 Phytochrome B Represses *Teosinte Branched1* Expression and Induces Sorghum Axillary Bud Outgrowth in Response to Light Signals

Tesfamichael H. Kebrom, Byron L. Burson, and Scott A. Finlayson*

Department of Soil and Crop Sciences (T.H.K., S.A.F.), and United States Department of Agriculture Agricultural Research Service (B.L.B.), Texas A&M University, College Station, Texas 77843–2474

Light is one of the environmental signals that regulate the development of shoot architecture. Molecular mechanisms regulating shoot branching by light signals have not been investigated in detail. Analyses of light signaling mutants defective in branching provide insight into the molecular events associated with the phenomenon. It is well documented that phytochrome B (phyB) mutant plants display constitutive shade avoidance responses, including increased plant height and enhanced apical dominance. We investigated the phyB-1 mutant sorghum (*Sorghum bicolor*) and analyzed the expression of the sorghum *Teosinte Branched1* gene (*SbTB1*), which encodes a putative transcription factor that suppresses bud outgrowth, and the sorghum dormancy-associated gene (*SbDRM1*), a marker of bud dormancy. Buds are formed in the leaf axils of phyB-1; however, they enter into dormancy soon after their formation. The dormant state of phyB-1 buds is confirmed by the high level of expression of the *SbDRM1* gene. The level of *SbTB1* mRNA is higher in the buds of phyB-1 compared to wild type, suggesting that phyB mediates the growth of axillary shoots in response to light signals in part by regulating the mRNA abundance of *SbTB1*. These results are confirmed by growing wild-type seedlings with supplemental far-red light that induces shade avoidance responses. We hypothesize that active phyB (Pfr) suppresses the expression of the *SbTB1* gene, thereby inducing bud outgrowth, whereas environmental conditions that inactivate phyB allow increased expression of *SbTB1*, thereby suppressing bud outgrowth.

The highly ordered arrangement of leaves and branches and the final shoot architecture are associated with a plant's developmental strategy to ensure its survival and productivity under continuously changing growing conditions. A complex developmental program that integrates genetic mechanisms, physiological processes, and environmental signals controls the overall form of the plant. The shoot architecture of crop plants has been modified during their domestication and improvement. One of the great achievements in the history of crop improvement, “The Green Revolution,” was due to dwarfing of wheat (*Triticum aestivum*) and other crops to increase nitrogen-use efficiency and reduce lodging. A more complete understanding of the developmental programs that control shoot architecture will help to further improve resource-use efficiency and yield of crop plants.

Shoot architecture is largely determined by the number of axillary shoots produced. Axillary shoot development begins with the initiation of an axillary meristem in the axil of a leaf to form a bud (for review, see Ward and Leyser, 2004; McSteen and Leyser, 2005). Then, depending on internal and environmental signals, the bud may continue growing to form an axillary shoot or enter into dormancy. Different approaches have been used to study the regulation of axillary shoot development. Early decapitation studies and application of plant hormones showed that auxin produced in the shoot apex inhibits the outgrowth of axillary buds (Thimann and Skoog, 1933). Further research indicated that other plant hormones, such as cytokinins and abscisic acid, are also involved in regulating branching (for review, see Stafstrom, 2000; Shimizu and Mori, 2001).

Molecular and genetic approaches have been used to study the mechanisms of action of plant hormones and to identify genes involved in regulating branching. Genes that control axillary meristem initiation have been identified in various species (Schumacher et al., 1999; Greb et al., 2003; Li et al., 2003). Studies using branching mutants of Arabidopsis (*Arabidopsis thaliana*), pea (*Pisum sativum*), and petunia (*Petunia hybrida*) are revealing the mechanisms of action of complex signaling networks of plant hormones that regulate shoot branching (Leyser et al., 1993; Stirnberg et al., 1999; Booker et al., 2005; Foo et al., 2005; Snowden et al., 2005). Investigations using these mutants suggest the presence of a novel, as yet unidentified, plant hormone-like signal that integrates hormonal action during branching.

A genetic analysis of the morphological differences between maize (*Zea mays* subsp. *mays*) and its wild
ancestor teosinte (*Zea mays subsp. parviglumis*) led to the cloning of the *Teosinte Branched1* (*TB1*) gene (Doebley et al., 1997). The *TB1* gene encodes a putative basic helix-loop-helix transcription factor that is involved in suppressing bud outgrowth (Doebley et al., 1997; Hubbard et al., 2002). An orthologous gene to maize *TB1* was cloned from rice (*Oryza sativa*; *OsTB1*) and functions in a manner similar to the maize *TB1* (Takeda et al., 2003). In both maize/teosinte and rice, *TB1* is expressed predominantly in the young axillary bud. Further studies of the functional properties of *TB1* will provide insights regarding the regulation of shoot branching at the molecular level.

Several genes that are specifically up-regulated or down-regulated during branching have been identified. The dormancy-associated genes of pea, *PsDRM1* (Stafstrom et al., 1998b) and *PsAD1* (Madoka and Mori, 2000), are among the genes identified in cDNA libraries from dormant axillary buds. The mRNA abundance of *PsDRM1* and *PsAD1* in axillary buds declines after decapitation. However, while the expression of these genes correlates strongly with bud dormancy, their functions are not known.

It is well established that light quality (red:far red [R:FR]) is one of the environmental signals that regulate shoot branching (Casal et al., 1986; Wan and Sosebee, 1998). However, little information exists regarding the molecular mechanisms regulating shoot branching in response to light. Light signals may interact with plant hormones to regulate plant growth and development (Halliday and Fankhauser, 2003). Recent findings indicate that light and auxin interact in the regulation of adventitious root formation in Arabidopsis (Sorin et al., 2005). To fully understand the regulation of axillary shoot development, the interaction of light and hormonal and other environmental signals should be investigated.

Plants use light signals perceived by photoreceptors to coordinate all stages of growth and development from germination to flowering. The phytochrome family of photoreceptors is involved in deetiolation, vegetative development, and flowering time in both dicots and monocots (Mathews and Sharrock, 1996; Sawers et al., 2005). Mutation in one of the family members, *PHYTOCHROME B* (*PHYB*), affects several developmental processes, including branching in sorghum (*Sorghum bicolor*) and Arabidopsis (Childs et al., 1992, 1997; Reed et al., 1993).

We used the *phyB* null mutant sorghum (*phyB-1*) as a model for studying the regulation of branching by light. The strong apical dominance of *phyB-1*, and the early formation and ease of excising axillary buds make sorghum useful for studying the role of light in axillary shoot development. We asked whether light signals perceived by phyB control shoot branching by regulating the expression of branching genes previously identified in other species. We characterized the enhanced apical dominance in *phyB-1* sorghum and investigated the expression of branching-related genes, including the sorghum homologs of the *TB1* (*SbTB1*) and dormancy-associated (*SbDRM1*) genes in *phyB-1* and wild-type axillary buds. In this article, we report the regulation of expression of the sorghum *SbTB1* and *SbDRM1* genes by light signals perceived by phyB, and phyB’s association with dormancy and outgrowth of axillary buds.

### RESULTS

#### Enhanced Apical Dominance in *phyB-1* Mutant Sorghum

The *phyB-1* mutant sorghum fails to produce branches during vegetative development, whereas the near-isogenic wild-type plants branch profusely. The branching deficiency in *phyB-1* could theoretically arise from either a defect in axillary meristem initiation or bud outgrowth. We found that the defect occurs...
in bud outgrowth since equivalent buds are formed early in the development in both phyB-1 and wild-type sorghum (Fig. 1). The buds in the axil of the first leaf of both phyB-1 and the wild type grow at the same rate until 7 d after planting (DAP). Then they begin to show different developmental fates (Fig. 2A). While the buds of the wild type continue elongation, bud outgrowth is inhibited in phyB-1. Axillary buds are formed at all nodes of the main shoot of phyB-1 (data not shown). These buds remain dormant and branching is observed only when the main shoot begins flowering. The phyB-1 seedlings were taller than the wild type, showing enhanced growth of the main shoot as a result of constitutive shade avoidance (Fig. 2B).

Expression of SbTB1 in Different Organs of Sorghum Seedlings

SbTB1 (accession no. AF322132) exists as a single copy in the sorghum genome with 93.9% nucleotide identity with the maize TB1 gene (Lukens and Doebley, 2001). The maize TB1 mRNA is 1.5 kb in size and accumulates in husks, axillary inflorescence primordia, and axillary meristems (Doebley et al., 1997; Hubbard et al., 2002). OsTB1 is expressed in the basal part of the shoot apical meristem, in vascular tissue in the pith, and in the entire axillary bud (Takeda et al., 2003). We investigated the abundance of SbTB1 in different parts of sorghum seedlings. The SbTB1 mRNA of about 1.7 kb in size was detected only in the buds (Fig. 3).

Expression of SbDRM1 in Different Organs of Sorghum Seedlings

PsDRM1 is expressed in dormant axillary buds, and its expression is suppressed by decapitation and auxin (Stafstrom et al., 1998b; Stafstrom, 2000). PsDRM1 has similar expression patterns to PdDRM1, PdDRM1, and AtDRM1, which have been shown to be involved in shade avoidance responses in plants (Stafstrom et al., 1998a; Tatematsu et al., 2005). We investigated the abundance of SbDRM1 mRNA in the axillary buds of both phyB-1 and wild-type seedlings. At 7 DAP, when their size was comparable (Fig. 2), SbDRM1 message is hardly detected in the buds of phyB-1 and the wild type (Fig. 6A). At 9 DAP, when the buds of the wild type were rapidly elongating while those of phyB-1 were suppressed, the mRNA level of SbDRM1 was more than 5-fold higher in the buds of phyB-1 than in the wild type (Fig. 6C).

SbTB1 and SbDRM1 mRNA Abundance in Axillary Buds of Wild-Type Seedlings Grown at High and Low Densities

The enhanced apical dominance of phyB-1 sorghum is consistent with a constitutive shade avoidance response. Shade avoidance responses are also observed in the natural environment when wild-type plants are grown at high density that lowers the R:FR. In an attempt to simulate the constitutive shade avoidance response of phyB-1 sorghum in the wild type, wild-type seedlings were grown at high (3,000 seedlings m\(^{-2}\)) and low (20 seedlings m\(^{-2}\)) densities.

### Figure 3

SbTB1 mRNA abundance in leaf, sheath, root, and bud of sorghum seedlings at 9 DAP. The sorghum ubiquitin gene (SbUBQ) was used as a loading control.

### Figure 4

Sequence similarity among sorghum, pea, and Arabidopsis dormancy-associated proteins (SbDRM1, PsDRM1, and AtDRM1, respectively).
We investigated the regulation of branching by light signals perceived by phyB in the branching-deficient phyB-1 null mutant sorghum. Mutants used previously to study the molecular mechanisms of branching are defective either in initiation of axillary meristems or outgrowth of buds (Napoli et al., 1999; Ward and Leyser, 2004). Our results show that the initiation of axillary meristems and formation of buds occur normally in phyB-1; however, the outgrowth of those buds was inhibited, indicating that the role of light signals in branching is limited to bud dormancy and outgrowth. Bud outgrowth was also inhibited in wild-type plants grown at high plant density or with supplemental FR light, demonstrating the regulation of axillary buds by environmental signals soon after their formation.

Supplemental FR light treatment of wild-type seedlings, started at 7 DAP, inhibited the outgrowth of buds in the first leaf axil of all treated seedlings. However, when FR treatment was started at 9 DAP, the
buds in the first leaf axil of some seedlings were arrested, while in others they escaped the inhibitory signal and elongated (data not shown). It is noteworthy that all the buds in the axil of the second leaves of those plants treated with FR at 9 DAP were arrested (data not shown). The inconsistency in the response of buds in the axil of the first leaf to delayed FR light treatment was observed in repeated experiments. The “escape” phenomenon may be similar to the regulation of bud dormancy and outgrowth by auxin (for review, see Cline, 1997), in which decapitation-induced bud outgrowth can be suppressed by exogenous auxin early after decapitation but not later. These results indicate the presence of a developmental window at which FR light and auxin can inhibit bud outgrowth. The data raise the question of whether the effect of light on bud dormancy and outgrowth is through auxin, or whether both light and auxin act on a common target that regulates the process.

Previous research has indicated that DRM1 expression correlates with bud dormancy, and we have used it here as an indicator of a bud’s physiological status. The inhibition of bud outgrowth in phyB-1 sorghum is reflected in the high level of SbDRM1 mRNA in these axillary buds (Fig. 6). The results are confirmed by the high level of SbDRM1 mRNA in the arrested buds of wild type grown at high plant density or with supplemental FR (Figs. 9 and 11). There was a small increase in SbDRM1 mRNA at 9 DAP compared to 7 DAP in wild type grown under standard conditions (Fig. 6) and in wild type grown at low densities (Fig. 9). These results may seem contradictory to the typical correlation of DRM1 expression with dormancy but may indicate an increase in the proportion of non-dividing cells in the rapidly elongating buds of the wild type.

The pattern of SbDRM1 accumulation agrees with that observed in pea and Arabidopsis, implying that conserved mechanisms of dormancy and outgrowth may operate in both monocots and dicots—an observation also supported by the orthologous function of MAX-related genes in rice (Ishikawa et al., 2005). Since auxin represses PsDRM1 in pea (Stafstrom, 2000), the suppression of SbDRM1 mRNA accumulation by light suggests that similar dormancy mechanisms are induced by both auxin and light. Phytochrome regulation of gene expression has been shown to be both auxin dependent and auxin independent (Tanaka et al., 2002). Whether the suppression of SbDRM1 mRNA by light is auxin dependent or auxin independent needs to be investigated. It is not known whether decapitation-induced and non-decapitation-induced bud outgrowth (i.e. via light) are regulated through the same pathway (Napoli et al., 1999). Further studies on the regulation of expression of SbDRM1 mRNA and related genes by light signals will help resolve such questions.

The TB1 gene was identified as one of five quantitative trait loci that distinguish the morphology of maize from its wild ancestor, teosinte (Doebly and Stec, 1991). Compared to teosinte, maize exhibits enhanced apical dominance. Sequence comparison of the maize and teosinte TB1 alleles indicated no difference in the predicted coding region of the two alleles (Doebly et al., 1997); however, the expression of the maize TB1 was twice that of teosinte, suggesting differences in the regulation of expression of the gene (Doebly et al.,...
Increased expression of the TB1 gene in axillary organs was correlated with suppression of growth of those organs (Doebley et al., 1997; Hubbard et al., 2002). Increased expression of OsTB1 was also found to suppress axillary bud outgrowth but not their formation (Takeda et al., 2003). The elevated SbTB1 mRNA abundance in phyB-1 axillary buds compared to wild type and in wild type grown with low R:FR suggests that SbTB1 gene expression is regulated by light signals perceived by phyB. Therefore, phyB may exert its influence on branching in part through modulation of SbTB1 mRNA levels.

Branching in maize is relatively insensitive to planting density; whereas branching in teosinte is reduced at high plant density and increases at low plant density. Doebley et al. (1995) suggested that TB1 expression in maize is decoupled from regulation by environmental signals and is constitutively expressed, while teosinte TB1 is expressed under unfavorable growing conditions and repressed under favorable growing conditions. Our results suggest that the regulation of TB1 expression in wild-type sorghum more closely resembles that proposed for teosinte, while the expression in phyB-1 sorghum more closely resembles that proposed for maize.

It should be noted that the response of wild-type sorghum seedlings to high planting density was not the same as their response to low FR light treatment during the experimental period. Although bud elongation was inhibited in both treatments, only FR induced an increase in seedling height, which is one of the typical shade avoidance responses. The pattern of accumulation of SbDRM1 mRNA at 9 DAP at high planting density and in the FR-treated seedlings was similar in each case, indicating both treatments inhibited bud outgrowth through similar downstream mechanisms. However, the level of SbTB1 mRNA at 9 DAP at high planting density was lower than at 7 DAP at both high and low planting densities (Fig. 8), while FR light treatment increased the level of SbTB1 mRNA at 9 DAP compared to the level at 7 DAP (Fig. 11). Takeda et al. (2003) reported that the branching habit of the fine culm 1 mutant rice, which contains a loss-of-function mutation of OsTB1, responds to planting density. They suggested that the regulation of branching in rice in response to density is independent of OsTB1 but dependent on other factors. Since high density did not affect plant height and the effect on SbTB1 expression is relatively small, it is unlikely that suppression of bud outgrowth in our experiment is mainly the result of a phyB-mediated shade avoidance response. In addition to changes in light quality, other undetermined density-derived cues may also inhibit bud outgrowth at high planting density.

SbTB1 mRNA is highest in the younger wild-type buds (7 DAP) and decreases with time, suggesting that early in development these buds acquire a signal that inhibits their outgrowth. The fate of these buds, whether to continue growth or enter into dormancy, is determined at a later stage depending on the perception of light and possibly other signals required for their elongation. The results imply that the absence of active phyB to suppress SbTB1 accumulation in the phyB-1 axillary buds leads to dormancy, while the suppression of SbTB1 accumulation in the wild-type axillary buds by active phyB leads to bud outgrowth.

Figure 9. A, Abundance of SbDRM1 mRNA in axillary buds in the axil of the first leaves of wild-type seedlings grown at high and low densities at 7 DAP and 9 DAP. B, SbDRM1 mRNA quantitation relative to buds of seedlings grown at low density at 7 DAP. The sorghum ubiquitin gene (SbUBQ) was used as a loading control.

Figure 10. Effect of supplemental FR light on bud outgrowth (A) and seedling height (B) of wild-type sorghum. Data are mean ± se; n = 7.
PhyB Represses TBI Expression and Induces Bud Outgrowth

Transgenic potato (Solanum tuberosum) plants overexpressing phyB produced more branches at high plant density (Boccalandro et al., 2003). These results are consistent with the hypothesis that active phyB suppresses accumulation of the TBI mRNA, thereby promoting bud outgrowth. Recently, a potato gene (sttcp1) with a function similar to that of the maize TBI gene was cloned (Fairev-Rampant et al., 2004). It would be interesting to study the level of expression of the sttcp1 gene in the phyB-overexpressing and wild-type potato.

Devlin et al. (2003) studied genome-wide gene expression changes in phyB mutant, phyA phyB double mutant, and wild-type Arabidopsis in response to shade and identified 301 genes that demonstrated altered expression. Some of these genes encode proteins that are known to function in light and hormone signaling, transcription regulation, and protein degradation. In some developmental programs, phytochrome action involves a direct interaction with transcription factors to regulate the expression of light-responsive genes. Phytochromes may also act through posttranslational regulation of the level of transcription factors by directed proteolysis to indirectly regulate gene expression (Quail, 2002). The mechanism by which phyB regulates SbTBI abundance remains to be discovered.

The TBI gene belongs to the TCP family of transcription factors with a noncanonical basic helix-loop-helix domain that is predicted to function in DNA-binding and protein-protein interactions (Cubas et al., 1999). Previous studies indicated that the TCP proteins stimulate or repress the growth of an organ in which they are expressed by regulating the expression of several cell cycle-related genes (Kosugi and Ohashi, 1997, 2002; Gaudin et al., 2000; Tremousaygue et al., 2003; Li et al., 2005). Since TBI represses the growth of organs in which it is expressed, it may directly or indirectly repress growth by interfering with the progression of cell division. We are investigating the expression of several cell cycle genes, such as PCNA, ribosomal protein genes, Cyclins, and CDKs, in the phyB-1 and wild-type sorghum axillary buds to elucidate the regulation of bud outgrowth by TBI in response to light.

It is not yet understood whether hormonal signals interact with TBI to suppress bud outgrowth (McSteen and Leyser, 2005). We have identified several sorghum expressed sequence tags with homology to key genes involved in regulating hormonal signals during axillary shoot development, including MAX related (Booker et al., 2005) and AXR1 (Leyser et al., 1993; Stirnberg et al., 1999), and we are investigating their patterns of expression in the sorghum system to understand the complex signaling networks involved in regulating axillary shoot development.

Tatematsu et al. (2005) identified cis-elements that regulate the expression of genes involved in axillary bud outgrowth in Arabidopsis by analyzing the transcriptome of axillary shoots collected from all positions. Since axillary shoots at different phyllotactic positions are at different developmental stages, it is difficult to delineate the role of those elements at the different stages. Using both the monocots such as sorghum where working with buds comparable in size and at similar positions is easier and dicots such as pea and Arabidopsis where decapitation, grafting, and genetic manipulation are easier, a more complete understanding of the regulation of plant architecture may be achieved.

**MATERIALS AND METHODS**

**Plant Materials and Growing Conditions**

Wild-type and phyB-1 mutant sorghum (Sorghum bicolor) seedlings were grown in a growth chamber with incandescent and fluorescent lamps, maintained at 31°C/22°C day/night temperatures with a 12-h photoperiod. The photosynthetically active radiation was approximately 600 μmol m⁻² s⁻¹ and the R:FR 3.0. Seeds were sown at a rate of 300 m⁻² on trays containing 7-cm-deep cells filled with growth medium prepared as described by Beall et al. (1991). To study the effect of plant density, seeds were sown at a rate of 300 and 3,000 m⁻² for the low and the high plant density, respectively. Seedlings near the perimeter of the trays were avoided during sampling. To study the effect of shade signals (R:FR) on bud outgrowth, seedlings were grown in 50-mL tubes, one seedling per tube, and the tubes were supported on a rack arranged at a seedling density of 900 m⁻². At this rate, shading due to density was not observed as detected by measurement of bud elongation until 10 DAP (data not shown). At 7 DAP, uniform seedlings were selected, divided into two groups, arranged on a rack at a seedling density of 300 seedlings m⁻², and then one group was treated with supplemental FR (FR, 9 DAP) while the other group was grown as control (control, 9 DAP). Supplemental FR was applied using FR light-emitting diode arrays directed from the sides starting at 7 DAP. FR-treated and control seedlings were grown in the same growth chamber under the same light conditions.
conditions except that the FR-treated seedlings had additional FR light from the sides.

**Histological Studies**

Young elongating shoots of phyB-1 and the wild type were collected about 5 mm from the base of seedlings and fixed in FAA (70% ethanol, 37% formaldehyde-acetic acid, 18:1:1) for at least 24 h, and then stored in 70% ethanol. The young stems were dehydrated in a tertiary butyl alcohol series, embedded in Tissueprep (Fisher Scientific), sectioned at 15 μm with a rotary microscope, and placed on microscope slides that were kept at 40°C to 50°C for at least 24 h. The slides were then stained in Safranin-O fast-green series using an HMS series programmable slide stainer (Carl Zeiss). Two drops of Permount mounting medium (Fisher Scientific) were placed on each slide and covered with a cover glass. The slides were then observed with a bright-field Zeiss microscope.

**Bud Length and Seedling Height Measurement**

Subtending leaves were removed, and buds in the axil of the first leaf were excised and their lengths measured under a dissecting microscope using a micrometer. A ruler was used to measure buds longer than 3 mm. Seedling height was also measured as the height from the base of the shoot to the tip of the tallest leaf.

**RNA Isolation and Northern Analysis**

For gene expression studies, seedlings were harvested, roots were washed, and buds in the axil of the first leaves were excised under a dissecting microscope using a micrometer. The roots of seedlings were kept moist during sampling. Sampling was done between 11 AM and 2 PM during the day. Total RNA was extracted using the Trizol method (Life Technologies), and then separated on a denaturing glyoxal agarose gel and transferred to a Hybond membrane (Amersham Biosciences). Membranes were exposed to an imaging plate and analyzed using a phosphor imager. After subtracting background, the target mRNA levels were quantified by a computer program.

**LITERATURE CITED**


Kosugi S, Ohashi Y (2005) DNA binding and dimerization specificity and potential targets for the TCP protein family. Plant J 30: 337–348


Stafstrom JP, Krueger MT, Stoudt W (1998a) Nucleotide sequence of AtDRM1-1 cDNA and genomic clones (accession nos. AF053746 and AF053747), an Arabidopsis homologue of a pea dormant bud-associated gene. Plant Physiol 117: 718


Thimmann KV, Skoog F (1933) Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. Proc Natl Acad Sci USA 19: 714–716


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