Field Crops Research 204 (2017) 242–248

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

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

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ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 19 January 2017
Accepted 27 January 2017

Keywords:
Bread wheat
Rht4
Rht-B1b
Plant height
Agronomic trait
Water regime

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.

1. Introduction

Wheat 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, 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; Mathews 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; Mathews 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.,

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.

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http://dx.doi.org/10.1016/j.fcr.2017.01.020
0378-4290/

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 (Mathews 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 and Richards, 2000; Rebetzke et al., 1999, 2004, 2011, 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 × 1.5 m wide.

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 m^2 (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, Miller, 1999), 0.25 m^2 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)