Effect of GA sensitive *Rht18* and GA insensitive *Rht-D1b* dwarfing genes on vegetative and reproductive growth in bread wheat

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Highlight

The GA sensitive dwarfing gene *Rht18* and the conventional, GA insensitive dwarfing gene *Rht-D1b* had similar effects on growth and partitioning of assimilates when assessed in backcross-derived lines with one notable exception: *Rht18* does not reduce coleoptile length and has potential to improve crop establishment in rainfed, semi-arid environments.

Abstract

Gibberellin (GA) insensitive dwarfing genes *Rht-B1b* and *Rht-D1b* that are responsible for the 'Green Revolution' have been remarkably successful in wheat improvement globally. However, these alleles result in shorter coleoptiles and reduced vigour and hence poor establishment and growth in some environments. *Rht18*, on the other hand, is a GA sensitive, dominant gene with potential to overcome some of the early growth limitations associated with *Rht-B1b* and *Rht-D1b*. We assessed progeny from both a biparental and backcross population that contained tall, single-dwarf and double-dwarf lines, to determine whether *Rht18* differs from *Rht-D1b* and hence verifying its value in wheat improvement. Progeny with *Rht18* had an almost identical height to lines with *Rht-D1b*, and both were about 26% shorter than the tall lines with the double dwarf 13% shorter again. However, coleoptile length of *Rht18* was 42% longer than *Rht-D1b*. We detected no differences in time to terminal spikelet, anthesis and few differences in stem or spike growth. Both dwarfing genes diverted more dry matter to the spike than tall lines from prior to heading. No differences were detected between *Rht18* and *Rht-D1b* that could prevent the adoption of *Rht18* in wheat breeding to overcome some of the limitations associated with the 'Green Revolution' genes.

Key words: early growth, stem internodes, assimilate competition, carbohydrate remobilisation

Introduction

The semi-dwarfing genes *Rht-B1b and Rht-D1b* in wheat, have been very successful in global breeding programs as they reduce crop height which reduces the risk of lodging. *Rht-B1b and Rht-D1b* are the most important and widely used semi-dwarfing genes and are now found in over 70% of current commercial wheat cultivars globally (Evans 1998). These genes reduce plant height by about 20% in favourable conditions (Youssefian *et al.* 1992b) and they were responsible for the 'Green Revolution' in wheat as more nitrogen fertiliser and irrigation could be applied without the crop lodging. They are referred to as GA insensitive dwarfing genes and they increased yield by 5-25% under favourable conditions (Youssefian *et al.* 1992b, Perkins 1997). The phenotypic traits in *Rht-B1b* and *Rht-D1b* are essentially indistinguishable in terms of height reduction, grain yield and harvest index (Fischer and Quail, 1990; Youssefian et al. 1992b; Flintham et al. 1997), despite *Rht-B1* and *Rht-D1* being on different genomes in hexaploid wheat. Their success is not only due to reduced lodging and increased fertiliser use but they have also been successful in regions where lodging is rare and nitrogen use is low such as Australia (Brennan 1989). The reduced height associated with the *Rht-B1b/D1b* also result in more fertile florets at anthesis resulting in more grains per unit area, a higher harvest index and greater yields (Gale *et al.* 1985, Siddique *et al.* 1989, Miralles *et al.* 1998). The increase in grain number in field environments is associated with an increase in spike dry weight per unit area with total above-ground dry weight at anthesis being unchanged (Fischer 2007). The generally accepted hypothesis for the increase in grain number and spike dry weight in semi-dwarf lines is the reduced competition between developing stems and spikes for limited resources at the time of stem elongation (Brooking and Kirby 1981, Fischer and Stockman 1986).

The critical period for spike development starts at stem elongation, i.e. around terminal spikelet (TS) and ends at anthesis; both the spike and the stem grow rapidly during this period using a limited carbon supply (Kirby 1988). It has been found that *Rht-B1b* and *Rht-D1b* reduce some organ dimensions by reducing cell length rather than cell number (Keyes *et al.* 1989) thereby reducing leaf, sheath and stem lengths. With the onset of stem and spike growth after TS, dry matter partitioning to the spike is greater in lines with *Rht-1* genes because stems are shorter and they do not require as much assimilate as those without *Rht-1* genes. This results in heavier spikes and less abortion of distal florets due to more carbon being available and thus more grains per unit area (Brooking and Kirby 1981, Fischer and Stockman 1986, Youssefian *et al.* 1992b, González *et al.* 2011).

Despite the advantages of the GA insensitive dwarfing genes in terms of grain number and grain yield over a broad range of both irrigated and rainfed environments globally, there are significant disadvantages in some environments, particularly those that are dry and/or when the season is short. The disadvantages are related to the reduced cell size that result in shorter coleoptiles and less early growth compared with their taller counterpart (Richards 1992b, Botwright et al. 2005). This can result in poor establishment and poor early vigour, which has led to a search for alternative dwarfing genes that have long coleoptiles and greater vigour (Rebetzke and Richards 2000, Richards and Lukacs 2002, Ellis et al. 2004). *Rht18,* a mutant originally identified in durum wheat, is a dominant dwarfing gene that shows potential. The gene was first identified in the tall durum cultivar Anhinga and deployed in cultivar Icaro (Konzak 1987). Later, *Rht18* was introduced into winter wheat where the gene reduced plant height by 12-25% in three different Chinese bread wheat cultivars and contributed to an increase in dry matter partitioning between spike and stem (Yang et al. 2015). Molecular studies have shown that the *Rht18* phenotype resulted from a different mechanism of height reduction to *Rht-B1b* and *Rht-D1b*. The *Rht18* mutation lowers GA content by removing GA precursors from the biosynthetic pathway that reduces stem growth but also seedling leaf growth rates (Ford et al., 2018). However, coleoptile length was not reduced by *Rht18* (Tang 2016). In contrast, *Rht-B1b* and *Rht-D1b* encode GA-insensitive forms of this negative regulator of GA signalling, which reduce early growth and coleoptile length (Peng et al., 1999).

Rht-B1b and *Rht-D1b* are reported to have no effect on the timing of developmental events such as floral initiation or anthesis but they change the rate of stem elongation (Youssefian *et al.* 1992a). Elongation of internodes is initiated sequentially, and longer distal internodes have faster growth rates than basal ones. The maximum rate of stem elongation occurs just before anthesis when the peduncle and penultimate internodes are elongating rapidly. *Rht-B1b*, *Rht-D1b* and a more extreme GA-insensitive allele, *Rht-B1c,* were found to slow down internode elongation rate, resulting in shorter and lighter internodes (Fischer and Stockman 1986, Youssefian *et al.* 1992b). However, spike growth does not follow the same pattern as the internodes. Rachis elongation initiates after TS and reaches full length before ear emergence, but linear spike growth in dry matter reaches its maximum rate at ear emergence. Fischer and Stockman (1986) found no difference in spike weight between *Rht-B1b*/*D1b* and tall, but the proportion of spike to stem was larger in dwarf lines in the 15 days preceding anthesis. Similarly, Youssefian *et al.* (1992b) reported no difference between *Rht-*

B1b, Rht-D1b, Rht-B1c and tall lines in terms of spike length, but lines with dwarfing genes showed greater ratios of spike weight to stem weight than tall lines even before TS. Thus, the key advantage for these dwarfing genes is their greater dry matter partitioned to the spike than to the stem and the distal internodes in the period before anthesis. No information is available on the growth dynamics of GA sensitive dwarfing genes such as *Rht18,* which could inform breeders about their potential to replace *Rht-B1b* and *Rht-D1b.*

The objective of this study was to contrast the dynamics of growth from the time of coleoptile emergence through to maturity in closely related lines differing in the presence of GA insensitive and GA sensitive dwarfing genes including tall (*Rht-D1a*), semi-dwarf (*Rht-D1b* or *Rht18*) and double dwarf (*Rht-D1b + Rht18*) sibs from biparental and backcross populations. These comparisons will help determine the potential advantages and/or disadvantages in deploying *Rht18* in wheat breeding programs and help to better understand how changes in carbon partitioning to the developing wheat ear and stem that can contribute to an increased grain number and harvest index (Rivera-Amado *et al.* 2019).

Materials and Methods

Genotyping

Families from both experiments were genotyped with markers for *Rht-D1b* (Ellis *et al.* 2002). *Rht18* is tightly linked with SSR marker *WMS4603* (Ford *et al.* 2018), and families with *Rht18* carried the 239 bp allele in contrast with 220 bp in families lacking *Rht18.* All families were fixed as Tall (*Rht-D1a,*-), *Rht18* Semi-dwarf (*Rht-D1a, Rht18*), *Rht-D1b* (*Rht-D1b,* -), Double dwarf (*Rht-D1b, Rht18*).

Plant material

The *Rht18* gene was transferred from durum cultivar Icaro into the hexaploid tall wheat Halberd. A backcross was made to Halberd and the line HI25M carrying *Rht18* was selected from a BC_1F_5 plant with high fertility (Rebetzke, pers. comm.). Lines used in this study were derived from the cross HI25M (*Rht18*) × Espada (*Rht-D1b*), which gave rise to lines of four genotypic classes. Espada is a successful cultivar grown in Australia with good yield, disease

resistance and grain quality and was bred by Australian Grain Technologies. Experiments were conducted using the four genotypic classes selected from each cross but from different generations which are described in Table 1. Espada and HI25M as parental controls were grown in both field and tray experiments. Unless otherwise mentioned, there were five families per genotype chosen based on their uniformity in height.

Two populations were used in this study: For Tray 2012, approx. 300 F_2 seedlings from the HI25M x Espada bi-parental population were screened with markers described in section 2.1. Between 12 and 14 homozygous lines were identified for each genotypic category. F_3 progeny with six seeds per line from the bi-parental population were evaluated for coleoptile length. For Tray 2014, a backcross population (BC_2F_4) was used for assessment of early vigour. Five families per genotype and 4-5 seeds per family were selected within weight range of 36 to 45 mg and sown in trays (see section 2.3.2) in a randomised design (Table 1).

Field experiments

In the field experiments, seed was limited and so each family was sown as a single 20 m row and each row was spaced 30 cm apart in ACT, (-35.270S, 149.105E) in 2012 and 2013. The experiments were planted on 18 September 2012 and 15 May 2013. Both Espada and HI25M are spring wheats and in Australia can be planted in winter or spring. The standard planting time for a spring wheat in Acton is early June to escape frost at flowering. The two different planting dates were chosen to maximise seasonal variation in growing conditions and crop duration. Each experiment was arranged in a split-plot design with genotypes as main plots and the five families of each genotype as sub-plots. This resulted in the formation of mini-canopies of each genotype as each family of each genotype had a similar height. There were two blocks of each genotype in each experiment. Basal nutrients were applied at sowing and later top dressed with urea. In 2014 BC_2F_5 seed were sown in plots (1.3 m × 6 m) at Ginninderra Experiment Station (GES, -35.199S, 149.085E), ACT on 10 June 2014. Four genotypes were sown in 2 blocks and lines were randomised within blocks.

The 2012 and 2013 experiments were irrigated, while the 2014 experiment was reliant on natural rainfall. Fungicides and herbicides were used to control diseases and weeds as required. Meteorological data was sourced from the main Canberra meteorological station, ACT about 10 km east of the experimental site in Acton (Table 2).

Tray experiments

Experiments in 2012 and 2014 were sown in wooden trays (55 × 28 × 12 cm) containing a potting mix of 50% compost and 50% vermiculite. Tray 2012 was wrapped in black plastic to prevent moisture loss and to block out light and placed at 4 °C for 2 days to remove any residual seed dormancy and ensure even germination. Trays from 2012 were then kept in the dark in a cabinet maintained at a constant 15°C for 14 days, while the Tray 2014 experiment was sown outside in July. Average maximum and minimum temperature during the growing period in July and August are provided in Table 2. Average daily solar radiation in these months was 10.7 MJ m^{-2}. These are representative of conditions during early seedling growth in Australia.

Morphological measurements and data analysis

Field experiments

Flowering time (GS65) was determined for each line in all field experiments (Tottman 1987) to record when each plot had 50% or more ears flowering (anthers visible).

Harvests were made on 13 occasions in 2012 and 15 in 2013 between terminal spikelet (TS) and maturity. In each experiment five plants were harvested randomly from each family and the main stem was processed to determine spike and internode length and weight. All length data was measured using a ruler and recorded in millimetres and all weight data were attained after drying at 65 °C for 48 hours using 4 digits scale and reported in milligrams. Plant height was measured from the soil surface to the tip of the spike. All internodes were measured after cutting at the node and the leaf sheath was removed. They were named in an order from top to bottom as: Peduncle, P-1, P-2 and P-3 as shown in Figure 1. Spikes were cut at the base of the ear (node between ear and peduncle) and spike length was measured from the base to the tip of the ear (not including awn). Spike length at terminal spikelet (TS) (39 DAS in 2012, and 85 DAS in 2013) was used to determine whether genotypes differed in their early development. Spike partitioning index (SPI) was calculated at each harvest up to flowering as the ratio of spike to above-ground dry weight of the main stem. Fruiting efficiency (FE) was determined as the quotient of grain number per spike at the maturity harvest and spike dry weight at anthesis. Remobilisation of stem carbohydrates after anthesis was calculated as the maximum stem weight less the stem weight at maturity. Maximum

weights were averaged over the first three harvests after anthesis in 2012 and the first four harvests after anthesis in Field 2013. Stem density for each internode was calculated as the weight per unit length of the internode. In 2014 five randomly chosen main stems from each plot were harvested 7 days after anthesis. Stems and spikes were treated the same as in 2012 and 2013.

Tray experiments

For Tray 2012, coleoptile length was measured as the distance from soil surface to the top of the coleoptile sheath. Six coleoptile length measurements from each line were ranked and the three longest values of each line (free from any abnormalities) were used to calculate the mean lengths.

For Tray 2014, plants were harvested before the fourth main stem leaf had fully expanded. The number of leaves and tillers was recorded. Leaf width and length of each main stem leaf was measured with a ruler or calliper. Leaf area was calculated using the formula 'Leaf area = 0.75 × Leaf length × Leaf width'(Rebetzke and Richards 1999). Leaves were dried at 60°C for 24 hours then weighed. Specific Leaf Area (SLA) was calculated as the ratio of leaf area to dry mass of the three main stem leaves. Total leaf area was calculated from the product of dry mass of the total leaves and SLA.

Data analysis

Statistical analyses were performed using ANOVA in Genstat (V16th Edition) and the least significant difference (l.s.d.) was provided. Thermal time (ºCd) was calculated by days from sowing multiplied by averaged daily temperatures (0 °C was used as base temperature) extracted from a temperature logger placed at each site starting from sowing day. Sigmoidal curves were fitted to plots of spike and internode lengths or weights versus thermal time using SigmaPlot (Ver.12). The main spike growth period (MSGP) in length and weight were determined using the corresponding thermal time for 80% of the total growth (between 10 and 90%) in spike length and weight.

Results

Meteorological data from May to December 2012-2014 showing minimum, maximum temperature and rainfall amount for each month is in Table 2.

Tray experiments are summarised in section 'Coleoptile length and early growth', while data from field experiments are described in the other sections below.

Plant height and internode length

Final plant height varied in the three experiments (Table 3) because of either sowing date (2012 and 2013) or available soil moisture (2014). Lines in the earlier planting (Field 2013-May 2013) are most typical of plant height in favourable field environments and 80 cm is typical for Espada grown in the field under favourable conditions (Richards unpublished). Averaged across three experiments the tall lines were 35% taller than the single gene dwarfs (*Rht18* or *Rht-D1b*) and 52% taller than the double dwarfs. The single dwarfs were 13% taller than the double dwarfs. There was no significant difference in plant height between *Rht18* and *Rht-D1b* in any experiment, suggesting both genes reduce height by the same amount even in contrasting environments.

The three distal internodes were longest in the tall (*Rht-D1a*) and shortest in the double dwarf (*Rht-D1b* + *Rht18*) (Supplementary Table 1a). The internodes of the single dwarfs *Rht18* and *Rht-D1b* were similar in length. When internode length was expressed as a percentage of the total stem length, there was almost no difference between *Rht18* and *Rht-D1b* in any of the internodes (Supplementary Table 1b). The greatest difference was for the double dwarf which had a shorter peduncle and a longer P-2 internode in percentage terms. Internode length varied according to genotype and environment (Supplementary Table 1a,b).

Time of terminal spikelet and flowering

Despite large differences in time of sowing no differences were detected among genotypes for flowering time in any year. Growth stage 65 occurred on Nov 28th in 2012 and on Oct 5th in 2013. Terminal spikelet of each line also occurred at the same time in all genotypes in both years (Oct 25th 2012 and Aug 10th 2013). There were no significant differences in the length of the young spike in 2012 at the time of TS although the tall

genotype was longer (2.8 mm) than the *Rht-D1b* (2.6 mm), Double dwarf (2.5 mm) and *Rht18* (2.2 mm). In 2013 the differences at TS were even less, as may be expected in BC_2 lines, with spike lengths ranging from 2.3 mm (Tall, *Rht18* and *Rht-D1b*) to 2.5 mm (Double dwarf).

Coleoptile length and early growth

Coleoptile length of *Rht18* lines were equivalent to standard tall (*Rht-D1a*) lines (Table 4). Coleoptiles of both genotypes were significantly longer than the semi-dwarf *Rht-D1b* and the double dwarf (*Rht-D1b* + *Rht18*). There was no difference in coleoptile length between *Rht-D1b* lines and double dwarf lines. Despite the long coleoptile of *Rht18* lines the length of the first three main stem leaves were shorter than the tall lines and equivalent to the double dwarf. Main stem leaves 2 and 3 of the *Rht-D1b* were also significantly longer than *Rht18*. On the other hand, the leaf area and plant dry weight at harvest (average main stem leaf number 3.5) of *Rht-D1b*, *Rht18* and the double dwarf were all lower than the tall genotype. There was no significant difference between *Rht18* and the other genotypes in the average width of the first three main stem leaves. The weight of each seed sown in the early seedling growth experiment were similar (Table 4), although some of the differences were significant. Differences of this magnitude are unlikely to influence vigour measurements. No differences in tiller number or leaf number were observed between the genotypes.

Spike and stem growth up to anthesis

Figure 2 shows the increase in the length of the spike and the stem as well as the weight of the spike and stem for the four height classes in 2012 and 2013. No difference in final spike length was found among the genotypes in both years, nor in 2014 (Table 5), although there were small differences in spike length early in development in the tall line in 2012. Spike length was about 2 cm longer in 2013 than 2012. In 2012, the rachis of the tall genotype elongated faster (Tt=650-900 °Cd) than other genotypes early in development but this was not found in the backcross population. Similarly, only small differences among genotypes were found in spike weight in the two years (Fig 2). In contrast to spike length and weight, the stems varied markedly for length and weight between genotypes with differences

generally matching plant height. Thus, the tall genotype had the longest and heaviest stems in both years whereas the double dwarf had the shortest and lightest stems. The two single dwarfs were intermediate and largely indistinguishable from each other. Similarly, the elongation rate of the stems (slope of the lines in Fig 2) was highest in the tallest lines and lowest in the shortest lines. Although there was some evidence that the *Rht18* lines from the biparental population were slower to elongate; this was not found in the BC population. There were large differences between 2012 and 2013 for stem weight, which was attributed to the earlier sowing time and longer stems in 2013. Results from 2014 were generally similar (Table 5) although spike weight of *Rht18* was less than other genotypes at the harvest time one week after anthesis.

In both years the maximum increase in spike length occurred before the maximum increase in spike weight. Whereas the fastest rate of stem elongation occurred at about the same time as the increase in stem weight in both years (Fig 2AB).

The main spike growth period (MSGP) in length and weight capturing 80% of the maximum period of growth of the spike is shown in each panel in Fig 2. Interestingly, the increase in spike length occurred before the main stem elongation period in both years whereas the increase in spike weight coincided exactly with the main increase in stem weight in both years. It was evident that the increase in spike length ceased before anthesis whereas stem length continued slightly after. The increase in spike weight after anthesis is due to grain growth in both years. Stem weight also continued to increase after anthesis especially in 2012.

Internode growth up to anthesis

To determine which internodes compete with spike growth, and if *Rht18* differs from *Rht-D1b* in length elongation and weight accumulation during the critical period, stems were partitioned into peduncle, P-1, P-2 and P-3 (or including lower internodes). Growth of each internode in each height class in the 2012 and 2013 experiments are shown in Figs 3 and 4. The elongation pattern of different internodes was largely similar for each of the genotypes (the tall genotype is not shown in the length figures) in both experiments. The basal internodes elongate first, and the peduncle elongates last. The elongation of the spike occurred at the same time as the elongation of P-2 in both experiments. There is little overlap

in elongation of the spike with the other internodes. In fact, the spike had reached its maximum length about the time the peduncle and P-1 commenced elongating. It is notable that there was little overlap in elongation between P-3, P-2 and P-1 and P-1 elongated about the same time as the peduncle in both years. As was expected the elongation of the double dwarf was lowest. There were notable differences between *Rht18* and *Rht-D1b* in the timing of internode elongation. *Rht18* was generally slower to commence elongation of most internodes in most years compared with *Rht-D1b* (Fig 3).

The increase in weight of each of the internodes followed the same pattern as the change in length with the basal internodes elongating first (Fig 4). The internode weight was always greatest in the tall genotype and lowest in the double dwarf. It is notable that the fastest dry matter growth of the spike was coincident with the increase in weight of the last three internodes (P-2, P-1 and the peduncle). Thus, the main competing sinks to the spike were P-2, P-1 and the peduncle. This was the case for all genotypes. It is also notable that the length of the internodes did not correspond with the weight of the internodes. The heaviest internode at anthesis was P-1 in 2013 (peduncle was equivalent to P-1in 2012), yet it was half the length of the peduncle. This was also observed in 2014 (Table 5) which was measured a week after anthesis. There was also evidence of a small delay in the increase in dry weight of the peduncle associated with *Rht18* in both years. This delay of *Rht18* in peduncle and P-1 led to a shorter and lighter peduncle and P-1 in 2014 (Table 5). There were no consistent differences between *Rht18* and *Rht-D1b* in growth of the different internodes in 2012 and 2013.

After anthesis there was further linear growth in the length of the peduncle. This was greater in the tall genotype than in the shorter genotypes (data not shown as the tall genotype was omitted in Fig 3). However, due to the slight delay of *Rht18*, the elongation rate remained higher than *Rht-D1b* and double dwarf in both 2012 and 2013. All stem internodes continued to increase in weight after anthesis.

Spike partitioning index (SPI) up to anthesis

The ratio of spike weight to spike plus stem weight from the period just after TS to just after flowering in 2012 and 2013 is shown in Fig 5. Changes in this ratio occurred over two phases. The first phase is when the spike is growing at a faster rate than the stems and this continues to just after heading i.e. the time the spike emerges out of the flag leaf sheath, and

before the main increase in weight of the peduncle and P-1. After this the SPI increases more slowly until just before anthesis because of the increase in weight of the stems. This is largely due to the growth of the peduncle and P-1 but also P-2. Fig 5 shows that the second phase is the period of greatest competition between the growing stems and spikes. The tall genotype had the lowest values for SPI at most time points, whereas the double dwarf had the highest values in both experiments. This was also found at one week after anthesis in 2014 (Table 5). SPI for *Rht18* was lower than *Rht-D1b* around heading in 2013 and the difference was maintained throughout anthesis, but this was not evident in 2012. It was also notable that in 2012 where the stems were shortest the SPI values were higher than in 2013. The SPI values in 2014 one week after anthesis were equivalent to those at the equivalent time point in 2013 (Table 5). Also, consistent with 2012 and 2013, the tall lines had the lowest SPI and double dwarf had the highest SPI. *Rht18* had the same SPI as *Rht-D1b* despite the lower spike weight and peduncle weight.

Fruiting efficiency at anthesis was determined in the three field experiments. Averaged over these experiments the highest values were obtained for *Rht18* (79.7) and the double dwarf (78.7) and the lowest value was for the tall genotypes (71.7) with *Rht-D1b* intermediate (74.1).

Stem carbohydrate storage and remobilisation after anthesis

Just after anthesis, stems had reached their full length, however, dry matter accumulation in the stems continued until a maximum was reached approximately 200 °Cd after anthesis (Figure 6). This maximum value was maintained for a longer duration in 2013 than 2012 before it declined to values similar to that at anthesis in 2012 but to values lower than those at anthesis in 2013 (Figure 6). On average, about 30% of the maximum stem weight was lost by maturity.

In both 2012 and 2013, *Rht18* had significantly larger maximum stem weights than *Rht-D1b* but they ended up with the same weight at maturity (Fig 6). The decline in dry weight of the total stem and each internode from the maximum value to maturity is shown in Table 6. There were large differences between 2012 and 2013 but the trends were the same. Although longer the peduncle stored less labile carbohydrate than internode P-1 and P-2 in 2012 and less than P-1, P-2 and P-3 in 2013. Averaged over both experiments the tall lines remobilised

more labile carbohydrate than *Rht-D1b* and the double dwarf but not significantly more than *Rht18*. Internode P-1 lost the most assimilates followed by P-2 and then the peduncle, indicating loss of dry matter of each internode is affected by linear density rather than length.

The change in internode density of the distal three internodes of *Rht18* and *Rht-D1b* from anthesis to maturity in 2012 and 2013 was compared (data not shown). The pattern of change in density for each internode was very similar. No differences between *Rht18* and *Rht-D1b* were detected in the peduncle in either experiment.

Discussion

The GA insensitive alleles *Rht-B1b* and *Rht-D1b* have been extensively used in wheat breeding globally. They reduce stem length that results in more assimilate for spike growth, more fertile florets and thus higher grain yield. The alternative dwarfing gene, *Rht18* was compared with *Rht-D1b* to determine whether different height reducing mechanisms had different effects on growth of single dwarfs and double dwarfs. This study enabled detailed comparisons of early growth, stem growth and spike growth in related progeny derived from biparental and backcross populations.

Rht18 was found to reduce plant height by about 25% compared with the tall *Rht-D1a*. The height reduction by *Rht18* is equivalent to that found for *Rht-B1b* and *Rht-D1b* in several studies. Richards (1992a) found that *Rht-B1b* or *Rht-D1b* reduced plant height approximately 23% in rainfed environments, which was larger than the 18% reduction of *Rht-D1b* under irrigated conditions (Fischer and Quail 1990). The individual internode lengths in lines with *Rht18* were essentially the same as in *Rht-D1b*. When expressed as a proportion of total stem length all lines were very similar including the tall and the double dwarf. The reduction in internode lengths attributed to *Rht18* in this study was greater in magnitude compared with that reported by Yang *et al.* (2015) but the overall trend was similar.

Combining *Rht-D1b* with *Rht18* reduced height by 11% in the double dwarf, i.e. the height of the double dwarf was 37% shorter than the tall. This is less than the reduction of combining the GA insensitive alleles, *Rht-B1b* + *Rht-D1b*, which reduced height of the tall isolines by 47% (Richards 1992a). This double dwarf combination of *Rht-D1b* and *Rht18* may be valuable to achieve more suitable plant height as typically the *Rht-B1b* or *Rht-D1b* can sometimes be too tall whereas *Rht-B1b* + *Rht-D1b* are almost always too short. The *Rht-B1b* or *D1b* + *Rht18* may prove a useful height under favourable conditions as we show here

that there is no compromise in spike growth. Double dwarf lines with the GA sensitive *Rht4* gene and *Rht-B1b*, were shown to not change plant height over *Rht-B1b* when *Rht4* was added (Liu *et al.* 2017). In this study further height reduction was achieved by combining *Rht18* with *Rht-D1b* indicating that the *Rht18* mutation was able to reduce GA content and therefore height in the presence of a mutant DELLA gene (*Rht-D1b*) which negatively regulates growth by perturbing GA signalling. The same result was observed when *Rht18* was combined with *Rht-B1b* in the cultivar Young background (Tang 2016).

We could find no differences in timing of development (terminal spikelet or anthesis) between *Rht18* and the other genotypes with *Rht-D1a* or *Rht-D1b* and this was also found in other studies on *Rht-B1* and *Rht-D1* (Richards 1991, Youssefian *et al.* 1992a). There was some evidence for a slightly delayed elongation of the stem in *Rht18*, but this was not so evident in the earlier sowing with more genetically related germplasm in 2013.

The reduced height of GA insensitive dwarfing genes is associated with reduced vigour and coleoptile length that often results in poor establishment and slow early growth (Richards 1992b). *Rht18* has a long coleoptile and therefore offers advantages over *Rht-B1b* and *Rht-D1b* in many environments where deep sowing is required and if soils are warm (Yang *et al.* 2015, Rebetzke *et al.* 2016). It may also have advantages over *Rht-B1b* and *Rht-D1b* if greater vigour is required to increase crop transpiration as a proportion of evapotranspiration or to outcompete weeds (Richards and Lukacs 2002). In this study we confirmed the long coleoptile associated with *Rht18* where it was 42% longer than *Rht-D1b* and was equivalent to tall wheats from the same population. Interestingly the double dwarf had the same coleoptile length as the single *Rht-D1b* semi-dwarf. An unexpected finding was that in contrast to the tall genotype, backcross-2 lines containing *Rht18* had reduced early vigour. This was evident from the smaller leaf area and plant dry weight at the 3.5 leaf stage of growth. This reduction in vigour must occur very early as the lengths of the first three main stem leaves in the *Rht18* lines, including the double dwarfs (*Rht18 +Rht-D1b*), were significantly shorter than the tall and not different to the *Rht-D1b* wheats. The finding that *Rht18* reduces plant height as well as the elongation of early leaves is similar to the findings of Paolillo Jr *et al.* (1991) who demonstrated that *Rht-B1b* and *Rht-D1b* alleles reduce stem length as well as the length of the extension zone in wheat seedling leaves and hence the rate of leaf elongation. Reduced early growth in *Rht18* is consistent with the finding that the gene lowers GA content by upregulating the expression of GA 2-oxidase (*GA2oxidaseA9)* throughout plant development, which reduces seedling growth and plant height (Ford *et al.*

2018). The contrast between *Rht18* and *Rht-D1b* is interesting as both reduce growth of seedling leaves whereas coleoptile length is unaffected in *Rht18* but reduced in *Rht-D1b*.

The success of *Rht-B1b* and *Rht-D1b* centres primarily on the growth of the spike relative to the stem. An objective of this study was to determine whether *Rht18* behaves like *Rht-D1b* as this will also determine the potential success of *Rht18*. Accordingly, the growth of the spike in relation to the growth of the stem and internodes was monitored weekly between terminal spikelet and flowering. As in other studies, despite the large difference in plant height, the length of the spike of all genotypes here was the same (Fischer and Stockman 1986). The elongation of the spike occurred earlier than the main elongation phase of the stem. The increase in spike weight on the other hand was delayed and this coincided with the increase in stem dry weight in distal internodes. Thus, there is competition for assimilates between growing stems and developing spikes as found by others (Brooking and Kirby 1981, Fischer and Stockman 1986). In this study, for the same increase in spike weight there were large differences in dry weight increase in stems of the different genotypes. Averaged over both experiments the increase in dry weight of stems during the main spike growth period was 695 mg for the tall, 515 mg for the semi dwarfs (*Rht18* and *Rht-D1b*) and 370 mg for the double dwarf (*Rht-D1b* + *Rht18*) demonstrating that for the same spike weight there was substantial variation in demand for assimilate by the stem as height changed. In these experiments the increase in dry weight of the spike slowed down around 50 to 100 ^{0}Cd before anthesis. This corresponded to about 1 week before flowering, which is the period that determines the competency of the florets to set seed (Kirby 1988). Averaged over both experiments there was an additional 250, 160 and 90 mg of additional growth in the tall, semi-dwarf and double dwarf stems indicating a further competition for assimilates according to height during the critical floret development stage. These dynamics are also shown in Fig 5 for the spike partitioning index (SPI). Similar results have been reported in winter wheat (Brooking and Kirby 1981) and spring wheats (Miralles *et al.* 1998). Interestingly SPI shows two phases, an early phase just after terminal spikelet when competition between the growing spike and the stems was small when spikes grew proportionately faster than stems, particularly in the lines with dwarfing genes, and then a later critical period between heading and anthesis when the SPI plateaued as the growing spikes versus faster growing stems contrast to the SPI from previous phase. The plateau is attributed to the growth of the peduncle and the P-1 internode which are strong sinks for additional assimilate. The competition for assimilates is also partly reflected in the fruiting efficiency which was lowest

in the tall line and highest in *Rht18* and the double dwarf. In this study, association of SPI with plant height (correlation coefficient = 0.92 for data in all experiments) was independent of the dwarfing genes.

To better understand the competition between spike and stem during the critical spike growth period, the maximum spike growth period (MSGP) capturing 80% of the spike growth was compared with the growth of each internode to determine which internodes overlap with spike growth and whether *Rht18* behaves differently compared with tall or *Rht-D1b*. Internodes grew sequentially in time from basal to distal and the number of internodes detected on the main stem varied with time of planting. In this study, plants sown in winter (2013) had one more internode than plants sown in spring (2012), thus the distal internodes (peduncle, P-1, P-2 and P-3) were the most consistent internodes to examine. We found the MSGP coincided with the growth of the three most distal internodes but more with P-1 and P-2 in weight and not just the peduncle. This is similar to Kirby (1988) although in their study further growth of internodes after anthesis was not examined. The peduncle was the longest internode and it was almost twice the length of P-1 which in turn was about twice the length of P-2. However, in terms of weight, P-1 tended to be the heaviest followed by P-2 and then the peduncle. This was most evident in 2013 and 2014 which are the most representative field environments in terms of planting time in this region (Table 5, Figs 3 and 4). The spike continued to increase in weight up to anthesis as did the internodes especially P-1 and the peduncle. This relates to the question posed by Richards (1996) as to whether the length of the peduncle could be shortened so as to reduce competition for assimilates in the period immediately before flowering, which is a main period that determines grain fertility and grain number. There is evidence that the GA sensitive dwarfing gene *Rht13,* in combination with *Rht8*, has a shorter peduncle to reduce competition to the growing spike (Rebetzke *et al.* 2011). There is also evidence here with the double dwarf (*Rht-D1b* + *Rht18*) that it invested far less in stem growth in the critical one-week period before anthesis.

The dry weight of the stem in all genotypes continued to increase after flowering despite no further increase in length and this was attributed to the storage of labile carbohydrates as these later declined to levels below those at anthesis in 2013, which closely matched the most suitable planting date in southern Australia. Although this increase in weight is expected (Bell and Incoll 1990), the surprising feature is that there appears to be surplus assimilates at the time when florets are being aborted just before flowering. There was some evidence that

Rht18 accumulated more labile carbohydrates than *Rht-D1b*. This may also account for the higher fruiting efficiency in *Rht18*.

Except for the coleoptile length of *Rht18*, the results presented here for lines in the Espada genetic background show that *Rht18* is very similar to the major GA insensitive dwarfing genes in detailed aspects of growth and development in spite of the different mechanism. They confirm the conclusion of Fischer and Stockman (1986) and Richards (1992a) that there is nothing intrinsically unique about the Green Revolution genes (*Rht-B1b, Rht-D1b*) other than they reduce plant height and this reduces the competition for resources between the spike and the growing stems and results in proportionately larger ears, a higher grain number and a greater harvest index and yield.

Conclusion

Comparing an alternative semi-dwarfing gene with a conventional semi-dwarfing gene in a related genetic background demonstrated that *Rht18* behaved almost identically to *Rht-D1b*. The most important difference is the long coleoptile associated with *Rht18* but a surprising finding was the reduction in seedling vigour associated with *Rht18*, similar to *Rht-B1b* and *Rht-D1b*. *Rht18*, therefore, is likely to limit genetic gain if greater early seedling vigour is essential for water-use efficiency in some environments (Richards, 1991). However, where early seedling vigour is not essential, no impediments to growth and yield were identified in germplasm containing *Rht18*. It is likely that the further reduction in height by combining *Rht-B1b/D1b* with *Rht18* could be important for very favourable environments.

Lines with *Rht18* were also shown to be very similar to lines with *Rht-D1b* in final plant height, internode lengths, and growth pattern pre- and post-anthesis in three field experiments varying markedly in sowing time and crop duration. Both *Rht18* and *Rht-D1b* varied from the tall and the double dwarf. *Rht18* may have delayed the growth of spike and distal internodes at the beginning of stem elongation more than *Rht-D1b*, although no differences were detected after anthesis. That resulted in the smaller SPI in *Rht18* than *Rht-D1b* pre-anthesis in one of the experiments. There was also some evidence for a greater storage of labile carbohydrates in *Rht18* stems just after anthesis and for a higher fruiting efficiency. During the critical period where spike and internodes are growing fast on limited carbon supply, maximum spike growth coincided with the peduncle, P-1 and P-2 in dry matter although

there was some evidence that the peduncle was less important in lines with dwarfing genes. The major advantage of the dwarfing genes was associated with the increased partitioning of dry matter to the growing spikes. This has been associated with an increase in harvest index, an increase in grain number because of more fertile florets and greater yields.

The study also reinforced the conclusion that there is nothing intrinsically unique about the GA insensitive dwarfing genes that leads to greater grain number, HI and yield but that any reduction in stem length may release more carbohydrates for ear growth and floret fertility. In addition, it is not just the growth of the peduncle that is important but the three distal internodes that are growing and competing with the spike during the final stages of spike growth.

Data availability statement

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

Acknowledgements

TT was supported by PhD scholarship from a research alliance that included CSIRO and Bayer CropScience. We thank Tony Fischer for useful comments on the manuscript.

Author contributions

TT contributed to investigation, formal analysis and writing of original draft. TBA contributed to supervision, review and editing. WS contributed to supervision, conceptualization, writing, review and editing. RAR contributed to conceptualization, supervision, formal analysis, writing, review and editing

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Table list

Table 1 Experiments and germplasm deployed in studies.

ID	Population	Parent 1	Parent 2/ Recurrent	Sowing pattern	Sowing time	Location
2012	F_5	HI25M	Espada	Field Row*	Sep-2012	Acton
2013	BC_2F_4	HI25M	Espada	Field Row*	May-2013	Acton
2014	BC_2F_5	HI25M	Espada	Field Plot	Jun-2014	GES
Tray 2012	F_3	HI25M	Espada	Tray	Apr-2012	Acton
Tray 2014	BC_2F_4	HI25M	Espada	Tray	Jul-2014	Acton

*Rows of different lines of the same genotype, each with a similar height, were sown adjacent to form a plot

Table 2 Temperature and rainfall for Canberra from 2012-2014

Year	2012			2013			2014		
Month	Min. T(°C)	Max. T(°C)	Rain (mm)	Min. T(°C)	Max. T(°C)	Rain (mm)	Min. T(°C)	Max. T(°C)	Rain (mm)
May	-0.2	15.6	30	1.3	17.4	12	2.7	17.6	24
Jun	0.6	12.9	51	1.6	13.9	108	2.8	13.2	75
Jul	-1.1	13.0	48	1.7	13.4	55	0.0	12.2	28
Aug	-0.5	13.6	46	2.4	14.8	29	-0.8	14.3	38
Sep	1.6	18.0	53	4.0	19.9	69	2.7	17.9	43
Oct	3.4	20.8	78	3.8	21.9	20	5.4	22.5	57
Nov	9.3	25.4	37	6.7	23.8	105	10.2	27.9	48
Dec	11.8	27.7	51	11.5	28.5	21	12.7	27.7	77

Table 3 Final plant height (cm) (including spike length) for each genotype in each field experiment.

Genotype	2012	2013	2014	Average
Tall	87.5	103.4	87.1	92.7
Rht18	57.8	82.7	67.2	69.2
Rht-D1b	57.4	81.2	67.5	68.7
Double dwarf	48.8	74.2	60.3	61.1
Average	62.9	85.4	70.5	

The l.s.d. was 2.0 (Expt***), 2.3 (Genotype***), and 4.0 (Expt × Genotype **). ***: $P<0.001$, **: $P<0.01$

Table 4 Early seedling growth of Espada backcross lines differing in dwarfing genes.

Genotype	Coleoptile length* (mm)	Seed wt (mg)	Leaf 1 length (mm)	Leaf 2 length (mm)	Leaf 3 length (mm)	Total leaf area (cm2)	Plant dry wt (mg)
Tall	117	42.1	69	93	120	20.2	112.4
Rht18	122	42.6	66	85	109	17.1	100.4
Rht-D1b	86	40.3	68	91	120	18.5	103.7
Double dwarf	89	39.9	64	83	107	16.9	96.3
l.s.d (P<0.05)	6	1.7	3	4	6	1.7	8.6

*Coleoptile length was from Tray 2012, and early vigour traits were from Tray 2014

Table 5 Spike length (SpL), spike weight (SpW), internode length and weight for peduncle (P) and P-1 and P-2, and ratio of spike to main stem (SPI) one week after anthesis (including grains) in 2014.

Genotype	SpL	SpW	PL	PW	P-1 L	P-1 W	P-2 L	P-2 W	SPI
	mm	mg	mm	mg	mm	mg	mm	mg	
Tall	93.4	758	376	468	213	579	120	450	0.28
Rht18	96.4	712	261	309	150	378	101	386	0.34
Rht-D1b	95.3	774	282	353	164	426	92.2	369	0.34
Double dwarf	94.4	803	246	304	145	383	89.4	358	0.37
l.s.d.	ns	60*	17**	32**	7**	41**	8.7**	30**	0.02**

*: $P<0.05$, **: $P<0.01$, ns not significant

Table 6 Estimate of loss of labile carbohydrates from the stem and internodes between maximum post-anthesis value and maturity. Values are averaged over 2012 and 2013.

	Stem (mg)	Peduncle (mg)	P-1 (mg)	P-2 (mg)	P-3[A] (mg)
Genotype					
Tall	556	123	176	127	130
Rht18	509	106	161	129	113
Rht-D1b	465	86	161	121	98
Double dwarf	400	64	137	110	89
l.s.d.	78***	29***	25*	ns	ns
Experiment					
2012	262	63	94	71	33
2013	703	126	223	172	182
l.s.d.	55***	20***	18***	8***	27 ***

*: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ns: not significant, [A] plants in 2012 have one less internode below P-2

Figure list

Figure 1 Stem was dissected into 4 sections recorded as peduncle, P-1, P-2 and P-3(+) (includes the lower internodes) in *Rht18*, *Rht-D1b*, Tall and Double dwarf. These plants were taken from experiment 2013.

Figure 2 Length and weight changes over time for spike and main stem in 2012 and 2013. Bars represent the standard error. The AN arrow shows anthesis (GS65). Green and blue horizontal bars indicate the main spike growth period in length and weight respectively. Spike and stem elongation time dots were fitted in a 3-parameter sigmoid model in SigmaPlot (Ver. 12). Note; the increase in spike weight after anthesis is due to the beginning of grain growth.

Figure 3 Change in internodes length over time in 2012 (left column) and 2013 (right column). Error bars represent the standard error. AN means anthesis. Curves were fitted in a 3 parameter sigmoid model in SigmaPlot (Ver. 12), red, black and blue curves indicate *Rht18*, *Rht-D1b*, and double dwarf respectively. Green bar indicates the main spike growth period for length. The tall genotype was excluded from the figure to give more resolution between lines with the dwarfing genes. The tall genotype far exceeded the length of the other genotypes, but the timing was similar.

Figure 4 Change in internode weight over time in 2012 (left column) and 2013 (right column). Bars represent the standard error. AN means anthesis. Red, black, green and blue lines indicate *Rht18*, *Rht-D1b*, tall and double dwarf respectively. Blue bar indicates main spike growth period in weight.

Figure 5 Change in spike partitioning index (SPI) before anthesis in 2012 and 2013. HE and AN refer to heading and anthesis respectively.

Figure 6 Dry weight changes per stem for *Rht18*, *Rht-D1b* and Double dwarf in 2012 and 2013 populations after anthesis. AN: anthesis, lower and upper graph represent 2012 and 2013respectively. The tall genotype (not shown) had similar trend as *Rht18* and the change between maximum post-anthesis value and maturity was not significantly different to *Rht18***.**

Figure-1

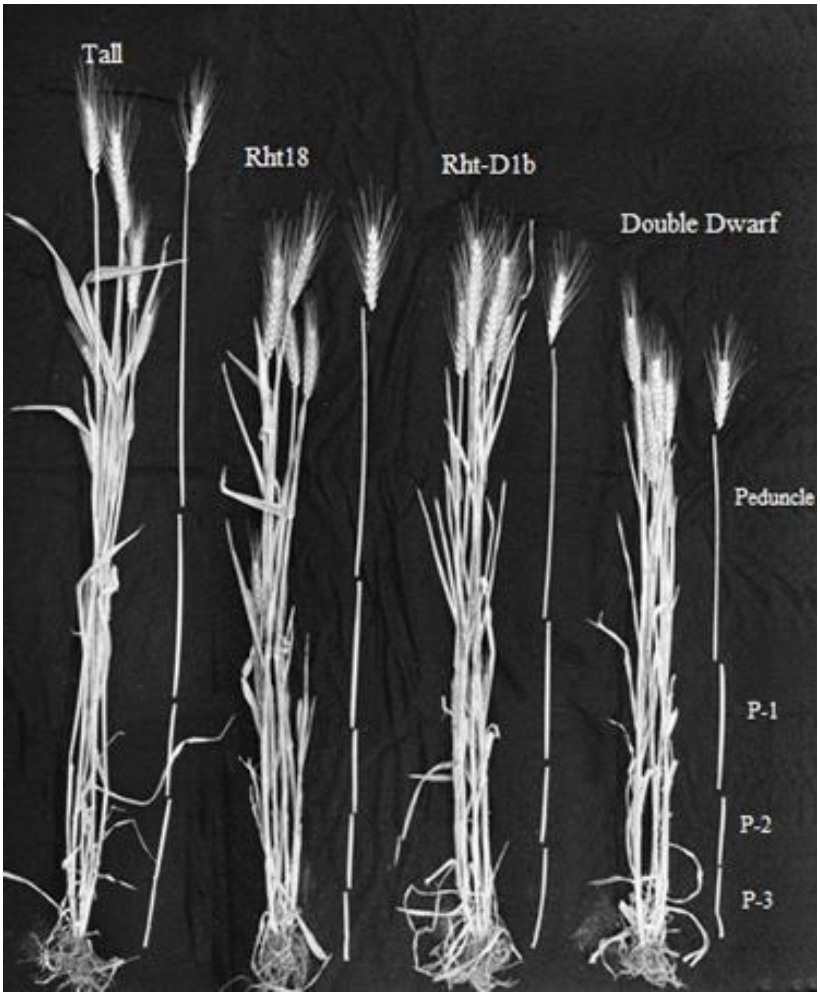


Figure-2

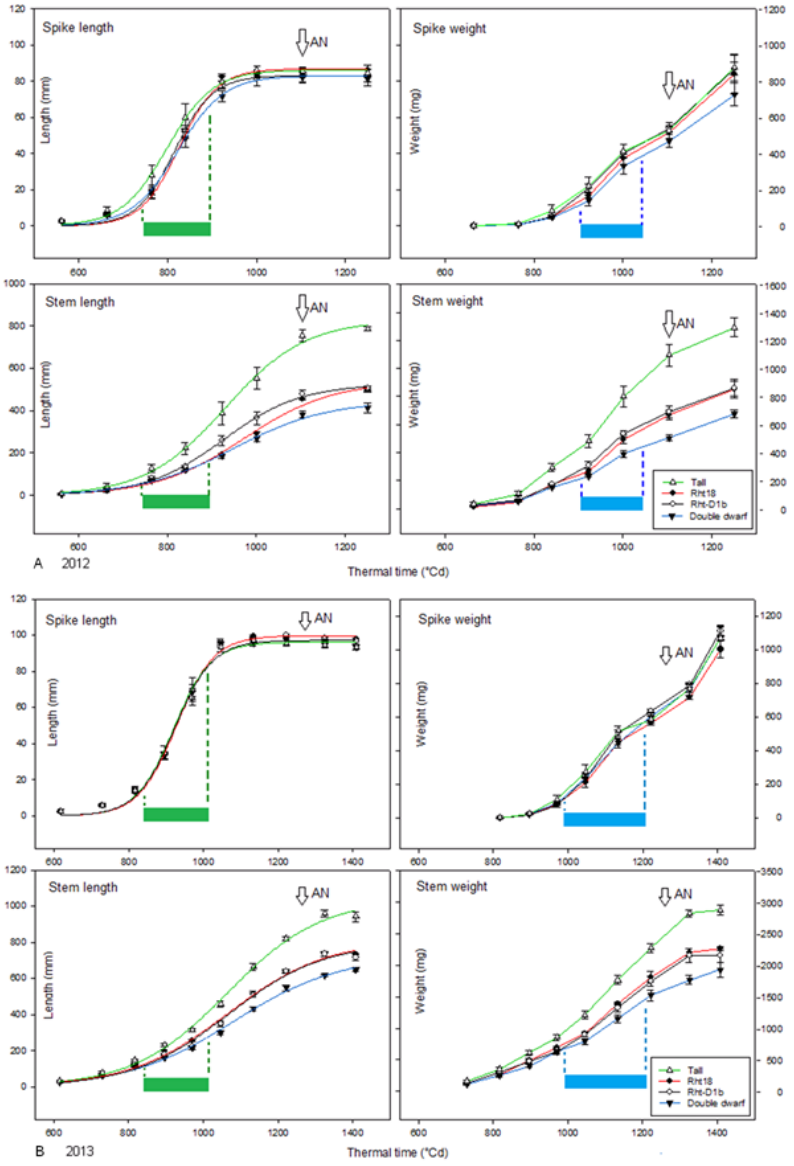

Spike length
AN
Length (mm)
Spike weight
Weight (mg)
Stem length
Stem weight
Tall
Rht18
Rht-D1b
Double dwarf
A 2012
Thermal time (°Cd)
B 2013

Figure-3

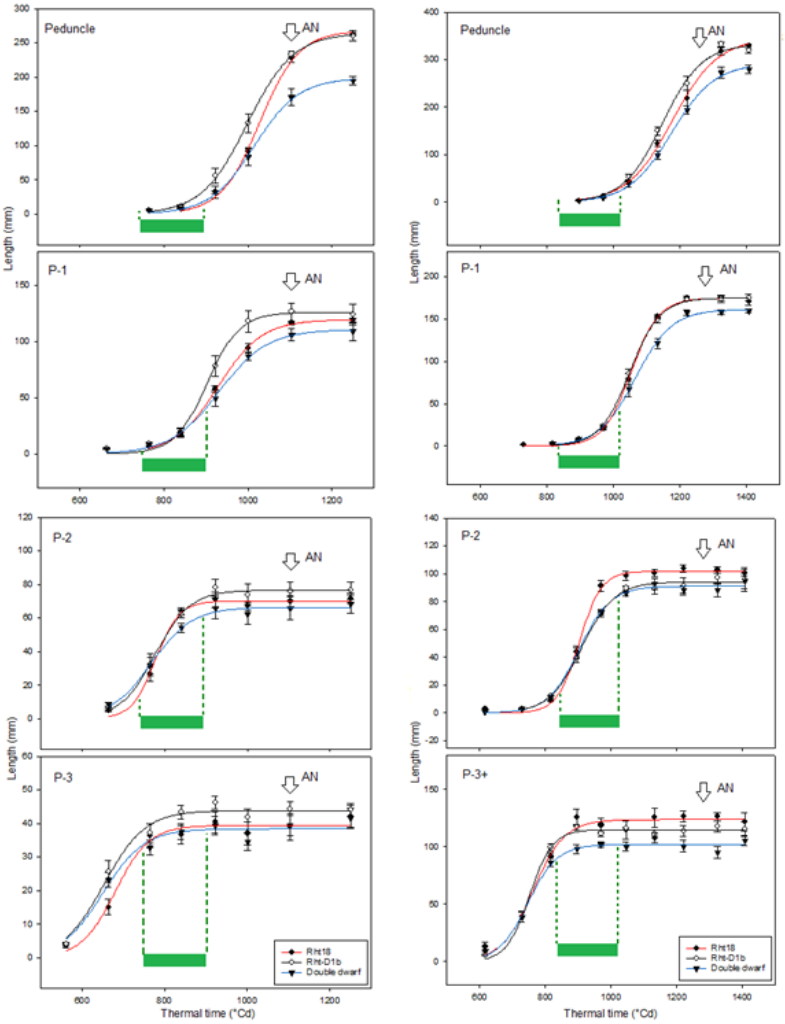

Peduncle
AN
P-1
P-2
P-3
P-3+
Length (mm)
Thermal time (°Cd)
Rht18
Rht-D1b
Double dwarf

Figure-4

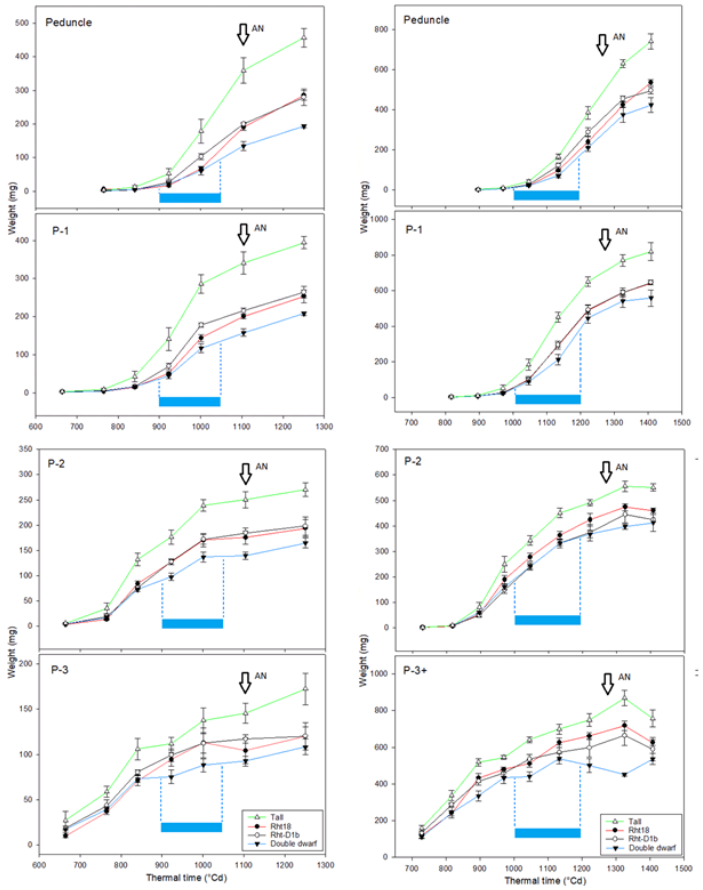

Peduncle
P-1
P-2
P-3
P-3+
AN
Weight (mg)
Thermal time (°Cd)
Tall
Rht18
Rht-D1b
Double dwarf

Figure-5

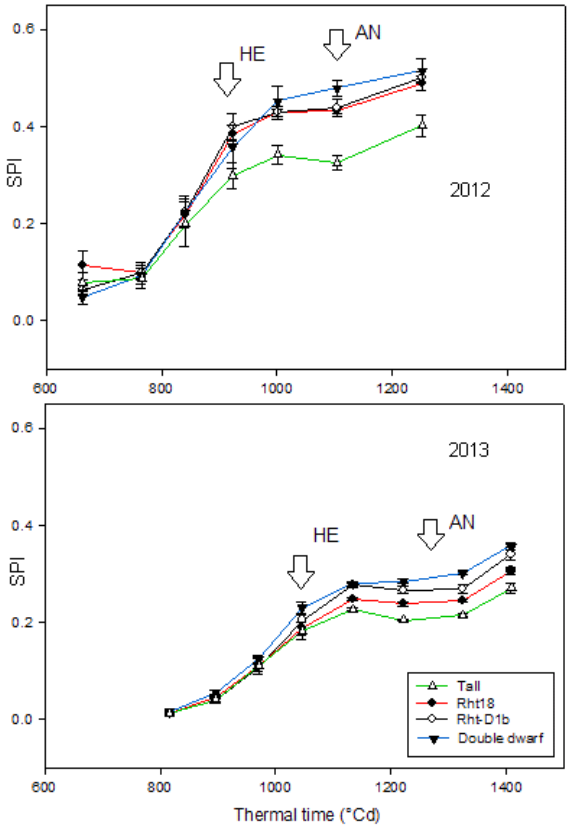
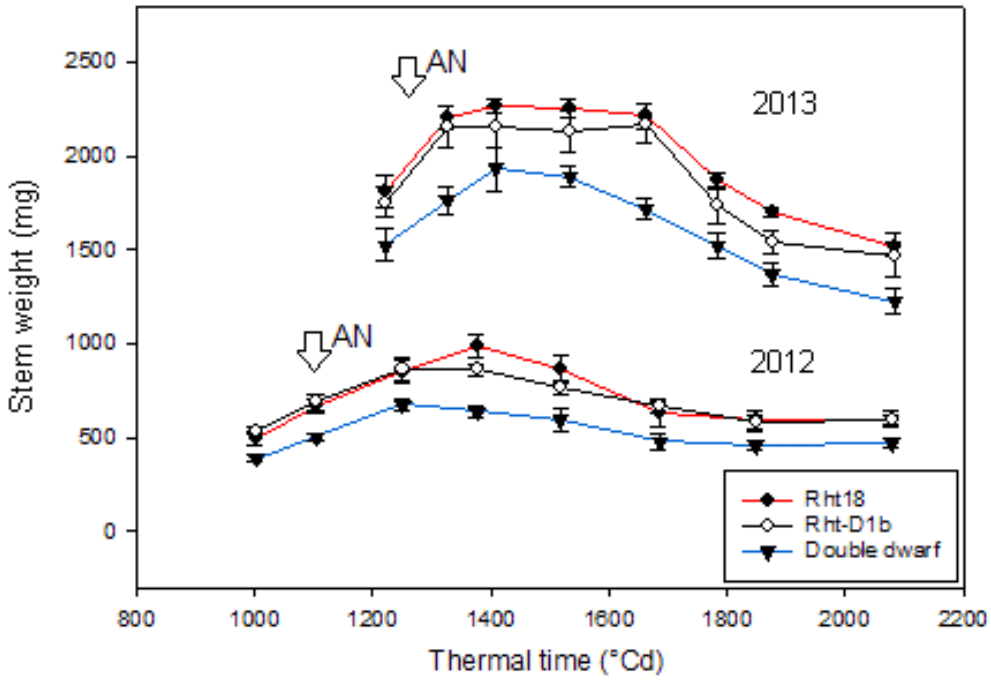

HE
AN
2012
SPI
0.6
0.4
0.2
0.0
600
800
1000
1200
1400
2013
HE
AN
SPI
Tall
Rht18
Rht-D1b
Double dwarf
Thermal time (°Cd)

Figure-6


AN
2013
AN
2012
Rht18
Rht-D1b
Double dwarf
Stem weight (mg)
Thermal time (°Cd)
0
500
1000
1500
2000
2500
800
1000
1200
1400
1600
1800
2000
2200