RESEARCH PAPER

**TEOSINTE BRANCHED1** regulates height and stem internode length in bread wheat

Laura E. Dixon¹,² Marianna Pasquariello¹ and Scott A. Boden¹,³,*

¹ Department of Crop Genetics, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK
² School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
³ School of Agriculture, Food and Wine, Waite Research Institute, Waite Research Precinct, University of Adelaide, Glen Osmond, SA, 5064, Australia

* Correspondence: scott.boden@adelaide.edu.au

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Abstract

Regulation of plant height and stem elongation has contributed significantly to improvement of cereal productivity by reducing lodging and improving distribution of assimilates to the inflorescence and grain. In wheat, genetic control of height has been largely contributed by the **Reduced height-1** alleles that confer gibberellin insensitivity; the beneficial effects of these alleles are associated with less favourable effects involving seedling emergence, grain quality, and inflorescence architecture that have driven new research investigating genetic variation of stem growth. Here, we show that **TEOSINTE BRANCHED1** (**TB1**) regulates height of wheat, with **TB1** being expressed at low levels in nodes of the main culm prior to elongation, and increased dosage of **TB1** restricting elongation of stem internodes. The effect of **TB1** on stem growth is not accompanied by poor seedling emergence, as transgenic lines with increased activity of **TB1** form longer coleoptiles than null transgenic controls. Analysis of height in a multiparent mapping population also showed that allelic variation for **TB1** on the B genome influences height, with plants containing the variant **TB-B1b** allele being taller than those with the wild-type **TB-B1a** allele. Our results show that **TB1** restricts height and stem elongation in wheat, suggesting that variant alleles that alter the expression or function of **TB1** could be used as a new source of genetic diversity for optimizing architecture of wheat in breeding programmes.

**Keywords:** Architecture, coleoptile, growth, height, **TEOSINTE BRANCHED1** (**TB1**), wheat (**Triticum aestivum**).

Introduction

Height is an important trait in cereals including wheat, rice, and barley, with genetic variation that reduces stem elongation having contributed significantly to the generation of superior yielding cultivars during the ‘Green Revolution’ (Hedden, 2003). Plants with reduced height benefit from improved lodging resistance, and the suppression of growth increases the ability of plants to tolerate higher inputs of nitrogen-based fertilizer; the decrease in stem elongation boosts partitioning of assimilates to the developing inflorescence, facilitating improved floret fertility and grain numbers per ear (Youssefian *et al.*, 1992a; Flintham *et al.*, 1997)

Genetic variation that contributes semi-dwarfing alleles in wheat, barley, and rice mostly involves modification of biosynthesis, metabolism, or signalling of the growth-promoting phytohormone, gibberellic acid (GA) (Hedden, 2003; Pearce *et al.,* 2011). For example, the semi-dwarfing **Reduced height-1** (**Rht-1; Rht-B1b** and **Rht-D1b**) alleles encode variants of the DELLA protein that no longer confer responsiveness to GA,
with the DELLA protein being a negative regulator of GA signalling that represses growth (Peng et al., 1999; de Lucas et al., 2008; Feng et al., 2008; Pearce et al., 2011). In rice and barley, the semi-dwarfing sd1 and shd1 alleles contain loss-of-function mutations in the key GA biosynthesis enzyme GA 20-oxidase 2 (GA20ox2)—the defective alleles reduce levels of the growth-promoting bioactive GAs (Spielmeyer et al., 2002; Jia et al., 2009; Xu et al., 2017). In addition to the beneficial height reduction effects of the semi-dwarfing Rht1 alleles, the decreased responsiveness to GA provokes unfavourable pleiotropic phenotypes including reduced seedling emergence, lower photosynthetic rate, and decreased grain size and grain protein content, which has encouraged research of novel Rht-B1 alleles that contain second site mutations and other dwarfing alleles, such as Rht8 and Rht18/Rht25 (Allan, 1980; Youssefian et al., 1992a, b; Schillinger et al., 1998; Rebetzke et al., 1999; Wu et al., 2011; Gasperini et al., 2012; Chandler and Harding, 2013; Casebow et al., 2016; Kowalski et al., 2016; Van De Velde et al., 2017; Ford et al., 2018; Mo et al., 2018; Jobson et al., 2019). Rht18 encodes a GA 2-oxidase, which is responsible for converting bioactive GA into inactive forms of the hormone (Ford et al., 2018). The effect of these Rht alleles on stem elongation and height is accompanied by changes in physical characteristics of the inflorescence; dwarfed plants develop compact spikes with an altered number of spikelets (Mo et al., 2018).

Inflorescence development is closely associated with stem elongation, as elongation of stem internodes 2 and 3 occurs simultaneously with the white anther and terminal spikelet stage, respectively, which are when floret and spikelet numbers are determined (Kirby, 1987; Reynolds et al., 2009; Guo and Schnurbusch, 2015). To identify genes that influence spikelet and floret formation, we and others have studied genes that alter the rate of inflorescence development, spikelet architecture, and/or floret fertility (Boden et al., 2015; Guo and Schnurbusch, 2015; Dixon et al., 2018; Sakuma et al., 2019). We showed that a wheat orthologue of the major maize domestication gene, TEOSINTE BRANCHED1 (TB1), is a key regulator of plant architecture and inflorescence development (Dixon et al., 2018). Increased dosage of TB-D1 (D genome homologue of TB1) delayed inflorescence development, restricted tiller growth, and promoted formation of paired spikelets, which are supernumerary spikelets characterized by the development of two spikelets at a single rachis node, rather than the typical single spikelet (Boden et al., 2015). These traits are all linked physiologically by the suppression of growth, particularly of axillary meristems including spikelet primordia and tiller buds, with a decrease in the rate of inflorescence development occurring at the terminal spikelet stage associated with elongation of stem internode 3 (Dixon et al., 2018). The effect of TB1 on growth is consistent with it being a class II TCP transcription factor, which regulates expression of genes involved in processes including cell proliferation, the cell cycle, as well as signalling and metabolism of hormones such as GA and jasmonic acid; TB1 and its Arabidopsis homologue, BRANCHED1 (BRC1), inhibit expression of cell cycle genes and those involved in GA signalling (González-Grandid et al., 2013; Dong et al., 2019). Based on the role of TB1/BRCl controlling cell proliferation, and its key role in regulating the rate of inflorescence development and plant architecture, we hypothesized that TB1 may influence the height of wheat plants.

In this study, we investigated height and stem internode lengths in wheat lines that contain increased dosage of TB-D1, including a pair of near-isogenic lines (NILs) developed from a MAGIC population that are tetrasomic for chromosome 4D and transgenic plants that express TB1 at higher levels (Dixon et al., 2018). We show that increased activity of TB1 restricts height and stem elongation in bread wheat, and that allelic diversity for TB-B1 influences height in an advanced mapping population. These results provide new insights into the genetic regulation of height in wheat, which may help breeders optimize plant architecture.

**Materials and methods**

**Plant materials and growth conditions**

Hexaploid wheat (*Triticum aestivum*) germplasm used in this study included the following genotypes: MAGIC line 0053 of the CSIRO four-way multirepeat advanced generation intercross (MAGIC) population, from which the NILs termed wild type (WT) and highly-branched (hb) were derived (see Dixon et al., 2018); transgenic lines expressing TB-D1 using the VRN1 promoter generated in the cv. Fielder genetic background (Dixon et al., 2018); and the hexaploid wheat TILLING line, Cadenza1721 (h-h) (Krailevá et al., 2017). The hb line is tetrasomic for chromosome 4D and contains an additional haploid copy of TB-D1, relative to its WT NIL that is diploid for 4D (see Dixon et al., 2018). The pvRNA1:TB1 transgenic lines expressed the TB-D1 allele from cv. Baxter that is present in hb and WT NILs of the MAGIC line 0053, under the control of the barley VRN1 promoter (Alonso-Peret et al., 2011). The ‘NIAB Elite MAGIC’ bread wheat population has been previously described (Mackay et al., 2014), and height data were collated from published data of plants grown under field conditions at the NIAB experimental farm in Cambridge, UK (52°13′19″N, 0°53′46″E) (Mackay et al., 2014; Scutari et al., 2014).

WT, hb plants, transgenic lines, and the F1 plants of the hb crosses to Cadenza, th-d1 (Cadenza1721; see Supplementary Fig. S1 at JXB online), that were used for phenotype analysis and gene expression studies were grown in controlled growth chambers under long-day (16 h light/8 h dark) photoperiods at 300 µmol m–2 s–1 [using Plantastar 400-W HQI bulbs (Oslam) and Maxim 60 W tungsten bulbs], with a day temperature of 20 °C and a night temperature of 15 °C.

**RNA extractions and quantitative real-time PCR analysis**

For experiments with WT and hb plants, RNA extraction was extracted from internode 2 stem, prior to internode elongation. For experiments using the pvRNA1:TB1 transgenic and null control lines, including cv. Fielder, RNA was extracted from internode 2 stem, prior to initiation of elongation. For transcript analysis of TB-B1 and TB-D1 in cv. Cadenza, RNA was extracted from internodes 1, 2, and 3 and the peduncle prior to initiation of elongation and from tiller buds, which are immature tillers that were transparent or light green in colour for which no leaf blade had begun to emerge. All RNA extractions were performed using the RNeasy Plant Mini Kit (Qiagen). Synthesis of cDNA and quantitative real-time PCR (qRT-PCR) were performed as previously (Boden et al., 2014). Oligonucleotides for qRT-PCR analysis are provided in Supplementary Table S1. Expression of TB-B1 and TB-D1 was normalized using Trag_6D5_BE8B5E56D.1 (gene ID) in IWGSC reference genome is TraesCS6D02G145100 (Borrill et al., 2016). All qRT-PCR data points are the average of at least four biological replicates, with two technical replicates performed in each reaction.
Analysis of coleoptile elongation

Coleoptile length measurements were performed using seeds of equal weight (between 40 mg and 50 mg), with individual seeds sown into pots (dimensions 90×90×180 mm) containing fertile compost-based potting mix at a sowing depth of ~5 cm. The soil was initially watered until saturation (~150 ml), and pots were placed in growth chambers and covered with lids wrapped in aluminium foil. Lights were turned off and the temperature was set to 20 °C/15 °C (day/night). Pots were watered equally every 2 d (15 ml). Coleoptile length was measured using a ruler, 12 d after sowing once the cotyledon had emerged from the coleoptile. Each experiment was performed at least three times, with 12 seeds per genotype per replicate.

Kompetitive allele-specific PCR analysis

Kompetitive allele-specific PCR (KASP-PCR) analysis was performed as per Dixon et al. (2018). Sequences for oligonucleotides used in KASP assays are provided in Supplementary Table S2.

Statistical analysis

Differences between genotypes were tested by two-tailed Student’s t-test. The number of replicates for each experiment are provided elsewhere in the Materials and methods and in the relevant figure legend.

Accession numbers

The gene IDs for TB-A1, TB-B1, and TB-D1 are TraesCS4A02G271300, TraesCS4B02G042700, and TraesCS4D02G040100, respectively.

Results

Increased dosage of TB-D1 reduces height and internode length in bread wheat

Our previous investigation of inflorescence and plant architecture included analysis of a pair of NILs derived from a single line of a four-way MAGIC population (Dixon et al., 2018). The NIL hb formed multiple paired spikelets on the inflorescence, relative to the WT NIL that formed an inflorescence with single spikelets at each racis node. The hb line is tetrasomic for chromosome 4D, relative to the euploid WT line. In addition to the inflorescence and tiller phenotypes, the hb line was shorter than the WT. To investigate this trait further and determine if TB1 influences stem growth, we measured height and internode length in hb plants grown under long-day photoperiods, relative to the WT. The hb plants were significantly shorter than the WT (Fig. 1A, B), and lengths of internode 1 and the peduncle were significantly shorter in hb compared with the WT, while there was no significant difference in the length of internodes 2 and 3. This result demonstrates that increased dosage of chromosome 4D restricts plant height in wheat. To test if this effect of tetrasomy 4D is caused by increased dosage of TB1 and not another gene on chromosome 4D, we crossed the hb line to a Cadenza TILLING mutant that contains a premature stop codon in TB-D1 (tb-d1) and analysed height and internode length of the first filial generation (F1) individuals, relative to F1 individuals from a cross between hb and Cadenza (Cadenza contains WT tall alleles of Rht-B1 and -D1). We observed that the F1 individuals of the hb×tb-d1 individuals were taller than those derived from the hb×Cadenza cross (Fig. 2A). The lengths of internodes 1 and 2 were longer in the hb×tb-d1 F1 individuals, relative to individuals of the hb×Cadenza cross, while there was no significant difference in the length of internode 3 (Fig. 2B). Taken together, these results indicate that tetrasomy for chromosome 4D restricts plant height and internode length in wheat, and that increased dosage of TB-D1 contributes to this effect on growth.

Expression analysis of TB-1 in stem tissue

To determine if the effect of TB-D1 on height and internode length in the hb plants could be explained by increased expression

Fig. 1. Tetrasomy for chromosome 4D reduces plant height. (A and B) hb plants that are tetrasomic for chromosome 4D are shorter than wild-type near-isogenic lines. (C) Internode lengths for wild-type and hb plants. I1, internode 1; I2, internode 2; I3, internode 3; Ped., peduncle. Scale bar=10 cm. Values are the mean ±SE of 10 biological replicates. *P<0.05; ***P<0.001.
of TB-D1, we measured TB-D1 transcript levels in internode 2 stem regions prior to elongation. qRT-PCR analysis detected TB-D1 transcripts in the stem internode 2 in both WT and hb plants, with transcript levels being ~2-fold higher in hb relative to the WT (Fig. 3A). Transcript analysis in Cadenza showed that TB-B1 and TB-D1 are expressed in internodes 1 and 2 prior to elongation of the internodes, and the levels are low relative to those measured in tiller buds which we demonstrated previously to express TB1 (Fig. 3B); transcript levels in Cadenza were slightly lower than those detected in the same internode of the WT NIL. We did not measure TB-A1 expression because our previous analysis has shown that this homoeoallele is not expressed (Dixon et al., 2018).

These results show that reduced height and internode lengths of the hb plants are associated with increased expression of TB-D1 in stem internode tissue prior to elongation, and that TB1 is expressed at low levels in stem segments prior to elongation of the internode.

**Analysis of height in pVRN1:TB-D1 transgenic lines**

To further test if increased dosage of TB-D1 restricts stem growth and height in wheat, we analysed transgenic lines that contain an additional copy of TB-D1 under control of the VERNALIZATION1 (VRN1) promoter (pVRN1:TB1) (Alonso-Peral et al., 2011). The pVRN1:TB1 transgenic lines were significantly shorter than control null transgenic lines [pVRN1:TB1(−)] and the untransformed plants (cv. Fielder) (Fig. 4A, B). The pVRN1:TB1 transgenic lines had shorter internodes than the null transgenic control lines and cv. Fielder, with length of internodes 1, 2, and 3 and the peduncle being significantly reduced, relative to the control lines (Fig. 4C). The stem growth and height phenotypes are associated with significantly higher transcript levels of TB-D1 in stem segments prior to stem elongation, relative to the null transgenic lines (Fig. 4D). Increased expression of TB-D1 is
consistent with *VRN1* transcript levels being relatively high in stem tissue compared with leaf tissue where it is expressed robustly (Supplementary Fig. S2). These results show that increased expression of *TB-D1* within developing stem tissue restricts internode elongation and plant height in wheat, which support the phenotypes of the *hb* line relative to the WT NIL.

**Increased dosage of TB-D1 does not restrict coleoptile length**

A limitation of using *Rht-b1b* and *Rht-D1b* dwarfing alleles in modern wheat is that the benefit of reduced stem elongation to reduce lodging is accompanied by unfavourable pleiotropic traits including poor emergence of seedlings from deep sowing, decreased meristem size, lower grain set, increased susceptibility to diseases, and reduced 1000-grain weight (Allan, 1980; Borner et al., 1996; Schillinger et al., 1998; Rebetzke et al., 1999; Ellis et al., 2004; Saville et al., 2012; Serrano-Mislata et al., 2017). We have shown that increased dosage of *TB1* alters spikelet architecture and delays stages of inflorescence development critical for floret formation (Dixon et al., 2018); here, we tested if increased dosage of *TB1* reduced coleoptile length to determine if it would restrict seedling emergence from soil. We found that the coleoptile length of *hb* plants was shorter than that of the WT, but was longer in two independent *pVRN1:TB1* transgenic lines, relative to null transgenic plants (Fig. 5A–C). These results show that tetrasomy for chromosome 4D reduces coleoptile length, which is consistent with increased dosage of
Rht-D1a (also located on 4D); however, increased dosage of TB1 alone does not restrict coleoptile growth or emergence of seedlings. These results indicate that increased activity of TB1 restricts plant height without negatively influencing seedling emergence, suggesting that alleles with increased activity of TB1 could be used as an alternative to Rht-B1b or Rht-D1b semi-dwarfing alleles.

Allelic variation for TB-B1 influences plant height in bread wheat

Our previous analysis of TB1-dependent regulation of inflorescence architecture identified a variant allele for TB-B1 (known as TB-B1b), which is predicted to be a weak loss-of-function allele that contains three amino acid substitutions and two synonymous mutations, relative to the reference allele (Dixon et al., 2018). Based on our results showing that increased activity of TB-D1 reduces height (Figs. 1, 4), and TB-B1 being expressed equally to TB-D1 (Fig. 3), we hypothesized that the TB-B1b allele would increase height of bread wheat, relative to the TB-B1a allele. To test this hypothesis, we analysed the height of lines from the eight-way UK MAGIC population that were fixed for the semi-dwarfing allele of Rht-D1 (Rht-D1b; aka Rht-2) but differed with respect to the TB-B1 allele (i.e. Rht-D1b;TB-B1a, or Rht-D1b;TB-B1b) (Scutari et al., 2014; Mackay et al., 2014). We did not analyse any lines with two dwarfing alleles (Rht-B1b/Rht-D1b) or any of the Rht-B1b semi-dwarf lines because they all contained the TB-B1a allele, which is consistent with Rht-B1 and TB-B1 being in close genetic linkage with each other (Dixon et al., 2018). We found that lines containing the TB-B1b allele were significantly taller than lines with the TB-B1a allele (Fig. 6), with TB-B1b genotypes being 4.6% taller (3.4±0.91 cm; $P<0.01$) than TB-B1a genotypes. These results support the hypothesis that TB-B1b alleles are weak loss-of-function alleles that can partially restore the height of lines that contain dwarfing alleles of Rht-1.

Discussion

The genetic regulation of height has contributed significantly to improvement of crop productivity, especially in wheat, rice, and barley, by reducing stem elongation to improve lodging resistance and enhance partitioning of resources to the inflorescence (Hedden, 2003). In wheat, these benefits have largely been provided by the semi-dwarfing Rht-B1b and Rht-D1b alleles (Peng et al., 1999; Pearce et al., 2011); however, due to their pleiotropic effects on seedling emergence, spikelet number, grain weight, and quality (for example), research has continued to investigate novel sources of genetic variation for control of height (Allan, 1980; Youssefian et al., 1992a, b; Schillinger et al., 1998; Rebetzke et al., 1999; Wu et al., 2011; Gasperini et al., 2012; Kowalski et al., 2016; Ford et al., 2018; Mo et al., 2018). Here, we have shown that increased expression of TB1 reduces stem elongation in bread wheat, and that allelic variation for TB-B1 influences plant height.

TB1 and its homologue in eudicots—BRANCHED1 (BRC1)—have been studied extensively due to their role in regulating shoot branching and apical dominance (Doebly et al., 1997; Aguilar-Martinez et al., 2007). TB1/BRC1 of
maize, rice, wheat, barley, tomato, pea, Arabidopsis, switchgrass, and cucumber have conserved roles in regulating outgrowth of lateral branches, with loss-of-function mutations increasing lateral branch number and length, while gain-of-function alleles suppress their growth (Doebley et al., 1997; Takeda et al., 2003; Aguilar-Martínez et al., 2010; Martin-Trillo et al., 2011; Ramsay et al., 2011; Braun et al., 2012; Seale et al., 2017; Bennett et al., 2016; Dixon et al., 2018; Liu et al., 2018; Zwirek et al., 2019; Shen et al., 2019). A conserved role for TB1/BRC1 in controlling height and stem elongation is, however, less clear; for example, brc1 and br1/bn2 Arabidopsis mutants are shorter than the WT, as are tomato plants with reduced expression of BRC1, potentially due to reduced apical dominance (Finlayson et al., 2010; Martin-Trillo et al., 2011; Bennett et al., 2016; Seale et al., 2017). The effect of tb1/bn1 loss-of-function alleles in eudicots is maintained in rice, with loss-of-function mutants of TB1, known as FINE CULM1 (FC1), shorter than WT plants (Minakuchi et al., 2010). Analysis in maize did not detect a role for allelic diversity of Tb1 in controlling height, and there is no statistical difference in height for barley and switchgrass lines that contain alleles of reduced function, relative to the gain-of-function alleles (Sakuma et al., 2017). Moreover, barley deficiens mutants that maintain protein stability of VRSS1 display reduced plant height, relative to NILs with WT VRSS1 alleles (Sakuma et al., 2017). In domesticated wheat, a mutation in the miR172- (miR172) binding site of an AP2-like transcription factor that underpins the Q locus have contributed to development of a compact spike and a free-threshing phenotype, relative to the elongated rachis of speliotwheat (Debernardi et al., 2017; Greenwood et al., 2017). Further mutations that disrupt binding of miR172 modify spike compactness further, and are associated with significant reduction in plant height (Debernardi et al., 2017; Greenwood et al., 2017). Inflorescence architecture and height are also associated in rice; for example, the SQUAMOSA PROMOTER BINDING-LIKE PROTEIN-14 (SPL14) gene that underpins the IDEAL PLANT ARCHITECTURE (IPA)/WEALTHY FARMERS PANICLE (WFP) locus influences panicle branching and plant height (Jiao et al., 2010; Miura et al., 2010). Mutations in a miR156-binding site increase expression of SPL14 to promote formation of a more highly branched panicle with improved lodging resistance and reduced tillering. Taken together, these results demonstrate an important link between the genetic regulation of inflorescence and plant architecture, and highlight the importance of considering pleiotropic effects of genes when determining genetic variation that may be used to improve crop performance.

The influence of TB1 on height in wheat, including our analysis suggesting that the variant TB-B1b allele partially restores stem length in semi-dwarfed germplasm, provides an opportunity to investigate genetic variation that will help improve plant architecture in breeding programmes (Fig. 6). Analysis of Rht-B1b and Rht-D1b alleles has shown that semi-dwarfism is associated with unfavourable pleiotropic traits, including reduced seedling emergence from deep sowing, decreased grain size, and lower grain protein content, which has stimulated investigation of alternative Rht alleles, such as Rht8, Rht12, and Rht18/25 (Youssefian et al., 1992a; b; Rebetzke et al., 1999; Gasperini et al., 2012; Kowalski et al., 2016; Mo et al., 2018). Rht8 is particularly interesting as it is likely to encode a protein that is independent of the GA metabolism and signalling pathways, and does not restrict coleoptile growth (Rebetzke et al., 2018). Analyses of TB1 in wheat have demonstrated roles for this gene in regulating inflorescence development and plant architecture, including height and tillering (Dixon et al., 2018). A link between inflorescence architecture genes and height has also been shown in other crops. For example, in barley, a major inflorescence trait is row-type architecture, with cultivated barley forming spikes with either two or six row arrangements of spikelets; this trait is regulated by at least five genes, VRSS1–VRSS5 (VRSS5 is a TB1 homologue) (reviewed in Gauley and Boden, 2019). Analysis of plant architecture in the vrss mutants has shown that some of the VRSS row-type alleles influence plant height—vrss4 and vrss4/5 double mutants are significantly shorter (Zwirek et al., 2019). Moreover, barley deficiens mutants that maintain protein stability of VRSS1 display reduced plant height, relative to NILs with WT VRSS1 alleles (Sakuma et al., 2017). In domesticated wheat, a mutation in the miR172- (miR172) binding site of an AP2-like transcription factor that underpins the Q locus have contributed to development of a compact spike and a free-threshing phenotype, relative to the elongated rachis of speliotwheat (Debernardi et al., 2017; Greenwood et al., 2017). Further mutations that disrupt binding of miR172 modify spike compactness further, and are associated with significant reduction in plant height (Debernardi et al., 2017; Greenwood et al., 2017). Inflorescence architecture and height are also associated in rice; for example, the SQUAMOSA PROMOTER BINDING-LIKE PROTEIN-14 (SPL14) gene that underpins the IDEAL PLANT ARCHITECTURE (IPA)/WEALTHY FARMERS PANICLE (WFP) locus influences panicle branching and plant height (Jiao et al., 2010; Miura et al., 2010). Mutations in a miR156-binding site increase expression of SPL14 to promote formation of a more highly branched panicle with improved lodging resistance and reduced tillering. Taken together, these results demonstrate an important link between the genetic regulation of inflorescence and plant architecture, and highlight the importance of considering pleiotropic effects of genes when determining genetic variation that may be used to improve crop performance.

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![Boxplots showing the height of semi-dwarfed eight-way MAGIC lines (genotype Rht-D1b) that contain either the TB-B1a (grey) or the TB-B1b (white) allele. Boxes extend from the lower to the upper quartile, with a line marking the median, and whiskers extend to 1.5 times the interquartile range. *P<0.01.](https://academic.oup.com/jxb/article-abstract/doi/10.1093/jxb/eraa252/5843696/280x749)
et al., 1999; Gasperini et al., 2012). Our results suggest that increased expression of TB1 has a similar effect—elevated activity of TB1 reduced height and stem internode length without negatively affecting coleoptile growth (Figs 4, 5). Alleles that increase expression of TB1 may therefore be useful in restricting stem elongation and tillering without decreasing seedling emergence, providing an alternative to other Rht alleles. The Rht-B1b and Rht-D1b alleles reduce height to ~86% and 83% of tall controls, respectively, and Rht8 dwarfing alleles decrease height by 11%, relative to tall NILs (Flintham et al., 1997; Kowalski et al., 2016). Based on the results shown here, alleles that increase expression of TB1 have potential to reduce height similarly to Rht8 (91% of the height of WT controls) in tetrasomic plants, while the more highly expressed pVRN1:TB1 transgenes decreased height by ~30% (Figs 1, 4). Alleles that increase expression of TB-B1 or -D1 may therefore benefit breeding programmes by providing a comparable reduction in height to existing Rht alleles. Alternatively, TB1 alleles that reduce functionality may also benefit wheat breeding. For example, the effect of TB1 on tillering involves hormones such as abscisic acid, jasmonic acid, and GA, indicating that TB1-dependent regulation of growth is at least partially separable from the molecular role of Rht1 (Peng et al., 1999; de Lucas et al., 2008; Feng et al., 2008; Dong et al., 2019). The TB-B1b allele, or variant TB-D1b allele, could be introduced into Rht-B1b and Rht-D1b semi-dwarfed backgrounds to help fine-tune plant architecture traits including height, tillering, and spikelet number for improved crop performance (Dixon et al., 2018). Due to the genetic linkage of TB1 and Rht1 on chromosome group 4, the most efficient breeding strategy would most probably involve combining reciprocal alleles on the B and D genomes, to form genotypes such as Rht-D1b:TB-B1b or Rht-B1b:TB-D1b. Further investigation of variant TB1 alleles in different semi-dwarfed backgrounds will be necessary to determine the optimal allelic combinations for improved plant architecture.

In summary, our results demonstrate a new role for TB1 in wheat, where increased activity reduces internode elongation and height (Figs 1, 2, 4). Together with our previous report showing that TB1 controls inflorescence development and tillering, these results show that TB1 is an important regulator of plant architecture—further investigation of TB1 will be critical for identifying pathways that act downstream to regulate tissue-specific phenotypes, which may provide opportunities to optimize key yield-related traits including tillering, spikelet number, and height.

**Supplementary data**

Supplementary data are available at JXB online. Fig. S1. Schematic of the hh×hb-d1 cross and the control hh×Cadenza cross. Fig. S2. Tissue-specific analysis of VRN1 expression in wild-type wheat (cv. Cadenza)

Table S1. Oligonucleotide sequences used for qRT-PCR assays.

Table S2. Oligonucleotide sequences used for KASP-PCR assays.

Table S3. TB-B1 genotype information of MAGIC elite lines used to investigate height.

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