

Unravelling the effects of GA-responsive dwarfing gene *Rht13* on yield and grain size

Drouyer G J-P, Bonnett DG, Ellis MH, Sirault XRR
CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia
Email: David.Bonnett@csiro.au

ABSTRACT

A set of near-isogenic lines (NILs) with dwarfing genotypes *Rht-B1b*, *Rht13* and tall was grown in a phytotron glasshouse experiment to examine differences in biomass accumulation and partitioning. The objective was to determine how *Rht13* backcross lines may have produced equivalent yields but lower levels of screenings (small grains) in multi-environment yield trials compared to sister lines carrying *Rht-B1b* or *Rht-D1b*. Compared to the tall, the *Rht13* NIL grew at a similar rate until late stem elongation; both faster than the *Rht-B1b* NIL. From late stem elongation until anthesis, growth of the peduncle and penultimate internode were strongly reduced with *Rht13* compared with both the *Rht-B1b* and particularly the tall NIL. In spite of the resulting large differences in length of the top two internodes, total water soluble carbohydrate contents were similar. Ear length was reduced in the *Rht13* NIL compared to both the *Rht-B1b* and tall NILs suggesting a possible reduction in grain number that may contribute to the lower screenings observed in the field. Further results from the phytotron experiment and parallel field trials should determine if differences in biomass accumulation and partitioning are associated with reduced screenings and equivalent yield of lines carrying *Rht13* of *Rht-B1b* or *Rht-D1b*.

INTRODUCTION

Work at CSIRO Plant Industry has identified dwarfing genes that, unlike *Rht-B1b* and *Rht-D1b* which are virtually ubiquitous in modern semidwarfs, do not make seedlings insensitive to gibberellic acid (GA) allowing increases in coleoptile length and/or seedling vigour (Ellis et al 2004; Rebetzke et al 1999). Multi-environment trialling of backcross material under conditions that would not favour lines with a long coleoptile or greater seedling vigour further determined that genes *Rht4*, *5*, *8*, and *13* gave comparable yields to sister lines carrying *Rht-B1b* and *Rht-D1b* (Bonnett unpublished). Encouragingly, none resulted in an increase in the percentage of screenings (small grains) and lines with *Rht13* produced substantially lower screenings than *Rht-B1b* and *Rht-D1b* sibs.

Based on seedling studies and the relative lengths of stem internode segments, it appears that *Rht13* begins acting at a later stage of development than other dwarfing genes with much of the effect concentrated in the two uppermost internodes. If this is true and *Rht13*

allows a wheat plant to grow like a tall through much of its life, there is likely to be more competition between the elongating stem and the developing ear affecting grain number and grain size.

This study examined a set of near-isogenic lines to assess the effect of *Rht13* on biomass accumulation and partitioning over the life of the plant and effects on grain number and size. The results presented are from a glasshouse experiment in which plants were grown at a density similar to that found in a typical Australian wheat crop and managed to achieve a yield level similar to a typical Australian dryland yield of around 2.5t/ha. A corresponding experiment has been established at two field sites to validate results from the glasshouse study.

MATERIALS AND METHODS

Lines LAN-1, LAN-3 and LAN-13 are backcross-derived near isolines (NILs) carrying genes *Rht-B1b*, *Rht-D1c* (*Rht3*) and *Rht13*, respectively in a spring version of the Russian winter wheat background Miranovskaya (M808S).

The NILs were sown in a phytotron glasshouse at CSIRO Canberra during March 2008. Single plants were sown in 8cm diameter tube pots. Pots were packed close together to achieve a density of 100 plants/m². A cold temperature treatment was applied for four weeks during the early seedling stage to overcome a minor vernalisation requirement.

Genotypes were randomized with blocking to account for spatial trends and removal of plants for destructive sampling over six sampling dates. Six plants were sampled at each destructive sampling time and buffer plants were placed around the perimeter of the experiment to prevent border effects.

All plants were photographed from two sides and above using a LemnaTec Scanalyzer. Scanning took place every week to ten days depending on growth stage including on the same days as each of the destructive sampling dates.

Destructive sampling dates reported here were at the following key growth stages:

1. Stem elongation
2. 15 days before flowering (booting)

3. Flowering

Water soluble carbohydrate levels in ears and the top two internodes of the main stems at booting were determined by anthrone extraction.

RESULTS

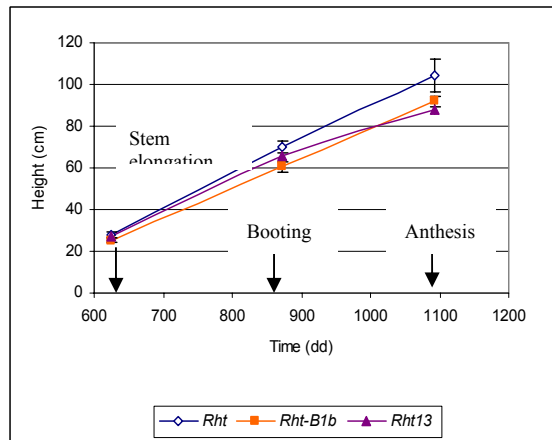
Rate of development of the tall, *Rht-B1b* and *Rht13* NILs were closely matched at all sampling dates (Table 1) indicating that any differences in growth rates and patterns are due to other factors.

Table 1. Zadoks growth stage of near isogenic lines (NILs) at three sampling times used for assessment of plant height, internode length and water-soluble carbohydrate levels.

NIL Genotype	Sampling date 1 21/05/2008	Sampling date 2 9/06/2008	Sampling date 3 26/06/2008
<i>Tall</i>	32.8	49.3	68.0
<i>Rht-B1b</i>	32.9	51.3	67.2
<i>Rht13</i>	33.1	50.1	67.1

Heights of the NILs over the three sampling dates spread from stem elongation through to flowering, showed that the *Rht13* line grew significantly faster than its *Rht-B1b* near-isoline and at a similar rate to the tall until around the booting stage (Figure 1). Although total biomass over this period has not yet been determined, it is expected to be strongly related to height through the stem elongation phase.

Figure 1. Height* of tall, *Rht-B1b* and *Rht13* near-isogenic lines over three sampling dates.



*height measured at the ligule of the uppermost leaf or top of the ear.

From the beginning of ear emergence, height of the *Rht13* NIL increased much slower than the *Rht-B1b* and particularly the tall. Much of the observed difference in growth is attributable to differences in elongation of the

peduncle (Figure 2) and internode immediately below (Figure 5) with *Rht13* having by far the lowest rate of peduncle elongation and ultimately, the shortest peduncle. Interestingly, ear length of the *Rht13* NIL increased significantly slower than in either the *Rht-B1b* or tall NILs which were similar to one another (Figure 3).

Figure 2. Peduncle growth of tall, *Rht-B1b* and *Rht13* near-isogenic lines over three sampling dates.

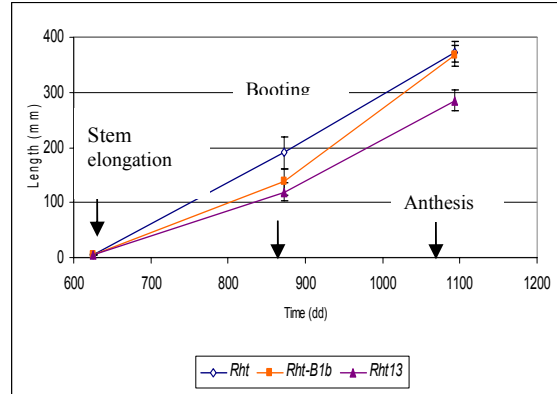


Figure 3. Ear length of tall, *Rht1* and *Rht13* near-isogenic lines over three sampling dates

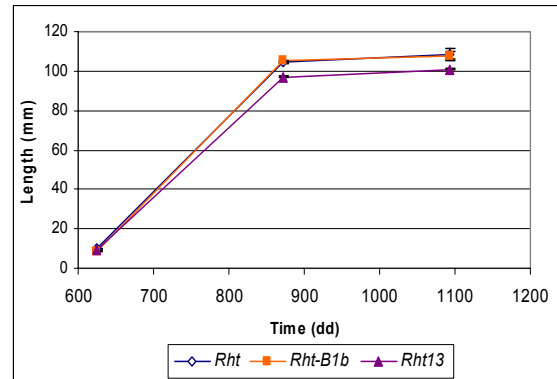
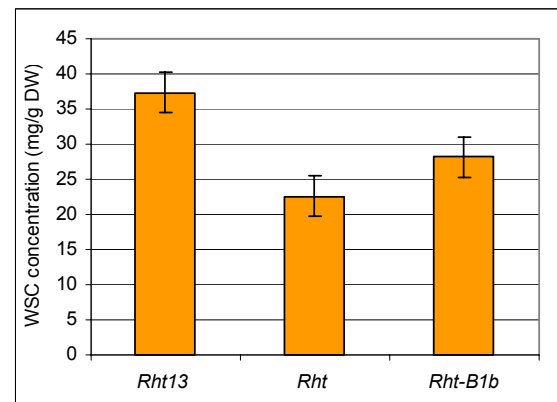
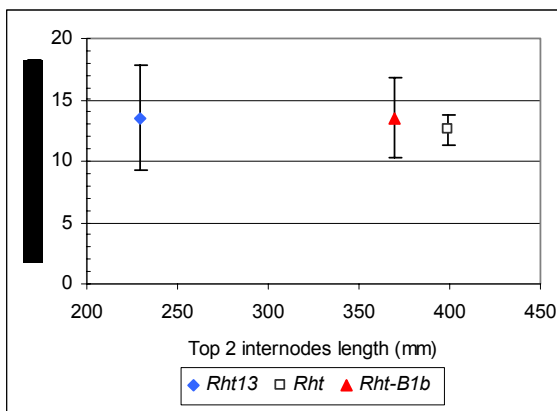


Figure 4. Water-soluble carbohydrate concentration (mg/g) in the top two internodes of tall, *Rht-B1b* and *Rht13* near-isogenic lines at booting (Z49).



Water soluble carbohydrate (WSC) concentrations in the top two internodes were greater in the *Rht13* NIL than in either the tall or *Rht-B1b* NILs (Figure 4). Because of differences in length, the total WSC content in the top two internodes was equivalent in all NILs (Figure 5).

Figure 5. Water-soluble carbohydrate content (mg) and length of the top two internodes of tall, *Rht-B1b* and *Rht13* near-isogenic lines at booting (Z49).



DISCUSSION

Our results show that *Rht13* has little or no height reducing effect until late in stem elongation, with the heights of tall and *Rht13* NILs increasing at a similar rate; both significantly faster than in a *Rht-B1b* NIL. From late stem elongation, the elongation rate of the *Rht13* NIL decreases sharply with much of this effect attributable to a reduction in length of the peduncle and the internode immediately below. This leads to these internodes being significantly shorter than both tall and *Rht-B1b* NILs.

Elongation rate of the developing ear was lower in the *Rht13* NIL than either the *Rht-B1b* or tall lines. It will be interesting to see if the different rates of ear elongation correspond to differences in ear biomass or grain number once these are determined. If *Rht13* produces a smaller ear, it is likely that there are either fewer grains set or more grains set per spikelet.

The findings from our glasshouse experiment so far do not give a clear indication that the different grow pattern conferred by *Rht13* is responsible for our field trial results equivalent yield but lower levels of screenings (small grains) compared with *Rht-B1b* or *Rht-D1b* sister lines. A lower grain number with an equivalent level of stored sugars, leaf area and water use are strong possibilities. Of these, the glasshouse experiment was not designed to compare water use or ensure equal water availability. The field trial will allow measurement of water use and should provide equal water availability to all genotypes to give a better indication of the effects of

the growth rate and partitioning differences to anthesis on yield and grain size.

REFERENCES

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- Rebetzke GJ, Richards RA, Fischer VM, Mickelson BJ. 1999. Breeding long coleoptile reduced height wheats. *Euphytica* 106: 159-168.