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NtBRC1 suppresses axillary branching in tobacco after decapitation

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ABSTRACT. Axillary branching is controlled by a very complex mechanism involving various endogenous and environmental factors. Previous studies have shown that *Tb1/BRC1* is the point of integration in the network of molecular mechanisms regulating axillary branching in plants. In this study, we cloned the *Tb1/BRC1* ortholog, *NtBRC1,* from *Nicotiana tabacum* and functionally analyzed its role in the control of axillary branching in tobacco. Overexpression of *NtBRC1* resulted in significant retardation of axillary branching, and downregulation of this gene resulted in significant acceleration of axillary branching after decapitation. This indicates a negative role for this gene in the regulation of axillary branching. In-line with previous reports, *NtBRC1* was found to be expressed predominantly in axillary buds. Additionally, as expected, expression was decreased 8 h following decapitation, which further confirms its role in the suppression of axillary

branching. Furthermore, the expression of *NtBRC1* was significantly downregulated by cytokinin, but was not affected by GR24, a synthetic strigolactone. Based on the data collected in the present study, we demonstrate that *NtBRC1* negatively regulates axillary branching in tobacco after decapitation and functions downstream of the cytokinin signaling pathway inside axillary buds.

Key words: Axillary branching; Decapitation; *NtBRC1*; Tobacco; Transgenic analysis

INTRODUCTION

Tb1/BRC1 belongs to the TCP family of transcription factors (TFs), which is named after the first characterized members (TB1, CYC, and PCFs) and encodes a basic helix-loop-helix-type TF (Cubas et al., 1999). The TCP family can be divided into two clades, classes I and II (Steiner et al., 2012); Tb1/BRC1 belongs to the TB1/CYC subgroup of class II (Aguilar-Martínez et al., 2007). Mutation of the *BRC1* gene in *Arabidopsis* resulted in a significantly higher number of rosette branches than observed in the wild-type. Mutation of the *Tb1* gene resulted in a profusion of tillers in maize and, similarly, mutation of the *BRC1* gene resulted in an increased shoot-branching phenotype in pea (Doebley et al., 1997; Aguilar-Martínez et al., 2007; Braun et al., 2012). Overexpression of *Tb1* reduced tiller and spike number in wheat (Lewis et al., 2008). *Tb1*/*BRC1* is expressed primarily in the axillary bud, and its expression is negatively correlated with bud activity, which is consistent with its function in the suppression of axillary bud outgrowth (Hubbard et al., 2002; Aguilar-Martínez et al., 2007). These observations strongly suggest that the *Tb1*/*BRC1* gene plays a critical role in controlling the outgrowth of the axillary bud, and represents a candidate integrator within the axillary bud, which responds to endogenous and environmental stimuli in the control of axillary branching.

Many endogenous and environmental factors are involved in the regulation of axillary branching in plants, including phytohormones, such as auxin, cytokinin (CK), and strigolactone (SL), and environmental factors, such as nutrients and light (Rameau et al., 2015). Although the cross-talk between these factors in the regulation of axillary branching has not been fully elucidated, numerous recent studies have shown that a common downstream molecular integrator, *Tb1*/*BRC1*, exists in this complicated network, and mediates the regulating functions of those factors. Auxin was the first phytohormone to be studied in the apical dominance, a term used to describe the phenomenon whereby the growing shoot apex inhibits the outgrowth of the axillary bud located below (Snow, 1937). Auxin, which is mainly synthesized in the intact shoot apex, is thought to be transported basipetally to suppress axillary bud outgrowth. However, exogenous application of auxin to the decapitated shoot does not seem to enter the axillary bud (Hall and Hillman, 1975; Morris, 1977; Cline, 1996), and direct application of auxin to axillary buds does not inhibit their outgrowth (Shimizu-Sato et al., 2009) suggesting that a secondary messenger mediate this process. CK has been proposed as one such candidate secondary messenger. CK exerts the opposite effect to auxin in apical dominance. Application of CK to axillary buds is sufficient to trigger axillary branching even in the presence of an intact growing shoot apex (Sachs and Thimann, 1967; Pillay and Railton, 1983; Ohkawa, 1984). Another putative second messenger acting downstream of auxin is SL, which was characterized in a series of studies using enhanced shoot branching mutants (Beveridge et al.,

1994; Beveridge, 2000; Gomez-Roldan et al., 2008). SL is primarily produced in the plant root and is translocated acropetally to suppress branching (Dun et al., 2012). The interaction between these three classes of phytohormones has been summarized in a model showing that auxin produced in the shoot apex moves down the plant to repress CK biosynthesis and induce SL biosynthesis, which subsequently suppresses the outgrowth of axillary buds (Domagalska and Leyser, 2011).

Previously, studies have shown that *BRC1* integrates endogenous and environmental signals. In pea, the *PsBRC1* gene acts downstream of the CK and SL signaling pathway to suppress axillary bud outgrowth as evidenced by its upregulation in response to GR24 (a synthetic SL analog) and its downregulation in response to 6- benzylaminopurine (Braun et al., 2012). Furthermore, in rice, *FC1*, an ortholog of *BRC1*, has been found to work downstream of CK and SL to inhibit axillary bud outgrowth, since treatment with 6-benzylaminopurine suppressed *FC1* expression and GR24 treatment did not repress tiller growth in the *fc1* mutant (Minakuchi et al., 2010). In accordance with these observations, the expression of *AtBRC1* has been shown to be reduced in *max* mutants in *Arabidopsis* (Aguilar-Martínez et al., 2007). More recently, the phytohormone gibberellin (GA) was reported to be a positive regulator in the control of axillary branching, and expression of *JcBRC1* declined within 24 h of treatment with GA or CK (Ni et al., 2015). Besides the phytohormones, sugar has been proposed as the initial regulator of axillary bud outgrowth following decapitation, and exogenous sucrose downregulates the expression of *PsBRC1* in pea (Mason et al., 2014). In addition, environmental factors, such as planting density and decapitation, were also found to regulate the expression of *AtBRC1* (Aguilar-Martínez et al., 2007).

In this study, we aimed to characterize an ortholog of *BRC1* in tobacco. We used the coding sequence (CDS) of *SlBRC1b* from tomato to BLAST against the Sol genomics database, and cloned its ortholog from tobacco, designated as *NtBRC1*. Phytohormone treatment and transgenic analysis suggested that this gene is involved in the regulation of axillary branching and functions downstream of the CK signaling pathway.

MATERIAL AND METHODS

Plant material and treatments

Wild-type and transgenic tobacco plants (*Nicotiana tabacum* 'Yunyan 87') were grown in a greenhouse for about 2 months. Samples of the shoot apex, root, leaf, stem, and axillary buds at nodes 1, 2, 3, 5, and 8 (the axillary bud at node 1 is located in the axil of the topmost fully expanded leaf and node 8 is mostly near the base) were collected from wild-type tobacco plants before decapitation and the axillary buds at node 1 were collected 0, 0.5, 1, 3, 5, 8, 24, 48, 72, and 96 h after decapitation for quantification of the gene expression. Three transgenic plants from each line were selected and the axillary bud in node 1 was collected for the quantification of gene expression before decapitation. The remaining transgenic plants from each line were decapitated and the lengths of branching shoots at node 1 were measured 16-days post decapitation. For SL and CK treatments, 10 μL 5 μM GR24, or 5 μM 6-benzylaminopurine was applied directly to the axillary bud at node 4 and the axillary buds were collected for quantification of gene expression after 3 h of treatment.

NtBRC1 cloning from tobacco

A BLAST search was performed against the genome database of *N. tabacum* (https://solgenomics.net/) using the cDNA sequence of *SlBRC1b* (GenBank accession No. HM597230). To obtain the full length cDNA, total RNA was extracted from axillary buds using the SuperPure Plantpoly RNA Kit (GeneAnswer, China) and cDNA was synthesized using SuperScriptTM Reverse Transcriptase (Invitrogen, USA) with an oligo (dT) primer. The primers used in the PCR were as follows: ATGTATCCGCCAAGCAACAG (forward primer), GCTATTGAAATCCTAAAAAAT (reverse primer). The PCR fragment was cloned into pCR®-BluntII-TOPO® (Invitrogen) for sequencing.

Generation of transgenic tobacco plants

Gateway technology was used to generate gene constructs using pK2G7.0 and PK7GW1WG2 as backbones for *NtBRC1* overexpression and knockdown (RNAi) respectively. The CDS of *NtBRC1* was first obtained by PCR from the axillary bud cDNA. The primers used to generate gene constructs were as follows: GGATCCATGTATCCGCCAAGCAACAG (forward primer for overexpression construct), CTCGAGCTATTGAAATCCTAAAAAAT (reverse primer for overexpression construct), GGATCCCAGGAGCTGTTACAAACAGTC (forward primer for RNAi construct), and CTCGAGAAAGTCTCATCCTTCGGTCTC (reverse primer for RNAi construct). The full-length CDS and partial fragment for RNAi constructs were first cloned in pCR®-BluntII-TOPO®, then cleaved and inserted into pENTR 2B, which was subsequently used to generate final gene constructs following the kit instructions (Gateway LR clonase™ II enzyme mix, Invitrogen, USA).

Transgenic plants were produced from the cultivar Yunyan 87 using standard *Agrobacterium tumefaciens*-based leaf disc transformation (Horsch et al., 1985). Initial primary transformants (T_0) were grown in the greenhouse. Transgenic plants were self-pollinated to set seeds of T_1 lines, from which the seedlings were screened on the growth medium with 100 mg/L kanamycin. Healthy seedlings were selected and grown in the greenhouse.

Real-time PCR for gene expression quantification

Total RNA was extracted as described above. qRT-PCR was performed using LightCycler 480 SYBR Green I Master (Rox) (Roche, Germany) on a LightCycler 4800 II (Roche, Germany) according to the manufacturer instruction. The *N. tabacum* actin gene was used as a control for normalization. Three technical replicates were performed using the following PCR program: 95°C for 5 min; 45 cycles of 95°C for 20 s, followed by 58°C for 15 s and 72°C for 15 s using the primer: CTTTCCCTTGATGCAGCTCG (forward primer), TGCCTCCTCCTTTATTGCCT (reverse primer). Axillary bud samples from wild-type or transgenic plants were analyzed with three or four biological replicates and gene expression was normalized against the reference gene: *actin*.

RESULTS

Identification of the tobacco *NtBRC1* gene

We searched for a *BRC1* ortholog in the genome database of *N. tabacum*

(https://solgenomics.net/) using the cDNA sequence of *SlBRC1b* and identified a cDNA sequence that was most similar to *SlBRC1b*. After this sequence was analyzed, we designed primers to clone the CDS of this gene from tobacco. A gene with a CDS of 984 nucleotides was cloned, which encoded a TCP TF of 328 amino acids. BLAST analysis of this protein against the NCBI database showed that it shares the highest sequence similarity (48% identical) with SlBRC1b from tomato. Thus, we designated it *NtBRC1*. To infer the evolutionary relationship between NtBRC1 and other TCP proteins in the CYC/TB1 subgroup, a phylogenetic tree was constructed using the maximum likelihood method in MEGA5.2. This shows that the NtBRC1 is most closely related to SlBRC1b (Figure 1) indicating that *NtBRC1* is an ortholog of *BRC1* in tobacco.

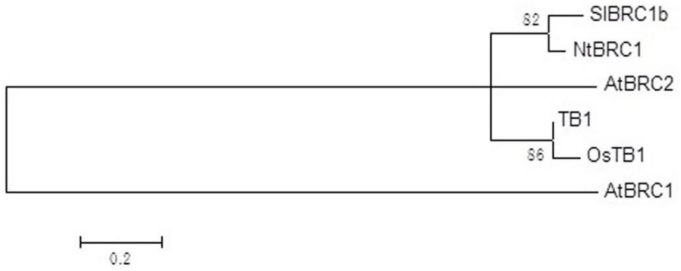

Figure 1. Phylogenetic tree of NtBRC1 and other TCP proteins belonging to the TB1/CYC subgroup of class II. AtBRC1 (CAL64010) and AtBRC2 (CAL64011) are from *Arabidopsis*, TB1 (Q93WI2) is from maize, OsTB1 (BAC54954) is from rice, and SlBRC1b (NP_001234572) is from tomato. The accession numbers in the parentheses are from the NCBI database.

Transgenic analysis of the role of *NtBRC1* in axillary branching

To explore the potential function of *NtBRC1* in the regulation of axillary branching, we generated eight T_1 lines transformed with an overexpression construct and seven T_1 lines transformed with an RNAi construct. When these transgenic plants had been grown for about 2 months in the greenhouse, we selected three plants from each line to investigate the levels of *NtBRC1* expression in node 1 axillary buds. Based on the results of qRT-PCR quantification, we selected three overexpression lines with significantly increased expression (about 2-7 fold) and three downregulated lines with significantly decreased expression (about 30-40% versus the vector control) (Figure 2) for further study. No obvious phenotypic differences were observed in those transgenic lines compared to the vector control before decapitation. In particular, no visible growth of the axillary buds was observed in the non-decapitated RNAi plants as we predicted. However, after decapitation, all the overexpression lines showed significantly repressed axillary bud outgrowth, and all the RNAi lines displayed significantly increased axillary bud outgrowth at node 1 (Figure 3) indicating that the *NtBRC1* gene negatively regulates axillary branching following decapitation in tobacco.

NtBRC1 is predominantly expressed in the axillary bud

Since *BRC1* has been reported to be almost exclusively expressed in the axillary bud (Martín-Trillo et al., 2011), we examined the transcript level of *NtBRC1* in different organs of tobacco. Figure 4 shows that *NtBRC1* is predominantly expressed in the axillary buds of intact tobacco plants, with similar levels of expression observed from the top to the basal axillary

buds. In other organs, such as the root, stem, and leaf, expression was barely detected. This expression pattern supports the role of *NtBRC1* in the control of axillary branching. Meanwhile, we observed weak expression in the main shoot apex (about 16-fold lower than in the axillary buds) suggesting that it may also function in the development of the shoot apical meristem.

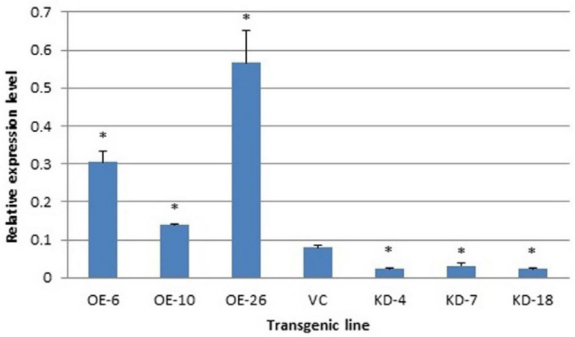

Figure 2. Expression of *NtBRC1* in transgenic lines. OE: overexpression line, KD: RNAi line, and VC: vector control. The values are reported as means ± SE (N = 3) and the asterisk indicates a significant difference compared to the VC by the Student *t*-test (P < 0.05).

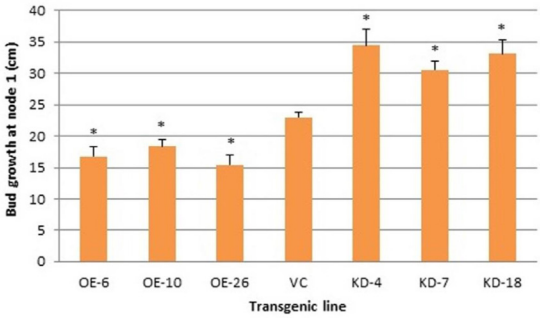

Figure 3. Length of the axillary branch at node 1 of transgenic plants 16 days after decapitation. OE: overexpression line, KD: RNAi line, and VC: vector control. The values are reported as means ± SE (N = 7-15) and the asterisk indicates a significant difference compared to the VC by the Student *t*-test (P < 0.05).

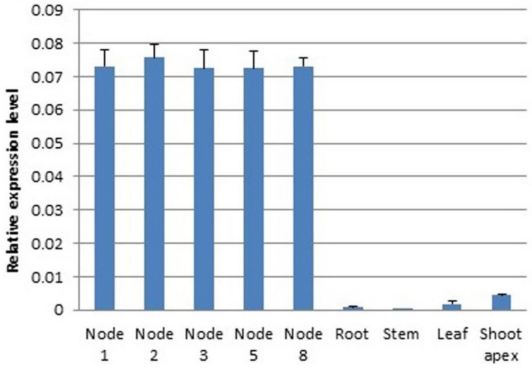

Figure 4. Expression pattern of *NtBRC1* in different organs of tobacco. The values are reported as means ± SE (N = 4).

Response of *NtBRC1* expression to decapitation

Since decapitation gives rise to the outgrowth of axillary buds in tobacco, we continued to examine the effect of decapitation on *NtBRC1* expression in the axillary buds over a 96-h time course. We selected the axillary bud at node 1 for this experiment, because at this position, it is the most responsive to decapitation as it grows more vigorously than other axillary buds situated below (data not shown). The results show that the expression of *NtBRC1* began to decrease at 8 h after decapitation (about 42% wild-type levels) and further decreased to about 30% of wild-type levels at 48 h. Subsequently, the expression of *NtBRC1* was maintained at this low level over the 96-h study period (Figure 5).

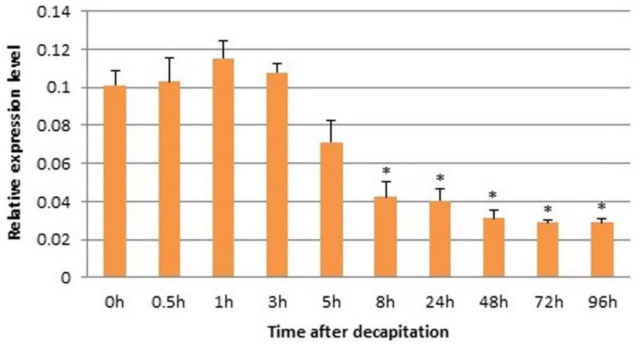

Figure 5. Response of *NtBRC1* expression to decapitation. The values are reported as means ± SE (N = 3) and the asterisk indicates a significant difference compared to the control (0 h) by the Student *t*-test ($P < 0.05$).

NtBRC1 is downregulated by CK

To determine whether *NtBRC1* is regulated by SL and CK, we examined the expression of this gene 3 h after treatment with GR24 (5 μM) or 6-benzylaminopurine (5 μM). As shown in Figure 6, *NtBRC1* was not significantly affected by GR24, but was significantly downregulated by 6-benzylaminopurine, with an approximate 2-fold reduction in transcript levels.

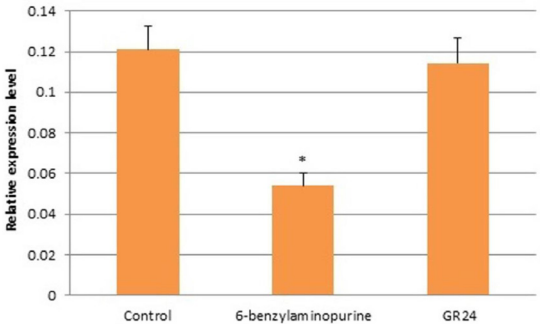

Figure 6. Expression of *NtBRC1* in response to 6-benzylaminopurine or GR24. The values are reported as means ± SE (N = 3) and the asterisk indicates a significant difference compared to the control by the Student *t*-test ($P < 0.05$).

DISCUSSION

Tobacco is one of the most widely cultivated non-food crops in China. Decapitation, which is a common agricultural practice in tobacco production, releases axillary buds from the apical dominance and results in axillary branching, which has to be removed by the tobacco farmers. Breeding for varieties with less or inhibited axillary branching has attracted the research interests of tobacco breeders, but little progress has been made in recent years.

It is well known that phytohormones play critical roles in the control of axillary bud outgrowth, especially auxin, CK, and SL, and a common molecular integrator, *BRC1*, has been suggested to mediate the functions of these phytohormones (Rameau et al., 2015). In this study, we cloned an ortholog of *Tb1/BRC1* in tobacco, designated as *NtBRC1*. NtBRC1 shares high amino acid sequence similarity (48% identical) with SlBRC1b from tomato. The tissue-specific expression pattern of *NtBRC1* reveals that its expression primarily occurs in the axillary buds (Figure 4), which is consistent with the expression pattern of its orthologs in other plant species (Aguilar-Martínez et al., 2007; Martín-Trillo et al., 2011) and its putative function as a regulator of axillary bud outgrowth. However, unlike *SlBRC1b*, which was reported to be expressed at variable levels in axillary buds at different node positions (the highest in the basal axillary buds far from the apex, and the lowest in the axillary buds closest to the apex) (Martín-Trillo et al., 2011), *NtBRC1* expression appeared to be independent of the stem position of the axillary buds based on the observation that expression in axillary buds at different nodes of tobacco plant remains relatively constant (Figure 4). This discrepancy may reflect an evolutionary divergence in the functional mechanism between these two orthologs, even though tomato and tobacco are very closely related species in the same family.

Because decapitation causes axillary bud outgrowth at node 1 in tobacco plants, we hypothesized that the expression of *NtBRC1* in this bud would be down regulated by decapitation. Consistent with this hypothesis, the expression of *NtBRC1* was found to decrease in response to decapitation with transcription levels declining significantly at 8 h after decapitation. Even after 96 h of treatment, the transcript level remained low and did not return to the control level (Figure 5). Since CK was found to negatively regulate the expression of *NtBRC1* (Figure 6), the downregulation of *NtBRC1* by decapitation may be mediated by the increase of CK in the buds (Turnbull et al., 1997). In addition, the observation that SL did not significantly affect the expression of *NtBRC1* 3 h after treatment may suggest that either *NtBRC1* functions independently of SL or that SL does not affect the function of *NtBRC1* via transcriptional control.

Even though these results strongly suggest that *NtBRC1* could function similarly to its orthologs in other plant species, transgenic functional analysis of this gene is still necessary to validate its role in the control of axillary branching in tobacco. For the T_1 transgenic lines, we anticipated some phenotype changes, at least in the RNAi lines with significantly repressed expression of *NtBRC1*. Unexpectedly, the transgenic plants showed no visible difference from the vector control plants before decapitation, in particular regarding the outgrowth of axillary buds in the RNAi lines. However, following decapitation, we observed a difference in axillary bud outgrowth in those transgenic lines compared to the vector control. Overexpression of *NtBRC1* significantly inhibited the axillary bud outgrowth and knocking-down the expression of *NtBRC1* promoted axillary bud outgrowth (Figure 3). This result confirmed that *NtBRC1* functions as a suppressor of tobacco axillary branching. The RNAi lines may not have exhibited increased axillary branching prior to decapitation, because 1) the expression of *NtBRC1* in RNAi plants was reduced to about one-third but was not completely inhibited, and

therefore, could function to repress axillary branching in non-decapitated tobacco plant; or 2) the common tobacco we used in this study was tetraploid, and should have homologous genes for *NtBRC1*, which may exhibit functional redundancy.

In summary, we cloned a *TB1/BRC1* ortholog, *NtBRC*1, from tobacco and examined the effects of its overexpression and knockdown. The data collected in this study clearly demonstrate that *NtBRC1* suppresses the outgrowth of axillary buds in tobacco plants following decapitation. In the future, *NtBRC1* may sever as a good candidate gene for use in the development of tobacco varieties with arrested axillary branching.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Aguilar-Martínez JA, Poza-Carrión C and Cubas P (2007). *Arabidopsis BRANCHED1* acts as an integrator of branching signals within axillary buds. *Plant Cell* 19: 458-472. http://dx.doi.org/10.1105/tpc.106.048934

Beveridge CA (2000). Long-distance signaling and a mutational analysis of branching in pea. *Plant Growth Regul.* 32: 193-203. http://dx.doi.org/10.1023/A:1010718020095

Beveridge CA, Ross JJ and Murfet IC (1994). Branching mutant *rms-2* in *Pisum sativum. Plant Physiol.* 104: 953-959. http://dx.doi.org/10.1104/pp.104.3.953

Braun N, de Saint Germain A, Pillot JP, Boutet-Mercey S, et al. (2012). The pea TCP transcription factor PsBRC1 acts downstream of Strigolactones to control shoot branching. *Plant Physiol.* 158: 225-238. http://dx.doi.org/10.1104/pp.111.182725

Cline MG (1996). Exogenous auxin effects on lateral bud outgrowth in decapitated shoots. *Ann. Bot. (Lond.)* 78: 255-266. http://dx.doi.org/10.1006/anbo.1996.0119

Cubas P, Lauter N, Doebley J and Coen E (1999). The TCP domain: a motif found in proteins regulating plant growth and development. *Plant J.* 18: 215-222. http://dx.doi.org/10.1046/j.1365-313X.1999.00444.x

Doebley J, Stec A and Hubbard L (1997). The evolution of apical dominance in maize. *Nature* 386: 485-488. http://dx.doi.org/10.1038/386485a0

Domagalska MA and Leyser O (2011). Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* 12: 211-221. http://dx.doi.org/10.1038/nrm3088

Dun EA, de Saint Germain A, Rameau C and Beveridge CA (2012). Antagonistic action of strigolactone and cytokinin in bud outgrowth control. *Plant Physiol.* 158: 487-498. http://dx.doi.org/10.1104/pp.111.186783

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, et al. (2008). Strigolactone inhibition of shoot branching. *Nature* 455: 189-194. http://dx.doi.org/10.1038/nature07271

Hall SM and Hillman JR (1975). Correlative inhibition of lateral bud growth in *Phaseolus vulgaris* L. timing of bud growth following decapitation. *Planta* 123: 137-143. http://dx.doi.org/10.1007/BF00383862

Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, et al. (1985). A simple and general method for transferring genes into plants. *Science* 227: 1229-1231. http://dx.doi.org/10.1126/science.227.4691.1229

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.

Lewis JM, Mackintosh CA, Shin S, Gilding E, et al. (2008). Overexpression of the maize *Teosinte Branched1* gene in wheat suppresses tiller development. *Plant Cell Rep.* 27: 1217-1225. http://dx.doi.org/10.1007/s00299-008-0543-8

Martín-Trillo M, Grandío EG, Serra F, Marcel F, et al. (2011). Role of tomato *BRANCHED1*-like genes in the control of shoot branching. *Plant J.* 67: 701-714. http://dx.doi.org/10.1111/j.1365-313X.2011.04629.x

Mason MG, Ross JJ, Babst BA, Wienclaw BN, et al. (2014). Sugar demand, not auxin, is the initial regulator of apical dominance. *Proc. Natl. Acad. Sci. USA* 111: 6092-6097. http://dx.doi.org/10.1073/pnas.1322045111

Minakuchi K, Kameoka H, Yasuno N, Umehara M, et al. (2010). *FINE CULM1* (*FC1*) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice. *Plant Cell Physiol.* 51: 1127-1135. http://dx.doi.org/10.1093/pcp/pcq083

Morris DA (1977). Transport of exogenous auxin in two-branched dwarf pea seedlings (*Pisum sativum* L.): Some implications for polarity and apical dominance. *Planta* 136: 91-96. http://dx.doi.org/10.1007/BF00387930

Ni J, Gao C, Chen MS, Pan BZ, et al. (2015). Gibberellin promotes shoot branching in the perennial woody plant Jatropha curcas. *Plant Cell Physiol.* 56: 1655-1666. http://dx.doi.org/10.1093/pcp/pcv089

Ohkawa K (1984). Effects of benzyladenine on bud break of roses. *Sci. Hortic. (Amsterdam)* 24: 379-383. http://dx.doi.org/10.1016/0304-4238(84)90123-7

Pillay I and Railton ID (1983). Complete release of axillary buds from apical dominance in intact, light-grown seedlings of *Pisum sativum* L. following a single application of cytokinin. *Plant Physiol.* 71: 972-974. http://dx.doi.org/10.1104/pp.71.4.972

Rameau C, Bertheloot J, Leduc N, Andrieu B, et al. (2015). Multiple pathways regulate shoot branching. *Front. Plant Sci.* 5: 741. http://dx.doi.org/10.3389/fpls.2014.00741

Sachs T and Thimann KV (1967). The role of auxins and cytokinins in the release of buds from apical dominance. *Am. J. Bot.* 54: 136-144. http://dx.doi.org/10.2307/2440896

Shimizu-Sato S, Tanaka M and Mori H (2009). Auxin-cytokinin interactions in the control of shoot branching. *Plant Mol. Biol.* 69: 429-435. http://dx.doi.org/10.1007/s11103-008-9416-3

Snow R (1937). On the nature of correlative inhibition. *New Phytol.* 36: 283-300. http://dx.doi.org/10.1111/j.1469-8137.1937.tb06917.x

Steiner E, Yanai O, Efroni I, Ori N, et al. (2012). Class I TCPs modulate cytokinin-induced branching and meristematic activity in tomato. *Plant Signal. Behav.* 7: 807-810. http://dx.doi.org/10.4161/psb.20606

Turnbull CGN, Raymond MAA, Dodd IC and Morris SE (1997). Rapid increase in cytokinin concentration in lateral buds of chickpea (*Cicer arietinum* L.) during release of apical dominance. *Planta* 202: 271-276. http://dx.doi.org/10.1007/s004250050128