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RESEARCH ARTICLE

Identification and expression profiling of *HvMADS57* and *HvD14* in a barley *tb1* mutant

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Abstract. MADS-box genes interact with *TB1* to regulate plant organ morphogenesis. In rice, *OsMADS57* interacts with *OsTB1* to control *OsD14* transcription. In this study, we aimed to determine the relationships among these genes in barley. We identified a natural mutant of *HvTB1* (*tb1*) formed by a C→A transition at position 230, which resulted in a premature stop codon. We cloned the *HvMADS57* and *HvD14* genes and studied their expression in the *tb1* mutant. The results showed that *HvMADS57* is a MIKC^c-type MADS-box gene, and the expression levels of both *HvMADS57* and *HvD14* were significantly reduced in the *tb1* mutant when compared to those in the wild-type gene. These results indicate that, *HvMADS57* regulates plant growth and development by interacting with *HvTB1* to suppress the transcription of *HvD14* in barley which is similar to the relationships among the orthologs of these genes in rice.

Keywords. spike; tiller; *HvTB1* gene; *HvMADS57* gene; *HvD14* gene; barley; MADS-box gene.

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops grown worldwide and is widely used in the food and feed industries. Tiller number (TN), kernel number per spike (KNS), and kernel weight (KW) are the important factors that affect the barley yield (Madić *et al*. 2005; Zhou *et al*. 2016; Shaaf *et al*. 2019). Spikes and tillers are the products of shoot branching, which plays an important role in the plant structure and directly contributes to grain yield (Steeves and Sussex 1989; Kebrom *et al*. 2013). Moreover, shoot branching is involved in plant plasticity in response to environmental cues and stresses (Mohapatra *et al*. 2011; Agusti and Greb 2013).

A recent analysis of shoot and inflorescence branching showed that shoot branches arise from axillary shoot meristems (Schmitz and Theres 2005). Inflorescence meristems in rice produce several primary-branch meristems, each of which generates next-order lateral meristems that acquire spikelet meristem identity. In maize, spikelets are paired, a feature unique to the tribe Andropogoneae. Unlike those of rice and maize, inflorescences of barley are unbranched, with the spikelets implanted directly on the main axis, and are characterized by a triple spikelet meristem at each rachis node (von Bothmer *et al*. 1985). The development of six-rowed spikes as well as the TN are controlled by *Vrs1* in barley (Komatsuda *et al*. 2007). The function of the *Vrs1.b* allele can be modulated up to 10 independent *INTERMEDIUM* (*INT*) genes, with *INT-C* having the largest effect (Lundqvist *et al*. 1997).

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Numerous genes related to shoot branching have been identified. The *Ls* gene of tomato was one of the earliest genes to be identified that regulate axillary meristem initiation. In an *ls* mutant, axillary meristem formation was completely blocked during the vegetative growth phase (Grot *et al*. 1994). The ortholog of this gene in *Arabidopsis*, *LAS*, also affects the axillary shoot number (Greb *et al*. 2003). *MONOCULM1* (*MOC1*) in rice is orthologous to *Ls*, and *moc1* mutants fail to form tiller buds (Li *et al*. 2003). Maize and its wild progenitor, teosinte, possess contrasting branching phenotypes; maize plants exhibit limited branching, whereas teosinte plants are highly branched (Doebley *et al*. 1997). *TEOSINTE BRANCHED1* (*ZmTB1*) regulates branching ability in maize (Hubbard *et al*. 2002), and its ortholog in rice, *OsTB1*, controls shoot branching (Takeda *et al*. 2003). The *INT-c* (*HvTB1*) gene in barley is orthologous to *ZmTB1* and *OsTB1* (Doebley *et al*. 1995; Ramsay *et al*. 2011). *TB1* in monocots and its homolog *BRANCHED1* (*BRC1*) in *Arabidopsis* and pea encode TB1/CYCLOIDEA/PCF (TCP), which is a transcription factor specifically expressed in inhibited axillary buds. OsTB1 acts as a negative regulator of lateral branching, presumably through expression in the axillary buds (Takeda *et al*. 2003). *OsTB1* might be involved in a network with two other genes, *OsD14* and *OsMADS57* (Guo *et al*. 2013). The function of OsD14 is reportedly repressed by OsMADS57 and, thus further, by interaction with OsTB1 (Guo *et al*. 2013).

OsMADS57 is a member of the MADS domain (yeast *MCM1*, plant *AGAMOUS* and *DEFICIENS*, and mammal serum response factor) gene family. It encodes a transcriptional regulator that is involved in diverse and important biological functions, including the initiation of flowering and the development of ovules, fruit, leaves, and roots (Shore *et al*. 1995). There are two distinct types of MADS-box genes in plants: type I and type II (MIKC^{c}-type). In addition, DWARF14 (D14), an α/β-fold hydrolase, has been identified as a strigolactone (SL) receptor in rice. It interacts with SCF^{MAX2} and negatively regulates tiller bud outgrowth (Liu *et al*. 2009). *HvD14* in barley encodes an α/β hydrolase and is involved in SL signalling; moreover, the *hvd14* mutant is insensitive to GR24, a synthetic SL analogue (Marzec *et al*. 2016). In the SL signalling pathway of rice, D14, D3 and D53 form a ubiquitin ligase complex, and D14-SCF^{D3}-dependent degradation of D53 is essential for SL signalling (Jiang *et al*. 2013; Zhou *et al*. 2013). Degradation of D53 in the presence of SL releases negative branching regulators (e.g. TB1/FC1/BRC1). Thus, TB1 and MADS57 are considered downstream components in the SL signalling pathway.

In the current study, considering the relationships among *OsD14*, *OsMADS57* and *OsTB1* in rice, we studied the potential involvement and functions of a *HvTB1*-mediated network in the growth and development of barley. Until now, we have screened mutant plants with modified tillering ability, and we cloned *HvTB1*, *HvMADS57* and *HvD14* and assessed the expression of the latter two in plants with a *tb1* mutant allele.

Materials and methods

Plant material

Twenty-two genotypes of *H. vulgare* L. subsp. *vulgare* and the mutants derived from them were obtained from the United States Department of Agriculture (table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). All genotypes were planted in glasshouses and fields at Sichuan Agricultural University, Wenjiang (103°41′E, 30°36′N), Sichuan, China. Each genotype in the glasshouse consisted of three replicates. Three plants, each in a different 2.0 L pot were used in each of the replicates. A randomized complete block design was used for each of the experiments. Inflorescence architecture (IA), flag leaf width (FLW), flag leaf length (FLL), plant height (PH), spike length (SL), tiller number (TN) and KNS were measured and used for analysis. According to the TN, we screened mutant plants with modified tillering ability compared to WT. *hvtb1* is a spontaneous mutant of the WT barley genotype CIho 14842.

Isolation of genomic DNA and RNA, and cDNA synthesis

Total genomic DNA (gDNA) was extracted from fresh leaf tissues of WT and mutant plants using a modified cetyltrimethylammonium bromide method (Allen *et al*. 2006). Leaf, root, stem, tiller-node, leaf-sheath, and spikelet samples were collected from WT and *tb1*-mutant plants at different growth stages (seedling stage, 1WAP, 2WAP, 3WAP, 4WAP, 5WAP, 6WAP and BS). Total RNA was extracted using a Baifeite RNA Extraction kit (ThermoFisher Scientific, Waltham, USA), according to the manufacturer's instructions. Total RNA was reverse-transcribed to cDNA (1 μg RNA per 20-μL reaction) using a PrimeScript RT Reagent kit with gDNA Eraser (Perfect Real Time) (RR047A; TaKaRa, Dalian, China).

Cloning of HvTB1, HvD14 and HvMADS57

Three rice genes (*OsTB1*, *OsD14* and *OsMADS57*) sequences were used to blast against the NCBI barley database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain homologous gene sequences in barley. Complete ORFs of *HvTB1*, *HvD14* and *HvMADS57* were PCR-amplified using gene-specific primers (table 2 in electronic supplementary material) in 50 μL reaction mixtures containing 4 μL template DNA or cDNA, 5 μL 10× Ex *Taq* Buffer, 4 μL $MgCl_2$, 6 μL dNTP, 0.5 μL TaKaRa Ex *Taq* (5 U μL^{-1}; RR001A, TaKaRa), 1 μL of each primer (5 mM), and 28.5 μL double distilled H_2O. Thermal cycles were as follows: initial

denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 1 min, and final extension at 72°C for 7 min. PCR products with the expected sizes were ligated into the pMD19-T vector (6013; TaKaRa). The ligation mixtures were transformed into competent *E. coli* DH5α cells (Tiangen, Beijing, China). Sequences were confirmed by DNA sequencing by Tsingke Biological Technology Company (Chengdu, China). Nucleotide sequences for each of the genes were determined from the sequencing results of five independent clones.

qPCR analysis

Primers for *HvTB1*, *HvD14*, *HvMADS57* and reference genes for qRT-PCR were designed using Primer Premier 5 (table 2 in electronic supplementary material). qRT-PCR experiments were performed using three biological and three technical replicates in 96-well plates using SYBR Premix Ex Taq II (Takara, RR820A), according to the manufacturer's instructions. The reaction mixtures (10 μL) contained 1 μL cDNA and 0.25 μL of each primer (5 mM). Thermal cycles were as follows: initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 10 s, 58°C for 30 s, and 72°C for 30 s. To verify the primer specificity for melt curve analysis, the PCR products were held at 65°C for 10 s and then heated up to 95°C at a rate of 0.1°C s^{-1}. *Actin* and *GAPDH* were used as endogenous controls for normalization (Solanki *et al*. 2016; Gines *et al*. 2018; Qiu *et al*. 2019). Relative expression levels were calculated as described by Pfaffl (2001). qRT-PCR experiments were performed using three biological and three technical replicates.

Statistical analysis

All the data are expressed as mean ± standard error. Comparison of phenotypic features between *tb1* mutant and the wild-type (WT) plant was conducted using a Student's *t*-test. Two-tailed *P* values < 0.05 were considered statistically significant. Student's *t*-test ($P < 0.05$) was also used to examine the differences in gene expression among the samples.

Results

Identification and phenotype of a barley hvtb1 mutant

To investigate the genetic control of TN in barley, mutant plants with modified tillering ability were selected. A barley gene sequence homologous to rice *OsTB1* was identified using BLAST. Variations of this sequence (NCBI accession number: JF904738.1) were examined in 18 WT genotypes as well as four mutant genotypes with modified tillering abilities, allowing the successful cloning of *HvTB1* (*INT-c*). The open reading frame (ORF) of *HvTB1* is 1074-bp long, and encodes a putative protein of 357 amino acids. Results from an earlier study showed that this gene controls lateral spikelet fertility (Ramsay *et al*. 2011) (table 1; figure 1 in electronic supplementary material). We found a spontaneous mutation in the WT genotype CIho 14842. Comparison of the WT and mutant sequences revealed a C→A transition at position 230, which caused a premature stop codon, resulting in a protein of 76 amino acids (figure 2a in electronic supplementary material).

Compared to WT plants, *tb1*-mutant plants showed substantially improved lateral spikelet fertility and produced nearly twice as many tillers (figure 1). In the elongation stage, the mean TN in *tb1*-mutant plants was 46.9% ($P < 0.0001$) higher than that in WT plants (12.3) (figure 1, a&b). Plant height, spike length, flag-leaf length, and flag-leaf width did not differ significantly between the two genotypes (table 1). In addition, *tb1*-mutant plants produced significantly more kernels than the WT plants due to the variation in inflorescence architecture (IA) ($P < 0.05$; table 1; figure 1, c&d).

Cloning and molecular characterization of HvD14 and HvMADS57

To determine the expression levels of *HvMADS57* and *HvD14* in plants with the *tb1* mutant allele, we cloned *HvD14* and *HvMADS57* based on sequences in the NCBI database (accession numbers KP069479 and AK363243.1, respectively) in WT. *HvD14* comprises two exons. The total length of this gene from the start to stop codon is 1055 bp, and encodes a putative protein of 303 amino acids (figure 2b in electronic supplementary material). The ORF of *HvMADS57* is 726 bp. It encodes a putative protein of 241 amino acids with an unknown function and belongs to the $MIKC^c$ group of MADS-box genes. A BLAST search against the NCBI database revealed that *HvMADS57* is an orthologue of *OsMADS57* in rice (accession number: AY177702.1), *ZmMADS57* in maize (accession number: KJ727680.1), and *WM32B* in common wheat (accession number: AM502905.1). The full-length protein encoded by *HvMADS57* shows 80.5% identity to that encoded by *OsMADS57* and 93.4% to that encoded by *WM32B* (figure 3 in electronic supplementary material).

Four single-nucleotide polymorphisms (SNP), including one C→G transversion and three A→G transitions (T→C, A→C, and A→G), were identified in *HvMADS57* in the *tb1* mutant when compared with the WT gene. Three of these were nonsynonymous mutations producing amino-acid substitutions, namely T→C at position 172 (serine → proline), A→C at position 541 (isoleucine → leucine), and A→G at position 571 (threonine → alanine). The *HvD14* sequence did not differ between the *tb1* mutant and the WT (figure 2, b&c in electronic supplementary material).

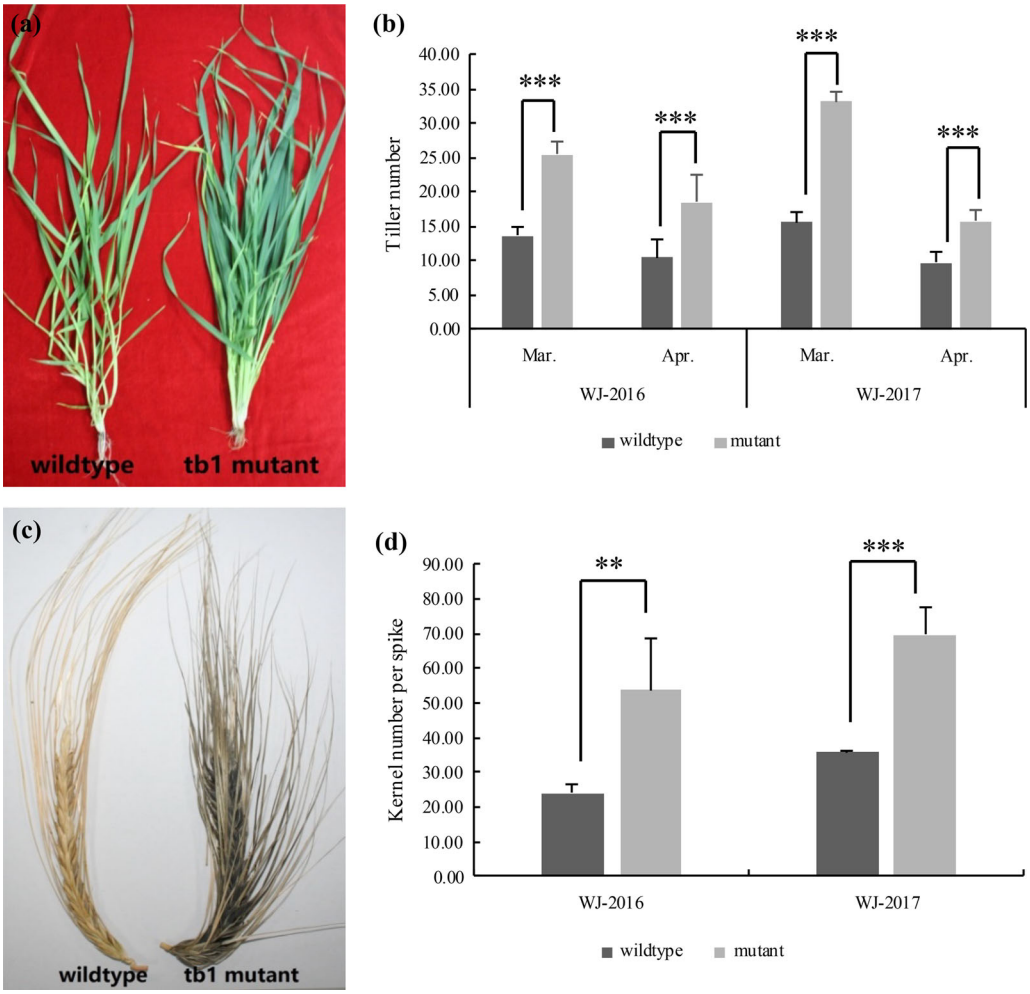

Figure 1. WT and *tb1*-mutant barley plants. (a) Morphological features and (c) inflorescence architecture of the WT and *tb1* mutant plants. Differences in (b) TN and (d) kernel number per spike between plants of the two genotypes. Data are the mean ± standard error ($n = 5$). $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t*-test).

Table 1. Phenotypic features of *tb1* mutant and the WT of cv. 'CIho 14842'.

	IA	Environment	PH (cm)	SL (cm)	FLL (cm)	FLW (cm)	KNS	TN of Mar.	TN of Apr.
WT	Two-rowed	WJ-2016	112.30±6.3	7.18±0.69	9.08±2.39	0.70±0.07	24.00±2.76	13.70±1.6	10.33±1.49
		WJ-2017	126.12±3.29	10.50±0.26	18.50±1.35	1.98±0.07	35.80±0.4	15.60±1.24	9.60±1.36
		Average	119.21	8.84	13.79	1.34	29.90	14.65	9.95
tb1 mutant	Deficient	WJ-2016	116.07±3.15	8.77±0.93	9.47±1.88	0.70±0.16	53.67±5.11	25.40±1.49	18.40±1.32
		WJ-2017	124.70±7.1	11.22±0.53	14.16±0.91	1.82±0.07	69.60±8.14	33.20±3.57	15.70±1.9
		Average	120.38	9.99	11.81	1.26	61.63	29.30	17.05
		P value	ns	ns	ns	ns	**	***	***

IA, inflorescence architecture; PH, plant height; SP, spike length; FLL, flag leaf length; FLW, flag leaf width; KNS, kernel number per spike; TN, tiller number; ns, not significant. *Significant differences between the WT and mutant as determined by the Student *t*-test. $^{**}P < 0.01$, and $^{***}P < 0.001$.

Tissue-specific expression of HvTB1, HvMADS57, and HvD14 in barley

Expression patterns of *HvTB1*, *HvMADS57* and *HvD14* in different tissues (including leaf, root, stem, tiller-node, leaf-sheath, and spikelet) and at different developmental stages (seedling stage, one week after planting (1WAP), 2WAP, 3WAP, 4WAP, 5WAP, 6WAP, and booting stage (BS)) were examined by quantitative reverse-transcription (qRT-)PCR in the cultivar CIho 14842. As it was impossible to collect leaf-

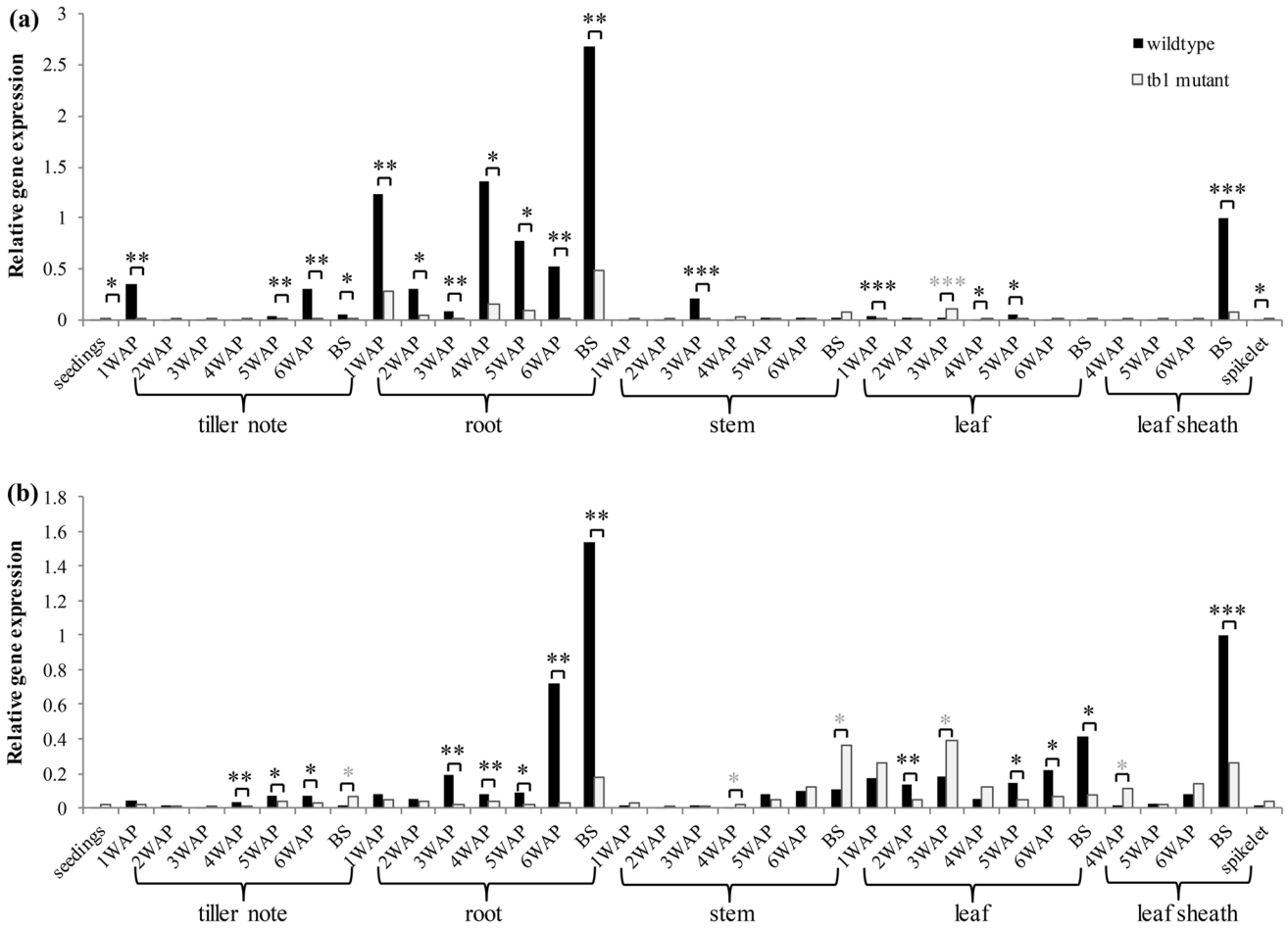

Figure 2. Relative mRNA expression levels of (a) *HvMADS57* and (b) *HvD14* in different tissues and stages of WT and *tb1*-mutant barley. WAP, week after planting; BS, boot stage. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Black asterisks represent significantly higher expression in the WT plants than in the *tb1* mutant plants. Grey asterisks represent significantly lower expression in the WT plants than in the *tb1* mutant plants.

sheath samples from 1WAP to 3WAP, only 34 of all possible tissue-stage combinations were assessed in this study. We found that all three genes were constitutively expressed in all analysed tissues and growth stages in WT plants, but the expression levels differed markedly from stage to stage and among tissues. In line with previously reported findings (Ramsay *et al*. 2011), *HvTB1* expression was generally low, except in the BS. *HvTB1* expression was significantly higher in the BS than in the 4WAP and 6WAP stages. *HvTB1* expression in the stem was significantly lower than that in the other tissues (figure 4 in electronic supplementary material). *HvMADS57* was highly expressed in roots, whereas its expression was weaker in stem and leaf samples ($P < 0.01$). *HvMADS57* expression was the highest in the BS, followed by that in the 1WAP stage (figure 5 in electronic supplementary material). *HvD14* transcripts were detected mainly in the root, leaf and leaf-sheath, and *HvD14* expression was significantly higher in the BS than in the 4WAP stage (figure 6 in electronic supplementary material). Clearly, all the three genes showed higher expression in the BS than in the other growth stages.

HvMADS57 and HvD14 show differential expression between the tb1-mutant and WT plants

To determine the effect of *HvTB1* on *HvMADS57* or *HvD14*, qRT-PCR was used to examine whether *HvMADS57* and *HvD14* are differentially expressed between *tb1*-mutant and WT plants. In *tb1*-mutant plants, *HvMADS57* expression was significantly reduced compared to that in WT plants in all tissues investigated (leaf, root, stem, tiller node, leaf-sheath and spikelet) at some growth stages ($P < 0.05$). *HvMADS57* expression levels in the *tb1* mutant plants were lower than those in the WT plants in most of the tissue stage combinations (figure 2a). *HvMADS57* expression in the root was significantly lower in the *tb1* mutant plant than in the WT plant at all growth stages examined ($P < 0.05$). *HvMADS57* expression levels in the *tb1* mutant were 21.6-fold and 15.9-fold lower in tiller nodes at the 1WAP and 6WAP stages, respectively, than those in the corresponding tissue-stage combinations in the WT ($P < 0.01$).

HvD14 expression in *tb1*-mutant plants was significantly decreased compared to that of WT plants ($P < 0.05$, figure 2b).

Among the 34 tissue samples, 14 showed significantly lower *HvD14* expression, and four displayed significantly higher expression in the *tb1* mutant than in the WT ($P < 0.05$, figure 2b). *HvD14* expression in the leaf-sheath of the *tb1* mutant at the BS was significantly downregulated compared to that in the corresponding tissue-stage combination in the WT. *HvD14* expression levels in the root in WT plants at the 6WAP and BS stages were 24.6-fold and 8.6-fold higher, respectively than those in the corresponding tissue–stage combinations in the *tb1* mutant. *HvD14* expression in tiller nodes of *tb1*-mutant plants at the 4WAP stage was 5.8-fold lower than that of the WT plants ($P < 0.01$). Thus, the expression of both *HvMADS57* and *HvD14* was lower in the *tb1* mutant than in the WT in most tissue-stage combinations.

Discussion

In the present study, we cloned the barley *HvTB1* gene. Based on the gene and protein sequences, barley genotypes could be divided into two groups differing at position 189. The *HvTB1* sequence in the first group, with 6-bp insertion at position 189 has been previously identified as an *INT-C.a* allele (Ramsay *et al*. 2011). We found a natural mutant of *HvTB1* among the 22 genotypes assessed in this study. A C→A mutation, which caused a premature stop codon was detected in the *tb1* mutant. Moreover, we cloned *HvMADS57* and found that its ORF is 726 bp and encodes 241 amino acids. In animals and fungi, the MADS-box gene family consists of two distinct gene types, namely SRF-like and MEF2-like, which can be further classified as many subfamilies (Alvarez-Buylla *et al*. 2000). In plants, the MIKC^c group of MADS-box genes encodes proteins that share a MIKC structure, with the highly conserved DNA-binding MADS domain at the amino terminus, followed by a weakly conserved I domain, a moderately conserved K domain and a poorly conserved carboxyl-terminal (C) region (Alvarez-Buylla *et al*. 2000). The K domain has shown to be important for protein–protein interactions and probably has a coiled-coil structure, whereas the C region might function as a *trans*-activation domain (Alvarez-Buylla *et al*. 2000). *HvMADS57* was found to encode a protein with a typical MIKC structure in this study, suggesting that it is a MIKC^c-type MADS-box gene.

OsTB1 and *OsMADS57* function as a negative and positive regulator, respectively, of lateral branching in rice (Takeda *et al*. 2003). It has been shown that *HvTB1* modifies *Vrs1*, which controls lateral spikelet fertility in barley (Ramsay *et al*. 2011). In plants, MADS-box genes are involved in flowering, floral organ identity and development, embryogenesis and meristem differentiation. A previous study revealed that the ABCDE model mainly controls flower development, and most ABCDE model genes are MIKC^c-type MADS-box genes (Melzer *et al*. 2010). In barley, the MIKC^c-type MADS-box short vegetative phase-like genes are expressed in vegetative tissues, but are repressed during floral development (Trevaskis *et al*. 2006). In maize, MADS and TCP (*TB1*) cofunction to determine meristem identity and control organ morphogenesis (Clark *et al*. 2004). In the current study, *tb1*-mutant plants had more lateral spikelets than WT plants, which might be related to the significantly lower expression of *HvMADS57*. Thus,

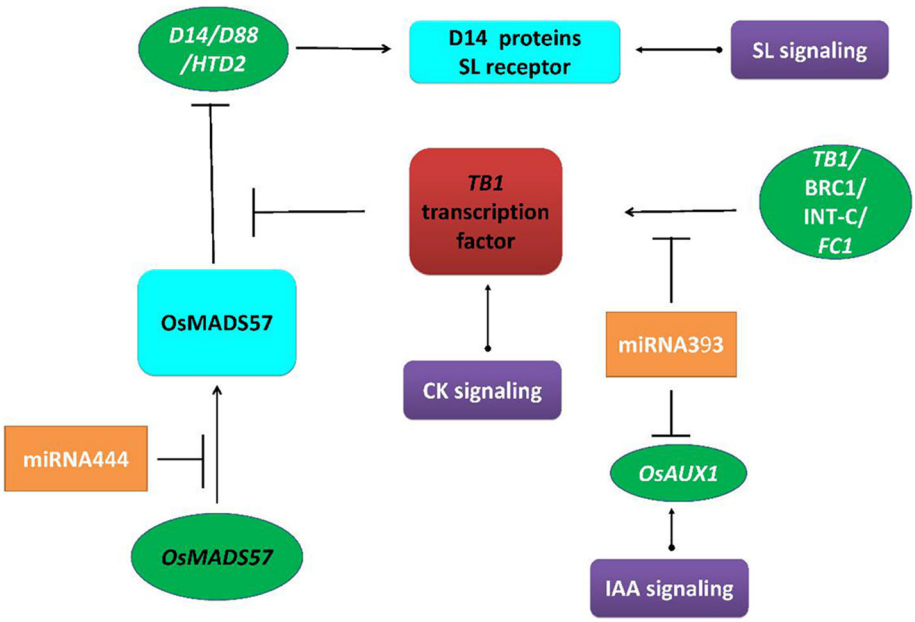

Figure 3. Gene model of *TB1*-mediated network for controlling tillering and inflorescence architecture. Blue boxes, proteins; green boxes, genes; orange boxes, microRNA; purple box, hormone signalling; red box, transcription factor, *TB1*.

similar to *MADS57* in rice and maize (Pelucchi *et al*. 2002; Clark *et al*. 2004), *HvMADS57* might cofunction with *HvTB1* to regulate spikelet fertility. *HvD14* and *HvTB1* were expressed at lower levels in the *tb1* mutant than in the WT. The products of these two genes are known to function as negative regulators of shoot branching (Takeda *et al*. 2003; Liu *et al*. 2009). Thus, the expression levels of *HvD14* and *HvTB1* in the *tb1* mutant are consistent with its increased TN phenotype (table 1).

As a negative regulator of lateral branching, *TB1* was mainly expressed in inhibited axillary buds. Xia *et al*. (2012) found that *OsAUX1* and *OsTB1* expression was downregulated upon overexpression of *OsmiR393*, which affected auxin transport and, consequently controlled tillering. In addition, *OsD14* expression in rice was reported to be directly repressed by *OsMADS57*, which was negatively regulated by *miR444a*, and the negative regulation by *OsMADS57* was further reduced by interactions between *OsD14* and *OsTB1* (Guo *et al*. 2013) (figure 3). In the current study, we found that the expression levels of both *HvMADS57* and *HvD14* were significantly decreased in the *tb1* mutant, suggesting that *HvTB1* might positively regulate the expression of *HvMADS57* and *HvD14*. Previous studies revealed that genes with similar expression profiles were more likely to encode interacting proteins (Ge *et al*. 2001), and that there was an overlap in gene expression patterns among nearly all interacting proteins in *Arabidopsis* (De Folter *et al*. 2005). Thus, the decreased expression of *HvMADS57* in the *tb1* mutant might be due to its interaction with *HvTB1*, as both genes exhibited a similar expression pattern. In addition, the effect of *HvTB1* deficiency in the *tb1* mutant might partially counteract the decrease in *HvMADS57* expression, leading to a decline in *HvD14* expression. In short, *HvMADS57* and *HvTB1* in barley have a similar expression pattern and cofunction to repress *HvD14*, consistently with the roles of their orthologs in rice.

Summary and outlook

We found a natural *tb1* mutant with increased tillering ability. Based on the sequence of a rice gene (*OsTB1*) controlling TNs, we cloned the barley *HvTB1* gene and found that it had a premature stop codon in the *tb1* mutant. As this gene is known to interact with both *OsMADS57* and *OsD14* in rice, we cloned their orthologs in barley (*HvMADS57* and *HvD14*). The expression levels of both genes were significantly lower in the *tb1* mutant than in the WT. These results suggested that *HvTB1* may affect *HvMADS57* and *HvD14* and that *HvMADS57* might interact with *HvTB1* to suppress *HvD14* transcription, similar to the roles of the orthologues of these genes in rice. Our findings provide novel insights that could serve as a foundation for future research on *TB1*-mediated networks and their associated regulatory mechanisms underlying barley tiller growth and development.

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References

Agusti J. and Greb T. 2013 Going with the wind–adaptive dynamics of plant secondary meristems. *Mech. Dev.* **130**, 34–44.

Alvarez-Buylla E. R., Pelaz S., Liljegren S. J., Gold S. E., Burgeff C., Ditta G. S. *et al.* 2000 An ancestral MADS-box gene duplication occurred before the divergence of plants and animals. *Proc. Natl. Acad. Sci. USA* **97**, 5328–5333.

Allen G. C., Flores-Vergara M. A., Krasynanski S., Kumar S. and Thompson W. F. 2006 A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nat. Protoc.* **1**, 2320.

Clark R. M., Linton E., Messing J. and Doebley J. F. 2004 Pattern of diversity in the genomic region near the maize domestication gene tb1. *Proc. Natl. Acad. Sci. USA* **101**, 700–707.

De Folter S., Immink R. G., Kieffer M., Pařenicová L., Henz S. R., Weigel D. *et al*. 2005 Comprehensive interaction map of the Arabidopsis MADS box transcription factors. *Plant Cell* **17**, 1424–1433.

Doebley J., Stec A. and Gustus C. 1995 teosinte branched1 and the origin of maize: evidence for epistasis and the evolution of dominance. *Genetics* **141**, 333–346.

Doebley J., Stec A. and Hubbard L. 1997 The evolution of apical dominance in maize. *Nature* **386**, 485.

Ge H., Liu Z., Church G. M. and Vidal M. 2001 Correlation between transcriptome and interactome mapping data from Saccharomyces cerevisiae. *Nat. Genet.* **29**, 482.

Gines M., Baldwin T., Rashid A., Bregitzer P., Maughan P. J., Jellen E. N. *et al*. 2018 Selection of expression reference genes with demonstrated stability in barley among a diverse set of tissues and cultivars. *Crop Sci.* **58**, 332–341.

Greb T., Clarenz O., Schäfer E., Müller D., Herrero R., Schmitz G. *et al*. 2003 Molecular analysis of the LATERAL SUPPRESSOR gene in Arabidopsis reveals a conserved control mechanism for axillary meristem formation. *Genes Dev.* **17**, 1175–1187.

Groot S. P., Keizer L. C., de Ruiter W. and Dons J. J. 1994 Seed and fruit set of the lateral suppressor mutant of tomato. *Sci. Hortic.* **59**, 157–162.

Guo S., Xu Y., Liu H., Mao Z., Zhang C., Ma Y. *et al*. 2013 The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. *Nat. Commun.* **4**, 1566.

Hubbard L., McSteen P., Doebley J. and Hake S. 2002 Expression patterns and mutant phenotype of teosinte branched1 correlate with growth suppression in maize and teosinte. *Genetics* **162**, 1927–1935.

Jiang L., Liu X., Xiong G., Liu H., Chen F., Wang L. *et al.* 2013 DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature* **504**, 401.

Kebrom T. H., Spielmeyer W. and Finnegan E. J. 2013 Grasses provide new insights into regulation of shoot branching. *Trends Plant Sci.* **18**, 41–48.

Komatsuda T., Pourkheirandish M., He C., Azhaguvel P., Kanamori H., Perovic D. *et al*. 2007 Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proc. Natl. Acad. Sci. USA* **104**, 1424–1429.

Li X., Qian Q., Fu Z., Wang Y., Xiong G., Zeng D. *et al.* 2003 Control of tillering in rice. *Nature* **422**, 618.

Liu W., Wu C., Fu Y., Hu G., Si H., Zhu L. *et al.* 2009 Identification and characterization of HTD2: a novel gene negatively regulating tiller bud outgrowth in rice. *Planta* **230**, 649–658.

Lundqvist U. 1997 New and revised descriptions of barley genes. *Barley Genet. Newslett.* **26**, 22–516.

Madić M., Paunović A., Đurović D., Kraljević-Balalić M. and Knežević D. 2005 The analysis of gene effect in the inheritance of kernel number per spike in barley hybrid. *Genetika* **37**, 261–269.

Marzec M., Gruszka D., Tylec P. and Szarejko I. 2016 Identification and functional analysis of the HvD14 gene involved in strigolactone signaling in Hordeum vulgare. *Physiol. Plant*. **158**, 341–355.

Melzer R., Wang Y. Q. and Theißen G. 2010 The naked and the dead: the ABCs of gymnosperm reproduction and the origin of the angiosperm flower. *Semin. Cell Dev. Biol.* **21**, 118–128.

Mohapatra P. K., Panda B. B. and Kariali E. 2011 Plasticity of tiller dynamics in wild rice Oryza rufipogon Griff.: a strategy for resilience in suboptimal environments. *Int. J. Agron*. **2011**, article ID 543237.

Pelucchi N., Fornara F., Favalli C., Masiero S., Lago C., Pè E. M. *et al.* 2002 Comparative analysis of rice MADS-box genes expressed during flower development. *Sex. Plant Reprod*. **15**, 113–122.

Pfaffl M. W. 2001 A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Res*. **29**, e45–e45.

Qiu C. W., Zhao J., Chen Q. and Wu F. 2019 Genome-wide characterization of drought stress responsive long non-coding RNAs in Tibetan wild barley. *Enviro. Exp. Bot.* **164**, 124–134.

Ramsay L., Comadran J., Druka A., Marshall D. F., Thomas W. T., Macaulay M. *et al.* 2011 INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. *Nat. Genet*. **43**, 169.

Schmitz G. and Theres K. Shoot and inflorescence branching 2005 *Curr. Opin. Plant Biol*. **8**, 506–511.

Shaaf S., Bretani G., Biswas A., Fontana I. M. and Rossini L. 2019 Genetics of barley tiller and leaf development. *J. Integr. Plant Biol*. **61**, 226–256.

Shore P. and Sharrocks A. D. 1995 The MADS-box family of transcription factors. *Eur. J. Biochem*. **229**, 1–13.

Solanki S., Ameen G., Richards J. S. and Brueggeman R. 2016 Modulation of integrated decoy R-genes/transcription factor assembly elicits wheat stem rust resistance responses in barley: rpg4/Rpg5-mediated Ug99 resistance. In *International Barley Genetics Symposium.* Minneapolis, USA.

Steeves T. A. and Sussex I. M. 1989 *Patterns in plant development*. Cambridge University Press, Cambridge.

Takeda T., Suwa Y., Suzuki M., Kitano H., Ueguchi-Tanaka M., Ashikari M. *et al*. 2003 The OsTB1 gene negatively regulates lateral branching in rice. *Plant J*. **33**, 513–520.

Trevaskis B., Hemming M. N., Peacock W. J. and Dennis E. S. 2006 HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status. *Plant Physiol*. **140**, 1397–1405.

von Bothmer R. and Jacobsen N. 1985 Origin, taxonomy, and related species. In *Barley* (ed. D. Rasmusson), pp. 19–56. ASA Monograph.

Xia K., Wang R., Ou X., Fang Z., Tian C., Duan J. *et al.* 2012 OsTIR1 and OsAFB2 downregulation via OsmiR393 overexpression leads to more tillers, early flowering and less tolerance to salt and drought in rice. *PLoS One* **7**, e30039.

Zhou F., Lin Q., Zhu L., Ren Y., Zhou K., Shabek N. *et al.* 2013 D14–SCF D3-dependent degradation of D53 regulates strigolactone signalling. *Nature* **504**, 406.

Zhou H., Liu S., Liu Y., Liu Y., You J., Deng M. *et al.* 2016 Mapping and validation of major quantitative trait loci for kernel length in wild barley (*Hordeum vulgare* ssp. spontaneum). *BMC Genet*. **17**, 130.

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