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The *Rht13* dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat

G.J. Rebetzke[a,*], M.H. Ellis[a,1], D.G. Bonnett[a,1], A.G. Condon[a], D. Falk[b], R.A. Richards[a]

[a] CSIRO Plant Industry, PO Box 1600, Canberra, ACT 2601, Australia
[b] Department Plant Agriculture, University of Guelph, Guelph, Ontario, N1G 2W1 Canada

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ABSTRACT

The green-revolution *Rht-B1b* and *Rht-D1b* dwarfing alleles are usually associated with increased wheat yields but are linked to reduced early growth and poor emergence if sowing conditions are unfavourable. Other dwarfing genes are available but not used in commercial breeding. The *Rht13* bread wheat donor, Magnif M1, produces uniquely short peduncle and penultimate internodes to reduce plant height. A set of near-isogenic (NILs) and recombinant inbred (RILs) lines varying for height were developed from the cross of Magnif M1 and the *Rht8c*-containing Chuan-mai 18, and evaluated for a range of agronomic characteristics across favourable environments. Reductions in plant height were associated with increased grain number ($r^2 = 0.35^{**}$) and harvest index ($r^2 = 0.62^{**}$) in the NILs. Reduced-height RILs containing the *Rht13*-linked, $Xgwm577_M$ microsatellite marker were significantly shorter, produced greater biomass, yield and harvest index, and increased spike and grain number than lines without the marker. Approximately 74 and 7% of the total phenotypic variance in plant height was accounted for by allelic differences in *Xgwm577* and *Rht8* loci, respectively. The peduncle and penultimate peduncle internodes of *Rht13*-containing lines were proportionately shorter than *Rht8c*-containing sibs and lines containing the *Rht-B1b* dwarfing allele. The unique height-reducing phenotype, increased grain number and yield associated with *Rht13* indicate considerable potential for use of this dwarfing allele for improving wheat performance.

1. Introduction

Reductions in plant height and earlier flowering have closely mirrored the commercial release of newer wheat varieties with greater yield and yield stability (Perry and D'Antuono, 1989). Development of wheats of semi-dwarf stature is a key objective of wheat breeding programs worldwide (Rebetzke et al., 1999; Mathews et al., 2006). Semi-dwarf wheats are less prone to lodging and have greater grain number to increase crop yields. Selection for reduced stature was simplified with the identification of the *Rht-B1b* and *Rht-D1b* dwarfing alleles from the Japanese wheat variety Norin 10. Their deployment and distribution has been highly successful [70% of the commercial wheat varieties grown globally contain either *Rht-B1b* or *Rht-D1b* (Evans, 1998)] and pivotal in yield increases arising out of the green revolution (Hedden, 2003). Yet despite their broad adoption, yield benefits of the *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) dwarfing alleles tend to be greatest in well-managed, irrigated environments and particularly those where fertiliser inputs are high (Pearman et al., 1978). Yield benefits are less obvious in lower-yielding environments where factors such as high temperatures or drought constrain crop growth to reduce yields (e.g. Brandle and Knott, 1986; Richards, 1992; Butler et al., 2005; Mathews et al., 2006).

The *Rht-B1b* and *Rht-D1b* dwarfing alleles reduce cell-expansion response to endogenous gibberellins (Hoogendoorn et al., 1990), and are the most widely deployed of a larger group of gibberellic acid-insensitive (GAI) dwarfing genes. Genotypes containing these alleles show less cell elongation and thus reduced cell length and width (Botwright et al., 2005; Keyes et al., 1989). Cell number remains approximately the same (Keyes et al., 1989; Hoogendoorn et al., 1990), so smaller cells contribute to reductions in internode length, including the peduncle, to affect final plant height (Hoogendoorn et al., 1990). It has been proposed that the reduced growth of the peduncle frees-up assimilate for partitioning to the growing ear, thereby allowing distal florets in the spikelet to continue to grow and undergo fertilisation (Miralles et al., 1998; Fischer and Stockman, 1986; Youssefian et al., 1992). Spikelet numbers remain unchanged but grain numbers per spike increase (Brooking and Kirby, 1981; Miralles et al., 1998; Fischer and Stockman, 1986;

Abbreviations: GAI, gibberellic acid-insensitive; GAR, gibberellic acid-responsive; PEDL, peduncle length; PPED, peduncle proportion of culm; RILs, recombinant inbred lines; NILs, near-isogenic lines.

[*] Corresponding author.
E-mail address: Greg.Rebetzke@csiro.au (G.J. Rebetzke).

[1] Current address: CIMMYT Int. Apdo. Postal 6-641, 06600 México, DF, Mexico.

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Nizam Uddin and Marshall, 1989) to raise grain number per unit area and grain yields (Brandle and Knott, 1986; Flintham et al., 1997).

The smaller cell size associated with the GAI dwarfing genes also contributes to reductions in coleoptile and subcrown-internode lengths, coleoptile tiller production and individual leaf area to reduce overall seedling vigour (Allan, 1989; Rebetzke et al., 2001, 2004; Botwright et al., 2005). Greater seedling vigour and an ability to emerge from deep sowing are common objectives of breeding programs targeting adaptation to water-limited environments (Richards et al., 2002), and are likely to increase in importance with increasing climate variability (Glover et al., 2008).

There is little opportunity in breeding more-vigorous GAI wheats (Allan, 1989; Rebetzke et al., 2007a). However, the prospect exists to develop reduced height wheats containing many minor or single, major gibberellic acid-responsive (GAR) dwarfing genes which permit selection of lines with greater seedling vigour (e.g. Schillinger et al., 1998; Rebetzke et al., 1999, 2007a,b). Accumulation of minor genes for reduced plant height from a biparental cross can be difficult owing to requirement for larger population sizes and potential for large genotype × environment interaction (Rebetzke et al., 1999). A number of major, GAR dwarfing genes have been identified with potential to reduce plant height without affecting seedling vigour (Ellis et al., 2004; Rebetzke et al., submitted for publication). Alleles at two of these loci, *Rht8* and *Rht13*, show promise for use in breeding owing to their effects on reductions in plant height and stem lodging without reducing seedling vigour (Rebetzke et al., submitted for publication). However, little is known of the effects of these genes on grain yield and grain yield components.

The aims of this study were to assess the influence of *Rht8c* and *Rht13* on plant height and associated effects on grain yield and its components. A secondary aim was to investigate the influence of *Rht8c* and *Rht13* on changes in internode length to affect final plant height and agronomic performance.

2. Materials and methods

2.1. Development of recombinant inbred and near-isogenic lines

A cross was generated between the *Rht8c*-containing Chuanmai 18 (hereafter 'CM-18') and the *Rht13*-containing Magnif M1. F_1 plants were allowed to self-pollinate to produce *ca.* 240 F_2 progeny. Further self-pollination without selection for three generations resulted in $F_{4:5}$ individuals. Seed from these plants were threshed and then sown in the winter of 2002 into rows for plant height assessment. A single head was harvested from *ca.* 200 lines homogenous for plant height. Seed from each head was increased in the summer of 2002–2003 to generate F_2-derived, $F_{5:7}$ recombinant inbred lines (RILs). From 30 $F_{4:5}$ lines segregating for plant height, five heads were sampled from each of putatively dwarf, semi-dwarf and tall individuals. These heads were individually threshed and sown into spaced $F_{5:6}$ rows in a nursery during the summer of 2002–2003 along with two near-isogenic wheat sets (the APD and KCD NIL sets described in Richards (1992)) containing 0, 1 or 2 GAI dwarfing genes. Eleven families were identified containing combinations of either doubled-dwarf (*Rht8c* + *Rht13*), semi-dwarf (*Rht8c* or *Rht13*), and/or tall, uniform progeny. These were harvested separately to produce sets of F_5-derived, $F_{6:8}$ near-isogenic pairs differing in plant height. Both NILs and RILs were genotyped for presence of each dwarfing gene using linked molecular markers [*Xgwm261* (*Rht8*) and *Xgwm577* (*Rht13*)] as described in Ellis et al. (2005).

2.2. Evaluating recombinant inbred and near-isogenic lines

Three experiments were conducted to investigate the influence of the *Rht8* and *Rht13* dwarfing genes on plant height and associated effects on agronomic performance:

(a) Experiment 1 evaluated agronomic performance, and ear and culm internode length in 167 CM-18/Magnif M1 RILs and 161 Cranbrook/Halberd DH lines (see Rebetzke et al., 2001) in two irrigated environments (Ginninderra Experiment Station (GES), Australian Capital Territory in 2003 and 2004). The Cranbrook/Halberd population varied for presence of the GAI *Rht-B1b* dwarfing allele.
(b) Experiment 2 evaluated agronomic performance for a set of genotyped NILs and a subset of the CM-18/Magnif M1 RILs (including seven doubled-dwarf, eight single-dwarf and seven tall $F_{5:7}$ selections). These were grown together in three environments: GES in 2003, and Griffith and Stockinbingal, NSW in 2004.
(c) Experiment 3 assessed changes in plant height from midway through stem elongation to midway through grain-fill in two NIL pairs: Bersee (tall) and GA-insensitive, semi-dwarf Bersee + *Rht-D1b*; and Magnif (tall) and GA-sensitive, semi-dwarf Magnif + *Rht13*. These were grown at GES in 2006.

In addition to the NILs, RILs and DH lines grown in the field, other evaluated entries included parents (CM-18 and Magnif M1), commercial semi-dwarf (H45, Janz, and Westonia) and tall (Halberd) varieties, and the APD set of near-isogenic, spring-habit lines varying for presence of *Rht-B1b* and *Rht-D1b* dwarfing alleles (Richards, 1992).

2.3. Phenotypic assessment

In all studies, entries were sown at an optimal 3–5 cm sowing depth into 6-m long, 0.17-m spaced, 5-row plots at a seeding rate of *ca.* 200 seeds per m^2. Lines were grouped into one of the three height classes to minimise competition, and height classes then allocated randomly throughout the experiment. Lines were unreplicated in an augmented design containing multiple replicates of the control check varieties Janz or H45. Nutrients were supplied at sowing as Starter 15® applied at 103 kg/ha. Plots at Stockinbingal were wholly reliant on pre- and growing-season rainfall whereas supplemental irrigation was supplied up to flowering and during grain-filling at GES and Griffith. Sowings were maintained free of diseases and weeds with the application of appropriate fungicide and herbicide control measures.

For each plot, phenological development near anthesis was recorded using a Zadoks score (Zadoks et al., 1974). Plant height was determined at maturity as the distance from the soil surface to the top of the ear (awns excluded) of the tallest culms for each plot. For all plots, between 80 and 120 culms were hand-cut at ground level at maturity using a 30-cm quadrat oriented across 3-bordered rows. Samples were air-dried at 35 °C for three days and weighed before and after threshing, and harvest index calculated as the ratio of grain to total above-ground biomass. Plots were end-trimmed to *ca.* 5.4 m length and the outside border rows removed before machine harvesting. 100-grain weight was measured for a sample of grain from each harvest index sample, and grain number (per m^2) subsequently calculated from plot yields. For the CM-18/Magnif M1 and Cranbrook/Halberd populations grown at GES in 2003 and 2004, additional measurements were made of numbers of fertile spikes, and lengths of the ear, peduncle and internodes below the peduncle on three to four random spikes cut at maturity.