in 1930's and Norman Borlaug and others as part of wheat improvement programs in America and at the CIMMYT (International Maize and Wheat Improvement Center) and then transferred to Europe and the rest of the world (Peng et al. 1999; Ellis et al. 2002). The Japanese cultivar Akakomugi has been used to introduce another semi-dwarf gene, *Rht8*, into Italian cultivars during 1920's and this gene has been widespread in European and Russian cultivars (Worland et al. 1998; Borojevic and Borojevic 2005a, 2005b).

Genes *Rht-B1* and *Rht-D1* are located on chromosomes 4B and 4D, respectively, both encoding DELLA proteins, which are involved in gibberellic acid (GA) sensitivity, being insensitive to exogenous GA (Peng et al. 1999; Hedden 2003). Polymorphisms in the DELLA domain results in different alleles, *Rht-B1b*, *Rht-B1c*, *Rht-B1d*, *Rht-B1e*, *Rht-D1b*, *Rht-D1c*, and *Rht-D1d*, and these alleles have a different effect on plant height (Börner et al. 1996). The perfect markers for *Rht-B1b* and *Rht-D1b* were used to detect the specific base-pair mutation responsible for the semi-dwarfing phenotype (Ellis et al. 2002). *Rht8* dwarfing gene located on chromosome 2D, is sensitive to exogenous GA (Börner et al. 1996). *Rht8* allele closely linked to *Xgwm261* is used as a marker for the detection of allelic variation at the *Rht8* locus (Worland et al. 1998). *Rht8* gene is also closely associated with the photoperiodic insensitivity gene *Ppd-D1a. Ppd-D1a* allele showed early heading and shorter plant height than *Ppd-D1b* allele (Blake et al. 2009; Sharma et al. 2012; Vinod et al. 2012).

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The *Rht-B1b* and *Rht-D1b* dwarfing genes reduced plant height by 15% and increase yield by 24% (Gale and Youssefian 1985; Flintham et al. 1997). These dwarfing genes influence yield increase in wheat grown under optimal conditions, but these genes have been considered for reduction in yield under heat and/or drought stress (Flintham et al. 1997; Korzun et al. 1998). *Rht8* dwarfing gene exhibits more resistance/tolerance to environments with low-inputs or abiotic stresses (Worland and Law 1986). This gene is known to reduce plant height by around 10% without significant negative effects on yield (Börner et al. 1993; Worland et al. 1998). *Rht8* alleles, like *Rht-B1b*, *Rht-D1b*, have been associated with increased floret fertility (Gale and Youseffian 1985).

Grain yield and early maturity have mainly been considered in Korean wheat breeding programs and wheat breeders have focused on selection of wheat lines with reduced plant height and improved lodging resistance. However, any genetic information on semi-dwarfing genes has not been available in Korean wheat cultivars, although Korean old landrace, Anzunbaengimil, could be the source of the origin for *Rht-B1b* and *Rht-D1b.* Therefore, the present study was conducted to investigate the distribution of semi-dwarfing genes, *Rht-B1*, *Rht-D1* and *Rht8* in Korean wheat cultivars using molecular markers and to elucidate the relationship between agronomic traits and allelic variation.

Matrials and methods

Plant materials

Twenty-seven Korean wheat cultivars were sown in Randomized Complete Blocks with 3 replications in the Upland Crop Experimental Farm of National Institute of Crop Science, Rural Development Administration (Korea) for five years, from 2010 to 2014 on 50% of clay loam soil. The seed was sawn on October 25, each plot consisted of three 2-m rows spaced 25 cm apart and plots were combine-harvested in June 20 every year. Fertilizer was applied at 5: 7: 5kg/10a (N: P: K) before sowing and weeds, insects and disease were stringently controlled. No supplemental irrigation was applied.

Agronomic traits

Agronomic traits namely, culm length (length from ground level to the base of the spike), spike length (length of spike excluding awn) and kernel number per spike (counted as number of grains at main spike). Culm length, spike length and kernel number per spike were determined from a sample of 10 main tillers selected randomly from each plot at maturity. The grain from each plot was dried by 14% moisture content using forced air driers and bulked from replications to provide grain for test weight and thousand kernel weight. Test weight and thousand kernel weight were measured from three samples from each plot using a Grain Scale (Seedburo Equipment Co., USA) and a Seed Counter (Pfeuffer GmbH, Germany), respectively. The measurement of culm length, spike length and kernel number per spike was repeated ten times, and test weight and thousand kernel weight were measured three times.

PCR conditions

Leaf tissue was collected from a single plant for each cultivar after germination for two weeks and snap-frozen in liquid nitrogen and stored at –80^{o}C until needed. 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. The primer *Rht-B1a* (5'-GGTAGGGAGGC GAGAGGCGAG-3'/5'-CATCCCCATGGCCATCTCG AGCTG-3'), *Rht-B1b* (5'-TCTCCTCCCTCCCCACCCC AAC-3'/5'-CATCCCCATGGCCATCTCGAGCTA-3'), *Rht-D1a* (5'-GGCAAGCAAAAGCTTCGCG-3'/5'-GGCCATCTCGAGCTGCAC-3') and *Rht-D1b* (5'-CGCGCAATTGGCCAGAGATAG-3'/5'-CCCCA TGGCCATCTCGAGCTGCTA-3') were designed based on the sequences of *Rht-B1* and *Rht-D1* described by Ellis et al. (2002) and Zhang et al. (2006). PCR was performed in an MJ Research PTC-200 thermal cycler (MJ Research Inc., USA) in a total volume of 25 µL containing 100 ng DNA, 1.5mM $MgCl_2$, 10 pmol of each primer, 0.2 mM of each dNTP, 1×Hotstar buffer and 1.0 unit of Hotstar *Taq* polymerase (Qiagen, Hilden,Germany). The PCR cycle consisted of an initial 5 min denaturation at 95^{o}C, followed by 38 cycles of 94^{o}C for 30 sec, 63^{o}C for 30 sec, and 72^{o}C for 30 sec, and 1 cycle of 72^{o}C for 5 min. Amplified PCR fragments were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized using UV light. The gene *Rht8* was detected with a microsatellite marker *Xgwm261* following the procedure of Korzun et al. (1998) with minor modifications. PCR reactions were performed in a total volume of 15 µL containing 100 ng genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit *Taq* DNA polymerase (Takara, Japan), and 0.2 µg/µl of each primer. The PCR cycle consisted of an initial 5 min denaturation

at 95°C, followed by 38 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 1 min and 1 cycle of 72°C for 5 min. The PCR products were analyzed with the QIAxcel Advanced system using a 12-capillary QIAxcel DNA high resolution Cartridge (Qiagen Co., Valencia, CA, USA).

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, and the genotype × year component was used as the error term. 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 tested loci for measured traits of 27 Korean wheat cultivars. Pearson correlations between pairs of traits were determined from the least square means of wheat cultivars at each year separately and over all environments, using the Corr procedure. Heritability (h^2) on a progeny mean basis was estimated from RCM analysis using the formula $h^2 = 1 - (M_2/M_1)$ proposed by Knapp et al. (1985), where M_1 is the mean square of genotype and M_2 is the mean square for genotype by year interaction (G × Y) in Korean wheat cultivars grown for five years.

Results and discussion

Allelic variations at different loci of dwarfing genes

Allelic variation at *Rht-B1*, *Rht-D1* and *Rht8* loci in Korean wheat cultivars are shown in Fig. 1 and summarized in Table 1. Among 27 Korean wheat cultivars, 19 carried *Rht-B1a* and 8 possessed *Rht-B1b* at *Rht-B1* loci, while 14 wheats carried *Rht-D1a* and 13 *Rht-D1b* at *Rht-D1* loci. Six cultivars carried both *Rht-B1a* and *Rht-D1a* alleles, 13 carried both *Rht-B1a* and *Rht-D1b* alleles while 8 contained both *Rht-B1b* and *Rht-D1a* alleles. Cultivars carrying both *Rht-B1b* and *Rht-D1b* alleles were not found among Korean wheats, in spite of Korean old landrace, Anzunbaengimil, could be as origin of source for *Rht-B1b* and *Rht-D1b* alleles (Cho et al. 1980). Korean wheat cultivars showed low frequency of *Rht-B1b* (29.6%) and high frequency of *Rht-D1b* allele (48.1%). Variations in the frequency of these genes are reported in Germany (6 and 38%, respectively), Japan (21.3 and 76.7%, respectively) and China (24.5 and 45.5%, respectively) (Yamada, 1990; Zhang et al. 2006; Knopf et al. 2008). But, only one Turkish wheat carried *Rht-D1b* allele, although 37% of Turkish wheats carried *Rht-B1b* allele (Yediay et al. 2011). Only 4% of wheats carried both *Rht-B1b* and *Rht-D1b* alleles among 172 genotypes originating from over 20 different countries (Tosovic-Maric et al. 2008). No semi-dwarf cultivar, carrying both *Rht-B1b* and *Rht-D1b* alleles, was found in Chinese wheats (Na et al. 2009), but Japanese wheats, Norin10 and Kokeshikomugi carried both *Rht-B1b* and *Rht-D1b* alleles (Yamada 1990). Korean experimental line, Suwon 86, carrying both *Rht-B1b* and *Rht-D1b*, and cultivars, Huixianhong and Yaobaomai are the primary source of *Rht-D1b* in Chinese wheats (Zhang et al. 2006). The frequencies of *Rht-B1b* and *Rht-D1b* increased from 8.6 to 32.2% and 36.2 to 53.4%, respectively, in comparing with landraces and leading cultivars in China (Zhang et al. 2006). Therefore, allelic variations in Korean wheat landraces and old experimental lines at *Rht* loci need to be evaluated to elucidate adaptation for improving grain yield and end-use quality through the hybridization with foreign cultivars.

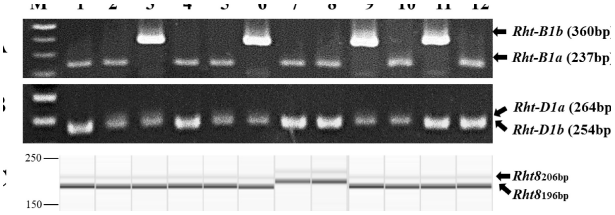

Fig. 1. PCR pattern of *Rht-B1* (A), *Rht-D1* (B) and *Rht8* alleles (C) in Korean wheat cultivars. M, molecular size marker; 1, Baekjoong; 2, Dajoong; 3, Goso; 4, Jeokjoong; 5, Joa; 6, Keumkang; 7, Namhae; 8, Ol; 9, Shinmichal; 10, Suan; 11, Sukang; 12, Younbaek

Two different types of allelic composition were identified at *Rht8* loci, of which 196bp fragment ($Rht8_{196bp}$) was frequently found in Korean wheat cultivars but two cultivars, Namhae and Ol showed 206bp ($Rht8_{206bp}$). Among about twelve alleles at *Rht8* locus, $Rht8_{165bp}$, $Rht8_{174bp}$ and $Rht8_{192bp}$ have been mostly found in common wheat, of which $Rht8_{192bp}$ allele has been prevalent among the southern European cultivars. $Rht8_{165bp}$ is often found in Australian, CIMMYT (Mexican) and New Zealand cultivars, while $Rht8_{174bp}$ allele is mostly present in the British, French, German, Turkish and American cultivars (Worland et al. 1998; Ahmad and Sorrells 2002; Yediay et al. 2011). Chinese wheats have often carried the $Rht8_{164bp}$, $Rht8_{174bp}$, $Rht8_{192bp}$ and $Rht8_{204bp}$ alleles (Liu et al.

Table 1. Allelic variations at *Rht* loci and mean of agronomic traits of Korean wheat cultivars grown for five years

Cultivar	Allelic variation			Culm length (cm)	Spike length (cm)	Kernel no./ spike	Test wt. (g)	Thousand kernel weight (g)
	Rht-B1	*Rht-D1*	*Rht8*					
Alchan	*a*	*b*	196bp	73.9	6.5	36.0	807.1	33.1
Anbaek	*b*	*a*	196bp	81.3	8.4	37.0	812.2	43.5
Baekjoong	*a*	*b*	196bp	83.4	7.2	33.2	795.3	41.5
Chunggye	*a*	*b*	196bp	78.4	7.3	36.9	817.1	33.6
Dahong	*a*	*b*	196bp	82.4	6.8	40.1	795.5	30.4
Dajoong	*a*	*a*	196bp	86.8	7.2	33.6	801.1	35.0
Eunpa	*a*	*b*	196bp	73.8	8.3	32.9	833.4	35.4
Geuru	*b*	*a*	196bp	78.6	7.2	34.0	813.3	45.8
Gobun	*a*	*b*	196bp	76.1	7.8	33.7	818.6	36.3
Goso	*b*	*a*	196bp	77.2	8.9	34.6	812.3	42.7
Jeokjoong	*a*	*b*	196bp	84.6	6.9	31.5	797.7	43.6
Jinpoom	*a*	*b*	196bp	81.8	8.7	37.3	818.4	36.8
Joa	*a*	*a*	196bp	77.9	8.0	33.9	778.1	42.1
Joeun	*a*	*a*	196bp	88.3	6.8	29.8	835.4	36.3
Jonong	*a*	*a*	196bp	76.5	7.0	28.4	809.3	43.2
Keumkang	*b*	*a*	196bp	83.1	7.5	33.1	826.7	44.6
Milsung	*b*	*a*	196bp	78.8	7.1	40.5	812.0	33.2
Namhae	*a*	*b*	206bp	80.0	8.5	37.9	786.3	35.7
Ol	*a*	*b*	206bp	82.4	7.4	39.5	789.9	35.9
Olgeuru	*a*	*a*	196bp	79.7	9.2	36.6	799.0	40.2
Saeol	*b*	*a*	196bp	72.0	7.0	34.4	818.6	37.0
Seodun	*a*	*b*	196bp	80.5	7.9	35.4	819.0	36.7
Shinmichal	*b*	*a*	196bp	78.2	8.1	32.9	801.6	35.8
Suan	*a*	*a*	196bp	92.2	7.4	31.7	821.5	43.8
Sukang	*b*	*a*	196bp	90.8	7.5	34.6	816.5	38.9
Uri	*a*	*b*	196bp	83.1	7.0	37.2	794.5	35.7
Younbaek	*a*	*b*	196bp	86.0	7.2	34.8	793.6	40.5
LSD[a]	-	-	-	1.4	0.3	1.8	0.9	0.2

[a]Least significant difference ($P < 0.05$)

2005). The GT→AC substitution at position 35 has been found at $Rht8_{174bp}$, $Rht8_{200bp}$ and $Rht8_{216bp}$ alleles and the AG insertion immediately at the end of CT-repeat region has also been found in $Rht8_{164bp}$, $Rht8_{174bp}$, $Rht8_{200bp}$ and $Rht8_{216bp}$ alleles (Liu et al. 2005). $Rht8_{165bp}$ and $Rht8_{174bp}$ alleles were primarily found in hard winter and soft red winter wheats, respectively (Bai et al. 2003). Chinese wheats showed high frequency of $Rht8_{192bp}$ (64%), but about 8% of all U.S. wheats carried $Rht8_{192bp}$ allele (Bai et al. 2003).

Year, genotype and their interactions significantly influenced agronomic traits of Korean wheat cultivars (Table 2), which agrees with the previous reports (Ledent and Stoy 1988; Perry and d'Antuono 1989; Brancourt-Hulmet et al. 2003). Year has accounted for the largest proportion of the variation in culm length, spike length, kernel number per spike and test weight (72.7-92.3%), but thousand kernel weight was largely affected by genotype (35.4%). Interaction of year and genotype has negligibly accounted for the proportion

Table 2. Mean squares and heritabilities for agronomic traits of 27 Korean wheat cultivars grown for five years

Traits	Mean squares[a]				Heritability
	Genotype (G)	Year (Y)	G × Y	Error	
Culm length	1236.1***	7214.7***	139.7***	12.7	0.89
Spike length	24.6***	74.7***	3.4***	0.6	0.86
Kernel number per spike	428.4***	6218.7***	93.9***	20.4	0.78
Test weight	3052.8***	23566.7***	903.5***	1.5	0.70
Thousand kernel weight	261.8***	462.1***	16.1***	0.1	0.94

[a]*** are significant at $P = 0.001$

of the variation in these traits (1.4-3.3%). The estimated heritability of Korean wheat cultivars for agronomic traits was over 0.70, and these values were similar to the results of Chinese, Japanese and American wheat populations (Hai et al. 2008; Heidari et al. 2011; Jia et al. 2013). These results indicate that culm length, spike length, kernel number per spike, test weight and thousand kernel weight were primarily influenced by genotype rather than cultivation year and/or their interactions between year and genotype, although the proportion of the variation in these traits has been mainly accounted by cultivation year. Increase in kernel number per unit area and/or kernels per spike could have contributed to improve the grain yield (Ma et al. 2007). Korean wheat cultivars grown in 2010 showed higher average culm length, spike length and kernel number per spike (88.6 cm, 8.3 cm and 42.9, respectively) than other years (< 83.5 cm, < 7.8 cm and < 34.8, respectively). Korean wheat cultivars grown in 2011 and 2013 exhibited higher thousand kernel weight or test weight (41.4g and 824.6g, respectively) than other years (< 40.0g and < 823.4g, respectively). Mean values of average culm and spike length were 81.0 cm and 7.6 cm, respectively. Range of averages of culm and spike length for cultivars over the years recorded were 72.0-92.2 cm and 6.5-9.2 cm, respectively. Mean value of kernel number per spike was 34.9 ranging from 28.4 to 40.5. Mean values of average test weight and thousand kernel weight recorded, were 808.3g and 38.4g, respectively, and ranges were 778.1-835.4g for test weight and 30.4-45.8g for thousand kernel weight.

Only the kernel number per spike showed negative correlation with test weight in Korean wheat cultivars grown over five years (r = -0.386, $P < 0.05$, Fig. 2). There was no significant relationship among other agronomic traits. However, plant height, including culm and spike length, was significantly correlated

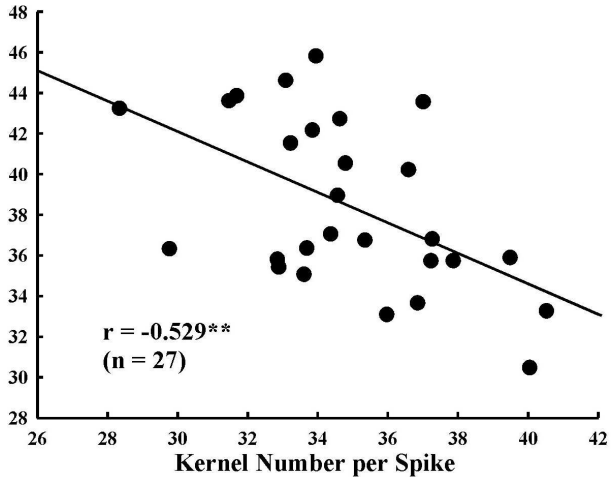

Fig. 2. Relationship between kernel number per spike and test weight in Korean wheat cultivars grown for five years

with kernel number per spike, thousand kernel weight or grain weight in American, Canadian, Chinese and CIMMYT wheat populations (Campbell et al. 2003; McCartney et al. 2005; Hai et al. 2008; Wang et al. 2009; McIntyre et al. 2010; Jia et al. 2013). Kernel number per spike was also correlated with thousand kernel weight or grain weight in Chinese and CIMMYT wheats (Hai et al. 2008; Wang et al. 2009; McIntyre et al. 2010; Jia et al. 2013). Thousand kernel weight correlated with test weight or grain weight in Australian and Canadian wheats (McCartney et al. 2005; Cuthbert et al. 2008). Thousand kernel weight also showed positive relation with kernel length and width in Chinese wheats (Sun et al. 2009; Cui et al. 2014).

Effects of allelic variation on agronomic traits

Korean wheat cultivars carrying *Rht-D1a* exhibited high thousand kernel weight (40.2g) than those with *Rht-D1b* (36.6g) (Table 3). Wheat cultivars carrying

Table 3. Difference in agronomic traits of Korean wheat cultivars carrying different combination of three alleles at *Rht* loci

Allelic variation			No	Culm length (cm)	Spike length (cm)	Kernel no./ spike	Test wt. (g)	Thousand kernel wt.(g)
Rht-B1a			19	81.5a[a]	7.5a	34.8a	805.8a	38.7a
Rht-B1b			8	80.0a	7.7a	35.1a	814.2a	40.2a
Rht-D1a			14	81.5a	7.7a	33.9a	811.3a	40.2a
Rht-D1b			13	80.5a	7.5a	35.9a	805.1a	36.6b
$Rht8_{196bp}$			25	81.0a	7.6a	34.6a	809.9a	38.6a
$Rht8_{206bp}$			2	81.2a	8.0a	38.7a	788.1b	35.8a
Rht-B1a	*Rht-D1a*		6	83.6a	7.6a	32.3b	807.4a	40.1a
Rht-B1a	*Rht-D1b*		13	80.5a	7.5a	35.9a	805.1a	36.6a
Rht-B1b	*Rht-D1a*		8	80.0a	7.7a	35.1a	814.2a	40.2a
Rht-B1a	$Rht8_{196bp}$		17	81.5a	7.5a	34.3b	807.9a	37.9a
Rht-B1a	$Rht8_{206bp}$		2	81.2a	8.0a	38.7a	788.1b	35.8a
Rht-B1b	$Rht8_{196bp}$		8	80.0a	7.7a	35.1ab	814.2a	40.2a
Rht-D1a	$Rht8_{196bp}$		14	81.5a	7.7a	33.9b	811.3a	41.6a
Rht-D1b	$Rht8_{196bp}$		11	80.4a	7.4a	35.4ab	808.2a	36.7a
Rht-D1b	$Rht8_{206bp}$		2	81.2a	8.0a	38.7a	788.1b	35.8a
Rht-B1a	*Rht-D1a*	$Rht8_{196bp}$	6	83.6a	7.6a	32.3b	807.4a	40.2a
Rht-B1a	*Rht-D1b*	$Rht8_{196bp}$	11	80.4a	7.4a	35.4ab	808.2a	36.7a
Rht-B1a	*Rht-D1b*	$Rht8_{206bp}$	2	81.2a	8.0a	38.7a	788.1b	35.8a
Rht-B1b	*Rht-D1a*	$Rht8_{196bp}$	8	80.0a	7.7a	35.1ab	814.2a	40.2a

[a]Values followed by the same letter are not significantly different at $P < 0.05$

$Rht8_{196bp}$ showed higher test weight (809.9g) than those with $Rht8_{206bp}$ (788.1g). But, no significant differences were observed in other traits based on the allelic variation at *Rht-D1* and *Rht8* loci. Also there was no difference between *Rht-B1a* and *Rht-B1b* alleles. Cultivars carrying *Rht-B1a* and *Rht-D1a* alleles showed lower kernel number per spike (32.3) than those with other alleles at *Rht-B1* and *Rht-D1* loci. But, there was no difference recorded in respect of other traits among *Rht-B1* and *Rht-D1* alleles. Four types of Korean wheat cultivars displayed allelic variation at *Rht-B1* or *Rht-D1* and *Rht8*. Cultivars Namhae and Ol, carrying *Rht-B1a* and $Rht8_{206bp}$ alleles produced higher kernel number per spike (38.7) than those with *Rht-B1a* and $Rh8_{196bp}$ alleles (34.3). Namhae and Ol also exhibited lower test weight (788.1g) than cultivars carrying different alleles at *Rht-B1* and *Rht8* loci. These cultivars also showed higher kernel number per spike than the cultivars with *Rht-D1a* and $Rh8_{196bp}$ alleles. The trend noticed in these cultivars was similar to the allelic variation at *Rht-B1*, *Rht-D1* and *Rht8.* These results indicate that $Rht8_{206bp}$ allele could be involved in reduction of test weight regardless the allelic compositions at *Rht-B1* and *Rht-D1* loci. On the other hand, cultivars carrying *Rht-B1a*, *Rht-D1a* and $Rht8_{196bp}$ showed lower kernel number per spike than those with other alleles. Korean wheats carrying *Rht-B1a* and *Rht-D1a* or $Rht8_{196bp}$ could be involved to reduce the kernel number per spike also.

Rht-B1b and *Rht-D1b* alleles reduced the plant height of wheat by approximately 20-30%, while *Rht8* by about 14-18% in Australian and Chinese wheats (Ellis et al. 2004, 2005; Na et al. 2009). Wheats carrying *Rht-D1b* were shorter in height and high yielding than wheat without this allele, but showed more susceptibility to *Fusarium* head blight, affecting their productivity in German cultivars (Knopf et al. 2008). It has been observed that these alleles had adversely affected the phenotypic characteristics of wheat, such as the reduction of the coleoptile length and leaf areas of seedlings which are most important traits at early stages under drought conditions (Rebetzke et al. 2007; Na et al. 2009). However, *Rht8* allele had less effect on the early growth of wheat (Ellis et al. 2004). No

significant difference in culm length between *Rht-B1a* or *Rht-D1a* and *Rht-B1b* or *Rht-D1b* alleles was recorded. Significant difference in culm length was also not observed between Rht_{196bp} and Rht_{206bp} alleles of *Rht8*.

Rht8 dwarfing gene has been widely used in South-Eastern European countries, Russian, Ukrainian and Chinese wheat breeding because it's closely associated with *Ppd-D1* gene, photoperiod genes related to heading time under field conditions in vernalized wheats, and increases grain yield without drastic reduction in height (Snape et al. 2001; Zheleva et al. 2006). The *Ppd-D1b* allele has been involved in reducing flowering time, plant height and number of spikelets per spike in European wheats (Langer et al. 2014) and only 9% of Indian wheats possessed this gene (Singh et al. 2013; Karman et al. 2014). $Rht8_{192bp}$ allele has been conferred as moderate to reduce plant height and an additional reduction when combined with the closely linked *Ppd-D1* gene (Worland et al. 1998). This allele has been an indicative of the more commercially favorable *Rht8* allele, while the other alleles at this locus are considered to be associated with various levels of height promotion (Worland et al. 1998). Recently, Ellis et al. (2007) showed that the presence of $Rht8_{192bp}$ allele is not always associated with *Rht8* gene and effecting reduction in height and hence proposed that its presence could be only indication of *Rht8* in wheat cultivars that have inherited this allele from Akakomugi or a Strampelli wheat ancestor.

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