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Effects of *Vrn-B1* and *Ppd-D1* on developmental and agronomic traits in *Rht5* dwarf plants of bread wheat

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ABSTRACT

Preliminary evidence indicates that *Rht5*, a gibberellin-responsive (GAR) dwarfing gene, could reduce plant height without affecting coleoptile length and seedling vigour. However, *Rht5* delayed ear emergence and anthesis, which would hinder its utilization in wheat improvement. To advance the flowering time of *Rht5* dwarf plants, the dominant vernalization gene *Vrn-B1* and the photoperiod-insensitive gene *Ppd-D1* were introduced through two crosses between Ningchun45 (*Vrn-B1*) and Jinmai47 (*Ppd-D1*) with Marfed M (*Rht5*), respectively. Fifty-nine and 71 $F_{2:3}$ and $F_{3:4}$ homozygous lines from the first and the second cross were used to evaluate the effects of *Vrn-B1* and *Ppd-D1*, respectively on the developmental and agronomic traits of *Rht5* dwarf plants during two growing seasons in Yangling, Shaanxi, China. The results showed that *Rht5* significantly reduced plant height (40.1% and 38.9%) but delayed the flowering time (14.0% and 4.8% in thermal time) in both populations. In general, *Rht5* significantly decreased the spike length by 22.6% and 16.7, grain number by 14.5% and 11.5%, 1000-grain weight by 24.1% and 18.4%, and grain yield by 35.1% and 21.5% in the two populations, respectively. *Vrn-B1* could not compensate for the negative effect of *Rht5* on spike development and the flowering time and had no significant effect on plant height or other agronomic traits. However, *Ppd-D1* was able to overcome the delaying effect of *Rht5* on spike development by shortening the duration of the reproductive phase (double ridge stage-anthesis) and finally promoting earlier flowering (8.7% in thermal time) in *Rht5* dwarf lines. *Ppd-D1* also reduced plant height (10.0%), and the combination of *Ppd-D1* and *Rht5* produced even shorter plants (45.0%), achieving dwarf plants with higher lodging resistance. Additionally, *Ppd-D1* increased the grain number (6.3% and 9.6%), 1000-grain weight (13.0% and 21.5%), plant yield (22.6% and 39.5%) and harvest index (31.1% and 49.6%) in both tall and dwarf plants, respectively. Clearly, the combination of *Rht5* and *Ppd-D1* had no negative effect on plant growth or grain yield, advanced spike development and the flowering time, and improved agronomic traits, which may be conducive to exploiting *Rht5* for wheat improvement.

1. Introduction

The most common dwarfing genes used in wheat breeding projects are GA-insensitive *Rht1* (*Rht-B1b*) and *Rht2* (*Rht-D1b*). *Rht1* and *Rht2* are effective in reducing plant height and have been widely adopted in wheat breeding programmes since their introduction in the 1960s, which resulted in the "Green Revolution" (Hedden, 2003). *Rht1* and *Rht2* increase the grain number and yield in favourable environments, however, they are associated with reduced coleoptile length and seedling vigour (Richards, 1992; Ellis et al., 2004; Rebetzke et al., 2004; Botwright et al., 2005) and performance in unfavourable environments (Rebetzke et al., 1999; Butler et al., 2005; Chapman et al., 2007). Longer coleoptiles may permit crops to be sown at the optimal time to increase biomass and yield (Shackley and Anderson, 1995), while deep sowing of short coleoptile *Rht1* and *Rht2* commonly results in fewer, later emerging seedlings with low relative growth rates, leaf area and biomass and ultimately with lower final biomass, fewer spikes and lower yield (Mahdi et al., 1998; Rebetzke et al., 2007, 2012b). Furthermore, *Rht1* and *Rht2* have been the major dwarfing genes used in wheat breeding since the "Green Revolution", and there is only a small number of dwarfing genes (*Rht1*, *Rht2* and *Rht8*) available in current wheat breeding programmes (Ellis et al., 2004; Botwright et al., 2005;

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Zhang et al., 2006; Swati et al., 2015). Disadvantages exist from the limited dwarf source in wheat production. However, preliminary evidence indicates the potential of gibberellin-responsive (GAR) dwarfing genes, *Rht4*, *Rht5*, *Rht8*, *Rht12*, *Rht13* and *Rht14*, to reduce plant height without affecting coleoptile length and seedling vigour (Rebetzke and Richards, 2000; Ellis et al., 2004). Opportunities exist for replacing *Rht1* and *Rht2* with these GAR dwarfing genes to improve wheat yield and lodging resistance (Rebetzke et al., 2007, 2012b; Ellis et al., 2005).

Rht5, a dominant GAR dwarfing gene of the EMS-induced mutant Marfed M of winter wheat, has been shown to be located on chromosome 3BS and associated with the molecular marker Xbarc102 (Ellis et al., 2005). It was reported that *Rht5* can significantly reduce plant height by about 25–55% and increase fertile tillers per plant by about 37.0% without reducing coleoptile length and seedling vigour (Daoura et al., 2013, 2014; Rebetzke et al., 2012b). However, *Rht5* was associated with a significantly delayed heading date and flowering, which possibly resulted in a reduction in grain number and grain weight and ultimately a decreased grain yield and harvest index (Daoura et al., 2013, 2014; Rebetzke et al., 2012b). This adverse effect on plant growth and spike development has limited its use in wheat improvement. Therefore, genes promoting development need to be used to advance the flowering time of *Rht5* dwarf lines to exploit their potential in wheat breeding programmes. The vernalization genes *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, which control the vernalization requirement (Law et al., 1976; Iwaki et al., 2002), have a major impact on the duration of the vegetative phase and flowering time. Plants achieved a spring growth habit when one of the three loci was dominant, and plants showed a winter growth habit when all three loci were recessive alleles (Yan et al., 2004; Chen et al., 2011). The dominant *Vrn-A1* allele provides complete insensitivity to vernalization (achieving the double ridge stage without low temperature), whereas the dominant *Vrn-B1* and *Vrn-D1* alleles each provide a reduced vernalization requirement compared to winter alleles (Pugsley, 1971). Thus, these genes can be used to modify the strong winter habit (requiring a longer time to finish the vegetative phase) and flowering time in wheat. Moreover, the photoperiod genes *Ppd-D1*, *Ppd-B1* and *Ppd-A1*, which are responsible for photoperiod sensitivity in wheat, also have a major impact on the flowering time. The dominant alleles confer photoperiod insensitivity, and in most cases, the effects ranking (measured as days to flowering) is as follows: *Ppd-D1* > *Ppd-B1* > *Ppd-A1* (Worland et al., 1998). The dominant allele of *Ppd-D1* (*Ppd-D1a*) is a major source of photoperiod insensitivity in wheat cultivars worldwide and can promote earlier ear emergence and flowering compared to its recessive allele *Ppd-D1b* (Worland et al., 1988; González et al., 2005; Wilhelm et al., 2013; Grogan et al., 2016). However, can *Vrn-B1* and *Ppd-D1* compensate for the negative effect of *Rht5* and accelerate flowering in the *Rht5* dwarf lines?

The objective of this work was to analyse whether *Vrn-B1* and *Ppd-D1* could overcome the delaying effect of *Rht5* on plant development, promote earlier flowering and improve the yield component in *Rht5* dwarf lines. Moreover, the interactive effects of *Vrn-B1* with *Rht5* and of *Ppd-D1* with *Rht5* on plant height and other agronomic traits were also assessed.

2. Materials and methods

2.1. General description

Experiments were carried out during the growing seasons of 2015–2016 and 2016–2017 in the experimental field of the Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling, Shaanxi, China (34°17′ N, 108°04′ E, elevation of 506 m). To avoid water stress, supplemental irrigation was provided as needed. Weeds were manually removed where necessary, and fungicides and insecticides were applied to prevent diseases and insect damage. Weather data were recorded at an automated weather station at the site.

2.2. Plant material

Two crosses were made between Ningchun45 (*Vrn-B1*, *ppd-D1*, *rht5*) and Jinmai47 (*vrn-B1*, *Ppd-D1*, *rht5*) with Marfed M (*vrn-B1*, *ppd-D1*, *Rht5*) as the pollen donor. Ningchun45 and Jinmai47 are tall wheat cultivars grown in Spring-sown and Autumn-sown areas in northern China, respectively. Marfed M, the donor of *Rht5*, is an EMS-induced mutant of Marfed. The dominant allele of *Vrn-B1* in Ningchun45 is *Vrn-B1a*; the dominant allele of *Ppd-D1* in Jinmai47 is *Ppd-D1a* (Grogan et al., 2016). The genotypes of those parents were confirmed by detection of the corresponding molecular markers.

The F_2 population of these two crosses was sown as spaced plants in the field in October 2014. The loci for *Vrn-B1*, *Ppd-D1* and *Rht5* in each F_2 individual were determined using the corresponding molecular markers, i.e., 2D-Ins-F1/R1/R2 for *Ppd-D1*, Intr1-B-F/Intr1-B-R3/Intr1-B-R4 for *Vrn-B1*, and BARC102 for *Rht5*, using the methods described by (Pugsley, 1983; Fu et al., 2005; Beales et al., 2007; Chen et al., 2013; Grogan et al., 2016). Then, individuals with the four homozygous genotypes of *Vrn-B1Vrn-B1Rht5Rht5* (abbreviated VVRR), *vrn-B1vrn-B1Rht5Rht5* (vvRR), *Vrn-B1Vrn-B1rht5rht5* (VVrr) and *vrn-B1vrn-B1rht5rht5* (vvrr) in the cross between Ningchun45 and Marfed M were selected and developed to the $F_{2:3}$ and $F_{3:4}$ lines as the *Vrn-B1* population for further analysis. Individuals with the four homozygous genotypes of *Ppd-D1Ppd-D1Rht5Rht5* (abbreviated as PPRR), *ppd-D1ppd-D1Rht5Rht5* (ppRR), *Ppd-D1Ppd-D1rht5rht5* (PPrr) and *ppd-D1ppd-D1rht5rht5* (pprr) in the cross between Jinmai47 and Marfed M were selected and developed to the $F_{2:3}$ and $F_{3:4}$ lines as the *Ppd-D1* population for further analysis. Finally, 59 $F_{2:3}$ homozygous lines and 59 $F_{3:4}$ lines from the cross between Ningchun45 and Marfed M and 71 homozygous $F_{2:3}$ lines and 71 $F_{3:4}$ lines from the cross between Jinmai47 and Marfed M were used to evaluate the effects of *Vrn-B1* and the effects of *Ppd-D1* on *Rht5* on developmental and agronomic traits, respectively. The lines and parents were sown in plots of three rows 2 m long and 25 cm apart, with seeds spaced 6.7 cm apart within rows. To avoid competitive effects between the tall and dwarf plants, the dwarf lines were randomly arranged in a dwarf plot, and the tall lines were randomly arranged in a separate tall plot. Each experiment was conducted with two replications.

2.3. Spike development and fertility

Spike differentiation was investigated on three randomly selected plants from each field plot. Beginning from the five-leaf stage (Z15), plants were sampled every 4 or 7 days, and the main shoot was dissected to determine the timing of the double ridge (DR) formation and the terminal spikelet (TS) initiation in the apical meristem, as described by (Gardner et al., 1985) using a digital Stereo Microscope (Nikon, SMZ1500). Pictures were taken using a digital camera linked to the microscope. The timing of heading (Z55) and anthesis (Z65) was visually determined when 50% plants per plot had reached these stages.

At anthesis, five plants in the central row of each plot were chosen to count the number of fertile florets in the main shoot spike. Florets were considered fertile when the stigmatic branches were spread wide, with either pollen grains present on them or with green anthers (Waddington et al., 1983).

Whenever thermal time was used to estimate developmental progress, 0 °C was chosen as the base temperature.

2.4. Plant height and internode characteristics

The number of leaves emerging on the main shoot was determined on five plants per plot (Haun, 1973). At maturity, ten plants for each plot were randomly selected and investigated to obtain the mean plant height as the distance from the soil surface to the top of the ear (awns