Dwarfing genes *Rht4* and *Rht-B1b* affect plant height and key agronomic traits in common wheat under two water regimes

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Abbreviations

PH, plant height; PL, peduncle length; I2L, length of the second internode from top; I3L, length of the third internode from top; I4L, length of the fourth internode from top; DSL, distance from spike to flag leaf ligule; LODS, lodging score; HD, heading date; SL, spike length; SN, spikelet number spike⁻¹; GN, grain number; SPN, spike number; TKW, thousand kernels weight; BIO, above-ground biomass; GY, grain yield; HI, harvest index; WW, well-watered; WS, water-stressed.

Abstract

To explore the potential utilization of *Rht4* in wheat improvement, we investigated and compared the effects of *Rht4* and *Rht-B1b* on the plant height and yield components using four groups of recombinant inbred lines derived from the cross between “IDO444” (tall, no known dwarfing alleles) and “Rio Blanco” (semi-dwarf, *Rht4+Rht-B1b*) grown under two water regimes.

Rht4, *Rht-B1b* and *Rht4+Rht-B1b* significantly reduced plant height by 11.5%, 19.3% and 18.2%, respectively. There were no additive effects on plant height in *Rht4+Rht-B1b* lines. Both *Rht4* and *Rht-B1b* significantly reduced internode length and lodging score with stronger effects by *Rht-B1b*. Water stress affected the plant height related traits of the single *Rht-B1b* lines more than that of the *Rht4* lines, except for distance from spike to flag leaf ligule.

Grain number was increased by *Rht4* and *Rht-B1b*, but higher spike numbers was only observed in lines with *Rht-B1b*. Both *Rht4* and *Rht-B1b* significantly reduced thousand kernels weight. *Rht-B1b* reduced above-ground biomass but increased grain yield and harvest index, while *Rht4* had less effect on these traits, compared with tall lines under both well-watered and water-

stressed conditions. The *Rht4+Rht-B1b* lines produced the highest yield under both water regimes, with more grain number, greater spike number and higher harvest index.

In conclusion, *Rht4* did not show advantages over *Rht-B1b* on grain yield, but *Rht4+Rht-B1b* did, in both water regimes, which suggests that *Rht4* should be combined with *Rht-B1b* in wheat improvement.

Introduction

Plant dwarfing genes have been classified into two categories, gibberellin-insensitive (GAI) and gibberellin-responsive (GAR), based on the phenotypic response to exogenous gibberellins. In wheat, the dwarfing genes *Rht-B1b* and *Rht-D1b* (previously known as *Rht1* and *Rht2*) are not affected by exogenous gibberellins so they are designated as GAI dwarfing genes (Wang et al., 2014). These two gibberellin-insensitive (GAI) dwarfing genes are used most widely, and they have major global impacts during the “Green Revolution” (Flintham et al., 1997; Hedden, 2003; Wang et al., 2014) and they are effective in reducing wheat plant height and lodging, as well as being associated with increases in grain number (GN), grain yield (GY), and harvest index (HI) (Richards et al., 1992a; Chapman et al., 2007). The effects of *Rht-B1b* and *Rht-D1b* on bread wheat (*Triticum aestivum* L.) vary with genetic background and growing environment (Butler et al., 2005; Mathew et al., 2006). Studies have demonstrated that the reduction of plant height (PH) by *Rht-B1b* and *Rht-D1b* is the highest in favorable environments, with reductions of about 20–25% compared with the wild types (Butler et al., 2005; Chapman et al., 2007; Mathew et al., 2006). These dwarfing genes perform better than the tall types in high yielding environments, but similar or the opposite results are observed in low yielding environments (Flintham et al., 1997; Butler et al., 2005). It has been reported that the high yield response associated with *Rht-B1* and

Rht-D1 dwarfing genes generally tends to be the greatest in well-irrigated, high-input growing conditions (Mathew et al., 2006). The reduced lengths of different internodes due to *Rht-B1b* and *Rht-D1b* contribute to the decrease in final PH, with the greatest reduction on the peduncle (Hoogendoorn et al., 1990; Keyes et al., 1989; Rebetzke et al., 2012b).

However, the reduced cell size associated with *Rht-B1b* and *Rht-D1b* has negative effects on the coleoptile length (reduced coleoptile length) and seedling vigor (Rebetzke et al., 1999, 2001, 2004; Wang et al., 2014). The shorter coleoptile attributable to these two genes can affect seed germination and seedling establishment when sufficient water could not be captured from the environment. In addition, it has been reported that in similar environments including Mediterranean environments, which rainfall occurs frequently but in small amounts, much of the water cannot be used effectively for crop canopy development (Cooper et al., 1983; French and Schultz, 1984; Siddique et al., 1990; Zhang et al., 1998; Rebetzke et al., 2012b).

Gibberellin-responsive (GAR) dwarfing genes, such as *Rht4*, *Rht5*, *Rht8*, *Rht12*, and *Rht13*, have the potential to reduce PH but without negatively affecting the CL and seedling vigor, unlike those GAI dwarfing genes (Ellis et al., 2004; Botwright et al., 2005; Rebetzke et al., 1999, 2004, 2012b; Wang et al., 2014; Wang et al., 2015). The reduced plant height associated with GAR dwarfing genes is correlated with a low lodging score (LODS), increased dry-matter partitioning to grain, and a higher grain number (Rebetzke et al., 2012b). These genes have negligible effects on aerial biomass, and some *Rht4*, *Rht12*, and *Rht13* semi-dwarf lines increase the proportional allocations of aerial biomass to increase grain yield, while *Rht4* reducing PH by about 17%, but increasing the grain number by 19% (Rebetzke et al., 2012b).

The objective of this study was to investigate and compare the effects of *Rht4*, *Rht-B1b*, and *Rht4+Rht-B1b* on PH and key agronomic traits using the wheat RILs grown under well-watered and water-stressed treatments in two locations in southeast Idaho, USA, to explore the potential application of dwarfing genes *Rht4* and *Rht-B1b* in wheat breeding.

2. Materials and methods

2.1. Plant materials

The materials used in this study were selected from a mapping population comprised of 159 F_{8,9} recombinant inbred lines (RILs) from a cross IDO444 x Rio Blanco (Li et al., 2011; Chen et al., 2012; Zhang et al., 2014). “IDO444” (no known dwarfing alleles, PI578278) is a tall winter wheat line with improved disease resistance and grain yield (Windes et al., 1995; Zhang et al., 2014), while “Rio Blanco” (*Rht4+Rht-B1b*, PI531244) is a semi-dwarf hard white winter wheat cultivar released by Agripro Biosciences Inc., Shawnee Mission, KS and widely used in wheat breeding programs (Wu and Carver, 1999; Martin et al., 2001; Carver et al., 2003; Haley et al., 2003; Zhang et al., 2014). The presence of the dwarfing genes *Rht4* and *Rht-B1b* in each RIL and two parents was determined using their linked molecular markers. Out of the 159 F₈-derived RILs, 51 lines were selected on their plant height in field experiments and the presence of *Rht4* and *Rht-B1b* using molecular markers and used in this study, which comprised of 14 tall lines, 10 single *Rht4* dwarf lines, 16 single *Rht-B1b* dwarf lines, and 11 *Rht4+Rht-B1b* double dwarf lines.

2.2. Field experiments

Field experiments were conducted during the growing seasons (September to July) of 2009–2010 and 2014–2015 with two water treatments at two locations in South eastern Idaho, i.e., Blackfoot (43.19° N, 112.35° W, elevation 1371 m) in harvesting year 2010 (10BFWW) and Aberdeen

(42.96° N, 112.83° W, elevation 1342 m) in harvesting years 2010 (10ABWS) and 2015 (15ABWW and 15ABWS). The 10BFWW and 15ABWW trials were well-watered (WW) in Blackfoot and Aberdeen, respectively. The 10ABWS and 15ABWS trials were performed under water-stressed (WS) at Aberdeen. The distributions of the monthly rainfall and irrigation at the four location-year trials during the wheat season are presented in Table 1. Fertilizer was applied based on a soil test before sowing.

A completely randomized block design was utilized for the RILs and two parents with two replicates for the 10BFWW and 10ABWS trials, and with three replicates for the 15ABWW and 15ABWS trials. The sowing weight of each line was calculated by 1000-kernel weight based on a density of 0.48 million seeds per hectare. All of the lines and parents were sown in plots with seven rows, which measured 3 m long $\times$ 1.5 m wide.

Table 1 Rainfall and irrigation (mm) at the 4 trials during the two wheat-growing seasons

Years	Trials	Sep. ~ Dec.	Jan. ~ Mar.	Apr.		May		Jun.		Jul.		Total
		R	R	R	IRR	R	IRR	R	IRR	R	IRR	
2009–2010	BFWW	77.5	50.8	53.1	/	58.2	203.2	29.7	203.2	3.3	101.6	780.5
	ABWS	50.7	39.1	39.6	/	32.5	25.4	16.8	142.2	3.0	40.6	390.1
2014–2015	ABWW	77.7	14.7	2.5	96.0	83.6	58.7	19.8	176.0	19.1	/	548.1
	ABWS	77.7	14.7	2.5	96.0	83.6	58.7	19.8	/	19.1	/	372.1

BFWW, well-watered in Blackfoot; ABWS, water-stressed in Aberdeen; ABWW, well-watered in Aberdeen; R, rainfall; IRR, irrigation water.

2.3. Genotyping of dwarfing genes

Total genomic DNA was extracted from young leaves using the CTAB method, as described by Aldrich and Cullis (1993). The DNA concentrations were estimated using a Nanodrop ND-1000 Spectrophotometer (Nanodrop, Wilmington, DE) and normalized to 80 ng μL^{-1} for marker

detection. The molecular markers WMC317, BF/MR1 and BF/WR1 (Ellis et al., 2002, 2005) were used to detect the presence of the *Rht4*, *Rht-B1b* (dwarfing allele) and *Rht-B1a* (wild allele) among the 159 RILs and the two parents in the USDA-ARS genotyping lab at Pullman, WA, and Raleigh, NC, USA. The two parents have the same genotype at *Rht-D1* locus.

2.4. Assessment of agronomic traits

The traits were investigated including heading date (HD), plant height (PH), distance from spike to flag leaf ligule (DSL), thousand kernels weight (TKW) and grain yield (GY) in the two growing seasons. Lodging score (LODS) were recorded only in the 2009–2010 growing season, as 0–9 scale, where 0 = no lodging and 9 = 100% plants lodged. The traits of the peduncle length (PL), length of the second internode from top (I2L), length of the third internode from top (I3L), length of the fourth internode from top (I4L), spike length (SL), spikelet number spike⁻¹ (SN), grain number per m² (GN), spike number (SPN), above-ground biomass (BIO), and harvest index (HI) were only recorded in the 2014–2015 growing season.

Heading date (HD) was recorded as the Julian date when 50% of the heads in the plot were completely emerged above the flag leaf collar. PH was measured from the soil surface to the tip of the spike (awns excluded). PL, DSL, I2L, I3L and I4L were investigated in ten main stems sampled at random.

At the maturity stage (Feekes 11.3–11.4), 0.25 m² of each plot was sampled for determination of seven yield-related traits, i.e., SL, SN, GN, TKW, SPN, BIO, and HI. All of the stems were cut by hand at ground level and packed in bags, and then air-dried at 40°C in the green house, and weighed to determine the BIO, and then the grain was obtained by threshing. HI was estimated as the proportion of grain relative to BIO. The threshed grain samples were used to measure the

TKW using the single-kernel characteristics system (SKCS 4100; Perten Instruments Inc., Springfield, IL, USA) based on single kernel weight of 100 seeds and converted to thousand kernels weight. Plots were harvested using a Wintersteiger Classic small plot combine (Wintersteiger Inc., Salt Lake City, USA) equipped with a Harvest Master weighing system (Juniper Systems, Inc., Logan, UT, USA). The GY was determined from the grain weight based on the 12% moisture.

2.5. Data analysis

The mean value was calculated for traits in all of the lines and two parents, significant differences were detected by analysis of variance (ANOVA) using JMP V12.0 statistical software from SAS. The relative effects of dwarfing genes were calculated as: $\text{effect} = (\text{Mean}_{\text{dwarf}} - \text{Mean}_{\text{tall}}) / \text{Mean}_{\text{tall}} \times 100\%$. Combined ANOVA and analysis of covariance over years and environments were tested for all of the agronomic characteristics using the Proc GLM procedure and MIXED procedure. Pearson's correlations coefficients between PH and other agronomic traits were determined using JMP V12.0. Significant differences among genotypes and water regimes were detected using Fisher's protected LSD at $\alpha = 0.05$ and $\alpha = 0.01$, respectively. Significant differences for correlations were determined as $^* p < 0.05$, $^{**} p < 0.01$, $^{***} p < 0.001$, and $^{****} p < 0.0001$, respectively.

3. Results

3.1. Comparison of the two parents IDO444 and Rio Blanco

A range of variations were detected in the investigated traits between IDO444 (tall) and Rio Blanco (*Rht4+Rht-B1b*) in the four location-year trials (Table2), indicating the performance of

the two parents was affected by rainfall and irrigation (Table 1). The average PH of IDO444 was significantly taller by 29.4% than that of Rio Blanco, with significantly longer DSL ($\alpha = 0.05$). Rio Blanco had a significantly lower LODS, and significantly higher GY than IDO444 ($\alpha = 0.05$), but IDO444 had a significantly heavier TKW ($\alpha = 0.05$).

Table 2 Plant height and agronomic traits of IDO444 (tall) and Rio Blanco (*Rht4+Rht-B1b*) in the four trials

ENV	Genotype	PH (cm)	DSL (cm)	LODS (0–9)	HD (day)	GY (t ha ⁻¹)	TKW (g)
10BFWW	IDO444	109.2	23.8	3.0	174.0	5.0	36.2
	Rio Blanco	86.4	15.8	2.0	164.0	5.5	35.4
10ABWS	IDO444	104.0	22.3	4.0	169.0	6.5	33.8
	Rio Blanco	81.2	12.8	2.0	163.0	6.1	32.6
15ABWW	IDO444	125.3	22.4	-	149.0	6.6	38.1
	Rio Blanco	88.1	17.2	-	144.0	6.8	37.4
15ABWS	IDO444	120.2	19.7	-	154.0	3.1	31.5
	Rio Blanco	85.5	13.3	-	144.3	5.7	25.5
Mean ^a	IDO444	114.7a	20.6a	3.5a	161.4a	5.3b	34.9a
	Rio Blanco	85.3b	15.5b	2.0b	153.8b	6.0a	32.7b

10BFWW, well-watered in Blackfoot in 2010; 10ABWS, water-stressed in Aberdeen in 2010; 15ABWW, well-watered in Aberdeen in 2015; 15ABWS, water-stressed in Aberdeen in 2015.

PH, plant height; DSL, distance from spike to flag leaf ligule; LODS, lodging score; HD, heading date; GY, grain yield; TKW, thousand kernels weight; -, not measured; ^a, means with year $\times$ environment.

The same small letter in the same column indicates that the difference is not significant between IDO444 and Rio Blanco at $\alpha = 0.05$.

3.2. Effects on PH and related traits

Plant height (PH), internode length and lodging score (LODS) were measured in RILs in four or two trials. As indicated in table 3, in general, PH was reduced by 11.5% in single *Rht4* lines, by 19.3% in single *Rht-B1b* lines, and by 18.2% in the double *Rht4+Rht-B1b* lines, compared with

the tall lines. This suggested that the effect of *Rht4* on PH reduction was less than that of *Rht-B1b*, there was no additive effect between the two dwarfing genes.

Table 3 Effects of *Rht4* and *Rht-B1b* on plant height, internode length and lodging score in RILs from IRO444 and Rio Blanco in the four⁴ or two² trials

Trait	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
PH ⁴ (cm)	108.1 ± 7.52a	95.7 ± 3.27 (−11.5%)b	87.2 ± 5.77 (−19.3%)c	88.4 ± 4.55 (−18.2%)c
PL ⁴ (cm)	41.3 ± 3.21a	37.9 ± 4.64 (−8.2%)b	34.2 ± 3.54 (−17.2%)d	35.6 ± 2.94 (−13.7%)c
I2L ² (cm)	30.7 ± 1.85a	25.7 ± 2.20 (−16.3%)b	24.0 ± 2.67 (−21.8%)c	23.8 ± 2.10 (−22.5%)c
I3L ² (cm)	17.6 ± 2.26a	15.1 ± 2.22 (−14.2%)b	13.3 ± 2.23 (−24.4%)c	12.7 ± 1.90 (−27.8%)c
I4L ² (cm)	12.6 ± 1.28a	11.1 ± 2.03 (−11.9%)b	10.3 ± 1.46 (−18.3%)c	10.4 ± 1.26 (−17.5%)c
DSL ⁴ (cm)	18.5 ± 3.94a	16.6 ± 3.59 (−10.3%)b	15.3 ± 3.04 (−17.3%)c	14.5 ± 2.73 (−21.6%)c
LODS ² (0-9)	4.4 ± 3.34a	3.1 ± 2.99 (−29.5%)b	1.7 ± 2.73 (−61.4%)c	2.9 ± 2.74 (−34.1%)b

The data represent as the mean ± standard deviation (SD), where the percentage in parentheses show the reduction compared with the tall genotype. The same small letter in the same row indicates that the difference is not significant at $\alpha = 0.05$.

PH, plant height; PL, peduncle length; I2L, length of the second internode from top; I3L, length of the third internode from top; I4L, length of the fourth internode from top; DSL, distance from spike to flag leaf ligule; LODS, lodging score.

The plant height (PH) reductions by dwarfing genes were attributable to the decreases in the lengths of the internodes, especially peduncle length (PL) and length of the second internode from top (I2L). Compared with the tall lines, PL was decreased by 8.2%, 17.2%, and 13.7% for single *Rht4*, single *Rht-B1b*, and double *Rht4+Rht-B1b* lines, respectively, which was stronger in the lines with either single *Rht-B1b* or its combination with *Rht4*. The lengths of I2L, I3L and I4L were reduced significantly by dwarfing genes, but the reduction by *Rht-B1b* was higher than by *Rht4*, compared with the tall lines ($\alpha = 0.05$). The DSL of either single *Rht4* or single *Rht-B1b* lines was shorter than that of the tall lines, and the greatest reduction (21.6%) in DSL was observed in the double *Rht4+Rht-B1b* lines. In addition, LODS was significantly lower in the

dwarfing lines than the tall lines ($\alpha = 0.05$), and the effect of *Rht-B1b* on lodging was stronger than that of *Rht4* (Table 3).

The reductions in plant height (PH), internode length and lodging score (LODS) by dwarfing genes under both well-watered (WW) and water-stressed (WS) conditions were similar as in the four or two trials (Table S1). However, PH and PL were significantly reduced for both the single *Rht-B1b* and tall lines under WS conditions compared with WW conditions, but were not significant for the single *Rht4* and *Rht4+Rht-B1b* lines ($\alpha = 0.01$). There were no significant differences for I2L, I3L, I4L and LODS among all lines between WW and WS conditions ($\alpha = 0.01$). However, DSL of all lines was significantly shorter under WS conditions than under WW conditions ($\alpha = 0.01$).

3.3. Effects on yield and yield components

The effects of *Rht4* and *Rht-B1b* on the grain yield (GY) and yield components were shown in Table 4. Significant higher GY was found in lines with either *Rht4+Rht-B1b* (30.4%) or single *Rht-B1b* (21.7%), while slightly higher GY in the single *Rht4* lines, compared with the tall lines ($\alpha = 0.05$). BIO was reduced significantly by *Rht-B1b* ($\alpha = 0.05$), whereas BIO of the single *Rht4* lines was similar as the tall lines. Significant increases in HI were observed in the double *Rht4+Rht-B1b* lines (43.3%), and single *Rht-B1b* lines (32.1%), small increase was found in the single *Rht4* lines ($\alpha = 0.05$). Though single *Rht4* lines reached the heading stage about 2 days later than tall lines, it was not significantly different with the *Rht-B1b* (*Rht-B1b*, *Rht4+Rht-B1b*) lines and the tall lines ($\alpha = 0.05$). SL was increased by *Rht4* and *Rht4+Rht-B1b*, whereas it was reduced by *Rht-B1b*, compared with the tall lines. There was no difference in SN among the three types of dwarfing lines, but it was significantly higher in dwarfing lines than the tall lines ($\alpha = 0.05$). Investigation on the yield components, found that GN was increased significantly in

all three types of dwarfing lines, and the highest increase was observed in the *Rht4+Rht-B1b* lines (26.7%), followed by the single *Rht-B1b* lines (12.1%) and single *Rht4* lines (10.3%); however, SPN were increased greatly only in lines with *Rht-B1b*, either *Rht4+Rht-B1b* lines (7.2%) or single *Rht-B1b* lines (3.8%), and only small increase was observed in the single *Rht4* lines (0.3%). TKW was reduced in all the dwarfing lines, with the greatest reduction observed in the single *Rht4* lines (7.5%), followed by the double *Rht4+Rht-B1b* lines (7.2%) and single *Rht-B1b* lines (3.9%).

Table 4 Effects of *Rht4* and *Rht-B1b* on yield components and grain yield in the RILs from IDO444 and Rio Blanco in the four⁴ or two² trials

Traits	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
GY ⁴ (t ha ⁻¹)	4.6 ± 1.07c	4.7 ± 1.31 (2.2%)c	5.6 ± 1.26 (21.7%)b	6.0 ± 1.28 (30.4%)a
BIO ² (t ha ⁻¹)	19.9 ± 2.41a	19.4 ± 3.13 (-2.5%)a	18.0 ± 2.73 (-9.5%)b	18.3 ± 2.75 (-8.0%)b
HI ² (%)	25.2 ± 6.72c	25.5 ± 7.79 (1.2%)c	33.3 ± 7.87 (32.1%)b	36.1 ± 5.83 (43.3%)a
HD ⁴ (day)	158 ± 11.74a	160 ± 11.96 (1.3%)a	158 ± 12.05 (0.0%)a	159 ± 11.72 (0.6%)a
SL ² (cm)	8.1 ± 0.56b	8.5 ± 0.40 (4.9%)a	7.9 ± 0.49 (-2.5%)c	8.6 ± 0.55 (6.2%)a
SN ²	14.3 ± 1.31b	15.0 ± 0.93 (4.9%)a	14.8 ± 1.15 (3.5%)a	15.1 ± 1.43 (5.6%)a
GN ² (m ²)	15584.8 ± 2499.20c	17191.6 ± 2875.35 (10.3%)b	17469.7 ± 2373.74 (12.1%)b	19751.8 ± 3331.92 (26.7%)a
SPN ² (10 ⁴ ha ⁻¹)	717.4 ± 129.34b	719.5 ± 116.21 (0.3%)b	745.0 ± 82.39 (3.8%)ab	769.4 ± 120.59 (7.2%)a
TKW ⁴ (g)	33.2 ± 3.90a	30.7 ± 3.14 (-7.5%)c	31.9 ± 4.26 (-3.9%)b	30.8 ± 4.19 (-7.2%)c

The data represent as the mean ± SD, where the percentage in parentheses denotes the reduction compared with the tall genotype. The same letter in the same row indicates that the means are not significantly different at $\alpha = 0.05$.

GY, grain yield; BIO, above-ground biomass; HI, harvest index; HD, heading date; SL, spike length; SN, spikelet number spike⁻¹; GN, grain number; SPN, spike number; TKW, thousand kernels weight.

The effects of dwarfing genes on yield-related traits under WW and WS conditions were further compared (Table 5). Water stress significantly affected yield components, significantly lower GY, BIO and HI were observed for all lines under WS condition than that under WW condition ($\alpha = 0.01$). Under water stress condition, GY, HI and GN of *Rht4+Rht-B1b* lines were

significantly higher than that of single *Rht-B1-b* lines, and GY and HI of single *Rht-B1-b* lines were significantly higher than that of single *Rht4* and tall lines. Compared with WW conditions, GN of either single *Rht-B1b* lines or double *Rht4+Rht-B1b* lines was significantly reduced, SPN of the double *Rht4+Rht-B1b* lines was significantly reduced, and TKW of all lines were significantly reduced ($\alpha = 0.01$) under WS conditions.

Table 5 Effects of *Rht4* and *Rht-B1b* on yield components in RILs from IDO444 and Rio Blanco under well-watered (WW) and water-stressed (WS) conditions

Trait	Treatment	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
GY (t ha ⁻¹)	WW	5.2 ± 0.87bA	5.5 ± 1.19bA	6.2 ± 1.12aA	6.6 ± 1.44aA
	WS	4.1 ± 0.97cB	4.0 ± 1.01cB	5.0 ± 1.07bB	5.5 ± 0.78aB
BIO (t ha ⁻¹)	WW	20.4 ± 2.07aA	19.9 ± 3.34abA	18.7 ± 2.73bA	19.0 ± 3.18bA
	WS	19.4 ± 2.64aB	18.9 ± 2.87aB	17.3 ± 2.58bB	17.7 ± 2.11bB
HI (%)	WW	28.5 ± 6.02bA	30.4 ± 6.95bA	37.9 ± 7.69aA	40.0 ± 4.91aA
	WS	22.0 ± 5.80cB	21.1 ± 5.64cB	29.1 ± 5.34bB	32.3 ± 3.79aB
GN (m ²)	WW	15929.3 ± 2713.69cA	17599.3 ± 3234.81bA	18112.7 ± 2338.64bA	20863.2 ± 3584.21aA
	WS	15125.4 ± 2150.65cA	16533.0 ± 2129.61bA	16698.1 ± 2208.23bB	18182.8 ± 2195.19aB
SPN (10 ⁴ ha ⁻¹)	WW	741.9 ± 109.00bA	720.3 ± 134.25bA	755.7 ± 76.90abA	800.4 ± 128.84aA
	WS	692.2 ± 144.38aA	718.6 ± 96.47aA	734.1 ± 87.11aA	738.5 ± 104.69aB
TKW (g)	WW	35.8 ± 2.74aA	32.8 ± 1.92cA	34.9 ± 2.93bA	33.7 ± 2.80cA
	WS	30.6 ± 3.05aB	28.6 ± 2.75bB	28.9 ± 3.08bB	27.8 ± 3.22bB

The data represent as the mean ± SD. The same small letter in the same row indicates that the difference is not significant between different genotypes at $\alpha = 0.05$. The same capital letter in the same column indicates that the difference is not significant between WW and WS treatments at $\alpha = 0.01$.

GY, grain yield; BIO, above-ground biomass; HI, harvest index; GN, grain number; SPN, spike number; TKW, thousand kernels weight; WW, well-watered; WS, water-stressed.

3.4. Correlations between PH and other agronomic traits

Correlation analysis between plant height (PH) and the length of internodes of the RIL lines showed PH had significant and positive correlations with the length of internodes, including PL, I2L, I3L, I4L, and DSL under both WW and WS conditions (Table 6). I2L had the highest

1 correlation with PH with $r = 0.77$ and 0.73 under WW and WS conditions, respectively,
 2 indicating that PH was more closely related to I2L.

3 **Table 6 Pearson's correlation coefficients (r) between plant height and the length of internodes in the RILs**
 4 **from IDO444 and Rio Blanco under well-watered (below diagonal) and water-stressed (above diagonal)**
 5 **conditions**

	PH	PL	I2L	I3L	I4L	DSL
PH	1	0.59****	0.73****	0.64****	0.49****	0.52****
PL	0.59****	1	0.52***	0.48****	0.41***	0.20*
I2L	0.77****	0.56****	1	0.74****	0.63****	0.31****
I3L	0.68****	0.49**	0.75****	1	0.67****	0.33****
I4L	0.53****	0.37***	0.55****	0.69****	1	0.22***
DSL	0.43****	0.20*	0.41****	0.29***	0.19*	1

6 PH, plant height; PL, peduncle length; I2L, length of the second internode from top; I3L, length of the third
 7 internode from top; I4L, length of the fourth internode from top; DSL, distance from spike to flag leaf ligule; * $p <$
 8 0.05 ; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

9
 10 To better understand the effects of dwarfing genes on yield components, pearson's correlation
 11 were conducted between plant height (PH) and yield traits of the RIL lines under well-watered
 12 (WW) and water-stressed (WS) conditions, respectively (Table 7). Under both water conditions,
 13 PH was negatively and significantly correlated with GY, HI and GN, but was positively and
 14 significantly correlated with BIO ($p < 0.05$). PH had significant and negative correlations with
 15 SPN, but positive correlations with TKW under WS conditions ($p < 0.05$). GY was positively
 16 and significantly correlated with HI, GN and TKW under WW conditions, but was significantly
 17 and positively correlated with HI, GN and SPN under WS conditions ($p < 0.05$).

18 **Table 7 Pearson's correlation coefficients (r) between plant height and yield traits in the RILs from IDO444**
 19 **and Rio Blanco under well-watered (below diagonal) and water-stressed (above diagonal) conditions**

PH	GY	BIO	HI	GN	SPN	TKW
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PH	1	−0.50 ^{****}	0.22 ^{**}	−0.55 ^{****}	−0.34 ^{**}	−0.23 ^{**}	0.13 [*]
GY	−0.50 ^{****}	1	0.10	0.79 ^{****}	0.25 [*]	0.16 [*]	0.11
BIO	0.21 ^{***}	0.12	1	−0.41 ^{****}	0.08	0.09	0.16 [*]
HI	−0.49 ^{****}	0.71 ^{****}	−0.56 ^{****}	1	0.14	0.10	−0.02
GN	−0.34 ^{****}	0.36 ^{****}	−0.07	0.29 ^{***}	1	0.80 ^{****}	−0.13
SPN	−0.13	0.15	−0.05	0.12	0.89 ^{****}	1	0.00
TKW	0.06	0.19 ^{**}	0.16	0.10	−0.08	0.01	1

PH, plant height; GY, grain yield; BIO, above-ground biomass; HI, harvest index; GN, grain number; SPN, spike number; TKW, thousand kernels weight; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

4. Discussion

The GAI dwarfing genes *Rht-B1b* and *Rht-D1b* have been used widely to decrease plant height (PH) to reduce plant lodging, and to increase the yield and harvest index (HI) in wheat (Richards, 1992a; Butler et al., 2005; Chapman et al., 2007). However, these genes also result in shorter coleoptiles, smaller leaf areas, and reduced BIO accumulation (Rebetzke et al., 2001; Botwright et al., 2005; Addisu et al., 2009; Uppal and Gooding, 2013). The GAR dwarfing gene *Rht4* reduced PH without affecting CL (Ellis et al., 2004) and with high yield (Rebetzke et al., 2012b). In this study, we selected the four classes of homozygous RILs, i.e., tall, single *Rht4*, single *Rht-B1b* and *Rht4+Rht-B1b* lines, to evaluate the effects of dwarfing genes *Rht4* and *Rht-B1b* on a range of agronomic traits under two water regimes, and to exploit their potential to improve lodging resistance and yield of wheat in different environments in southeastern Idaho, USA.

A reduction in plant height (PH) of 11.5% was associated with *Rht4* in the present study, which principally resulted from shortening of peduncle length and second internode length. This effect of *Rht4* on PH reduction was weaker than that reported previously, i.e., 17–20% (Rebetzke et al. 2012b), which may be due to the differences in the genetic backgrounds and growth environments. The reduction in PH associated with single *Rht4* was small, which might not be

sufficient to prevent lodging, as slightly lower LODS were observed in single *Rht4* lines than the tall lines in this study. Single *Rht-B1b* reduced PH by 19.3%, which was slightly weaker than that reported by Rebetzke et al (2012a).

The greater fertility of distal florets was associated with dwarfing genes during the development of spikes with reduced height (Miralles et al., 1998). Both *Rht4* and *Rht-B1b* reduced PH and increased GN and GY, which are consistent with previous studies (Rebetzke et al., 2012a, 2012b). Although longer SL was observed for single *Rht4* lines, similar SN and GN was observed in the single *Rht4* and single *Rht-B1b* lines, suggesting longer SL does not mean that SN was increased, which may be resulted from spike compactness. GN was greatly increased by 10.3% and 12.1%, while SPN was increased found by 0.3% and 3.8% in the single *Rht4* and single *Rht-B1b* lines, respectively. Therefore, these two dwarfing genes had stronger effect on GN than SPN. Significant reduction in TKW was found to different extent by *Rht4* and *Rht-B1b* compared with tall lines, confirming previous results (Li et al. 2006). However, *Rht4* had higher adverse effect on TKW than *Rht-B1b*, though lower TKW was partly compensated by increased GN by single *Rht4*, this can only obtain similar yield of tall lines. This resulted in a slight increase in GY by single *Rht4*, while previous study reported that a greater increase in GY was associated with *Rht4* (Rebetzke et al. 2012b), which was possibly due to the different genetic background and environments. The higher GY of single *Rht-B1b* lines than that of single *Rht4* lines mainly came from significantly higher SPN, TKW and HI, though TKW was also significantly decreased compared with the tall lines. While the highest GY of the *Rht4+Rht-B1b* lines resulted from significant increases in both GN and HI compared with the single *Rht-B1b* lines, suggesting that to some extent, the combination of *Rht4* and *Rht-B1b* may have additive effects on further increasing GN and HI.

1 As reported by Rebetzke et al. (2012b), *Rht4* and *Rht-B1b* increase grain yield (GY) by
 2 increasing the partitioning and aerial biomass, but we found that both *Rht4* and *Rht-B1b* reduced
 3 the above-ground biomass (BIO) to some extent but increased GY. The differences may be due
 4 to different plot size investigated on BIO, as this study was at the 0.25-m² level whereas
 5 Rebetzke et al. (2012a) measured the BIO at the plot level. But those results were similar as
 6 reported by Nizam and Marshall (1989), that both tall lines and semi-dwarf lines produced
 7 significantly more dry matter than the dwarf lines, but there were no significant differences in
 8 GY under both water regimes. However, we found that dwarfing lines had higher BIO under
 9 WW condition, which was contrary to results by Innes et al. (1984) that dwarf lines had higher
 10 BIO before flowering than tall lines under drought stress but lower BIO after flowering. The
 11 differences may be due to this study being conducted under WS and WW treatments throughout
 12 growth stages or BIO was measured only at the maturity stage.

13 Nizam and Marshall (1989) found that dwarfing lines exhibited the greatest reduction in yield
 14 compared with tall lines under drought stress, whereas dwarfing and semi-dwarfing lines
 15 produced higher yields than tall lines under irrigated and rain-fed conditions. In this study,
 16 dwarfing lines with *Rht-B1b* (*Rht-B1b*, *Rht4+Rht-B1b*) had higher GY under both WW and WS
 17 conditions than tall lines, while the single *Rht4* lines had similar yield as the tall lines. Dwarfing
 18 lines produced less yields under WS condition, which was consistent with that reported by
 19 Mathew et al., (2006). However, in WW condition, single *Rht4* had higher yield, but with higher
 20 lodging risk as PH reduction was not sufficient, while the single *Rht-B1b* increased HI and GY,
 21 with reduced LODS, and double *Rht4+Rht-B1b* maintained similar PH as that in WS condition,
 22 but increased GN, BIO, HI and GY in both water conditions.

In conclusion, the higher grain number (GN) but slight reduction in lodging, significantly reduced thousand kernels weight (TKW) and similar harvest index (HI) were observed in the single *Rht4* lines, resulted in slightly increased grain yield (GY). However, a significantly higher GN, highly significant reductions in lodging, and much greater HI associated with high GY were found in the single *Rht-B1b* lines. While the highest GN, more SPN and greatest HI were observed in the *Rht4+Rht-B1b* lines, which resulted in the highest GY among those lines under both WW and WS conditions. Thereby *Rht4* did not show much advantage than *Rht-B1b*, *Rht-B1b* may partly compensate for the slight effect of *Rht4* on SPN and HI, and the combination of *Rht4* and *Rht-B1b* had few but benefit advantage to improve wheat yield under both WW and WS conditions in this study. These results related with *Rht4* were from a single biparental population, and different results on *Rht4* may be obtained while using other populations, so further study is still needed using some other populations and under more conditions.

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Table 1 Rainfall and irrigation (mm) at the 4 trials during the two wheat-growing seasons

Years	Trials	Sep. ~ Dec.	Jan. ~ Mar.	Apr.		May		Jun.		Jul.		Total
		R	R	R	IRR	R	IRR	R	IRR	R	IRR	
2009–2010	BFWW	77.5	50.8	53.1	/	58.2	203.2	29.7	203.2	3.3	101.6	780.5
	ABWS	50.7	39.1	39.6	/	32.5	25.4	16.8	142.2	3.0	40.6	390.1
2014–2015	ABWW	77.7	14.7	2.5	96.0	83.6	58.7	19.8	176.0	19.1	/	548.1
	ABWS	77.7	14.7	2.5	96.0	83.6	58.7	19.8	/	19.1	/	372.1

BFWW, well-watered in Blackfoot; ABWS, water-stressed in Aberdeen; ABWW, well-watered in Aberdeen; R, rainfall; IRR, irrigation water.

Table 2 Plant height and agronomic traits of IDO444 (tall) and Rio Blanco (*Rht4+Rht-B1b*) in the four trials

ENV	Genotype	PH (cm)	DSL (cm)	LODS (0–9)	HD (day)	GY (t ha ⁻¹)	TKW (g)
10BFWW	IDO444	109.2	23.8	3.0	174.0	5.0	36.2
	Rio Blanco	86.4	15.8	2.0	164.0	5.5	35.4
10ABWS	IDO444	104.0	22.3	4.0	169.0	6.5	33.8
	Rio Blanco	81.2	12.8	2.0	163.0	6.1	32.6
15ABWW	IDO444	125.3	22.4	-	149.0	6.6	38.1
	Rio Blanco	88.1	17.2	-	144.0	6.8	37.4
15ABWS	IDO444	120.2	19.7	-	154.0	3.1	31.5
	Rio Blanco	85.5	13.3	-	144.3	5.7	25.5
Mean ^a	IDO444	114.7a	20.6a	3.5a	161.4a	5.3b	34.9a
	Rio Blanco	85.3b	15.5b	2.0b	153.8b	6.0a	32.7b

10BFWW, well-watered in Blackfoot in 2010; 10ABWS, water-stressed in Aberdeen in 2010; 15ABWW, well-watered in Aberdeen in 2015; 15ABWS, water-stressed in Aberdeen in 2015.

PH, plant height; DSL, distance from spike to flag leaf ligule; LODS, lodging score; HD, heading date; GY, grain yield; TKW, thousand kernels weight; -, not measured; ^a, means with year × environment.

The same small letter in the same column indicates that the difference is not significant between IDO444 and Rio Blanco at $\alpha = 0.05$.

Table 3 Effects of *Rht4* and *Rht-B1b* on plant height, internode length and lodging score in RILs from IRO444 and Rio Blanco in the four⁴ or two² trials

Trait	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
PH ⁴ (cm)	108.1 ± 7.52a	95.7 ± 3.27 (−11.5%)b	87.2 ± 5.77 (−19.3%)c	88.4 ± 4.55 (−18.2%)c
PL ⁴ (cm)	41.3 ± 3.21a	37.9 ± 4.64 (−8.2%)b	34.2 ± 3.54 (−17.2%)d	35.6 ± 2.94 (−13.7%)c
I2L ² (cm)	30.7 ± 1.85a	25.7 ± 2.20 (−16.3%)b	24.0 ± 2.67 (−21.8%)c	23.8 ± 2.10 (−22.5%)c
I3L ² (cm)	17.6 ± 2.26a	15.1 ± 2.22 (−14.2%)b	13.3 ± 2.23 (−24.4%)c	12.7 ± 1.90 (−27.8%)c
I4L ² (cm)	12.6 ± 1.28a	11.1 ± 2.03 (−11.9%)b	10.3 ± 1.46 (−18.3%)c	10.4 ± 1.26 (−17.5%)c
DSL ⁴ (cm)	18.5 ± 3.94a	16.6 ± 3.59 (−10.3%)b	15.3 ± 3.04 (−17.3%)c	14.5 ± 2.73 (−21.6%)c
LODS ² (0-9)	4.4 ± 3.34a	3.1 ± 2.99 (−29.5%)b	1.7 ± 2.73 (−61.4%)c	2.9 ± 2.74 (−34.1%)b

The data represent as the mean ± standard deviation (SD), where the percentage in parentheses show the reduction compared with the tall genotype. The same small letter in the same row indicates that the difference is not significant at $\alpha = 0.05$.

PH, plant height; PL, peduncle length; I2L, length of the second internode from top; I3L, length of the third internode from top; I4L, length of the fourth internode from top; DSL, distance from spike to flag leaf ligule; LODS, lodging score.

Table 4 Effects of *Rht4* and *Rht-B1b* on yield components and grain yield in the RILs from IDO444 and Rio Blanco in the four⁴ or two² trials

Traits	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
GY ⁴ (t ha ⁻¹)	4.6 ± 1.07c	4.7 ± 1.31 (2.2%)c	5.6 ± 1.26 (21.7%)b	6.0 ± 1.28 (30.4%)a
BIO ² (t ha ⁻¹)	19.9 ± 2.41a	19.4 ± 3.13 (-2.5%)a	18.0 ± 2.73 (-9.5%)b	18.3 ± 2.75 (-8.0%)b
HI ² (%)	25.2 ± 6.72c	25.5 ± 7.79 (1.2%)c	33.3 ± 7.87 (32.1%)b	36.1 ± 5.83 (43.3%)a
HD ⁴ (day)	158 ± 11.74a	160 ± 11.96 (1.3%)a	158 ± 12.05 (0.0%)a	159 ± 11.72 (0.6%)a
SL ² (cm)	8.1 ± 0.56b	8.5 ± 0.40 (4.9%)a	7.9 ± 0.49 (-2.5%)c	8.6 ± 0.55 (6.2%)a
SN ²	14.3 ± 1.31b	15.0 ± 0.93 (4.9%)a	14.8 ± 1.15 (3.5%)a	15.1 ± 1.43 (5.6%)a
GN ² (m ²)	15584.8 ± 2499.20c	17191.6 ± 2875.35 (10.3%)b	17469.7 ± 2373.74 (12.1%)b	19751.8 ± 3331.92 (26.7%)a
SPN ² (10 ⁴ ha ⁻¹)	717.4 ± 129.34b	719.5 ± 116.21 (0.3%)b	745.0 ± 82.39 (3.8%)ab	769.4 ± 120.59 (7.2%)a
TKW ⁴ (g)	33.2 ± 3.90a	30.7 ± 3.14 (-7.5%)c	31.9 ± 4.26 (-3.9%)b	30.8 ± 4.19 (-7.2%)c

The data represent as the mean ± SD, where the percentage in parentheses denotes the reduction compared with the tall genotype. The same letter in the same row indicates that the means are not significantly different at $\alpha = 0.05$.

GY, grain yield; BIO, above-ground biomass; HI, harvest index; HD, heading date; SL, spike length; SN, spikelet number spike⁻¹; GN, grain number; SPN, spike number; TKW, thousand kernels weight.

Table 5 Effects of *Rht4* and *Rht-B1b* on yield components in RILs from IDO444 and Rio Blanco under well-watered (WW) and water-stressed (WS) conditions

Trait	Treatment	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
GY (t ha ⁻¹)	WW	5.2 ± 0.87bA	5.5 ± 1.19bA	6.2 ± 1.12aA	6.6 ± 1.44aA
	WS	4.1 ± 0.97cB	4.0 ± 1.01cB	5.0 ± 1.07bB	5.5 ± 0.78aB
BIO (t ha ⁻¹)	WW	20.4 ± 2.07aA	19.9 ± 3.34abA	18.7 ± 2.73bA	19.0 ± 3.18bA
	WS	19.4 ± 2.64aB	18.9 ± 2.87aB	17.3 ± 2.58bB	17.7 ± 2.11bB
HI (%)	WW	28.5 ± 6.02bA	30.4 ± 6.95bA	37.9 ± 7.69aA	40.0 ± 4.91aA
	WS	22.0 ± 5.80cB	21.1 ± 5.64cB	29.1 ± 5.34bB	32.3 ± 3.79aB
GN (m ²)	WW	15929.3 ± 2713.69cA	17599.3 ± 3234.81bA	18112.7 ± 2338.64bA	20863.2 ± 3584.21aA
	WS	15125.4 ± 2150.65cA	16533.0 ± 2129.61bA	16698.1 ± 2208.23bB	18182.8 ± 2195.19aB
SPN (10 ⁴ ha ⁻¹)	WW	741.9 ± 109.00bA	720.3 ± 134.25bA	755.7 ± 76.90abA	800.4 ± 128.84aA
	WS	692.2 ± 144.38aA	718.6 ± 96.47aA	734.1 ± 87.11aA	738.5 ± 104.69aB
TKW (g)	WW	35.8 ± 2.74aA	32.8 ± 1.92cA	34.9 ± 2.93bA	33.7 ± 2.80cA
	WS	30.6 ± 3.05aB	28.6 ± 2.75bB	28.9 ± 3.08bB	27.8 ± 3.22bB

The data represent as the mean ± SD. The same small letter in the same row indicates that the difference is not significant between different genotypes at $\alpha = 0.05$. The same capital letter in the same column indicates that the difference is not significant between WW and WS treatments at $\alpha = 0.01$.

GY, grain yield; BIO, above-ground biomass; HI, harvest index; GN, grain number; SPN, spike number; TKW, thousand kernels weight; WW, well-watered; WS, water-stressed.

Table 6 Pearson's correlation coefficients (*r*) between plant height and the length of internodes in the RILs from IDO444 and Rio Blanco under well-watered (below diagonal) and water-stressed (above diagonal) conditions

	PH	PL	I2L	I3L	I4L	DSL
PH	1	0.59 ^{****}	0.73 ^{****}	0.64 ^{****}	0.49 ^{****}	0.52 ^{****}
PL	0.59 ^{****}	1	0.52 ^{***}	0.48 ^{****}	0.41 ^{***}	0.20 [*]
I2L	0.77 ^{****}	0.56 ^{****}	1	0.74 ^{****}	0.63 ^{****}	0.31 ^{****}
I3L	0.68 ^{****}	0.49 ^{**}	0.75 ^{****}	1	0.67 ^{****}	0.33 ^{****}
I4L	0.53 ^{****}	0.37 ^{**}	0.55 ^{****}	0.69 ^{****}	1	0.22 ^{***}
DSL	0.43 ^{****}	0.20 [*]	0.41 ^{****}	0.29 ^{***}	0.19 [*]	1

PH, plant height; PL, peduncle length; I2L, length of the second internode from top; I3L, length of the third internode from top; I4L, length of the fourth internode from top; DSL, distance from spike to flag leaf ligule; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

1 **Table 7 Pearson's correlation coefficients (r) between plant height and yield traits in the RILs from IDO444**
2 **and Rio Blanco under well-watered (below diagonal) and water-stressed (above diagonal) conditions**

	PH	GY	BIO	HI	GN	SPN	TKW
PH	1	-0.50****	0.22**	-0.55****	-0.34**	-0.23**	0.13*
GY	-0.50****	1	0.10	0.79****	0.25*	0.16*	0.11
BIO	0.21**	0.12	1	-0.41****	0.08	0.09	0.16*
HI	-0.49****	0.71****	-0.56****	1	0.14	0.10	-0.02
GN	-0.34****	0.36****	-0.07	0.29***	1	0.80****	-0.13
SPN	-0.13	0.15	-0.05	0.12	0.89****	1	0.00
TKW	0.06	0.19**	0.16	0.10	-0.08	0.01	1

3 PH, plant height; GY, grain yield; BIO, above-ground biomass; HI, harvest index; GN, grain number; SPN, spike
4 number; TKW, thousand kernels weight; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

5

Table S1 Effects of *Rht4* and *Rht-B1b* on the plant height, internode length and lodging score in the RILs from IRO444 and Rio Blanco under well-watered (WW) and water-stressed (WS) conditions

Traits	Environment	Tall	<i>Rht4</i>	<i>Rht-B1b</i>	<i>Rht4+Rht-B1b</i>
PH (cm)	WW	111.4 ± 7.70aA	95.8 ± 3.11bA	88.6 ± 6.49cA	89.2 ± 4.40cA
	WS	104.8 ± 5.73aB	95.6 ± 3.45bA	85.9 ± 4.58dB	87.6 ± 4.59cA
PL (cm)	WW	42.2 ± 3.20aA	38.4 ± 4.23bA	35.1 ± 3.71cA	35.1 ± 3.15cA
	WS	40.4 ± 2.99aB	37.5 ± 5.01bA	33.4 ± 3.20cB	36.0 ± 2.75bA
I2L (cm)	WW	30.5 ± 1.91aA	25.8 ± 2.11bA	23.9 ± 2.62cA	23.9 ± 1.86cA
	WS	30.9 ± 1.79aA	25.5 ± 2.32bA	24.1 ± 2.74cA	23.8 ± 2.35cA
I3L (cm)	WW	17.3 ± 2.08aA	15.2 ± 2.00bA	13.0 ± 2.17cA	12.8 ± 2.08cA
	WS	17.9 ± 2.44aA	15.0 ± 2.45bA	13.6 ± 2.27cA	12.6 ± 1.73cA
I4L (cm)	WW	12.5 ± 1.53aA	11.3 ± 2.06bA	10.2 ± 1.40cA	10.6 ± 1.28bcA
	WS	12.7 ± 0.98aA	10.9 ± 2.01bA	10.3 ± 1.53bcA	10.2 ± 1.21cA
DSL (cm)	WW	19.9 ± 4.55aA	17.9 ± 3.96bA	16.3 ± 3.36cA	15.7 ± 2.86cA
	WS	17.0 ± 2.49aB	15.4 ± 2.68bB	14.3 ± 2.28cB	13.3 ± 1.97dB
LODS (0-9)	WW	4.6 ± 3.03aA	3.0 ± 2.84abA	2.2 ± 3.14bA	3.1 ± 2.73abA
	WS	4.1 ± 3.63aA	3.3 ± 3.20aA	1.2 ± 2.14bA	2.8 ± 2.82abA

The data represent as the mean ± SD. The same small letter in the same row indicates that the difference is not significant between different genotypes at $\alpha = 0.05$. The same capital letter in the same column indicates that the difference is not significant between WW and WS treatment at $\alpha = 0.01$.

PH, plant height; PL, peduncle length; I2L, length of the second internode from top; I3L, length of the third internode from top; I4L, length of the fourth internode from top; DSL, distance from spike to flag leaf ligule; LODS, lodging score; WW, well-watered; WS, water-stressed.