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Functional Plant Biology, 2017; 44(5):525-537

Originally published at: http://dx.doi.org/10.1071/FP16262

Overgrowth (*Della*) mutants of wheat: development, growth and yield of intragenic suppressors of the *Rht-B1c* dwarfing gene

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SUMMARY TEXT

The increased wheat yields that occurred during the Green Revolution were made possible by incorporating semi-dwarfing alleles of the wheat *Della* gene into new wheat varieties. These alleles are still in widespread use in current wheat varieties, but we have now isolated many new mutants of this gene, and characterised their effects on growth, grain dormancy and yield. The results provide insight into regulation of growth by the DELLA protein and indicate particular alleles of potential value in wheat breeding.

ABSTRACT

A suppressor screen using the dwarf *Rht-B1c Della* mutant of wheat (*Triticum aestivum* L.) led to the isolation of 'overgrowth' mutants, which retained the original dwarfing gene but grew at a faster rate because of a new mutation elsewhere in that gene. 46 alleles were identified, which included amino acid substitutions, premature stop codons, and splice site alterations. The sites of amino acid substitution were primarily localised around conserved motifs in the DELLA gene, and these mutants showed a wide range in their extent of growth recovery (dwarf, semidwarf, tall). Detailed growth comparisons were made on a wide height range of back-crossed overgrowth alleles, comparing stem and spike growth, leaf size, tillering, phenological development, coleoptile length, grain dormancy, and grain yield. There were large and reproducible differences between alleles for some traits, whereas others were largely unaffected or varied with growth conditions. Some of the overgrowth alleles offer promise as alternatives to the *Rht-B1b* and *Rht-D1b* dwarfing genes, allowing a wider range of height control, improved grain dormancy and equivalent grain yield. The collection of mutants will also be valuable as a resource to study the effect of height on different physiological/agronomic traits, and in elucidating DELLA protein function.

INTRODUCTION

In wheat, naturally occurring semi-dwarf mutants at the *Rht-1* locus were the basis for the Green Revolution in this species. *Rht-1* denotes the single *Della* gene of wheat, with homoeologues in each of the A-, B- and D-genomes. The DELLA proteins they encode are essential components in signalling by the hormone gibberellin (GA) (Peng *et al.*, 1999). The tall ('wild type') alleles are designated *Rht-A1a*, *Rht-B1a* and *Rht-D1a*, and mutants of these genes are indicated by '*b*', '*c*', '*d*', etc. Two semi-dwarfing alleles at this locus (*Rht-B1b* and *Rht-D1b*) have been extensively used in the breeding of short-statured wheat varieties that combine high yield with resistance to lodging.

DELLA proteins belong to the GRAS transcription factor family, a group of plant-specific proteins with diverse roles in development and growth. Their amino acid sequences are closely related to other GRAS proteins in the central and C-terminal regions of the protein, but the N-terminal region distinguishes the DELLA sub-family and is characterised by two conserved sequence motifs (DELLA and TVHYNP) that are essential for DELLA's involvement in GA signalling. Typically, bioactive GA binds to the GA receptor (Ueguchi-Tanaka *et al.*, 2005; Nakajima *et al.*, 2006) inducing a conformational change that then

allows binding of DELLA protein, via the conserved N-terminal sequence motifs, to the receptor-GA complex (Murase *et al.*, 2008; Shimada *et al.*, 2008). Subsequent binding of specific F-box proteins to the GRAS domain of DELLA (Sasaki *et al.*, 2003; McGinnis *et al.*, 2003) leads to polyubiquitination by the $SCF^{SLY1/GID2}$ ubiquitin E3 ligase complex, and degradation of DELLA by the 26S proteasome. Mutations in the conserved N-terminal sequence motifs of DELLA disrupt its binding to the receptor-GA complex, and its subsequent degradation by the proteasome.

DELLA proteins are involved in growth repression, and recent studies indicate that a range of different mechanisms may be involved. DELLAs lack a DNA binding domain, but influence gene expression by binding to different classes of transcription factors. In some cases DELLA is proposed to function as a transcriptional co-repressor by 'sequestering' or 'titrating' transcription factors for genes involved in growth (Feng *et al.*, 2008; de Lucas *et al.*, 2008; Bai *et al.*, 2012). In other cases DELLA is proposed to function as a transcriptional co-activator, leading to promotion of gene expression, and presumably including genes involved in growth repression (Hirano *et al.*, 2012; Fukazawa *et al.*, 2014; Yoshida *et al.*, 2014). A direct physical interaction has also been shown between DELLA proteins and the prefoldin complex, a co-chaperone involved in tubulin folding and microtubule organisation, implying that DELLA may participate directly in growth repression (Locascio *et al.*, 2013). There is a growing awareness that DELLA proteins play an important role in integrating a range of inputs, both intrinsic (e.g. hormone networks) as well as extrinsic (e.g. environmental variables, biotic and abiotic stress) to regulate growth (Harberd *et al.*, 2009; Sun, 2011; Schwechheimer, 2012; Claeys *et al.*, 2014; Xu *et al.*, 2014).

Growth repression caused by DELLA is presumably the outcome of a dynamic equilibrium involving DELLA binding to different protein partners, and will depend on the availability and binding affinities of each partner. Some interactions involve the same N-terminal conserved motifs that are required for binding to the receptor-GA complex (Hirano *et al.* 2012), raising the possibility of direct competition for DELLA once the receptor has bound an active GA. This might explain the increase in growth observed in F-box (*sly1* and *gid2*) dwarf mutants of Arabidopsis and rice when the GA receptor was overexpressed, despite no change in the amount of DELLA protein (Ariizumi *et al.*, 2008; Ueguchi-Tanaka *et al.*, 2008). Most other cases involve DELLA binding to protein partners via conserved motifs in their GRAS domain, including LHRI and SAW (Bai *et al.*, 2012; Fukazawa *et al.*, 2014; Yoshida *et al.*, 2014). The presence of these same motifs in other (non-DELLA) GRAS

proteins in the cell will likely compete with DELLA interactions, resulting in a complex equilibrium.

The role of particular amino acid residues in DELLA binding to protein partners was revealed by extensive *in vitro* mutagenesis/yeast two-hybrid studies of rice DELLA (SLR1) and its interactions with both the GA receptor (GID1) and F-box (GID2) proteins. Specific residues in four of the conserved motifs (VHIID, LHRII, PFYRE and SAW) were shown to be involved in DELLA binding to one, or the other, or to both of these proteins (Hirano *et al.*, 2010).

The *Rht-1* semi-dwarf mutants of wheat involve premature stop codons that occur just after the DELLA motif (Peng *et al.*, 1999; Pearce *et al.*, 2011). It was proposed that translation reinitiates shortly after the premature stop codons in a region rich in methionine, generating an N-terminally truncated protein that lacked the DELLA motif. A more severely dwarfed allele at this locus, *Rht-B1c*, resulted from a 2 kb insertion into the gene, which after splicing resulted in an in-frame insertion of 30 amino acids immediately following the DELLA motif (Pearce *et al.*, 2011; Wu *et al.*, 2011). For *Rht-B1c*, it was proposed that the 30 amino acid in-frame insertion disrupts the normal function of the DELLA motif. Yeast two-hybrid assays confirmed that physical interaction between the receptor-GA complex and DELLA fails to occur for both the proposed N-terminally truncated RHT-B1B protein, and for RHT-B1C (Pearce *et al.*, 2011; Wu *et al.*, 2011). The dwarfism of these lines presumably results from accumulation of mutant DELLA protein acting as a growth repressor.

New sets of *Della* mutants with enhanced growth relative to their dwarf parents were identified following dwarf mutant suppressor screens in barley and wheat (Chandler and Harding, 2013). In barley (*Hordeum vulgare* L.), a diploid species, all but one of the 'overgrowth' mutants were localised to the single *Della* gene, and growth promotion was observed in a range of dwarf backgrounds, including mutants defective in GA biosynthesis, or in GA receptor function, or in DELLA function. In wheat, an allohexaploid species, overgrowth mutants were selected in the dwarf *Rht-B1c* background, where dwarfism was due to a single mutant gene, and loss-of-function mutants were identified on the basis of increased growth. This experimental system offers the potential for isolating large numbers of mutants in a single gene that has important roles in plant and crop growth.

This paper describes the isolation and detailed characterisation of a large set of overgrowth mutants in bread wheat. We determine the nature of mutations in the *Rht-B1* gene, and make a detailed analysis of plant development and growth, which indicates that overgrowth lines are well-suited to investigating the effects of single gene differences in plant

height on a range of traits. We also explore the potential for semi-dwarf overgrowth alleles to be used in wheat breeding, investigating their effects on coleoptile length, grain dormancy, and yield in field plots, and conclude that some alleles offer promise as alternatives to *Rht-B1b* and *Rht-D1b*.

MATERIALS & METHODS

Plant material

The tall (*Rht-B1a*) and dwarf (*Rht-B1c*) isolines in a Maringá bread wheat background were described previously (Chandler and Harding, 2013). In this paper the semi-dwarf *Rht-B1b* and *Rht-D1b* Maringá isolines were also used, obtained from the original source (Australian Winter Cereal Collection, Horsham, Victoria, Australia). Confirmatory sequencing of the *Della* genes of these two lines revealed the correct genotype for *Rht-B1b*, but the *Rht-D1b* isoline was actually *Rht-B1b*. Since this isoline is one of two important semi-dwarf controls, we obtained other samples of Maringá *Rht-D1b* from Australian and Argentinean wheat researchers, but they were also *Rht-B1b*, as was a stock obtained from the Germplasm Resources Unit, John Innes Centre, UK. We constructed an authentic Maringá *Rht-D1b* using plants of the 'double' semi-dwarf Maringá isoline (*Rht-B1b* + *Rht-D1b*; *Della* sequences confirmed), obtained from Dr Guillermo Santa Maria, National University of General San Martín, Instituto de Investigaciones Biotecnológicas (IIB-INTECH), Argentina. This line was crossed with the tall Maringá parent and F_2 seedlings were screened to identify an individual that was homozygous for both *Rht-B1a* and *Rht-D1b*. Progeny from this plant were retested to confirm their genotype, and formed the basis of a new *Rht-D1b* stock.

Twenty overgrowth derivatives of *Rht-B1c* were described previously (Chandler and Harding, 2013), and this paper reports an additional 15 alleles isolated in the same mutant screen.

The preliminary characterisation of overgrowth lines utilised inbred lines from the initial mutant selection, usually M_5 or M_6 generation. For more detailed characterisation most of the overgrowth alleles were back-crossed through two generations with Maringá tall before reselecting the homozygous overgrowth derivative, thereby removing the majority of 'background' mutations.

Coleoptile measurements

Coleoptile lengths were determined on seedlings of back-crossed overgrowth lines after 14 days of growth in the dark at 15^0C. Per genotype there were three replicate trays with 7 seedlings each.

Stem and spike growth analysis

Six overgrowth derivatives of Maringá *Rht-B1c*, with heights ranging from 50% to 100% of wild-type *Rht-B1a* plants, together with *Rht-B1c* (dwarf), *Rht-B1a* (tall) and *Rht-B1b* (semi-dwarf) were grown in a Canberra field nursery in 2013 during the typical growth season (sown late May, harvested mid-December). The plots were single rows of about 200 plants, and there were two rows per genotype which were arranged in a randomised design. Temperature was recorded with a data logger at 20 min intervals to determine thermal time. Three plants per row were sampled at eight time points between terminal spikelet and anthesis (growth stages 31 and 65 on the decimal scale of Zadoks *et al.* (1974)) and at maturity, and lengths and dry weights of stem internodes and spikes were determined.

Controlled environment experiment

To monitor the effect of mutant alleles on plant development we selected genotypes that represented a range of potential phenotypes and which covered the whole range of plant heights. There were two independent back-crossed lines for most of the overgrowth alleles (Supplementary Table S1). There were two pots per overgrowth line and three pots per control isoline, organised in a complete randomised design. Plants were grown in 20 cm pots with four plants per pot in a Conviron PGW40 growth room at 20^0C day and 15^0C night temperature and a photoperiod of 16 h at 450 µM m^{-2} s^{-1}. The leaf, developmental and height measurements were performed on the main shoot of all plants. Leaf area was estimated using the formula: length x width x 0.835 (Miralles and Slafer, 1991). At maturity, grain yield was determined per pot.

Field experiment

Field trials were conducted at Leeton, NSW, Australia (34° 36′ S, 146° 22′ E, 138 m elevation) in 2014. Nitrogen was applied at 15 kg per hectare pre-sowing, and then again at 80 kg per hectare prior to booting. The site received near-average rainfall of 376 mm (the long-term average is 400 mm), and the irrigated trial received two applications of water before and after anthesis. In the irrigated trial both the original (inbred) overgrowth mutant lines and the derived back-crossed lines were included, while the rainfed trial only contained

the original inbred lines. In both trials the lines were arranged in a randomised block design with two replicate blocks, with each replicate containing an independent representative for each allele. Plots were sown May 22nd , with 2,000 grains in 10 rows at 18 cm spacing and were 5.5 m in length. No significant lodging was observed. Grain yield per plot (corrected for moisture content) was assessed at maturity. Further yield trials involving the back-crossed lines and appropriate controls grown under rainfed or irrigated conditions were conducted at a nearby site (Yanco) in 2015, sown June 4th. Rainfall in 2015 was 25% lower than in 2014. There was lodging of tall lines in the irrigated plots, so yield data was restricted to semi-dwarf and dwarf lines.

Assessment of grain dormancy

Plants were grown in single rows in a field nursery environment (Canberra), sown early in June, and harvested late December. Individual heads were harvested as soon as they reached physiological maturity (loss of all green colouration in the upper peduncle and spike, Zadoks growth stage 89). They were further dried in a laboratory fume hood for 48 hours, hand threshed, and the germinability of the grains immediately assessed (T_0) by placing 100 grains (embryo uppermost) on moist Whatman 3MM paper in a 20^0C cabinet with constant low intensity fluorescent lighting. Germination was recorded over a period of seven days, and expressed either as percentage germination, or as a weighted germination index (GI) which was the sum of the percent germination on day 1, plus half of the percent germination from day 1 to day 2, ... plus $1/6^{th}$ the percent germination from day 6 to day 7. The remaining grains were stored in manila envelopes in a lab environment and germination was assessed at weekly intervals until it reached >95%. There were two or three replicates for standard isolines and overgrowth alleles.

Gene amplification and sequencing

DNA was prepared from leaf material by the method of Ellis *et al.* (2005). The *Rht-B1c* gene was amplified in four overlapping fragments using primer pairs of which one was B-genome specific and the other conserved between *Rht-1* homeologues (Supplementary Table S2). Amplified fragments were purified using the ExoSAP protocol and then sequenced using a BigDye Terminator sequencing kit (Applied Biosystems).

Statistical analysis

Analysis of variance (ANOVA) and correlations by linear regression were performed using GenStat software (Payne *et al*., 2011).

SUPPLEMENTARY M&M

Overgrowth derivatives of Rht-B1c in a Halberd wheat genetic background

Maringá *Rht-B1c* was intercrossed with Halberd, a tall Australian bread wheat variety. F_1 plants were then crossed with Halberd as recurrent parent through three generations, and then inbred to generate a BC_3 Halberd *Rht-B1c* stock. Approximately 10,000 grains of this line were treated with sodium azide as previously described (Chandler and Harding, 2013), sown in the field and M_2 grains harvested. M_2 plants were screened in the field at maturity, and single heads were selected from individual plants that were substantially taller than their sibs. The *Rht-B1c* gene of these lines was sequenced to identify overgrowth mutations. 23 different overgrowth alleles were identified, 12 of which were identical to alleles already described in Maringá, and 11 of which were novel (*Rht-B1c.40 – Rht-B1c.50*; Supplementary Table S3).

Protein alignment

DELLA protein sequences of barley SLN1 [Q8W127], maize (*Zea mays)* D8 [Q9ST48] and D9 [ABI84226], *Brachypodium distachyon* SLN1 [XP_003560731], rice SLR1 [NP_001051032], *Sorghum bicolor* DELLA [XP_002466594], and Arabidopsis GAI [CAA75492], RGA [CAA72177], RGL1 [NP_176809], RGL2 [NP_186995] and RGL3 [NP_197251] were recovered from NCBI. A multiple sequence alignment was generated using the ClustalW algorithm (Thompson *et al.*, 1994).

RESULTS

Overgrowth mutants

From a screen of approximately 1.6 million M_2 plants there were 400 plants initially selected that were allowed to inbreed prior to testing M_3 families. This resulted in approximately 300 families that were uniform in height (within a family), but all taller than the *Rht-B1c* parent. About 150 of these lines were shown to be deletions of the *Rht-B1c* gene (Miraghazadeh *et al*., 2016). The remaining 150 lines retained the *Rht-B1c* gene, and they were further characterised by sequencing the entire *Rht-B1c* coding region. In each case a new mutation was found elsewhere in the *Rht-B1c* gene. From the distribution of mutational events among

five different sub-populations that comprised the M_2 generation we established a minimum of 72 independent mutational events. These defined 35 new alleles of *Rht-B1c*, implying independent occurrences of the same mutational events. On average there were two (72/35) independent isolates representing each allele, although the actual values ranged from single to as many as four independent isolates for a particular allele. A similar screen in Halberd *Rht-B1c* resulted in the isolation of 23 independent overgrowth derivatives, 12 of which were identical to alleles previously isolated in the Maringá screen, and 11 of which were new alleles (Supplementary Table S3). All of these alleles are derivatives of *Rht-B1c*, and notated as *Rht-B1c.1* to *Rht-B1c.35* in Maringá (and in 12 cases Halberd as well), and *Rht-B1c.40* to *Rht-B1c.50* in Halberd.

Three different categories of mutation were identified; amino acid substitutions, premature stop codons, and splice site alterations (Table 1). The amino acid substitutions resulted in a wide range in the extent of growth recovery, and different alleles ranged in height from dwarf through to tall. The premature stop codons resulted in almost complete growth recovery, suggesting that the mutant DELLA protein characteristic of the *Rht-B1c* dwarf parent is largely absent. A set of five mutant alleles specifically involved the two nucleotides on either side of the donor and acceptor splice sites; these probably result in defective splicing of the single large intron from the *Rht-B1c* transcript, resulting in less of the mutant DELLA protein. The splice site mutants were generally intermediate in their degree of growth recovery.

The effects of different overgrowth alleles on plant height are shown in Table 1 for plants grown under field conditions, although as shown below (e.g. Fig 2B) the effects of an allele on relative height are largely independent of growth conditions. A close correlation was observed between the effects of an allele on height and its effect on coleoptile length (Table 1, Supplementary Fig 1).

The overgrowth alleles were crossed with both the original dwarf parent (*Rht-B1c*) and with the tall wild type (*Rht-B1a*) to allow dominance or recessiveness of overgrowth alleles to be determined. The results (Supplementary Table S4) are consistent with overgrowth alleles showing partial dominance; in the heterozygous condition, the presence of a dwarfing *Rht-B1c* allele causes a reduction in height, and the presence of an *Rht-B1a* allele an increase in height.

Stem and spike growth in a field nursery environment

To determine how differences in height of overgrowth lines relate to differences in the rate or timing of stem internode elongation, six overgrowth lines representing a wide range in plant height (approx.50, 60, 70, 80, 90 and 100% of tall) and three control isolines *Rht-B1a*, *Rht-B1b* and *Rht-B1c* were grown in the field nursery. Plants were sampled and internode and spike lengths and dry weights determined at eight time points during stem elongation between the terminal spikelet stage (Zadoks GS31 at 550^0Cd) and anthesis (Zadoks GS65 at 1179^0Cd).

The differences in stem length between lines were consistent throughout development (Fig. 1A), indicating no major differences between alleles in the timing of stem elongation, despite the large differences in final stem length. Similar patterns were observed for dry weight accumulation and for each individual internode (data not shown). There were also no major differences in the timing of spike elongation, although all lines achieved a similar final spike length (Fig. 1B), in contrast to stem length. Each stem internode comprised a comparable percentage of the mature stem, despite the large differences between alleles at maturity (Fig. 1C). These results show that the differences in height between lines carrying different *Rht-B1* alleles are not caused by differences in either the timing of stem internode elongation, or by changes in the number of stem internodes, but by differences in growth rate.

Development and growth of back-crossed lines in controlled environment

A selection of back-crossed overgrowth lines was grown in a controlled environment for detailed investigation of the effects of *Della* mutations on plant development and morphology. The lines covered the whole range of available heights (Supplementary Table S1) and mutational categories (premature stop codon, amino acid substitution, and splice site alteration). The three control isolines plus the newly reconstructed *Rht-D1b* isoline were also included.

As observed in the field nursery, the lengths of stem and stem internodes were all reduced proportionally under controlled environment conditions (Fig. 2A). However, unlike the field nursery experiment, spike length was correlated to total stem length (R^2=0.254, P<0.001); thus taller lines had longer spikes. When heights of cabinet-grown lines were directly compared with consensus heights based on all previous field and field nursery experiments (see Table 1), a high correlation was observed (Fig. 2B).

To clarify the presentation of results in the Figures below, the 17 lines are assigned to three height classes (tall: 90-100% of *Rht-B1a*; semi-dwarf: 70-89% of *Rht-B1a*; dwarf: <70% of *Rht-B1a*), and within each class they are displayed in order of decreasing height.

Overall development Development was scored at approximately the 4-leaf stage, 5-leaf stage, 7-leaf stage, booting and anthesis. Initially the short lines developed slightly faster, the fourth and fifth leaf were on average further emerged at the first two time points (Fig. 3A), but by the 7-leaf stage the tall lines had caught up and subsequently developed faster throughout the rapid stem elongation phase (between flag leaf emergence and anthesis). With the exception of *c.27*, the tall lines reached booting and anthesis significantly faster than the dwarf lines, with the semi-dwarf lines intermediate in development (Fig. 3B). The delayed anthesis of dwarf lines seen in this controlled environment condition has not been observed in field grown plants where no consistent differences in time to anthesis have been seen (data not shown).

Tillering There were slight but significant differences between lines in maximum tiller number ($P<0.01$), which was reached before the rapid stem elongation phase. However, these differences did not translate into a higher effective tiller number at maturity (Fig. 4). In fact, the genotype with the highest number of heads at maturity, *c.6*, actually had one of the lowest maximum tiller numbers, suggesting a degree of independence between tiller development and tiller survival.

Leaves All lines developed nine leaves on the main shoot, and the length, width and area of the top three leaves are shown in Figure 5. The relationship between plant height and leaf length varied between the three different leaves (Fig. 5A). For the first to the seventh emerged leaf there is a strong positive association ($P<0.001$) between plant height and leaf length (see Supplementary Fig. S2 for lengths of leaves 4 and 5), whilst for the flag leaf (leaf 9) the pattern is the opposite, with tall lines having the shortest flag leaves. Lengths of the penultimate leaf (leaf 8) show no consistent relation to plant height. Leaf width shows a consistent pattern for all three leaves, with short lines having wider leaves than tall lines (Fig. 5B). Overall, this results in shorter lines having a larger calculated leaf area for the top two leaves, often by a considerable amount; for instance there is an average 17% increase in calculated L8 area of dwarf lines compared to tall, and for the FL the difference is 58% (Fig. 5C). Lines that stand out in this regard are *c.6*, which has both the longest and widest leaves and thus the highest leaf area, and *c.27*, which has very short leaves and therefore the lowest leaf area.

Grain yield Grain yield per pot (and therefore per plant) was associated with plant height, with tall lines generally having a higher grain yield (Fig. 6A). This result also contrasts with field observations (see below).

Grain yield in field plots

A field trial at Leeton (NSW) was performed in 2014 under both rainfed and irrigated conditions. In the irrigated trial both the inbred overgrowth lines and the derived back-crossed lines were included, while the rainfed trial only contained the inbred lines.

Grain yields in the irrigated field experiment showed the expected higher yield of semi-dwarf lines compared to tall lines (Fig. 6B and 6C), which is in agreement with previous results of near-isogenic lines in Maringá (Miralles and Slafer 1995) and four UK wheat varieties (Flintham *et al*., 1997). Heights of greater than about 95-100 cm resulted in a progressive and large yield penalty, despite no significant lodging being recorded. Dwarf and semi-dwarf overgrowth lines achieved similar grain yields to the two widely used standard semi-dwarfing genes, *Rht-B1b* and *Rht-D1b*, as did the *Rht-B1c* dwarf. It is noteworthy that the back-crossed lines generally yielded higher than their parent inbred lines, by an average of 13% (paired *t*-test $P<0.001$), although there were no differences in height (Fig 6B).

In the rainfed trial yields were lower overall compared to the irrigated trial, but nearly all dwarf and semi-dwarf overgrowth lines were equivalent in yield to *Rht-B1b* (Fig. 6C).

These results were largely confirmed in the 2015 growing season, involving the back-crossed overgrowth lines. In the irrigated trial, yields of the dwarf and semi-dwarf overgrowth alleles were equivalent to the standard semi-dwarfs (data not shown), but lodging occurred in taller lines, preventing accurate height and yield measurements. In the rainfed trial there was a considerable reduction in yield associated with the drier season, and dwarf and semi-dwarf overgrowth alleles performed as well as the standard semi-dwarfs, but were not higher yielding as suggested by the 2014 data.

Grain dormancy

The Maringá *Rht-B1c* isoline exhibited much higher grain dormancy than either the tall (*Rht-B1a*) or semi-dwarf (*Rht-B1b*) isolines, requiring 6-7 weeks (compared to 1 week) after-ripening to achieve 50% germination. The different overgrowth alleles showed a considerable range in their extent of loss of this extra grain dormancy (Fig. 7A). Taller overgrowth lines generally showed much less dormancy than their dwarf parent, and were similar to the *Rht-B1a* and *Rht-B1b* control isolines, whereas shorter overgrowth lines retained higher levels of dormancy. Germination index (GI) is a more useful measure for assessing potential resistance to pre-harvest sprouting. The *c.23* and *c.26* semi-dwarfing alleles showed a lower GI than the standard *Rht-B1b* semi-dwarf allele (Fig. 7B), and similar behaviour has been observed in three successive field nursery seasons.

Sites of amino acid substitution in DELLA protein

The overgrowth alleles defined in barley and wheat identify a total of 42 amino acid substitutions, involving 31 distinct sites within the consensus DELLA amino acid sequence (Fig. 8). The distribution of these sites reveals regions that are more likely to result in identifiable 'loss-of-function' DELLA phenotypes, particularly localised around conserved motifs such as LHR1, VHIID and PFYRE.

DISCUSSION

We have isolated 46 different overgrowth alleles of the *Rht-B1c* dwarfing gene in wheat, each associated with enhanced growth compared to the parental dwarf line. They result from single second-site mutational events in the original *Rht-B1c* dwarfing gene that presumably result in DELLA proteins that are either less effective at growth repression, or that are present in lower amounts. From the same screen we isolated an equivalent number of lines that were deleted for the *Rht-B1c* gene; they involved loss of a minimum of about 50 genes, but included lines with loss of the whole short arm of chromosome 4B, or even loss of the whole chromosome 4B (aneuploidy; Miraghazadeh *et al*., 2016).

In view of the important role DELLA proteins play in growth regulation, the overgrowth mutants are a useful resource for investigating DELLA function, particularly in defining specific amino acid residues involved in interactions with other proteins. These mutants also represent a set of well-defined height isolines that will be of value in further physiological investigations of the relationship between plant/crop height and agronomic performance first highlighted by the Green Revolution. Some alleles potentially represent improved semi-dwarfing genes that may be of use in wheat breeding.

The mutants, together with earlier ones described in barley, define 31 amino acid residues important in DELLA function (Fig. 8). Most of these positions are fully conserved in DELLA amino acid sequences from both cereals and Arabidopsis (Suppl. Fig 3), and all but one are fully conserved in cereal DELLA sequences. Many of the substitutions occur in conserved sequence motifs (LHRI, PFYRE, VHIID and SAW) already characterised in the GRAS domain of DELLA proteins and implicated in direct physical interactions of DELLA with other proteins. A crystal structure has been recently reported (Li *et al*., 2016) for the GRAS domain of *Scarecrow-like7* from rice, corresponding to the C-terminal 370 amino acids of the wheat DELLA protein. In this region there is 35% amino acid sequence identity

between wheat DELLA and the SCARECROW-LIKE7 protein, spread relatively uniformly throughout and suggesting that the main structural features of the rice protein will be preserved in wheat DELLA. The mutations associated with *ovg* alleles occur in many of the structural elements (α-helices, β-strands), but are notably over-represented (20/29) in the 5 α-helices of the 'cap' structure, which includes the LHRI and PFYRE motifs (Li *et al*., 2016) implying an important role for this region in DELLA function.

Based on studies in Arabidopsis and rice, it is likely that some of the amino acid substitutions cause reduced binding of DELLA to other interacting proteins. In the case of transcription factors, this would result in less co-activation or co-repression, leading to growth promotion. For proteins directly involved in growth (e.g. prefoldin), the reduced binding of DELLA mutants would also likely result in growth promotion. DELLA mutants with lower binding to protein partners may also be associated with changed post-translational modification and/or stability. A major limitation in wheat and barley is that the identity of these DELLA-interacting protein partners is largely unknown, although studies in Arabidopsis and rice predict potential candidates for future investigation. A set of five overgrowth alleles caused changes in the two nucleotides immediately flanking either the donor or the acceptor splice sites, and these are likely to lower the splicing efficiency of the *Della* transcript and result in less DELLA protein.

Mutagenesis typically results in the induction of many more mutational events than the causal one responsible for a mutant phenotype. Mutants in the single *Rht-B1c* gene were recovered at a frequency of 1 per 280 M_1 plants (1 per 22,000 M_2 plants). If we assume 30,000 genes for each of the three sub-genomes of wheat, it is likely that most M_2 plants will contain at least several homozygous 'genic' mutations. Only about 2% of the total wheat DNA is accounted for by 'genes', so there will be many other mutations in each overgrowth line with potentially deleterious effects, especially on complex traits (e.g. yield) that integrate the action of many genes. For this reason nearly all alleles were taken through two rounds of back-crossing to reduce the presence of 'background' mutations prior to undertaking detailed phenotyping. It is of interest that a direct comparison of the yields of field plots (inbred lines versus back-crossed lines) showed the latter to have an average 13% higher yield; of 27 different alleles where a direct comparison was made, there were 24 cases where the yield of the back-crossed line was greater than that of the inbred line.

Overgrowth lines ranged in height from about 50-100% of the tall isoline, compared to 43% for their dwarf parent. For the majority of alleles, the effect on final height was largely independent of growth conditions, which ranged from field to controlled environment

(Fig. 2B). Consistent with what has been established for the standard *Rht-1* alleles (Youssefian *et al.*, 1992), the differences in height of the overgrowth lines were associated with different growth rates of stem internodes, rather than to different durations of growth or different numbers of internodes. There was much less effect of overgrowth alleles on the length of the spike (rachis internode length), although this varied between field and controlled environment growth conditions.

Detailed growth comparisons were made on a wide height range of back-crossed overgrowth alleles under controlled environment conditions, monitoring leaf growth, tillering, anthesis, and grain production. Overall, development was very similar between tall, semi-dwarf and dwarf categories, although statistically significant differences were observed. Dwarf lines were slower in development than tall lines at booting and anthesis. This may be an effect of the growth environment, as we have not observed height-related differences in anthesis date in field grown plants. An interesting finding was the increase in calculated leaf area for the flag leaf and penultimate leaf of dwarf compared to tall lines (Fig. 5C), since these two leaves are expected to be an important source of photosynthate for grain filling. The behaviour of two alleles (*c.6* and *c.27*) was notable, as these lines showed the highest and lowest leaf areas respectively, and had correspondingly high and low grain production under cabinet conditions when compared to lines of similar height (Fig. 6A).

There was a positive association between plant height and grain yield per plant under controlled environment conditions (Fig. 6A). In contrast, the field experiments showed that tall lines had lower grain yields than both dwarf and semi-dwarf lines (Figs. 6B and 6C). Field studies with Maringá isolines in Argentina, Canada and Mexico found that the dwarf lines *Rht-B1b+Rht-D1b* and *Rht-B1c* yielded less than the semi-dwarf *Rht-B1b* but more than the tall *Rht-B1a* (Miralles and Slafer, 1995; Ehdaie and Waines, 1996; Manske et al., 2002). In our field studies over several years the yield of the *Rht-B1c* isoline has nearly always been intermediate between the semidwarf and tall isolines, although often closer to the semidwarf value than to the tall. In the 2014 season the yield of the dwarf isoline was equivalent to that of the semi-dwarf.

Under field conditions in two different seasons we observed that dwarf and semi-dwarf overgrowth lines yielded as well as the standard semi-dwarfs. It is notable that tall overgrowth lines showed progressively lower yields as their height increased, up to that of the tall isoline. This result, in which a high yielding dwarf line differs by a single nucleotide substitution from a low yielding line, provides a direct experimental confirmation of the negative relationship between long stems and grain yield.

Apart from their wide range in height, the different overgrowth alleles are remarkably similar to each other in most respects. There were occasional apparent allele-specific effects noted, where a significant difference in a trait occurred for a particular allele in one experiment. Some of these have not been reproducible, and for others it requires further study to determine whether such effects are due to the *Della* mutation or to background mutations. But overall, the uniformity makes them a useful set of height isolines for detailed study of the relationship between plant/crop height and traits of interest to crop physiologists, ranging from efficiencies of resource use (e.g. light, water, nitrogen, CO_2) and disease susceptibility, through to biomass production, harvest index and grain yield. This apparent uniformity, apart from the effects on height, differs from our earlier experience in barley, where we encountered examples of allele-specific differences for other GA-related traits (e.g. GA-dependence of alpha-amylase production, grain size) despite there being a much larger number of wheat alleles. It is likely that the presence of normal DELLA function in the wheat overgrowth mutants, arising from the *Rht-A1a* and *Rht-D1a Della* genes, masks some of the 'loss-of-function' phenotypes of the B-genome *Della* mutants. This would not occur in barley, being diploid. To test this possibility we have recently generated wheat lines with null mutations in the *Della* gene of all three genomes, and have constructed lines for future phenotyping of overgrowth alleles in genetic backgrounds that have zero, one or two functional *Della* genes.

The potential usefulness of overgrowth alleles in wheat breeding depends on their effects on a range of traits that are important in the field. The widely used semi-dwarfing genes *Rht-B1b* and *Rht-D1b* were compared to the overgrowth alleles using the appropriate Maringá isolines. The shorter coleoptiles associated with the standard semi-dwarfing genes are a disadvantage when grains are sown deep to maximise moisture availability, as they result in poorer emergence (Schillinger *et al*,. 1998). The relationship observed between coleoptile length and plant height (Table 1) indicates that the overgrowth alleles probably offer no benefit compared to the standard semi-dwarfing genes for this trait. However, overgrowth alleles offer a much wider range of height control than the standard semi-dwarfing genes, and this might allow particular alleles to be targeted for specific environments. Field trials showed that overgrowth alleles yielded as well as the standard semi-dwarfing genes under irrigated conditions and dryland conditions. The *Rht-B1c* parent of the overgrowth lines has exceptional grain dormancy, which would likely be effective in minimising grain damage in situations of pre-harvest sprouting. This contrasts with the standard semi-dwarfing *Rht-B1b* gene, which offers no improvement in dormancy compared

538 to the tall isoline (Fig. 7; Gooding *et al*., 2012). Some of the semi-dwarf overgrowth alleles
539 e.g. *c.23* and *c.26*, retain a considerable part of the dormancy characteristic of their parent
540 allele, a trait potentially of value in environments where sprouting is a significant risk. These
541 two alleles also yielded well in field experiments: in rainfed conditions yields of the inbred
542 *c.23* and *c.26* lines were 313 and 297 g m^{-2} relative to 284 for the *Rht-B1b* control, and under
543 irrigated conditions, the back-crossed *c.23* and *c.26* lines yielded 438 and 443 g m^{-2}
544 compared to 409 for the *Rht-B1b* control.

545 Independent but parallel studies on selected overgrowth alleles in a range of elite
546 spring wheat varieties have recently been reported by Van De Velde *et al*., 2017. Their results
547 are generally consistent with the findings reported here, and establish that overgrowth
548 phenotypes are robust in their expression in other environments and in different genetic
549 backgrounds. The increase in grain dormancy associated with the *Rht-B1c.23* and *Rht-B1c.26*
550 overgrowth alleles was confirmed, and extended to include increased resistance to in-ear
551 sprouting under high humidity.
552

ACKNOWLEDGMENTS

We thank the Grains Research and Development Corporation for funding the initial characterisation of the overgrowth mutants, and for Ph.D. scholarship funding (A.M.).

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FIGURE and TABLE LEGENDS

Table 1. Overgrowth lines, mutations, stem length at maturity (% *Rht-B1a*) and coleoptile length (% *Rht-B1a*) in wheat cv. Maringá. Stem lengths are the means of different field nursery and field experiments conducted between 2009 and 2014, with typically about 10 independent values for each allele. ND: not determined. The nucleotide and amino acid sequences are under accession no. KC434134.

Table S1. Lines included in the controlled environment experiment.

Table S2. Primers used for amplification and sequencing of wheat *Rht-B1c*.

Table S3. Overgrowth lines and their mutations in wheat cv. Halberd.

Table S4. Stem length (cm) of parent and F_1 plants of overgrowth alleles crossed to *Rht-B1c*, and of F_2 plants of overgrowth alleles crossed to *Rht-B1a*. Genotypes of F_2 plants were confirmed by sequencing of the *Rht-B1* gene. Shown are the means of the three tallest stems of three plants. ND: not determined.

Fig. 1. Stem (A) and spike (B) growth until anthesis, and mature internode lengths (% of total stem length; C) of the main shoot of representative overgrowth lines and *Rht-B1a*, *Rht-B1b* and *Rht-B1c* in wheat cv. Maringá grown in the field nursery in 2013. Shown are the means of six plants plus SE.

Fig. 2. (A) Lengths of spike, peduncle, internode P-1 and lower internodes in relation to final length of the main shoot of a selection of back-crossed overgrowth lines grown under controlled environment conditions. The lines are arranged from the tallest to the shortest. Shown are the means of two to four replicate pots with four plants per pot. (B) Relationship between consensus plant height, based on multiple field and field nursery observations (see Table 1) and plant height in the controlled environment experiment.

Fig. 3. Development of the main shoot of a selection of back-crossed overgrowth lines grown under controlled environment conditions. Shown are the number of emerged leaves (A) and when final leaf number has been reached the Zadoks growth stage (B). The lines are arranged from the tallest to the shortest with tall lines in dark grey, semi-dwarf lines in light grey and dwarf lines in white. Axis-labels are the growth stage of the average plant. Shown are the means plus SE of two to four replicate pots with four plants per pot.

Fig. 4. Maximum and final number of shoots per plant of a selection of back-crossed overgrowth lines grown under controlled environment conditions in 2014. The lines are arranged from the tallest to the shortest with tall lines in dark grey, semi-dwarf lines in light grey and dwarf lines in white. Axis-labels are the stage of the average plant. Shown are the means plus SE of two to four replicate pots with four plants per pot.

Fig. 5. Length (A), width (B) and area (C) of the top three leaves of the main shoot of a selection of back-crossed overgrowth lines grown under controlled environment conditions. The lines are arranged from the tallest to the shortest with tall lines in dark grey, semi-dwarf lines in light grey and dwarf lines in white. Shown are the means plus SE of two to four replicate pots with four plants per pot.

Fig. 6. (A) Relationship between plant height and grain yield per pot of a selection of back-crossed overgrowth lines grown under controlled environment conditions. Values are the means plus SE of two (overgrowth) or three (control) pots (B-C) Relationship between crop height and grain yield of the original inbred overgrowth lines, back-crossed (BC) overgrowth lines and control isolines grown in field plots in 2014 under irrigated (B) and rainfed (C) conditions. Values are the means plus SE of from one to four lines per overgrowth allele, and

three replicates for each control isoline (B), and two replicates for each overgrowth line and four replicates of control isolines (C).

Fig. 7. Percent germination (A) and germination index (B) of grains of a selection of back-crossed overgrowth lines and control isolines in wheat cv. Maringá grown in the field nursery in 2013. Overgrowth lines are in grey, and control isolines are in black. Values are the means plus SE of two or three replicates.

Fig. 8. Sites of amino acid substitutions of barley and wheat overgrowth mutants in the DELLA protein. The top row of arrows represent barley mutations, and the bottom row the mutations in wheat (cv. Maringá and Halberd). Conserved regions are indicated in black, non-conserved regions in white, and the 30 amino acid insertion in *Rht-B1c* is shown as a crossed box.

Suppl. Fig. S1. Relationship between plant height and coleoptile length of the overgrowth mutant collection in wheat cv. Maringá.

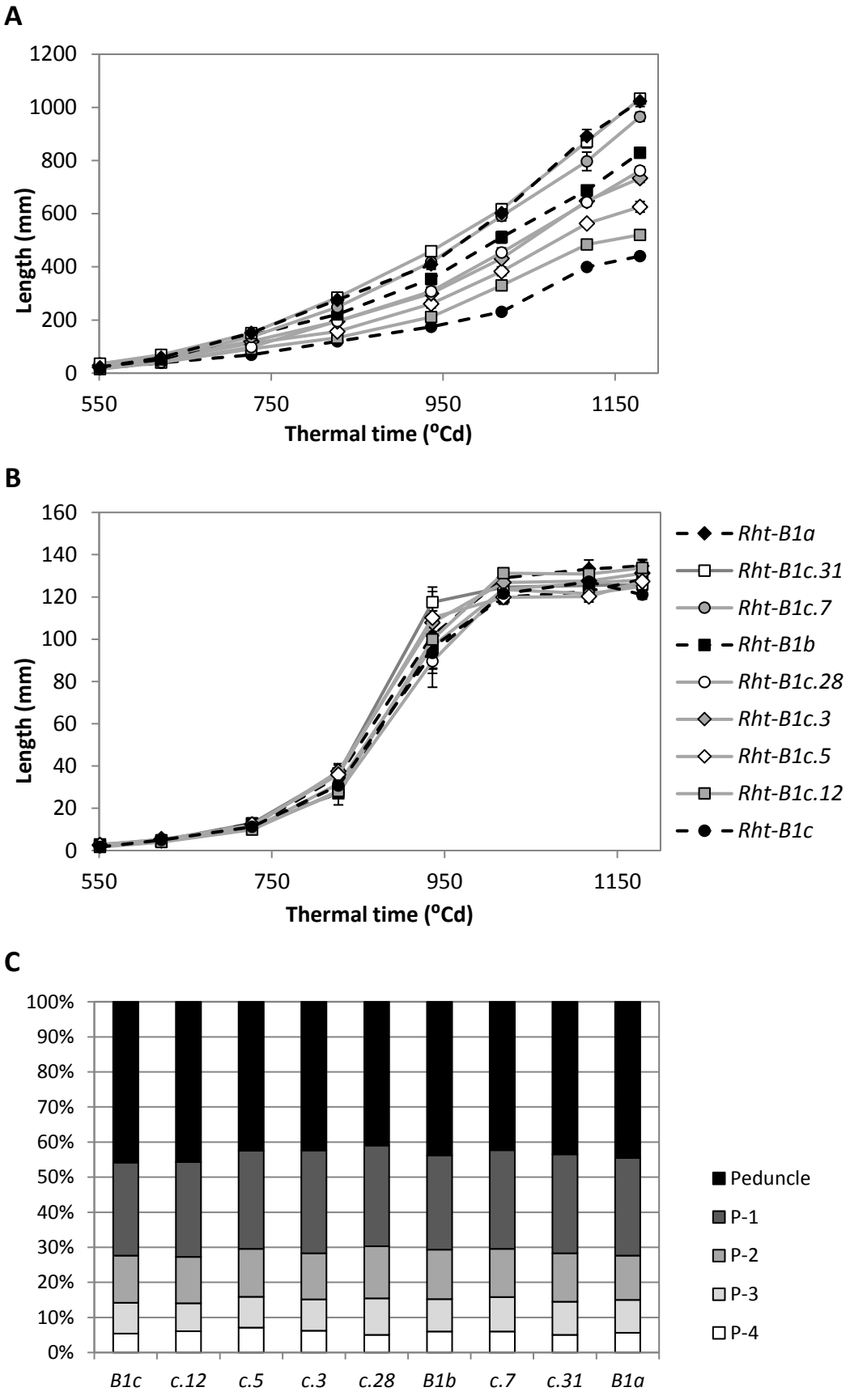
Suppl. Fig. S2. Length of the fourth and fifth emerged leaf of the main shoot of a selection of overgrowth lines grown under controlled environment conditions in 2014. The lines are arranged from the tallest to the shortest with tall lines in dark grey, semi-dwarf lines in light grey and dwarf lines in white. Shown are the means plus SE of two to four replicate pots with four plants per pot.

Suppl. Fig. S3. Alignment of cereal and Arabidopsis DELLA proteins. Conserved domains are indicated in yellow with characteristic motifs in bold. Sites of amino acid substitution in wheat and barley are highlighted in green. The arrow indicates the site of the 30 amino acid insertion in *Rht-B1c*.

TABLE 1

Allele	Overgrowth mutation		Length (% *Rht-B1a*)		Reference
	Nucleotide	Amino acid	Stem	Coleoptile	
Rht-B1a			100	100	*Hoogendoorn et al., 1988*
Rht-B1b			82	91	*Hoogendoorn et al., 1988*
Rht-B1c			43	67	*Hoogendoorn et al., 1988*
Rht-B1c.1	G2715A	G260E	93	107	*Chandler and Harding, 2013*
Rht-B1c.2	G2726A	V264M	95	97	*Chandler and Harding, 2013*
Rht-B1c.3	G2747A	A271T	71	90	*Chandler and Harding, 2013*
Rht-B1c.4	G2829A	G298D	67	79	*Chandler and Harding, 2013*
Rht-B1c.5	G2831A	A299T	64	70	*Chandler and Harding, 2013*
Rht-B1c.6	G2849A	A305T	67	76	*Chandler and Harding, 2013*
Rht-B1c.7	C2865T	A310V	91	107	*Chandler and Harding, 2013*
Rht-B1c.8	C2966T	P344S	60	84	*Chandler and Harding, 2013*
Rht-B1c.9	C2972T	L346F	87	88	*Chandler and Harding, 2013*
Rht-B1c.10	G3065A	G377R	63	72	*Chandler and Harding, 2013*
Rht-B1c.11	G3076A	W380ter	99		*Chandler and Harding, 2013*
Rht-B1c.12	C3117T	P394L	53	72	*Chandler and Harding, 2013*
Rht-B1c.13	G3190A	W418ter	95		*Chandler and Harding, 2013*
Rht-B1c.14	C2447T	P171S	56	77	*Chandler and Harding, 2013*
Rht-B1c.15	G3477A	R514H	90	105	*Chandler and Harding, 2013*
Rht-B1c.16	C3507T	T524I	91	98	*Chandler and Harding, 2013*
Rht-B1c.17	C3519T	S528F	76	83	*Chandler and Harding, 2013*
Rht-B1c.18	G3624A	G563D	94	97	*Chandler and Harding, 2013*
Rht-B1c.19	G3697A	W587ter	94	104	*Chandler and Harding, 2013*
Rht-B1c.20	G3874A	W646ter	96		*Chandler and Harding, 2013*
Rht-B1c.21	G2792A	V286M	59	84	This paper
Rht-B1c.22	CC2108TA	P58ter	87	99	This paper
Rht-B1c.23	G3047A	D371N	75	82	This paper
Rht-B1c.24	G2864A	A310T	81	92	This paper
Rht-B1c.25	C3071T	Q379ter	100		This paper
Rht-B1c.26	G3671A	E579K	76	81	This paper
Rht-B1c.27	G148A	splice	78	85	This paper
Rht-B1c.28	G148T	splice	81	89	This paper
Rht-B1c.29	G147A	splice	82	76	This paper
Rht-B1c.30	G2084A	splice	84	92	This paper
Rht-B1c.31	G2335A	W133ter	99	100	This paper
Rht-B1c.32	G2083A	splice	92	90	This paper
Rht-B1c.33	G3841A	W635ter	98		This paper
Rht-B1c.34	G3290T	E452ter	96		This paper
Rht-B1c.35	C2705T	Q257ter	93		This paper
Rht-D1b			82	83	This paper

FIGURE 1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Thermal time (°Cd)
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Thermal time (°Cd)
Rht-B1a
Rht-B1c.31
Rht-B1c.7
Rht-B1b
Rht-B1c.28
Rht-B1c.3
Rht-B1c.5
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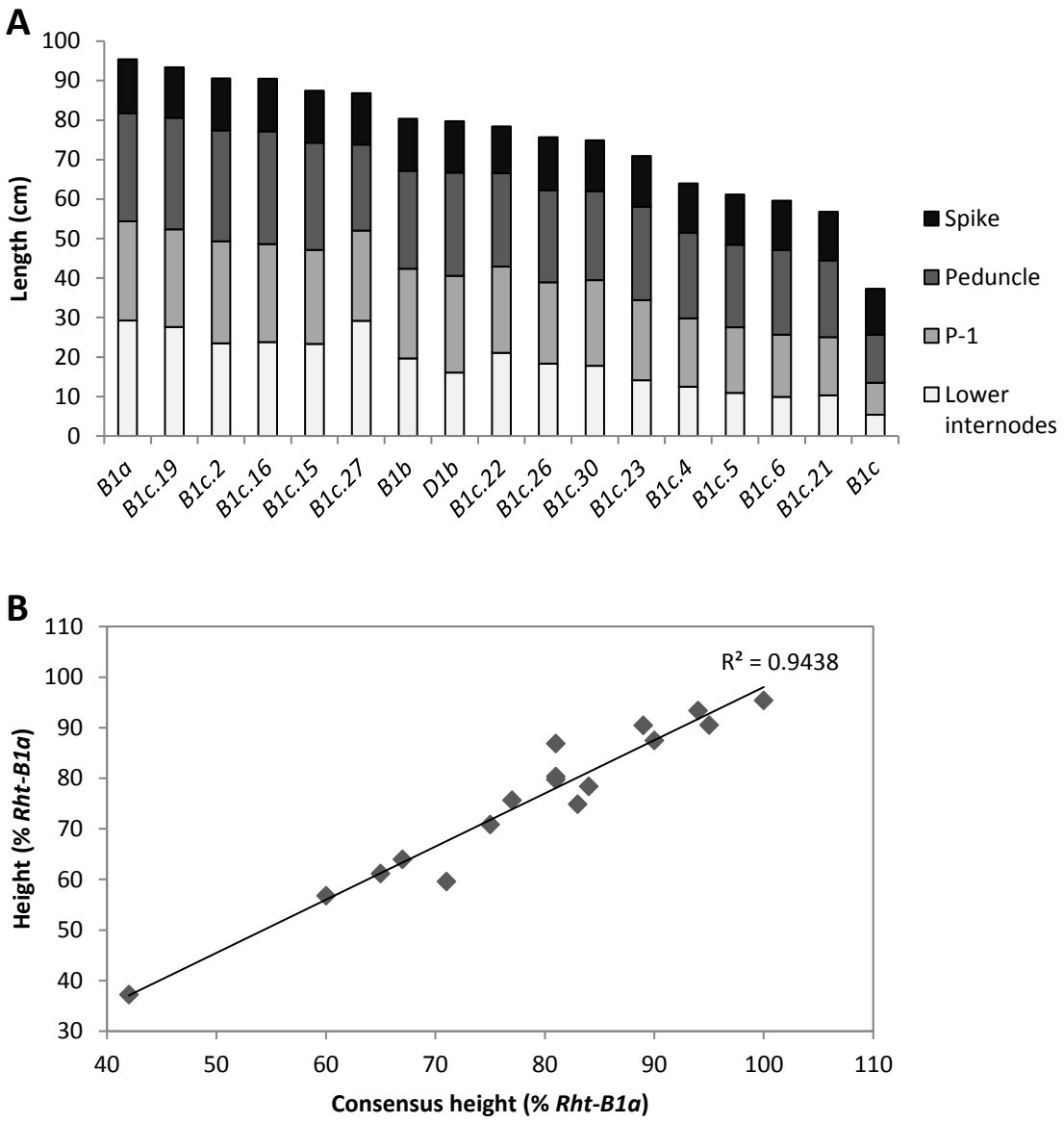
Peduncle
P-1
P-2
P-3
P-4

FIGURE 2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B1a
B1c.19
B1c.2
B1c.16
B1c.15
B1c.27
B1b
D1b
B1c.22
B1c.26
B1c.30
B1c.23
B1c.4
B1c.5
B1c.6
B1c.21
B1c
Spike
Peduncle
P-1
Lower internodes
B
Height (% Rht-B1a)
110
100
90
80
70
60
50
40
30
R² = 0.9438
40
50
60
70
80
90
100
110
Consensus height (% Rht-B1a)

FIGURE 3

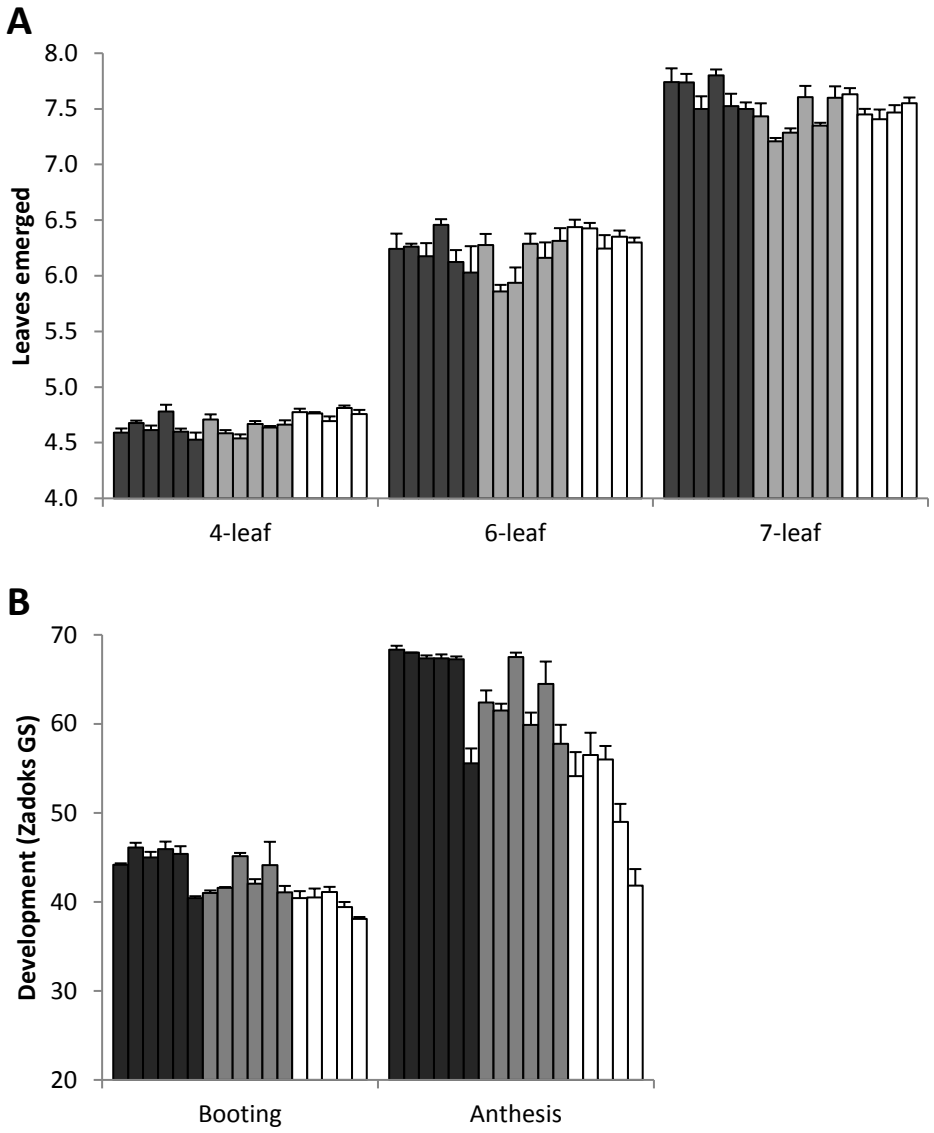

A
Leaves emerged
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
4-leaf
6-leaf
7-leaf
B
Development (Zadoks GS)
70
60
50
40
30
20
Booting
Anthesis

FIGURE 4

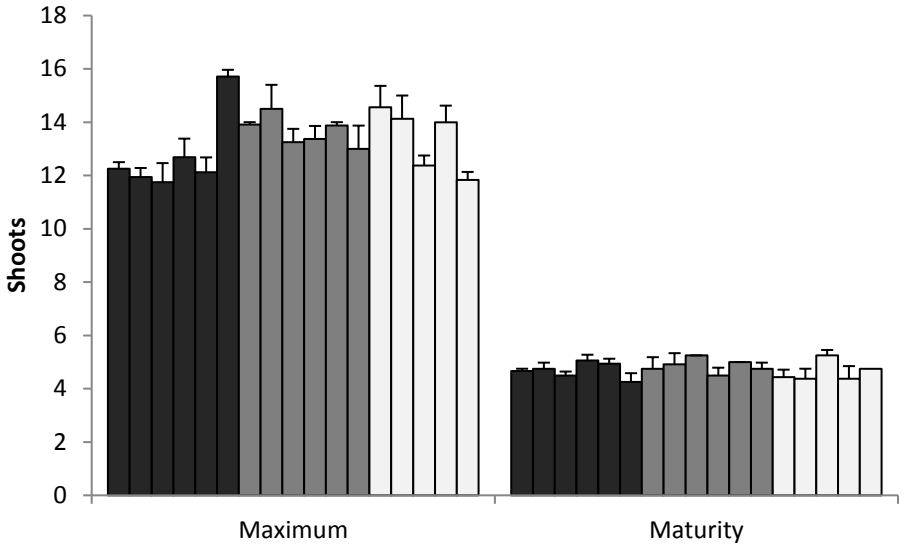


FIGURE 5

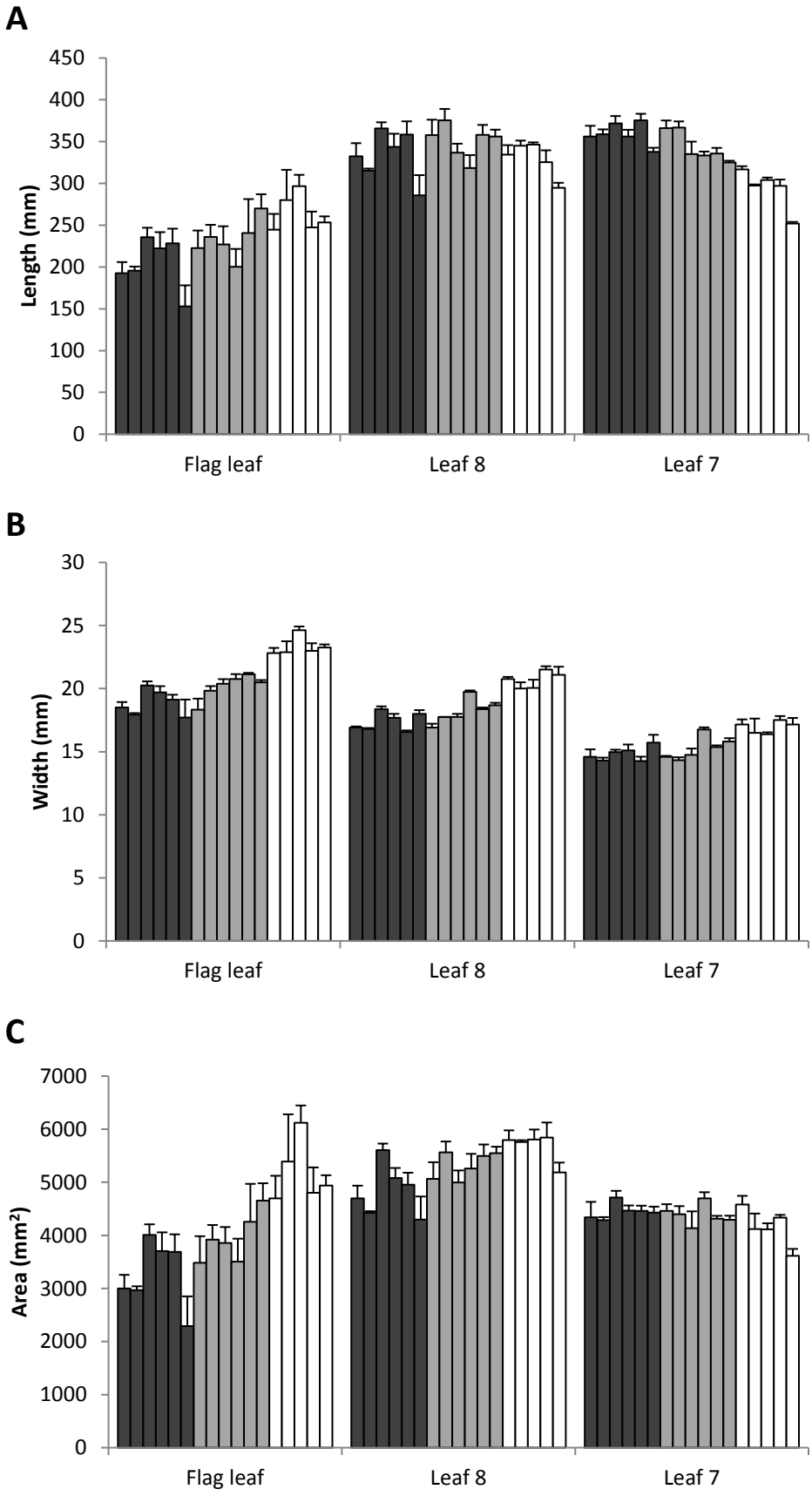

A
450
400
350
300
250
200
150
100
50
0
Length (mm)
Flag leaf
Leaf 8
Leaf 7
B
30
25
20
15
10
5
0
Width (mm)
Flag leaf
Leaf 8
Leaf 7
C
7000
6000
5000
4000
3000
2000
1000
0
Area (mm²)
Flag leaf
Leaf 8
Leaf 7

FIGURE 6

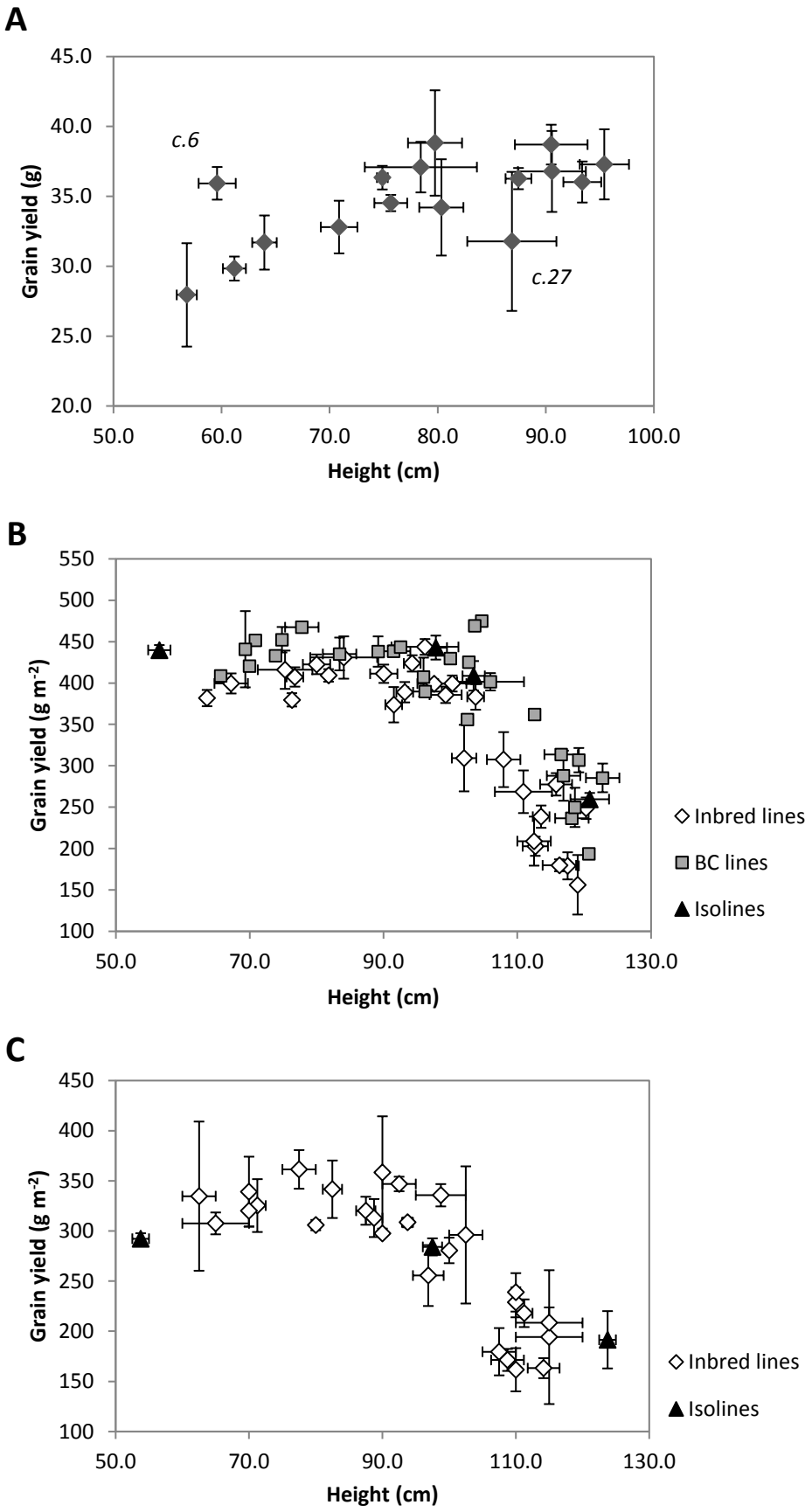

A
45.0
40.0
35.0
30.0
25.0
20.0
Grain yield (g)
c.6
c.27
50.0
60.0
70.0
80.0
90.0
100.0
Height (cm)
B
550
500
450
400
350
300
250
200
150
100
Grain yield (g m-2)
50.0
70.0
90.0
110.0
130.0
Height (cm)
Inbred lines
BC lines
Isolines
C
450
400
350
300
250
200
150
100
Grain yield (g m-2)
50.0
70.0
90.0
110.0
130.0
Height (cm)
Inbred lines
Isolines

FIGURE 7

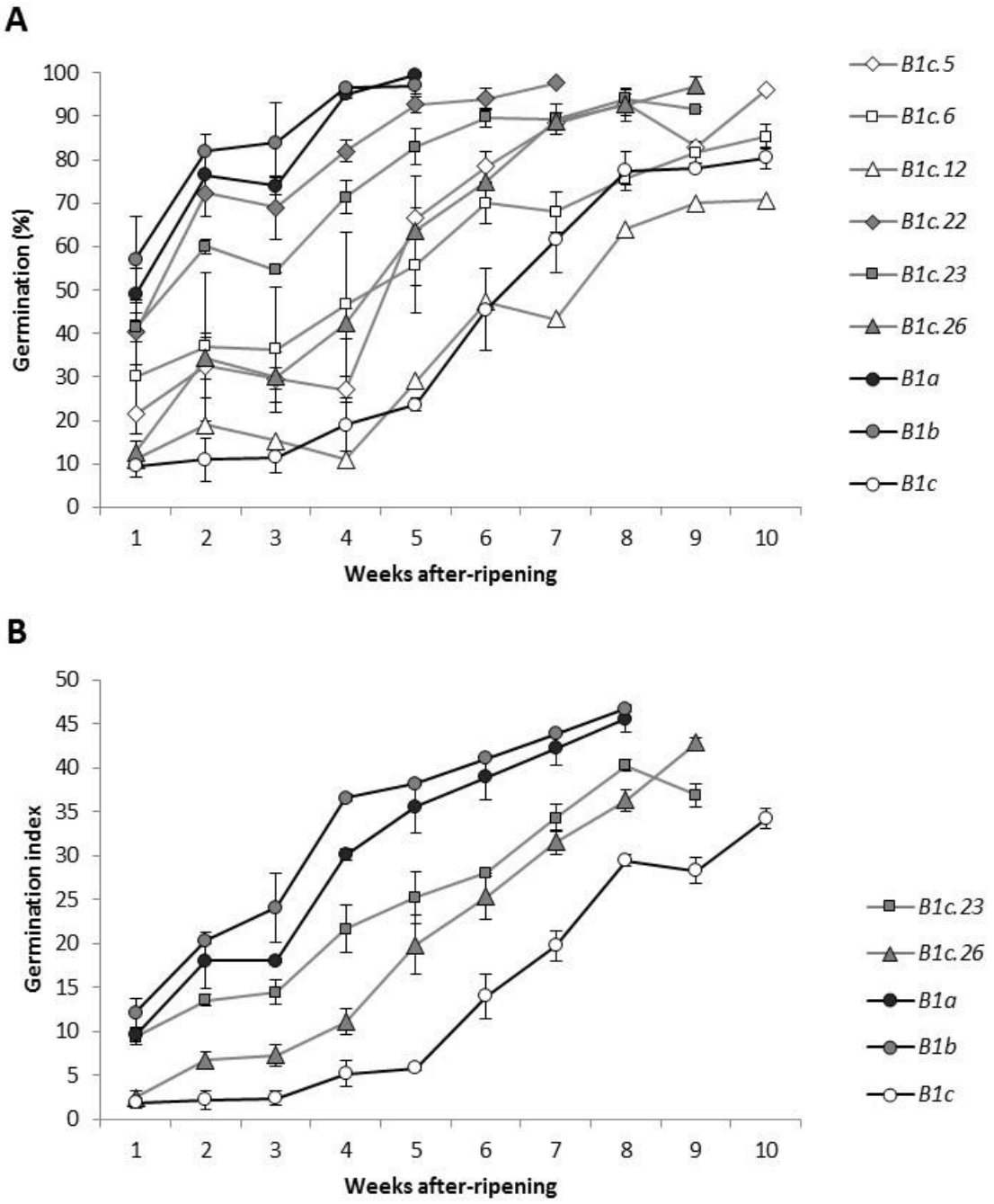
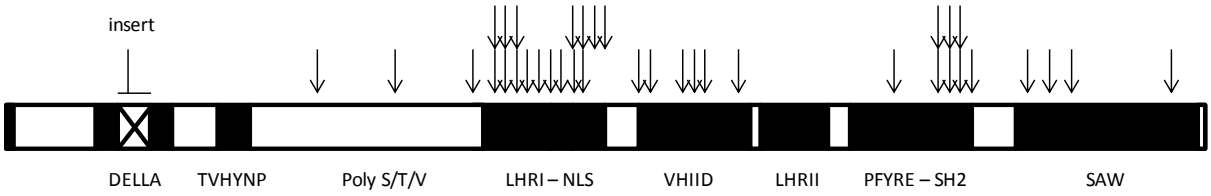
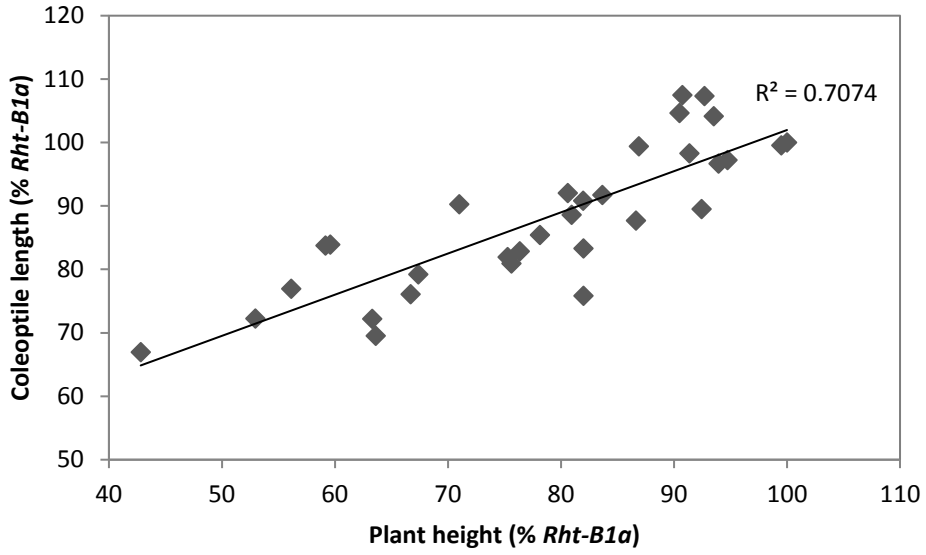

A
Germination (%)
Weeks after-ripening
B1c.5
B1c.6
B1c.12
B1c.22
B1c.23
B1c.26
B1a
B1b
B1c
B
Germination index
Weeks after-ripening
B1c.23
B1c.26
B1a
B1b
B1c

FIGURE 8

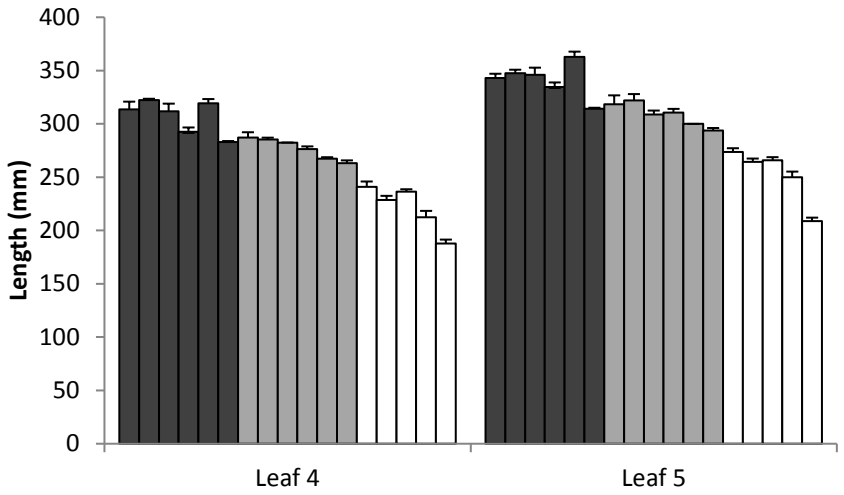

insert
DELLA
TVHYNP
Poly S/T/V
LHRI – NLS
VHIID
LHRII
PFYRE – SH2
SAW

SUPPLEMENTARY FIGURE 1



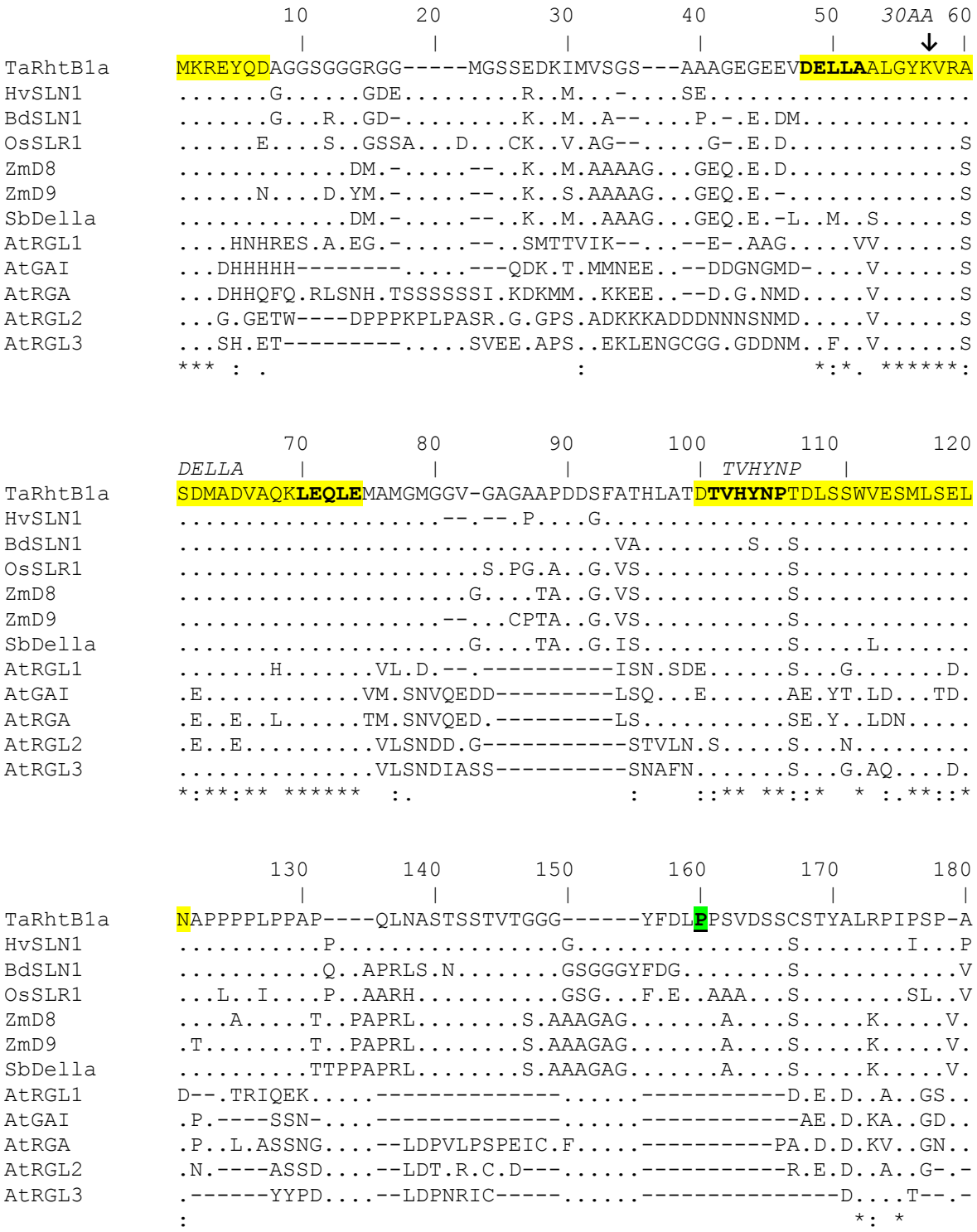
Suppl. Fig. S1. Relationship between plant height and coleoptile length of the overgrowth mutant collection in wheat cv. Maringá.

SUPPLEMENTARY FIGURE 2



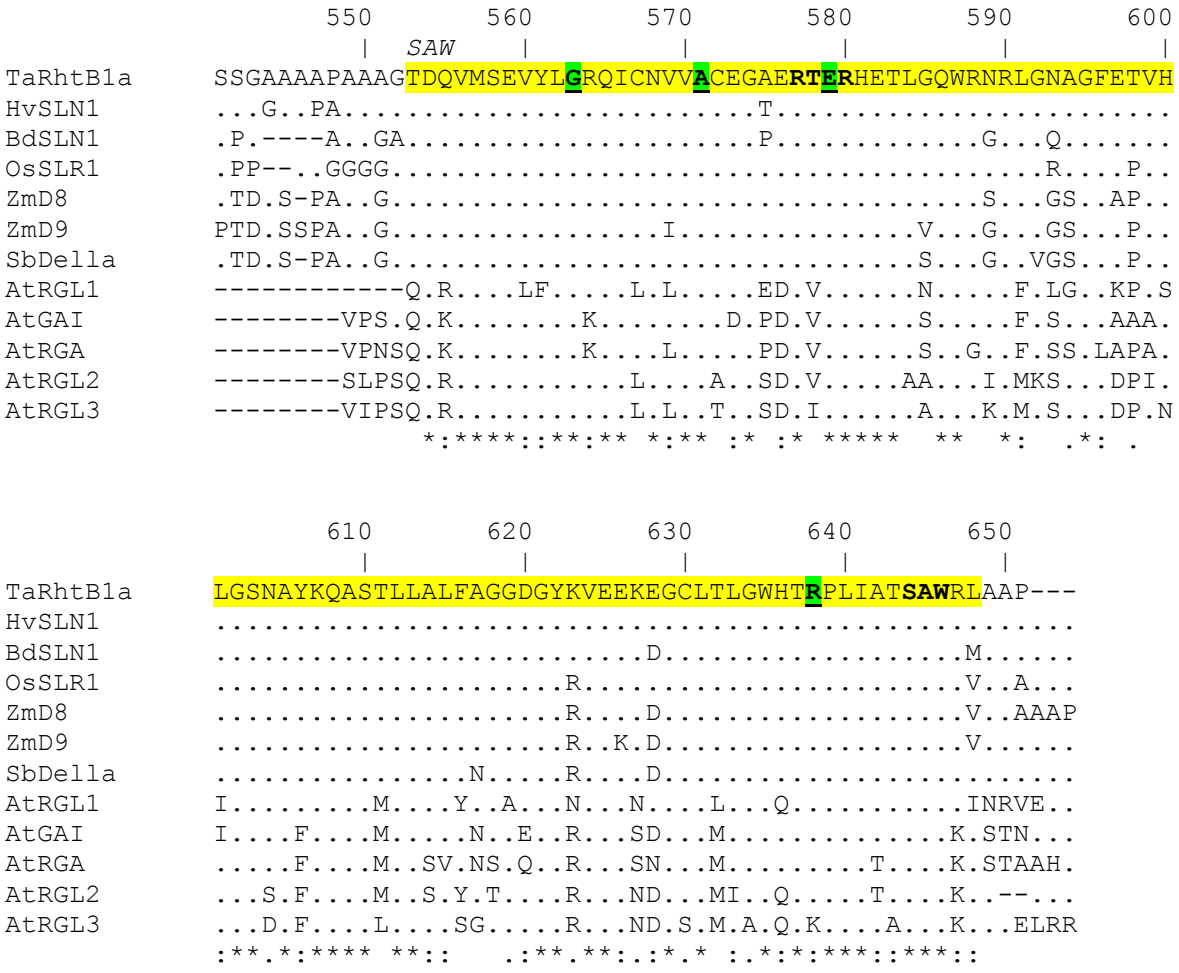
Suppl. Fig. S2. Length of the fourth and fifth emerged leaf of the main shoot of a selection of overgrowth lines grown under controlled environment conditions in 2014. The lines are arranged from the tallest to the shortest with tall lines in dark grey, semi-dwarf lines in light grey and dwarf lines in white. Shown are the means plus SE of two to four replicate pots with four plants per pot.

SUPPLEMENTARY FIGURE 3



```
                    190       200       210       220       230       240
                     |         |         |         |         |         |
TaRhtB1a     VAPADLSADSVVRDPKRMRT----GGSSTSSSSSSS-LGGGGARSSVVEAAP---PVAA
HvSLN1       ..........-.........................S....A.................
BdSLN1       .T..EP...P-A.E............G..........S.....TM.........PAA.AS.
OsSLR1       ..T..P..ADSA..T...........G..........S....AS.G.......PATQG..
ZmD8         APS..P.-TDSA.E............G..........SMD..RT.........PATQAS.
ZmD9         A.S..P.-PDSA.E............G..........SMD..RT.........PATQA--
SbDella      ..S..P.STDST.E............G..........SMD..RT.........PATQAP.
AtRGL1       .Y.R.EH---.T.RS..T.-....---IE.EL..TR..----------------..----
AtGAI        ILNQFAIDSASSSNQG----...G..DTYTTNKRLKCSN.-----------V-..VETTT
AtRGA        IYQFPAIDS.SSSNNQNK.LKSCSSPD.MVT.T.TGTQI..--VIGTT.TTT-..TTTTT
AtRGL2       -LS.FPKEEE.FDEEA----....-----.---.KRIR..-------------..SWCES
AtRGL3       -----DDDECCSSNSN----....-----.---NKRIR..-------------..PWCDS

                    250       260       270       280       290       300
                     |  LHRI   |         |         |         |         |
TaRhtB1a     AAGAPALPVVVVDTQEAGIRLVHALLACAEAVQQENFSAAEALVKQIPLLAASQGGAMRK
HvSLN1       ..A.............................L.....................
BdSLN1       ..N.............................L.....................
OsSLR1       ..N...V.....................................A.........T..........
ZmD8         ..NG..V.....................................M..S.....
ZmD9         .-NG..V.....................................D.......V..S.....
SbDella      ..NG..V....M.....................D.......M..S.....
AtRGL1       --------...L.S..T.V.............N.LKL.D....HVG...S..A.....
AtGAI        .TAESTRH..L..S..N.V.............K..LTV.....GF..V..I.....
AtRGA        ..AESTRS.IL..S..N.V......M.....I..N.LTL.....GC..V..A.....
AtRGL2       SD-ESTRS..L..S..T.V......V.....IH...LNL.D....RVGT..G..A...G.
AtRGL3       VTSESTRS..LIE--.T.V...Q..V.....L..L.L.D....RVG......A...G.
                    *:::: * *:***:**:*****:: :*: *:****::  ** ** *** *

                    310       320       330       340       350       360
                  NLS   |         |         |         | VHIID   |         |
TaRhtB1a     VAAYFGEALARRVFRFRPQP--DSSLLDAAFADLLHAHFYESCPYLKFAHFTANQAILEA
HvSLN1       ...............................................................
BdSLN1       .......................................................
OsSLR1       .............Y....-A....T.....................................
ZmD8         .............Y....P.....................................
ZmD9         .............Y.L..A....G.....................................
SbDella      .............Y....T....T......V..F.....................
AtRGL1       ..T..A.G....IY.IY.--..RDDVALSS.S.T.QI.....................V
AtGAI        ..T..A......IY.LS.SQ..--.PI.HSLS.T.QM....T.....
AtRGA        ..T..A......IY.LS.PQ..--NQI.HCLS.T.QM....T.....
AtRGL2       ..T..AQ.....IY.DYTAETDVCAAVNPS.EEV.EM.....
AtRGL3       ..T..A......IY.IH.SA..--AAI.PS.EEI.QMN..D.....
             **:**.:.****::*  .           .. : *. :**::****************.
```

```
                    370        380        390        400        410        420
                     |          |          |          |          | LHRII    |
TaRhtB1a      FAGCRRVHVVDFGIKQGMQWPALLQALALRPGGPPSFRLTGVGPPQPDETDALQQVGWKL
HvSLN1        ............................................................
BdSLN1        ............................................................
OsSLR1        ....H.......................................................
ZmD8          ............................................................
ZmD9          ............................................................
SbDella       ..................L..............................H.............
AtRGL1        ..TAEK...I.L.LNH.L.....I.......N...D.....I.YSLT.----I.E.....
AtGAI         .Q.KK....I..SMS..L.....M...........V.....I...A..NF.Y.HE..C..
AtRGA         .E.KK....I..SMN..L.....M......E....T.....I...A..NS.H.HE..C..
AtRGL2        VTTA.....I.L.LN........M.................I....TENS.S...L....
AtRGL3        VTTS.V...I.L.LN........M.....................N.--SNREGI.EL....
              .   . ***:*:.:.:*:*****:****** .*** *****:* .  .    ::::* **

                    430        440        450        460        470        480
                     |          |          |          | PFYRE    |          |
TaRhtB1a      AQFAHTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEMHRLLAQPG
HvSLN1        ............................................................
BdSLN1        ............................................................S...
OsSLR1        ..................................A.A..............L.........
ZmD8          ..................................D-.TDD............L.........
ZmD9          ..............................R...DG.TDD..........C.L.........
SbDella       ..................................D-.KD.............L.........
AtRGL1        G.L.S..G.N.EFKSIALNN.S..K.E..DIRPG------L.SV.......L.....H..
AtGAI         .HL.EA.H.E.E...F..N.....DAS..ELRPS-----.I.SV.......L.K..GR..
AtRGA         ..L.EA.H.E.E...F..NS....DAS..ELRPS-----DT.AV.......L.K..GR..
AtRGL2        ....QNMG.E.EFK..A.ES.S....E.FETRP------.S.TLV......L.....RS.
AtRGL3        ..L.QA.G.E.KFN..TTER.S....D.FETRT------.S.TLV......L.PV.S...
              .::*  : *:*::..:.   *:**.. *:  .          * :.**** *:* :*.:.*

                    490        500        510        520        530        540
                     |          |SH2-like |          |          |          |
TaRhtB1a      ALEKVLGTVRAVRPRIVTVVEQEANHNSGTFLDRFTESLHYYSTMFDSLEGGSSGGPSEV
HvSLN1        ..........................S...........................
BdSLN1        ..........................S.....................AG..-Q..I
OsSLR1        .........H................S.....................-QA.L
ZmD8          ..........................................AGA.--.GQ
ZmD9          T.D.......................................AG..--.GQ
SbDella       ..........................................AG----.GQ
AtRGL1        SID.F.S.IKSI..D.M.........GTV............SL......PP----.--
AtGAI         .ID....V.NQIK.E.F......S....PI............L......---------
AtRGA         GI.....V.KQIK.V.F......S...GPV............L......---------
AtRGL2        SI..L.N..K.IK.S...........GIV.....N.A.....SL.....DSY-------
AtRGL3        SI..L.A..K..K.GL..........GDV.....N.A.....SL.....D.V-------
               ::*.* .:. ::* :.******:***.  *****.*:*****::*****.
```



Suppl. Fig. S3. Alignment of cereal and Arabidopsis DELLA proteins. Conserved domains are indicated in yellow with characteristic motifs in bold. Sites of amino acid substitution in wheat and barley are highlighted in green. The arrow indicates the site of the 30 amino acid insertion in *Rht-B1c*.

SUPPLEMENTARY TABLE S1

Rht allele	Height (% *Rht-B1a*)	Independent BC lines	Total pots
B1c.2	*95*	2	4
B1c.4	*67*	2	4
B1c.5	*64*	1	2
B1c.6	*67*	2	4
B1c.15	*90*	2	4
B1c.16	*91*	2	4
B1c.19	*94*	2	4
B1c.21	*59*	2	4
B1c.22	*87*	1	2
B1c.23	*75*	2	4
B1c.26	*76*	2	4
B1c.27	*78*	1	2
B1c.30	*84*	1	2
B1a	*100*		3
B1b	*82*		3
B1c	*43*		3
D1b	*82*		3

SUPPLEMENTARY TABLE S2

Fragment	Name	Sequence	Used for	B-genome specific?
1	Rht3 F40	GGCAAGCAAAAGCTTGAGATAGAT	PCR & sequencing	Yes
1	Rht3 R5	GCGTCCGGTGGAGTTGCC	PCR & sequencing	No
2	Rht3 F54	GACAGCACCAGACGCTCAC	PCR & sequencing	Yes
2	Rht3 R2	GCTCTCGACCCAGGAGGAG	PCR & sequencing	No
3	Rht3 F46	GTGCTAACAAGGTGCGGG	PCR	Yes
3	Rht3 R8	TAGGGGCAGGACTCGTAGAA	PCR & sequencing	No
4	Rht3 F13	GCGCTGGTGAAGCAGATAC	PCR & sequencing	No
4	Rht3 R40	TTCAAACTCGCGGTCACG	PCR & sequencing	Yes
4	Rht3 F16	CGAGGAGCCCGAGGTAAT	Sequencing	No
4	Rht3 R10	AGGAATGTGCCGGAGTTGT	Sequencing	No

SUPPLEMENTARY TABLE S3

Allele	Overgrowth mutation	
	Nucleotide	**Amino acid**
Previously found in Maringa		
Rht-B1c.2	G2726A	V264M
Rht-B1c.6	G2849A	A305T
Rht-B1c.7	C2865T	A310V
Rht-B1c.10	G3065A	G377R
Rht-B1c.11	G3076A	W380ter
Rht-B1c.13	G3190A	W418ter
Rht-B1c.15	G3477A	R514H
Rht-B1c.25	C3071T	Q379ter
Rht-B1c.27	G148A	splice
Rht-B1c.31	G2335A	W133ter
Rht-B1c.32	G2083A	splice
Rht-B1c.33	G3841A	W635ter
New in Halberd		
Rht-B1c.40	C3504T	S523F
Rht-B1c.41	C3489T	S518F
Rht-B1c.42	C3476T	R514C
Rht-B1c.43	G3437A	E501K
Rht-B1c.44	C3077T	P381S
Rht-B1c.45	C3648T	A571V
Rht-B1c.46	G3849A	R638H
Rht-B1c.47	C3078T	P381L
Rht-B1c.48	C2804T	P290S
Rht-B1c.49	C2676T	P247L
Rht-B1c.50	G2610A	G225D

SUPPLEMENTARY TABLE S4

	Rht1-B1c cross		*Rht-B1a* cross F_2		
Allele	**Parent**	**F_1**	***B1a***	***Ovg/B1a***	***Ovg***
Rht-B1a	94				
Rht-B1b	72				
Rht-B1c	30				
Rht-B1c.1	91	50	122	113	120
Rht-B1c.2	86	46	103	89	98
Rht-B1c.3	66	45	104	76	66
Rht-B1c.4	49	37	99	79	61
Rht-B1c.5	52	45	109	72	49
Rht-B1c.6	53	38	107	79	58
Rht-B1c.7	88	43	120	110	108
Rht-B1c.8	45	31	115	76	54
Rht-B1c.9	72	43	107	94	78
Rht-B1c.10	46	36	103	74	52
Rht-B1c.11	97	44	ND	ND	ND
Rht-B1c.12	30	32	105	68	43
Rht-B1c.13	93	49	ND	ND	ND
Rht-B1c.14	42	36	93	80	56
Rht-B1c.15	84	50	109	90	100
Rht-B1c.16	87	51	104	101	94
Rht-B1c.17	59	43	104	89	68
Rht-B1c.18	87	48	104	85	92
Rht-B1c.19	89	48	93	97	85
Rht-B1c.20	90	47	ND	ND	ND
Rht-B1c.21	48	37	101	82	45
Rht-B1c.22	85	39	96	85	78
Rht-B1c.23	65	41	101	83	74
Rht-B1c.24	70	40	101	86	72
Rht-B1c.25	97	46	96	84	72
Rht-B1c.26	65	44	ND	ND	ND
Rht-B1c.27	75	37	101	90	77
Rht-B1c.28	ND	ND	88	82	71
Rht-B1c.29	ND	ND	102	86	80
Rht-B1c.30	75	41	96	83	72
Rht-B1c.31	96	54	97	92	92
Rht-B1c.32	86	40	96	92	106
Rht-B1c.33	ND	ND	ND	ND	ND
Rht-B1c.34	84	51	ND	ND	ND
Rht-B1c.35	86	44	ND	ND	ND