Transcriptome Profiling of Tiller Buds Provides New Insights into PhyB Regulation of Tillering and Indeterminate Growth in Sorghum

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Phytochrome B (phyB) enables plants to modify shoot branching or tillering in response to varying light intensities and ratios of red and far-red light caused by shading and neighbor proximity. Tillering is inhibited in sorghum genotypes that lack phytochrome B (phyB-1) until after floral initiation. The growth of tiller buds in the first leaf axil of wild-type (100M, PHYB) and phyB-1 sorghum genotypes is similar until 6 d after planting when buds of phyB-1 arrest growth, while wild-type buds continue growing and develop into tillers. Transcriptome analysis at this early stage of bud development identified numerous genes that were up to 50-fold differentially expressed in wild-type/phyB-1 buds. Up-regulation of terminal flower1, ACC oxidase, and TPPI could protect axillary meristems in phyB-1 from precocious floral induction and decrease bud sensitivity to sugar signals. After bud growth arrest in phyB-1, expression of dormancy-associated genes such as DRM1, GT1, AF1, and CKX1 increased and ENOD93, ACC oxidase, ARR3/6/9, CGA1, and SHY2 decreased. Continued bud outgrowth in wild-type was correlated with increased expression of genes encoding a SWEET transporter and cell wall invertases. The SWEET transporter may facilitate Suc unloading from the phloem to the apoplast where cell wall invertases generate monosaccharides for uptake and utilization to sustain bud outgrowth. Elevated expression of these genes was correlated with higher levels of cytokinin/sugar signaling in growing buds of wild-type plants.

The extent of tillering in crops affects plant density, leaf area, and the interception of light by the crop canopy. Modeling of light interception by crop canopies indicates that current genotypes often intercept an excess of light at the top of the canopy (Zhu et al., 2010; Drewry et al., 2014). Energy sorghum hybrids tiller to a greater extent than grain sorghum genotypes, often producing canopies with excess leaf area index (greater than 7; Olson et al., 2012). A more optimal distribution of light interception could be achieved by growing energy sorghum with reduced propensity for tillering at lower plant density with leaves having reduced leaf angles (Truong et al., 2015). The reduced leaf area of low tillering varieties would conserve soil moisture for the grain-filling stage in drought-prone regions (Islam and Sedgley, 1981; Kebrom and Richards, 2013). Reduced tillering associated with stay-green drought tolerance loci is also important for grain sorghum production in water-limited environments (Borrell et al., 2014). A more complete understanding of the genetic and biochemical basis of tiller production in sorghum will accelerate progress toward optimal crop canopy architectures and higher yield.

Shade is one of the major factors modifying the extent of shoot branching or tillering in crops grown at high planting density (Kebrom and Brutnell, 2007). Plants detect shade as a reduction in the intensity of red light (R) and decreases in the ratio of red (R) to far-red (FR) light. The R/FR of sunlight is about 1.2 (Holmes and Smith, 1975). The R/FR light within a canopy is reduced due to the absorption of R light by leaf chlorophyll. The microenvironment of plants grown at high density is also enriched in FR reflected from nearby plants (Ballaré et al., 1990). Plants continuously monitor R/FR using the phytochrome family of photoreceptors to detect neighbor proximity and potential competitors for light and other resources (Smith, 1995). Sorghum encodes three phytochromes (phyA, phyB, phyC), whereas other flowering plants such as Arabidopsis have up to five types of phytochromes (phyA, phyB, phyC, phyD, phyE) that regulate growth and development from germination to maturity (Sharrock and Quail, 1989; Clack et al., 1994; Mathews and Sharrock, 1996). Shade signaling is mainly regulated by phytochrome B [(phyB); Franklin and Whitelam, 2005; Martinez-Garcia et al., 2010; Casal, 2013]. Like the other phytochromes, phyB resides in an inactive R-light-absorbing form in...
darkness. Upon absorbing R light, phyB’s conformation changes into an active FR-absorbing form that can be converted to the R-absorbing form by FR or darkness. The ratio of active to inactive phyB is proportional to the R/FR in a consistent illumination environment at steady state. When a plant is shaded it perceives relatively higher FR than R and proportionally more phyB becomes inactive. Upon perceiving low R/FR light, plants display a set of growth and developmental changes that help them cope with or avoid shade (Martinez-Garcia et al., 2010; Casal, 2013). These responses include increased stem elongation that moves leaves above neighboring plants; inhibition of tillering, which prioritizes resources for elongating established shoots; and early flowering to escape from the detrimental effect of shade (Kebrom et al., 2006).

These responses include increased stem elongation that moves leaves above neighboring plants; inhibition of tillering, which prioritizes resources for elongating established shoots; and early flowering to escape from the detrimental effect of shade (Kebrom and Brutnell, 2007). This set of phenotypes is known as the “shade avoidance syndrome” (SAS; Smith and Whitelam, 1997). PhyB-deficient plants in diverse species constitutively display the SAS (Reed et al., 1993; Childs et al., 1997; Kebrom et al., 2006; Finlayson et al., 2010). The early flowering and stem elongation responses have been investigated in-depth and are mediated in part by changes in the level or action of most of the major plant hormones (Carabelli et al., 1993; Finlayson et al., 1999; Morelli and Ruberti, 2000; Cerdán and Chory, 2003; Carabelli et al., 2007; Sheehan et al., 2007; de Lucas et al., 2008; Kim et al., 2008; Tao et al., 2008). The molecular and physiological basis of phyB- and SAS-mediated reduction in branching is less well characterized.

Shoot branches or tillers develop from buds in the axil of leaves. The development of a branch begins with the initiation of a bud meristem and formation of a boundary zone surrounding the meristem (Janssen et al., 2014). Bud development and growth then reaches a transition stage or checkpoint where growth arrest/dormancy or outgrowth fates are determined by intrinsic and environmental factors (Shimizu-Sato and Mori, 2001; Dun et al., 2006). A plant may fail to develop a branch due to defects in axillary meristem initiation or inhibition of axillary bud outgrowth (Ward and Leyser, 2004). Reduced tillering in response to shading is due to bud growth arrest and inhibition of outgrowth because axillary buds are formed normally in phyB mutants of sorghum (phyB-1) and Arabidopsis (Kebröm et al., 2006; Finlayson et al., 2010). Sorghum genotypes that lack phyB such as 58M (phyB-1) have only one stem because axillary buds become dormant during the vegetative phase (Kebröm et al., 2006, 2010). In Arabidopsis, inactivation of phyB reduces but does not eliminate branching possibly due to the action of phyD and phyE that are not present in sorghum (Finlayson et al., 2010).

Research on the evolution of enhanced apical dominance in maize relative to its wild ancestor teosinte led to the discovery that teosinte branched1 (tb1) is expressed in tiller buds and inhibits bud outgrowth (Doebely et al., 1995, 1997; Hubbard et al., 2002). Tilling in Wheat (Triticum spp.) was suppressed through transgenic overexpression of the maize tb1 gene (Lewis et al., 2008), and mutants of rice tb1 orthologs (fc1) and Arabidopsis (brc1) are highly branched, indicating a role for tb1-like genes in the regulation of shoot branching in these species (Takeda et al., 2003; Aguilar-Martínez et al., 2007; Finlayson, 2007). Comparison of the phyB mutant (phyB-1) and wild-type (PHYB) sorghum demonstrated that expression of the sorghum ortholog of tb1 (SbTB1) was approximately 2.5-fold higher in phyB-1 buds 7–9 d after planting (Kebröm et al., 2006). Phytochrome regulation of tb1-like genes was also demonstrated in Arabidopsis (Aguilar-Martínez et al., 2007; Finlayson et al., 2010). Cytokinin has been found to down-regulate expression of FC1 (tb1) in rice (Minakuchi et al., 2010) and PsBRC1 in pea (Braun et al., 2012). Therefore, differential regulation of tb1 expression in phyB-1/wild-type buds could be mediated by altered cytokinin level or signaling. Maize mutants of grassy tillers1 (gt1), a gene that encodes an HD-Zip transcription factor, are highly branched (Whipple et al., 2011). Teosinte gt1 expression was induced by FR light, and sorghum SbGT1 expression increased in buds of phyB-1 plants between 7 and 9 d after planting, but not in wild-type plants (Whipple et al., 2011). Expression of gt1 is down-regulated in tb1 mutants, therefore tb1 may regulate tillering by controlling the expression of this gene (Whipple et al., 2011).

Strigolactones (SLs) are key regulators of tillering that bind to a MAX2/D14/DAD2 complex and act in part through the BRC1/GT1 pathway (Janssen et al., 2014). MAX2 encodes an F-box protein required for SL-mediated inhibition of bud outgrowth (Stirnberg et al., 2002, 2007). The Arabidopsis max2 mutant and mutants of MAX2 orthologs in pea (RMS4) and rice (D3) are highly branched, indicating this gene regulates axillary bud outgrowth in diverse species (Stirnberg et al., 2002; Ishikawa et al., 2005; Johnson et al., 2006). Increased expression of SbMAX2 in buds of shaded plants and phyB-1 genotypes (Kebröm et al., 2010) could increase SL-mediated repression of bud outgrowth, possibly by disrupting auxin transport (Crawford et al., 2010; Domagalska and Leyser, 2011). SbMAX2 expression in axillary buds was increased by defoliation treatments that inhibit tiller outgrowth (Kebröm et al., 2010). The rose (Rosa Hybrid) RwMAX2 was expressed at lower levels in buds supplied with Suc (Barbier et al., 2015b). These results indicate phyB and sugar-sensing pathways coregulate genes involved in SL sensing that modulate tiller outgrowth.

Cytokinin (CK) promotes, and abscisic acid (ABA) inhibits, bud outgrowth through their individual actions within the bud (Pillay and Railton, 1983; Chatfield et al., 2000; Shimizu-Sato and Mori, 2001). CKs enable growing buds to escape dormancy (Müller et al., 2015). The level of CK in the stem is reduced by auxin-mediated suppression of a key CK biosynthesis gene, whereas ABA appears to inhibit bud outgrowth independently of auxin (Chatfield et al., 2000; Tanaka et al., 2006; Beveridge et al., 2009). Exposure of Arabidopsis to low R/FR illumination repressed bud outgrowth and increased expression of genes involved in ABA.
biosynthesis and signaling in buds (González-Grandío et al., 2013; Reddy et al., 2013). The expression of these genes was decreased when buds were stimulated to grow by R light.

An important role for sugar in regulating shoot branching has been identified. In the tiller inhibition (tin) mutant of wheat, diversion of Suc away from buds to elongating internodes inhibits bud outgrowth (Kebrom et al., 2012). Sugar also stimulates bud outgrowth in pea and rose (Rabot et al., 2012; Mason et al., 2014; Barbier et al., 2015b). The expression of the Suc-starvation-inducible Gln-dependent Asn synthase (ASN) gene was increased and Suc-inducible PFP-gene encoding pyrophosphate-Fru-6-phosphate 1-phosphotransferase was down-regulated in dormant buds of tin in wheat and in buds repressed by defoliation in sorghum (Kebrom et al., 2012; Kebrom and Mullet, 2014). The molecular mechanism regulating Suc levels, and the specific signaling pathways mediating sugar sensing in buds, remain to be discovered (Barbier et al., 2015a).

The elegant studies described above have identified, among other discoveries, a group of genes expressed in sorghum buds (i.e. TB1/BRC1, GT1, MAX2) that help repress the outgrowth of dormant buds until light conditions, sugar, and cytokinin levels/signaling are permissive for tiller outgrowth. In nonshaded conditions, phyB-signaling down-regulates the expression of TB1, GT1 and MAX2 in buds, increasing the propensity for tiller outgrowth. Less well understood is how lack of phyB-signaling leads to the initial arrest of bud growth. Moreover, additional information about the molecular and biochemical processes involved in bud outgrowth in wild-type plants under permissive conditions would help provide a more comprehensive understanding of bud development. Therefore, in this study, transcriptome profiles of tiller buds from phyB-deficient (phyB-1) and wild-type (control) sorghum plants were obtained at a critical early stage of bud development when buds of phyB-1 plants arrest growth and transition into dormancy, whereas buds of control plants grow into tillers. RNA-seq analysis was used to characterize differences in bud gene expression that are associated with phyB-regulated tillering in sorghum plants.

RESULTS
Development of Tillers in Near-Isogenic phyB-1 and Wild-Type Sorghum Genotypes

The phytochrome B null mutant sorghum genotype (58M, phyB-1) produces a main shoot that is devoid of tillers during the vegetative phase while the near-isogenic wild-type sorghum genotype (100M, PHYB) produces many tillers (Fig. 1A). Buds in phyB-1 remain dormant during the vegetative phase, but after floral initiation, axillary buds can develop into tillers (Fig. 1B). Like most genotypes of sorghum with an indeterminate or perennial-like growth habit, phyB-1 genotypes continue to develop tillers and panicles as long as conditions are favorable for growth and flowering (Fig. 1C).

Overall Transcriptome Dynamics during Tiller Bud Development in phyB-1 and Wild-Type Sorghum

Bud growth in the first leaf axil is similar in wild-type and phyB-1 through the first 6 d after planting (DAP; Fig. 2A) Kebrom et al., 2006, 2010]. After 6 DAP, the buds in phyB-1 plants show no or very slow growth, whereas buds of wild-type plants continue to steadily increase in size (Fig. 2A). Expression of SbDRM1, a marker of bud dormancy, was low and similar in buds of both genotypes at 6 DAP, then increased differentially approximately 3-to-5-fold in the phyB-1 relative to wild-type controls by 7/8 DAP (Fig. 2B), as previously reported (Kebrom et al., 2006).

RNA for RNA-seq analysis was obtained from buds dissected from the first leaf axil of wild-type and phyB-1 at 6 DAP, 7 DAP, and 8 DAP and used to prepare the cDNA template for sequencing. The average reads from a total of three biological replicates was 40.1 million, of which 93.9% mapped to the sorghum reference sequence Sbicolor-v2.1_255 (Phytozome; http://www.phytozome.net), which encodes 33,032 annotated genes and 39,441 transcripts (Supplemental Table S1). RNA-seq data from biological replicates were found to co-cluster when assessed using principal component analysis (Supplemental Fig. S1). Transcriptomes of buds of phyB-1 and wild-type plants increasingly diverged between 6 DAP and 8 DAP. Relative gene expression was estimated by analyzing RNA-seq data in CLC Bio Genomics Workbench (CLC, Aarhus, Denmark), using reads per kilobase per million (RPKM) mapped read values, and a false discovery rate (FDR) of <5% (Supplemental Table S2). Relative expression results from RNA-seq were confirmed using quantitative real-time PCR (qRT-PCR; Figs. 2, B and C, and Supplemental Fig. S2), and were consistent with prior qRT-PCR analysis of bud gene expression (Kebrom et al., 2006, 2010; Whipple et al., 2011). For example, SbDRM1 mRNA levels in phyB-1 and wild-type buds analyzed by RNA-seq (RPKM) and qRT-PCR showed that RNA abundance in buds was similar at 6 DAP and that by 8 DAP, 5-fold higher in phyB-1 buds relative to wild-type, confirming prior results (Fig. 2, B and C).

Genes that were differentially expressed in at least one of the time points analyzed are listed in Supplemental Table S2. There were 127 (6 DAP), 315 (7 DAP), and 611 (8 DAP) genes up- or down-regulated ≥2-fold (RPKM ≥ 2) in buds of phyB-1 plants relative to the corresponding wild-type controls (Supplemental Fig. S3). A total of 71 genes were consistently differentially expressed in phyB-1 buds relative to wild-type controls at all time points assayed. A large number of the differentially expressed genes categorized by MapMan software (Thimm et al., 2004) function in
hormone metabolism and signaling, response to stress, transcription (RNA synthesis), protein synthesis, signaling, development, and transport (Supplemental Table S3). Differentially expressed genes with biological relevance based on established knowledge of regulation of dormancy and outgrowth of axillary buds, flowering time, and vegetative meristem identity maintenance in diverse species and novel genes that are up- or down-regulated greater than 5-fold in the phyB-1 buds relative to the corresponding wild-type controls, were analyzed in more detail beginning with genes differentially expressed at 6 DAP.

Differential Expression of Cell Cycle Genes and Peter pan in Growth-Arrested Buds at 6 DAP

Bud growth arrest occurs in phyB-1 at 6 DAP, although seedling shoot and root growth continues (Kebrom et al., 2006). Differential bud growth arrest in phyB-1 could be due to differences in bud gene expression caused by the absence of phyB-signaling during the first 6 d of bud growth leading to the depletion of a gene product required for cell division or growth, or the buildup of one or more repressors. To examine this possibility, we searched for genes involved in the cell cycle and that were differentially expressed in buds of phyB-1 compared to wild-type plants at 6 DAP.

Expression of cell cycle genes such as SbHis4, SbCycD2, SbCDKB, SbCycB, and SbPCNA was previously found to be similar in buds of phyB-1 and wild-type (Kebrom et al., 2010). In this study, genes involved in the cell cycle were expressed at similar levels in buds of both genotypes at 6 DAP, and showed minimal change in relative expression from 6 DAP to 8 DAP consistent with prior analysis based on an overall survey of genes involved in the cell cycle and cell division (Supplemental Figs. S4 and S5). The expression of an E2F3-like gene Sb08g003670.1 was reduced approximately 3-fold in phyB-1 buds at 8 DAP (Fig. 3A). The E2F transcription factor is a component of the...
Figure 3. Expression of A, the cell cycle gene E2F3 (Sb08g003670.1); B, Peter pan-like genes Sb01g035700.1; C, Sb01g035720.1 determined by RNA-seq in buds in the first leaf axil of the phytochrome B mutant (phyB-1) and wild-type (PHYB) controls at 6, 7, and 8 DAP. Data are mean RPKM mapped reads ± s; n = 3 biological replicates.

retinoblastoma/E2F/Dp pathway of the cell cycle that controls progression from G1 to S-phase by activating genes required for the s-phase of the cell cycle (Inzé and De Veylder, 2006). In Arabidopsis, AtE2FA is required for lateral root initiation and vascular patterning (Berckmans et al., 2011).

A broader survey for genes that could regulate growth identified Sb01g035700.1, a gene expressed at greater than 100-fold lower levels in phyB-1 buds compared to wild-type (Fig. 3B). This sorghum gene is homologous to Peter pan, a member of the SSF1/2 gene family, first identified in Drosophila as a gene required for normal mitotic growth (Migeon et al., 1999) and ribosome biogenesis (Eisenhaber et al., 2001). Sorghum encodes two Peter pan-like genes and while Sb01g035700.1 was nearly completely silenced in phyB-1 buds, another sorghum gene homologous to Peter pan, Sb01g035720.1, was expressed at similar levels in both phyB-1 and wild-type axillary buds (Fig. 3C).

Differential Expression of Genes that Regulate Meristem Differentiation and Gibberellin and T6P Levels

SbCN2 (Sb04g021650.1), a homolog of TFL1 (ZCN2, OsRCN2), was expressed at approximately 34-fold higher levels in phyB-1 buds at 6 DAP, and 86-fold higher levels compared to wild-type buds by 8 DAP (Fig. 4). TFL1’s role in preventing floral induction is well known (Lifschitz et al., 2014) and expression of ZCN2 (TFL1/SbCN2) was previously reported in axillary buds of maize (Danilevskaya et al., 2010). Elevated expression of SbCN2 in buds of phyB-1 plants indicates that in the absence of phyB-signaling, increased expression of TFL1 enhances the barrier to floral induction. Expression of SbCN4 (ZCN4, OsRCN4) is differentially up-regulated in phyB-1 buds starting at 8 DAP (Fig. 4). ZCN4 is expressed in maize root apical regions (Danilevskaya et al., 2010). The increase in expression of this gene in phyB-1 at 8 DAP may indicate that nascent root meristems of the bud have developed and require protection from premature differentiation.

Several additional genes that regulate the transition from the vegetative to reproductive phase were differentially expressed to a limited extent in phyB-1 buds (Fig. 4). Genes that promote flowering such as LEAFY3-like (LFY3, Sb06g027340.1) and flowering promoting factor1 (FPF1; sb03g044960.1) were expressed at 2-fold higher levels in phyB-1 buds. In addition, two splice variants of a gene similar to the Arabidopsis MADS-box, SHORT VEGETATIVE PHASE (Sb01g044810.1 and Sb01g044810.2) that encodes a repressor of flowering (Hartmann et al., 2000), were expressed at 2-fold higher levels in phyB-1 buds at 7 DAP and 8 DAP. The expression of APETALA1-like (Sb02g038780.1) was reduced approximately 3-fold in phyB-1 buds at 8 DAP. The expression of the APETALA1-like and LFY3 is repressed by TFL1 in different domains of the Arabidopsis shoot apical meristem (Conti and Bradley, 2007).

Gibberellin (GA) plays an important role in regulating floral transition (Sun, 2008) and many other aspects of growth and development (Claeyts et al., 2014). GA biosynthesis/turover, signaling, and expression of GA-responsive genes have been associated directly or indirectly with variation in shoot branching (Lo et al., 2008; Mauriat et al., 2011; Ni et al., 2015). Genes encoding GA2ox1 were expressed at 7-fold to 18-fold higher levels in phyB-1 buds compared to wild-type at all time points examined (Fig. 4). Elevated expression of GA2ox1 genes in phyB-1 buds indicates that GA levels are maintained at low levels in the absence of phyB-signaling, creating an additional barrier to floral induction and growth.

Reduction in trehalose-6-phosphate inhibits flowering and growth (Schluemmann et al., 2003; van Dijken et al., 2004; Delatte et al., 2011; Wahl et al., 2013). Therefore, elevated expression of a TPP1-like gene in phyB-1 buds at 6 DAP (9-fold) through 8 DAP (34-fold) compared to buds of wild-type plants could impact bud physiology (Fig. 4). Other members of the TPP and TPS gene family were also differentially expressed in phyB-1 versus wild-type buds, but to a lesser extent and for most, starting after 6 DAP (Fig. 4).

Additional Genes Differentially Expressed More Than 5-Fold in phyB-1/Wild-Type Buds at 6 DAP

Genes with diverse and in some cases, unknown functions also showed high levels of differential expression in phyB-1 buds at 6 DAP through 8 DAP.
For example, genes encoding an F-box protein and VQ-motif protein are expressed approximately 80-fold and approximately 30-fold higher in phyB-1 buds at 6 DAP. The gene family encoding F-box proteins is very large (>600; Xu et al., 2009) and these proteins function in numerous pathways including hormone signaling and Glc-delayed seed germination (Song et al., 2012). Proteins with VQ-motifs interact with WRKY-transcription factors and MAP-kinases (Weyhe et al., 2014) and regulate seedling deetiolation (Li et al., 2014), endosperm growth (Wang et al., 2010), and responses to biotic and abiotic stress (Weyhe et al., 2014). A gene encoding an AAP3-like gene showed consistently higher expression in buds of phyB-1 (Supplemental Fig. S6). AAP3 amino-acid permeases are located in the plasmalemma and import amino acids from the apoplast into the phloem (Okumoto et al., 2004), but the exact role and localization of the bud-expressed member of the AAP3 gene family is unknown. Genes encoding a haloacid dehalogenase-like hydrolase and UDP-glucosyl transferase 85A2 were both expressed at higher levels in buds of phyB-1. The gene encoding UDP-glucosyl transferase 85A2 was expressed at approximately 7-fold higher levels in phyB-1 buds at 6 DAP, and at 19-fold and 55-fold higher levels at 7 DAP and 8 DAP, respectively (Supplemental Fig. S6). This enzyme mediates synthesis of DIBOA-glucoside, a compound involved in plant defense (Dick et al., 2012). DIBOA-glucoside is cleaved to form DIMBOA by a plastid-localized beta-glucosidase. A gene (sb08g007610) encoding a plastid beta-glucosidase that may be involved in DIMBOA synthesis was expressed at approximately 9-fold lower levels in phyB-1 at 6 DAP. DIMBOA is a compound used by plants for defense against herbivores; therefore, increased expression of this gene may be to protect buds from pests/pathogens (Frey et al., 1997; Dick et al., 2012). Genes encoding CTP-synthase, vacuolar iron transporter, and heat shock proteins are expressed at lower levels in phyB-1 buds compared to wild type at 6 DAP. Reduced expression of these genes may be a consequence of growth inhibition.

Differential Gene Expression in phyB-1 Buds Beginning at 7/8 DAP

DRMI is a marker for the bud dormancy program (Kebrom et al., 2006). After arrest of bud growth at 6 DAP in phyB-1, this gene is differentially induced.
approximately 3-fold at 7 DAP and approximately 6-fold by 8 DAP (Fig. 2). Numerous genes with expression kinetics similar to DRM1 in phyB-1 buds were identified (i.e. DIN1), including genes encoding transcription factors previously shown to regulate bud outgrowth that will be described below (Kebrom et al., 2006, 2010, 2012). Increased expression of ASN (Sbo1g038460; approximately 5–8-fold) and decreased expression of a gene encoding asparaginase (Sbo6g030910; 51-fold) is consistent with increased N-storage/decreased amino-acid utilization in growth-inhibited phyB-1 buds at 8 DAP (Fig. 5). In addition, several SbENOD93-like genes were expressed at 6-fold to 90-fold lower levels in phyB-1 buds compared to wild-type starting at 7 DAP (Fig. 5). Expression of ribosomal protein genes in buds of the two genotypes was similar at 6 DAP; however, transcript levels of several nuclear genes encoding plastid ribosomal proteins decreased approximately 2- to 3-fold in phyB-1 compared to wild-type buds by 8 DAP (Supplemental Fig. S7). In pea and Arabidopsis, the expression of genes encoding ribosomal protein genes is lower in dormant buds (Devitt and Stafstrom, 1995; González-Grandío et al., 2013).

Differential Expression of Genes during Wild-Type Bud Outgrowth at 7/8 DAP

Axillary buds of wild-type continue growing from 6 DAP to 8 DAP and will escape dormancy and form a tiller under the growth conditions used in this study. Increased expression of an MtN3 gene that encodes a SWEET sugar transporter between 6 DAP and 7 DAP in wild-type plants could help supply Suc from the phloem of the main plant for continued growth and development of buds (Fig. 6). Increased expression of two genes (Sb0067s002110.1 and Sb0067s002130.1) encoding cell wall invertases (cwINV) in buds of wild-type between 6 DAP and 7 DAP could cleave Suc unloaded into the apoplast to Glc and Fru for uptake by developing buds (Fig. 6; Sherson et al., 2003; Weschke et al., 2003). A gene encoding an RNase T2 protein expressed at low levels in both phyB-1 and wild-type buds at 6 DAP, was up-regulated greater than 10-fold in growing buds of wild-type at 7 DAP and 8 DAP (Supplemental Table S2). RNase T2 could be involved recycling phosphate required for outgrowth (MaIntosh et al., 2010; Hillwig et al., 2011).

Differential Expression of Genes Encoding Transcription Factors

Transcriptome analysis revealed a large number of genes encoding transcription factors that are induced or repressed in phyB-1 buds relative to wild-type with varying time courses (Fig. 7). Genes differentially expressed by 6 DAP included EEL, LSH3, GT1, protodermal factor1, HB1, and a WRKY transcription factor (Fig. 7). EEL (DPBF4/AtbZIP11) and ABI5 are bZIP transcription factors that interact with G-box motifs and regulate genes involved in the biosynthesis of Asn- and Asp-derived amino acids in response to sugar levels/ABA (Ufaz et al., 2011). Expression of EEL/DPBF4/AtbZIP12 was approximately 8-fold higher at 6 DAP and 16-fold higher in buds of phyB-1 at 8 DAP (Fig. 7). Sorghum genes homologous to LSH3 (Sbo2g002650.1), a gene involved in shoot organ boundary formation (Cho and Zambrayski, 2011), and protodermal factor1 (Sb04g008270.1), were expressed at 5- to 16-fold higher levels in buds of phyB-1 plants. SbgT1 RNA was 2-fold higher in buds of phyB-1 at 6 DAP and accumulated further by 8 DAP resulting in 3- to 5-fold differential expression relative to wild type (Kebrom et al., 2006, 2010; Whipple et al., 2011). SbTB1

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Figure 5. Gene expression associated with induction of bud dormancy identified by RNA-seq in buds in the first leaf axil of phytochrome B mutant (phyB-1) sorghum relative to wild-type (PHYB) controls at 6, 7, and 8 DAP. Boxes with green color indicate genes expressed at higher levels in buds of phyB-1/wild-type, and red color indicates genes expressed at lower relative levels in phyB-1 buds. Values in boxes are fold-differences in expression in phyB-1 relative to wild-type controls, and represent the mean of three biological replicates with a corresponding FDR less than 5%. Blank boxes represent values less than 2-fold and/or a corresponding FDR greater than 5%.
Using qRT-PCR, SbTB1 RNA levels were detected at approximately 2-fold higher in phyB-1 buds relative to wild type (Supplemental Fig. S8). Expression analysis via qRT-PCR utilized cDNAs prepared using random hexamer priming. In contrast, RNA-seq libraries were prepared from mRNA purified using poly-T oligo-attached magnetic beads. Similarly, tb1 was not detected in cDNA prepared from two-week-old maize plants using T7 Oligo (dT) primers (Vega-Arreguín et al., 2009). Therefore, it is possible that tb1 transcripts in maize and sorghum lack polyA tracts long enough for binding to the oligo(dT) primers used to prepare cDNA.

The sorghum AtHB1-like gene (Sb04g029080.2) was expressed at lower levels in phyB-1 buds at 6 DAP (4-fold), 7 DAP (12-fold), and 8 DAP (15-fold). Transgenic expression of Arabidopsis AtHB1 in tobacco indicated a role in leaf development (Aoyama et al., 1995). Arabidopsis AtHB1 is regulated by phytochrome/PIF1 signaling and also plays a role in activating hypocotyl elongation (Capella et al., 2015). Therefore, down-regulation of SbHB1 in phyB-1 buds is consistent with growth arrest and bud dormancy. A gene encoding a WRKY transcription factor (Sb04g33240) was also expressed at lower levels in phyB-1 buds from 6 DAP to 8 DAP, and expression of a bZIP transcription factor (bZIP44, Sb07g01540.1) was reduced in phyB-1 buds at 7 DAP and 8 DAP (Fig. 7). Six genes encoding NAC domain-containing proteins, including genes similar to the Arabidopsis ANAC029, showed increased expression in phyB-1 buds at 8 DAP (Fig. 7). The expression of the Arabidopsis ANAC029 is associated with leaf senescence (Guo and Gan, 2006). Several homeobox transcription factors (WOX, Knotted-like, and Bell) were differentially expressed in phyB-1 buds at 7/8 DAP (Fig. 7). These transcription factors function in meristem maintenance and leaf development.

Differential Expression of Genes Involved in Plant Hormone Metabolism and Signaling

Plant hormones play a key role in regulating bud outgrowth, and variation in hormone level or signaling could be causing some of the differences in gene expression observed in buds (Beveridge et al., 2009; Müller and Leyser, 2011). While analysis of hormone transcripts were not detected by RNA-seq. However, using qRT-PCR, SbTB1 RNA levels were detected at approximately 2-fold higher in phyB-1 buds relative to wild type (Supplemental Fig. S8). Expression analysis via qRT-PCR utilized cDNAs prepared using random hexamer priming. In contrast, RNA-seq libraries were prepared from mRNA purified using poly-T oligo-attached magnetic beads. Similarly, tb1 was not detected in cDNA prepared from two-week-old maize plants using T7 Oligo (dT) primers (Vega-Arreguín et al., 2009). Therefore, it is possible that tb1 transcripts in maize and sorghum lack polyA tracts long enough for binding to the oligo(dT) primers used to prepare cDNA.

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pathways in seedling leaves and roots was beyond the scope of this study, we conducted an analysis of genes involved in hormone biosynthesis or signaling that are differentially expressed in phyB-1 and wild-type buds (Fig. 8). CKs promote bud outgrowth (Müller et al., 2015). The expression of a CK-responsive GATA transcription factor (Sb04g007750.1) similar to the Arabidopsis CGA1 (Chiang et al., 2012) was induced in wild-type buds by approximately 8- and 15-fold at 7 DAP and 8 DAP, respectively (Fig. 8, S9). CK levels in phyB-1 buds could be lower than wild-type due to elevated expression of a CK-deactivating gene, cytokinin oxidase/dehydrogenase1 (CKX1, Sb03g045410.1) in phyB-1 buds relative to wild-type controls from 6 DAP (2-fold) to 8 DAP (6-fold; Fig. 8, S9). In addition, four genes encoding CK-inducible type-A A response regulators (ARRs; D’Agostino et al., 2000) were expressed at 3- to 4-fold lower levels in phyB-1 buds as early as 6 DAP (Fig. 8). Expression of SbARR3 (Sb04g026750) decreased approximately 7- to 11-fold between 6 DAP and 8 DAP in buds of phyB-1 but not in wild type (Fig. 8, S9). The expression of sorghum SHY2/IAA3 (Sb01g005110.1) decreased in phyB-1 buds relative to wild-type controls between 7 DAP and 8 DAP (Fig. 8). Expression of SHY2/IAA3 in Arabidopsis roots that is induced by CKs (Dello Ioio et al., 2008) therefore reduced expression of SHY2/IAA3 in phyB-1 buds, which is consistent with lower CK levels in phyB-1 tiller buds relative to wild type.

Down-regulation of several genes encoding ACC oxidase1 that catalyze the last step of ethylene biosynthesis may reduce ethylene production in phyB-1 buds (Fig. 8). In particular, the expression of Sb03g026000.1

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Figure 8. Differential expression of genes involved in plant hormone metabolism and signaling identified by RNA-seq in buds in the first leaf axil of phytochrome B mutant (phyB-1) sorghum relative to the corresponding wild-type (PHYB) controls at 6, 7, and 8 DAP. A, CK; B, ethylene; C, ABA; D, auxin. Boxes with green color indicate genes that are expressed at higher levels in phyB-1/wild-type, and red color indicates genes expressed at lower relative levels in phyB-1. Values in boxes are fold-differences in phyB-1 relative to wild-type controls, and represent the mean of three biological replicates with a corresponding FDR less than 5%. Blank boxes or asterisks indicate values less than 2-fold and/or a corresponding FDR greater than 5%. **
was 10-fold lower in phyB-1 buds relative to the corresponding wild-type controls, at 7 DAP and 8 DAP. The expression of an ethylene-responsive protein-related gene, Sb04g032150.1, was 28.6-fold lower in phyB-1 buds at 8 DAP. AP2/EREBP-like genes, Sb01g045060.1 and Sb10g005520.1, were also down-regulated in phyB-1 buds. The expression of the sorghum orthologs of Arabidopsis AP2/EREBP genes (At1g72360 and At1g25190) is induced by ethylene (Yang et al., 2011; Zhang et al., 2011). Therefore, down-regulation of AP2/EREBP and ethylene-responsive genes in phyB-1 buds could be due to decreased expression of several ACC oxidase1 genes involved in ethylene biosynthesis.

Inhibition of bud outgrowth by FR in Arabidopsis has been linked to an increase in the expression of ABA biosynthesis and signaling genes and ABA level (González-Grandío et al., 2013; Reddy et al., 2013). Analysis of differentially expressed genes in phyB-1/wild-type buds identified several changes in gene expression related to ABA synthesis or signaling between 6 DAP and 8 DAP (Fig. 8). A gene similar to the Arabidopsis aldehyde oxidase (AAO2) that catalyzes the conversion of abscisic aldehyde into ABA (Leydecker et al., 1995; Seo et al., 2000) was reduced approximately 4- to 6-fold in phyB-1 buds relative to the corresponding wild-type controls at all time points. A gene encoding abscisic acid 8′-hydroxylase (Sb02g026600.1) that de-activates ABA, and a protein phosphatase 2C (ABP1, Sb09g022410.1) that is a negative regulator of ABA signaling (Gosti et al., 1999; Bensmihen et al., 2002), were also expressed at a higher levels in phyB-1 buds. However, the expression of two ABA-inducible genes, Sb01g021730.1 (mediator of ABA-regulated dormancy1; He and Gan, 2004) and Sb05g017940.1 (HVA22-like; Shen et al., 2001), was higher in phyB-1 buds relative to the corresponding wild-type controls. A NAC domain-containing transcription factor (Sb03g041920.2) homologous to Arabidopsis AtAF1 (ANAC002) was up-regulated by about 18.6-fold in phyB-1 buds at 8 DAP. The AtAF1 gene in Arabidopsis increases ABA level by directly up-regulating the expression of the ABA biosynthesis gene NINE-CIS-EPoxyCAROTENOID DIOXYGENASE3 (NCED3; Jensen et al., 2013). NCED3 was among ABA-related genes up-regulated in Arabidopsis buds grown under low R/FR and down-regulated at high R/FR (Reddy et al., 2013). The expression of the sorghum ortholog of Arabidopsis NCED3, Sb01g013520, was slightly elevated in phyB-1 buds at 8 DAP, but not significantly different from the expression level in wild-type buds (Fig. 8).

Relatively few genes involved in SL and auxin responses were differentially expressed in phyB-1/wild-type buds. SbMAX2 showed 2-fold higher expression in phyB-1 buds at 8 DAP as previously reported. Supplemental Table S2. The auxin-responsive SAUR genes and dwarf in light1 (DFL1) were differentially expressed to some extent (Fig. 8). The DFL1 gene, a homolog of G3, inhibits cell elongation and lateral root formation in light in response to auxin (Nakazawa et al., 2001). Expression of two DFL1-like genes (Sb03g036680.1 and Sb09g024710.1) increased in buds of phyB-1 at 8 DAP (Fig. 8).

DISCUSSION

Auxiliary meristems are initiated in leaf axils and develop into tiller buds that can grow into tillers or become dormant in nonpermissive growth conditions (Fig. 9). Research on bud outgrowth has identified numerous factors that promote outgrowth such as CKs, sugars, and phyB-signaling, and inhibitors such as SL, auxin, and ABA (Beveridge et al., 2009; Müller and Leyser, 2011; Kebrom et al., 2013; Janssen et al., 2014; Wang and Wang, 2015; Yuan et al., 2015; Barbier et al., 2015a). This study focused on early steps in bud development that lead to growth arrest and dormancy in phyB-1 or outgrowth in wild-type seedlings (Fig. 9). Bud auxillary meristem formation and growth were similar in phyB-1 and wild-type until 6 DAP, when bud growth differentially arrests in phyB-1 (Fig. 9). Dormant buds of phyB-1 plants can grow out after floral initiation, indicating that bud growth arrest is reversible. A similar seedling growth checkpoint, mediated by ABI5/ABA, occurs during the first 3 d after seed germination (Lopez-Molina et al., 2001). Differences in gene expression in axillary buds of phyB-1 and wild-type at 6 DAP could contribute to growth arrest of buds in phyB-1 (Fig. 9, lower left). After growth arrest, buds of phyB-1 transition into dormancy, a developmental process associated with further changes in gene expression between 6 DAP and 8 DAP (Fig. 9, lower right). In contrast, in the wild-type, tiller growth and development continues between 6 DAP and 8 DAP leading to tiller outgrowth (Fig. 9, top). Analysis of wild-type bud transcriptomes during this interval identified increased expression of MtN3, encoding a SWEET transporter, and two cslNVS as key events during tiller outgrowth (Fig. 9, top right). After 9 DAP, developing tillers of wild-type plants no longer respond to shade signals by inhibiting tiller growth, but instead accelerate growth in a manner similar to the shade avoidance response of the main shoot (Kebrom et al., 2006). Thus, the interval from 6 DAP to 8 DAP corresponds to a transition stage when bud growth occurs in the wild type but can still be inhibited by shading, and bud dormancy development occurs in growth-arrested buds of phyB-1. Since phyB-1 plants lack phyB-signaling from leaves, leaf sheaths, and buds from germination through 8 DAP, differences in bud gene expression in phyB-1/wild-type could be a direct (in buds) or indirect (via leaf phyB-signaling) consequence of lack of phyB in phyB-1 seedlings. Analysis of leaf transcriptomes of phyB-1/wild-type during this same phase of development is planned to investigate the possible role of leaf-derived phyB-signaling that could modulate bud growth and development. In addition, while this study identified a large number of phyB-regulated changes in gene expressions that are associated with bud


growth and development. In addition, while this study identified a large number of phyB-regulated changes in gene expressions that are associated with bud
development, phyB is also known to regulate gene expression at posttranscriptional levels.

**Maintenance of Bud Vegetative Meristems in phyB-1 Sorghum**

Elevated expression of *TFL1*, *GA2oxidases*, and *TPPI* in buds of *phyB-1* compared to wild-type plants could result in enhanced protection of axillary buds from floral transition. Expression of *TFL1* (*SbCN2*) was approximately 30-fold higher in buds of *phyB-1* plants at 6 DAP and approximately 80-fold higher by 8 DAP. *TFL1* is a member of the phosphatidylinositol ethanolamine-binding protein gene family, and members of this family in Arabidopsis include *FLOWERING LOCUS T*, MOTHER OF FT AND TFL1 (MFT), and CENTRORADIALIS homolog (ATC), genes that activate flowering (Karlgren et al., 2011). *TFL1*-like genes encode proteins that are expressed in meristems and bind to leaf-derived FT to prevent FT-induced flowering (Lifschitz et al., 2014). The *AadTFL1* in *Arabidopsis alpina* prevents young shoots from transitioning into flowering, thereby maintaining a perennial growth habit (Wang et al., 2011). The indeterminate growth habit is lost in *TFL1* mutants of *Arabidopsis* and tomato (*SELF-PRUNING*; Bradley et al., 1996; Pnueli et al., 1998).

*TFL1* is expressed in the shoot apical meristem and axillary meristems of *Arabidopsis* (Conti and Bradley, 2007), maize (Danilevskaya et al., 2010), and sorghum. The proposed function of elevated expression of the *TFL1*-like gene *SbCN2* in *phyB-1* sorghum tiller buds is to protect these vegetative axillary meristems from florigenic signals in a manner similar to perennial plants. In annual crops, such as wheat, the transition from vegetative to the reproductive phase converts all apical and axillary meristems from vegetative to inflorescence meristems (Hay and Kirby, 1991; Evers et al., 2006; Kebrom et al., 2012). In perennial plants, vegetative meristems in axillary buds do not transition to flowering before forming a vegetative shoot during the next growth cycle, which ensures a perennial growth habit (Rohde and Bhalerao, 2007). Grain sorghum is classified as weakly perennial (Dewet and Huckabay, 1967), but sorghum germplasm can exhibit a wide range of annual to perennial growth habits. *Sorghum* and maize florigens are produced in leaves by *ZCN8/ SbCN8* and *ZCN12/SbCN12* (Danilevskaya et al., 2010; Murphy et al., 2011). Flowering in sorghum is inhibited in long day photoperiods by repressing the expression of *SbCN8* and *SbCN12* through a pathway that requires phyB-signaling (Murphy et al., 2011; Yang et al., 2014). Therefore, *phyB-1* or shaded plants have greater propensity to produce FT, and increased expression of *TFL1* (*SbCN2*) in axillary buds of these plants may be needed to prevent precocious floral transition.

Genes encoding *GA2ox1* were expressed at approximately 7- to 18-fold higher levels in buds of *phyB-1* plants. Elevated expression of these genes is expected to reduce GA levels and increase DELLA activity in *phyB-1* buds. GA is an important regulator of meristem development (Ubeda-Tomas and Bennett, 2010), growth, and flowering (Xu et al., 2014). GA levels are elevated in leaves of *phyB-1* mutants and shade-treated plants in diverse species (Rood et al., 1990; Lee et al., 1998; Hisamatsu et al., 2005). Therefore, increased expression of genes encoding *GA2ox1* in *phyB-1* buds may be required to prevent GA-mediated growth and floral promotion. In maize, rice, and Arabidopsis, *GA2oxidase* is expressed around the subapical region of the shoot apical meristem (SAM) during the vegetative phase and

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**Figure 9.** A summary of patterns of differential expression of genes associated with bud outgrowth or dormancy in phytochrome B null (*phyB-1*, 58M, red) and wild-type (*PHYB*, 100M, green) sorghum genotypes at 6, 7, and 8 DAP. The bud in wild type continues growing and develops into a tiller whereas the growth of the bud in the *phyB-1* is arrested and becomes dormant. Symbols “>” and “>” indicate up-regulated and dramatically up-regulated genes, respectively; “<” and “<” indicate down-regulated and dramatically down-regulated genes, respectively. Arrows indicate the timing of differential expression of genes. Genes with a short arrow are differentially expressed at 8 DAP; genes with a long arrow are differentially expressed at 7 and 8 DAP; and genes without an arrow, such as the *Peter pan*, *HB1*, etc., are differentially expressed at 6, 7, and 8 DAP.

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**Table 9.** Summary of patterns of differential expression of genes associated with bud outgrowth or dormancy in phytochrome B null (*phyB-1*, 58M, red) and wild-type (*PHYB*, 100M, green) sorghum genotypes at 6, 7, and 8 DAP. Symbols “>” and “>” indicate up-regulated and dramatically up-regulated genes, respectively; “<” and “<” indicate down-regulated and dramatically down-regulated genes, respectively. Arrows indicate the timing of differential expression of genes. Genes with a short arrow are differentially expressed at 8 DAP; genes with a long arrow are differentially expressed at 7 and 8 DAP; and genes without an arrow, such as the *Peter pan*, *HB1*, etc., are differentially expressed at 6, 7, and 8 DAP.
deactivates GA derived from young leaves and leaf primordia, thereby protecting the SAM from precocious transition into flowering (Sakamoto et al., 2001; Jasinski et al., 2005; Bolduc and Hake, 2009). The expression of GA2oxidase at the base of the SAM in maize is maintained in part by KNOX and BELL transcription factors (Bolduc and Hake, 2009). The expression of the Knox and BELL genes was 2- to 4-fold higher in the phyB-1 buds at 8 DAP, which may enhance or maintain elevated expression of GA2oxidases. The FPF1 mediates GA3-induced flowering (Kania et al., 1997). FPF1 and LFY3, genes that promote flowering, are expressed at 2- to 3-fold higher levels in buds of phyB-1 plants. These genes are also up-regulated in apical meristems induced to flower by shading or inductive photoperiods that would reduce phyB-signaling (Blázquez and Weigel, 1999). Therefore, increased expression of TFL1 and genes encoding GA2ox1 in phyB-deficient plants may help antagonize the floral-promoting effects of these genes. GA2ox1-mediated reduction in GA level and elevated DELLA activity in phyB-1 buds may have additional functions related to bud growth arrest and induction of dormancy (Choubane et al., 2012).

**Growth Arrest and Maintenance of Cell Cycle Gene Expression in phyB-1 Buds**

The molecular mechanism causing the arrest of bud growth in phyB-1 is unknown, but strongly reduced expression of a gene homologous to Peter pan may be involved. The sorghum genome encodes two Peter pan-like genes and expression of one of the Peter pan-like genes was reduced by approximately 100-fold in buds of phyB-1 compared to wild-type. Peter pan was first identified as a gene required for larval growth in *Drosophila* (Migeon et al., 1999). Down-regulation of both Peter pan genes (SSF1 and SSF2) in yeast leads to cell division arrest (Yu and Hirsch, 1995). In *Xenopus laevis*, ppan and pse1 interact and regulate ribosome biogenesis, cell proliferation, apoptosis, and gene expression (Tecza et al., 2011). Expression of PPAN and PES1 in developing pronephros is dependent on wtnt4 and fzd3 function, key regulators of cell growth and cell cycle progression (Tecza et al., 2011; Pfister et al., 2015). While the function of Peter pan has not been examined in plants, strong down-regulation of this gene in growth-inhibited buds of phyB-1 plants at 6 DAP, suggests that low Peter pan expression may contribute to inhibition of growth and cell division in buds of these plants.

Transcriptome analysis showed similar levels of cell cycle gene expression in buds of phyB-1 and wild-type plants at 6 DAP, consistent with prior analysis (Kebrum et al., 2010). Continued expression of cyclins and cyclin-dependent protein kinases indicates the cell division machinery in growth-arrested buds of phyB-1 is on standby until at least 8 DAP, similar to cambial meristem dormancy in hybrid aspen (Espinosa-Ruiz et al., 2004). Reduced expression of a sorghum homolog of *AtE2F3* in phyB-1 buds starting at 8 DAP could contribute to further inhibition of cell division as part of the bud dormancy program. Cell cycle gene expression was decreased in Arabidopsis buds inhibited by shade signals in a BRC1-dependent manner (González-Grandío et al., 2013). Over-expression of *AtE2FA* in Arabidopsis induces mature leaf cells to reenter the cell cycle (Rossignol et al., 2002), and *AtE2FA* silencing reduces cotyledon and root growth and cell proliferation (Magyar et al., 2012). Decapitation increases cell cycle and ribosomal protein gene expression in dormant buds of Arabidopsis and pea and stimulates bud outgrowth (Devitt and Stafstrom, 1995; Tatematsu et al., 2005). Taken together, growth arrest at 6 DAP is not associated with decreased expression of genes involved in the cell cycle, but reduced expression of a sorghum homolog of *AtE2FA* at 8 DAP and elevated expression of *SbTB1* in phyB-1 buds may lead to reduced expression of genes involved in the cell cycle as the dormancy program becomes more fully established after 8 DAP.

**PhyB-Regulated TPP Expression—Possible Impact on Flowering, Growth, and Sugar-Signaling**

The dormancy-versus-growth fates of axillary buds in diverse species have been linked to the level of Suc/sugar-signaling and to changes in the expression of Suc-responsive genes in buds (Kebrum et al., 2012; Rabot et al., 2012; Kebrum and Mullet, 2014; Mason et al., 2014). Sugar signaling occurs through pathways involving hexokinase, trehalose-6-phosphate/SnRK1, and TOR, and by direct effects of sugars on growth and metabolism (Rolland et al., 2006; Lunn et al., 2014; Ruan, 2014). In this study, transcriptome analysis revealed that the trehalose phosphate phosphatase TPPI-like gene was expressed at 9-fold higher levels in buds of phyB-1 at 6 DAP and 34-fold higher at 8 DAP. Other members of the TTP/TPS gene family were differentially expressed, including a 2-fold to 8-fold increase in the expression of five class II TPS genes in phyB-1 buds at 8 DAP. The class II TPS genes do not have TPS or TPP activities (Lunn et al., 2014). The expression of the Arabidopsis and maize class II TPS genes is induced by Suc starvation (Henry et al., 2014; Yadav et al., 2014).

Trehalose-6-phosphate is required for growth (Schulmeiann et al., 2003) and flowering (van Dijken et al., 2004; Wahl et al., 2013) and can modify inflorescence branching (Satoh-Nagasawa et al., 2006), among other functions (Vandesteene et al., 2012). *Ramosa3*, a member of the TTP-gene family in maize, is expressed in domains subtending axillary inflorescence meristems, and mutations in *Ramosa3* increase the number of long branches in tassels (Satoh-Nagasawa et al., 2006). Loss of TTP activity in tassal meristems is thought to increase T6P levels, thereby promoting development of axillary branches (Tsai and Gazzarrini, 2014). Arabidopsis plants over-expressing TTP showed...
increased apical dominance. Plants lacking TPS activity develop slowly and are arrested at the torpedo stage (Schluempmann et al., 2003). Plants with reduced TPS activity and low T6P levels also showed delayed flowering (Wahl et al., 2013). Furthermore, T6P levels become elevated in plants that accumulate Suc and high levels of T6P inhibit SnRK1, a central regulator of growth and metabolism under low energy/sugar conditions (Ghillebert et al., 2011; Tsai and Gazzarrini, 2014). Low T6P levels signal a low sugar metabolic state, increasing SnRK1 activity and inhibiting the use of sugars for growth (Eastmond et al., 2003; Schluempmann et al., 2003).

Elevated expression of TPPI in phyB-1 buds relative to wild type at 6 DAP is not correlated with changes in expression of the sugar-responsive ASN1 and TP5-genes, because these genes show differential expression starting at 7 DAP (TPS11) or 8 DAP (ASN1, TP55, TPS9). Expression of ASN1 is increased in buds of tiller inhibition tin mutant wheat and defoliated wild-type sorghum plants (Kebrom et al., 2012; Kebrom and Mullet, 2014). ASN1 expression is also elevated in darkness under conditions of low sugar status (Fujiki et al., 2001). Therefore, increased TPPI expression in phyB-1 buds at 6 DAP is more likely a consequence of reduced phyB-signaling. Increased trehalose phosphate phosphatase activity could reduce T6P levels, thereby contributing to bud growth inhibition and reducing the propensity of buds to undergo floral transition.

Gene Expression Associated with Induction of Dormancy in phyB-1 Buds

Induction of bud dormancy involves coordinated inhibition of growth of nascent leaves, roots, and other tissues; altered metabolism; and the creation of barriers to outgrowth. Previous studies have identified TB1 (BRCI), GT1, and MAX2 as key regulators that increase bud resistance to outgrowth (Kebrom et al., 2006, 2010; Whipple et al., 2011). These genes also help mediate bud growth inhibition caused by SL (Aguilar-Martínez et al., 2007; Finlayson, 2007; Braun et al., 2012). Elevated and differential expression of GT1 (approximately 3- to 5-fold from 6 to 8 DAP), TB1 (approximately 2- to 3-fold from 6 to 8 DAP), and MAX2 (2-fold at 8 DAP) was observed in phyB-1 buds in this study. Elevated expression of these genes in buds of phyB-1 plants is consistent with prior studies showing that TB1, MAX2, and GT1 expression is regulated by phyB-signaling (Kebrom et al., 2006; Shen et al., 2007; Finlayson et al., 2010; Kebrom et al., 2010; Whipple et al., 2011), and that demonstrated a role for these genes as inhibitors of bud outgrowth (Doebely et al., 1995, 1997; Stirmberg et al., 2002; Takeda et al., 2003; Aguilar-Martínez et al., 2007; Finlayson, 2007; Lewis et al., 2008; Whipple et al., 2011).

Reduced expression of sorghum genes homologous to HB1, WRKY, bZIP44, ENOD93, and ACCoxidase in buds of phyB-1 may also help coordinate growth arrest and the induction of bud dormancy. AtHB1 regulates increased hypocotyl growth in response to light signaling through PIPI1 (Capella et al., 2015). ENOD93 genes are involved in nodule development, and these genes are expressed in vascular bundles as well as the epidermis (Bi et al., 2009; Yan et al., 2015). Decreased expression of ENOD93 may inhibit the development of the vascular system in phyB-1 buds. Seeds of Arabidopsis bZIP44 mutants germinate at a reduced rate and bZIP44 promotes germination by enhancing the expression of endo-beta-mannanase (AtMAN7) that softens tissues surrounding the embryo (Iglesias-Fernández et al., 2013). Therefore, down-regulation of bZIP44 in phyB-1 buds might lead to hardening of leaves surrounding axillary meristem. Decreased expression of genes encoding ACC oxidases may reduce the level of ethylene in phyB-1 buds. Because ethylene biosynthesis increases in growing young leaves and leaf primordia (Hunter et al., 1999), a low level of ethylene in the phyB-1 bud might be associated with suspension of the growth of leaf primordia and young leaves enclosing the bud meristem.

Transcriptome analysis indicated that cytokinin levels and expression of genes involved in CK-signaling are reduced in buds of phyB-1 plants. Higher expression of a gene encoding CKX1 in phyB-1 buds may reduce bud cytokinin levels since transgenic expression of cytokinin oxidases/dehydrogenase (CKX) reduces CK-levels (Werner et al., 2001; Galuszka et al., 2004). In addition, genes encoding several type-A response regulators (ARR3/6/9) were expressed at lower levels in phyB-1 compared to wild-type buds. CKs stimulate bud outgrowth in pea when directly applied to the bud (Pillay and Railton, 1983) and decapitation-induced bud outgrowth in chickpea was associated with an increase in the level of CKs (Turnbull et al., 1997). Therefore, reduced expression of genes involved in CK signaling in phyB-1 buds may increase resistance to bud outgrowth.

The transition of buds in phyB-1 into dormancy was associated with an increase in the expression of NAC-domain proteins and dormancy-associated auxin repressed/stress-related genes at 7/8 DAP (Fig. 7). The NAC domain-containing proteins are involved in the regulation of stress tolerance (Puranik et al., 2012). Inhibition of bud outgrowth by shade signals in Arabidopsis is associated with increased expression of genes involved in ABA biosynthesis/response in plants (González-Grandío et al., 2013; Reddy et al., 2013). Few genes in the ABA pathway were differentially expressed at 6 DAP. However, by 7/8 DAP, several genes involved in ABA metabolism and signaling were expressed at higher levels in buds of phyB-1 [ABI1, CYP707A4 (ABA 8'-hydroxylase), mediator of ABA-regulated dormancy1, HVA22, AtAFL, NCED3]. For example, expression of an AtAFL-like gene (Sb03g041920.2) that induces NCED3 expression (Jensen et al., 2013) increased approximately 19-fold in phyB-1 buds at 8 DAP (Fig. 8). Therefore, it is likely that ABA levels increase in phyB-1 buds at later stages of bud dormancy, consistent with prior studies showing a role for ABA in
shade-induced bud dormancy (González-Grandío et al., 2013; Reddy et al., 2013).

**Induction of Genes for SWEET Sugar Transport and cwINVs during Bud Outgrowth**

The expression of genes encoding an MtN3/SWEET-transporter and cwINV was very low in phyB-1 and wild-type buds at 6 DAP, but strongly (>50-fold) and differentially increased in wild-type buds at 7/8 DAP. MtN3 encodes a SWEET transporter, a member of a family of Suc/sugar transporters that are involved in phloem sugar loading/unloading and efflux of sugars from maternal tissues for seed filling and pollen development (Chen et al., 2012; Guo et al., 2014; Chen et al., 2015). CwINVs cleave apoplastic Suc into Glc and Fru for import into cells (Sherson et al., 2003; Weschke et al., 2003). Increased expression of the SWEET transporter in the phloem could facilitate unloading of Suc into the apoplasm where cwINVs generate Glc and Fru for uptake by developing buds. The increase in Suc transport and cleavage into Glc and Fru in the bud apoplasm appears to be a key event in tiller development in wild-type plants. Aposlastic sugars provide feed-forward signaling to genes involved in the cell cycle, growth, and auxin signaling, and by increasing levels of T6P (Wang and Ruan, 2013). Increased expression of genes encoding an MtN3/SWEET transporter and cwINVs could increase the supply of sugars from the shoot for continued growth of buds of wild-type plants until growth can be sustained by tiller leaf photosynthesis. Mechanisms that regulate expression of the SWEET-gene family are largely unknown (Chen et al., 2015). The expression of genes that encode cwINVs can be induced by sugar (Huang et al., 2007). Therefore, it is possible that increased cwINV gene expression is a consequence of increased sugars supplied by leaves and/or from elevated SWEET activity. The expression of cwINVs is also induced by cytokinins (Ehness and Roitsch, 1997). Changes in gene expression indicate that the level of cytokinin increases in wild-type buds between 6 DAPS and 8 DAP because the CK-responsive genes CGA1 and SHY2/IAA3 are induced in buds during this time frame (Supplemental Fig. S9). In Arabidopsis, cytokinin acting through ARR1 increases SHY/IAA3 expression in root meristems (Peng et al., 2013). Increased expression of CGA1 mediates activation of genes involved in chloroplast development in rice (Hudson et al., 2013). Genes involved in chloroplast development including several genes encoding LHClII show higher expression in buds of wild-type plants by 8 DAP, as would be expected after increased expression of CGA1 (data not shown). The lack of induction of genes encoding a SWEET-transporter and cwINVs in buds of phyB-1 could be caused by several factors including elevated expression of CKX1 that degrades CK, with lower expression of ARR3/6/9 response factors that may mediate CK-induced bud outgrowth (Supplemental Fig. S9; Müller et al., 2015).

In both phyB-1 and wild-type plants, initiation of auxiliary meristems and growth of auxiliary buds in the first leaf axils was similar until 6 DAP (Fig. 9). It is likely that the buds in both genotypes were supplied with sugars symplastically through plasmodesmata at least until 6 DAP. Lack of phyB-signaling in phyB-1 plants resulted in the many large differences in bud gene expression by 6 DAP compared to the wild-type that could contribute to coordinated growth cessation, increased protection of bud meristems from floral transition, increased barriers to outgrowth, and induction of bud dormancy. In contrast, buds of wild-type plants continued to grow and showed increased expression of genes encoding a SWEET transporter and cwINVs at 7 DAP. The timing of this change in gene expression is critical and occurs during a transition stage from 6 to 8 DAP where bud growth can still be inhibited by shading (Kebrom et al., 2006). We propose that during the transition stage, increased expression of the SWEET transporter and cwINV creates an apoplastic supply of sugar stimulating further bud development that enables the acquisition of photosynthetic capacity and escape from dormancy-inducing factors.

**MATERIALS AND METHODS**

**Plant Materials and Growing Conditions**

Seeds of phyB-1 (also known as 58M) and near-isogenic phytochrome B (PHYB; wild type, also known as 100M) were grown in flats containing cells filled with growing mix prepared from peat moss, vermiculite, and perlite and fertilizers as described in Beall et al. (1991). The seedlings were grown in a growth chamber using 14/10 h light/dark periods, 31°C/22°C day/night temperatures and 50% relative humidity. The length of the bud in the first leaf axil of phyB-1 and wild type was measured under a dissecting microscope at 6, 7, and 8 d after planting (DAP).

**RNA-seq Library Preparation and Sequencing**

RNA-seq library was prepared from RNA isolated from three biological replicates of phyB-1 and wild-type axillary buds at 6, 7, and 8 DAP. Total RNA was isolated from at least six axillary buds per sample using the TRIZol method (Invitrogen, Carlsbad, CA). RNA-seq libraries were prepared using the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA), following the recommended protocol. Briefly, mRNA was purified from 400-ng total RNA using poly-T oligo-attached magnetic beads. The mRNA was eluted, fragmented, and primed for cDNA synthesis. After first- and second-strand synthesis, the cDNA was end-repaired, adenylated, and unique adapters were ligated to each sample and the DNA fragments were amplified before sequencing. The quality of each RNA-seq library was assessed using a bioanalyzer and sequenced using HiSeq2500 (Illumina) at the TX A&M Genomics and Bioinformatics Services Center (College Station, TX).

**RNA-seq Data Analysis and Bioinformatics**

The sequence reads were aligned with the sorghum transcript sequence Sbicolor-v2.1_255 (Phytozone, http://www.phytozone.net) using the CLC Genomics Workbench (CLC, Aarhus, Denmark). The Sbicolor-v2.1_255 includes 33,032 genes and 39,441 transcripts. To identify differentially expressed genes, RNA-seq datasets were analyzed using the Edge statistical test in the CLC Main Workbench Ver. 7 (CLC). Genes differentially expressed in axillary buds of phyB-1 relative to the corresponding wild-type controls at 6, 7, and 8 DAP were determined using the following criteria: a false discovery rate ≤ 0.05, and reads per kilobase per million ≥ 2 in the three biological replicates of either phyB-1 and/or corresponding wild-type control. Genes differentially expressed
(up- or down-regulated) at 2-fold or higher were included in the analysis. The function of most of the genes was annotated using MapMan (http://mapman.gabipd.org) based on their homologs in Arabidopsis and rice.

Analysis of Gene Expression by Quantitative Real-Time PCR
To analyze the expression of genes by quantitative real-time PCR (qRT-PCR), cDNAs were prepared from 400 ng of total RNA from three independent biological replicates for each sample. The cDNAs were suspended in 320 μL of 10 mM Tris–HCl (pH 8.3) and 50 mM KCl and reverse transcribed using SuperScript III (Invitrogen). The cDNA was suspended in 320 μL of 2× LIF SuperScript III Platinum qRT-PCR Reaction Mix (Life Technologies, Carlsbad, CA). The av-cDNAs were prepared from 400 ng of total RNA from three independent biological replicates for each sample. In addition to the cDNA template the qRT-PCR reaction included 2 μL of 250 ng primer pairs and 6 μL of 2× LIF SuperScript III Platinum qRT-PCR Reaction Mix (Life Technologies, Carlsbad, CA). The fold change between two samples was calculated using the slightly modified −ΔΔCt method as described in Kebrom et al. (2010). The primers for sorghum ortholog of the (SbTBI), SIMAX2, SbDRM1, SbPCNA, SbCycD2, SbCycE, and 18S rRNA used for qRT-PCR were published in Kebrom et al. (2010). The primers for SbGT1 were published by Whipple et al. (2011) and those for SbASN1 and SbFPP were published by Kebrom and Mullet 2014.

Accession Numbers
Supplemental Data
The following supplemental materials are available.
Supplemental Figure S1. Principal component analysis of RNA-seq data.
Supplemental Figure S2. qRT-PCR confirmation of RNA-seq results.
Supplemental Figure S3. Venn diagram showing the number of differentially expressed genes.
Supplemental Figure S4. The expression level of cell cycle-related genes determined by RNA-seq.
Supplemental Figure S5. MapMan display of cell division-, cell cycle-, and development-related genes.
Supplemental Figure S6. Differentially expressed genes (>5-fold).
Supplemental Figure S7. MapMan display of differentially expressed plastid ribosomal protein genes.
Supplemental Figure S8. Expression of SbTBI (tosiote branchedI) in buds.
Supplemental Figure S9. CK-related differentially expressed genes.
Supplemental Table S1. RNA-seq analyses of transcriptome dynamics.
Supplemental Table S2. List of MapMan-classified differentially expressed genes.
Supplemental Table S3. MapMan functional categorization of genes differentially expressed in buds.

Received January 7, 2016; accepted February 13, 2016; published February 18, 2016.

LITERATURE CITED


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Tuong SK, McCormick RF, Rooney WL, Mullie J (2015) Harnessing genetic variation in leaf angle to increase productivity of Sorghum bicolor. Genetics 201: 1229–1238


