KNOX1 genes regulate lignin deposition and composition in monocots and dicots

Brad T. Townsley
University of California - Davis

Neelima R. Sinha
University of California - Davis

See next page for additional authors

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Recommended Citation
Townsley, Brad T.; Sinha, Neelima R.; and Kang, Julie, "KNOX1 genes regulate lignin deposition and composition in monocots and dicots" (2013). Faculty Publications. 23.
https://scholarworks.uni.edu/bio_facpub/23

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INTRODUCTION
Lignin is a complex polymer and is a fundamental component of cell walls in higher land plants (Gifford and Foster, 1988). Lignin polymers are localized to secondary cell walls found primarily in the xylem (vessel elements and tracheids), phloem fibers, sclerenchyma, and periderm (Esau, 1965). The hardened thickenings of these secondary cell walls, where lignin is covalently attached to carbohydrate components, are necessary to maintain the structural integrity of the plant body. Lignin was a key innovation in the evolution of land plants, allowing for the development of these rigid support structures and robust vessels that are required for the transport of water and minerals throughout the organism. The lignified fibers provide structural support to the vascular system, which allowed for much larger body plans and colonization of a broader range of environments.

The primary driver of carbon-fixation and metabolism on Earth is photosynthesis and a significant majority of all terrestrial photosynthetic activity can be attributed to the angiosperms. The primary chemical forms of reduced carbon that are available from the products of photosynthesis are polysaccharides, proteins, and lipids. These macromolecules form the major structural and functional components of living plant cells. Plant secondary cell walls are deposited mostly in vascular tissues such as xylem vessels, tracheids, and fibers. These cell walls are composed of a complex matrix of compounds including cellulose, hemicellulose, and lignin. Lignin functions primarily to maintain the structural and mechanical integrity of both the transport vessel and the entire plant itself. Since lignin has been identified as a major source of biomass for biofuels, regulation of secondary cell wall biosynthesis has been a topic of much recent investigation. Biosynthesis and patterning of lignin involves many developmental and environmental cues including evolutionarily conserved transcriptional regulatory modules and hormonal signals. Here, we investigate the role of the class I Knotted-1-like homeobox (KNOX) genes and gibberellic acid in the lignin biosynthetic pathway in a representative monocot and a representative eudicot. Knotted1 overexpressing mutant plants showed a reduction in lignin content in both maize and tobacco. Expression of four key lignin biosynthesis genes was analyzed and revealed that KNOX genes regulate at least two steps in the lignin biosynthesis pathway. The negative regulation of lignin both in a monocot and in a monocot and a eudicot by the maize Kn1 gene suggests that lignin biosynthesis may be preserved across large phylogenetic distances. The evolutionary implications of regulation of lignification across divergent species are discussed.

Keywords: Knotted1, KNOX, gibberellic acid, lignin, maize, tobacco, tomato

Plant secondary cell walls are deposited mostly in vascular tissues such as xylem vessels, tracheids, and fibers. These cell walls are composed of a complex matrix of compounds including cellulose, hemicellulose, and lignin. Lignin functions primarily to maintain the structural and mechanical integrity of both the transport vessel and the entire plant itself. Since lignin has been identified as a major source of biomass for biofuels, regulation of secondary cell wall biosynthesis has been a topic of much recent investigation. Biosynthesis and patterning of lignin involves many developmental and environmental cues including evolutionarily conserved transcriptional regulatory modules and hormonal signals. Here, we investigate the role of the class I Knotted-1-like homeobox (KNOX) genes and gibberellic acid in the lignin biosynthetic pathway in a representative monocot and a representative eudicot. Knotted1 overexpressing mutant plants showed a reduction in lignin content in both maize and tobacco. Expression of four key lignin biosynthesis genes was analyzed and revealed that KNOX genes regulate at least two steps in the lignin biosynthesis pathway. The negative regulation of lignin both in a monocot and in a monocot and a eudicot by the maize Kn1 gene suggests that lignin biosynthesis may be preserved across large phylogenetic distances. The evolutionary implications of regulation of lignification across divergent species are discussed.

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suggest that BP might function to repress lignin biosynthetic enzymes thus suppressing lignin deposition (Mele et al., 2003). Therefore, the involvement of KNOX genes, such as BP, in controlling lignin biosynthesis strongly suggests that KNOX genes have the potential to be effective modulators of lignification in plant tissues. One regulatory mechanism by which KNOX genes can modulate development and downstream gene expression is through the regulation of small molecule hormones such as gibberellic acid (GA) and cytokinin (Rodriguez et al., 2010). Through this method of regulation, local KNOX gene expression can have cell non-autonomous effects on development. GA is a known regulator of lignin biosynthesis and morphogenesis (Luquita et al., 2009). Increasing the levels of bioactive GA leads to higher a decrease in plastochron index (Palatnik et al., 1990) resulting in increased nodal spacing. The effects on growth and lignification caused by modulating the expression of GA biosynthesis and degradation genes differ depending on the portion of the biosynthetic pathway being affected, with down-regulation of the catabolic enzyme GA2-oxidase showing stronger phenotypic consequences than up-regulation of a GA biosynthetic enzyme GA20-oxidase (Dayan et al., 2010). Direct transcriptional repression of GA20-oxidase and up-regulation of GA2-oxidase genes has been demonstrated in eudicot (Schaposnik et al., 1958; Rodriguez et al., 2010; Spießli et al., 2011) and monocot (Palatnik et al., 2007; Matros et al., 2010) model systems, respectively. Gibberellin is a potent target for plant breeding and crop improvement since some agronomic traits such as fiber quality (Larkan et al., 2013), tree height, and growth rate (Ye et al., 2012) can be improved by increasing endogenous GA levels. Other traits such as changes to plant architecture responsible for the green revolution result from decreases in GA levels and/or GA signaling.

In this study, we have used phylogenetically distant model species, maize (monocot) and tobacco (eudicot), to determine how class I KNOX genes are involved in the process of lignification. We also examine the effect of GA on lignification in these species and discuss the evolutionary implications of this regulatory role in the lignin biosynthetic pathway.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

Maize (Zea mays) lines with overexpression mutations of Kn1 (Knotted1), Gnt1 (Gn1-like), and Rs1 (Roughsheath1) were used in this study. Maize plants were grown under field conditions at the University of California, Davis, CA, USA. Tobacco (Nicotiana tabacum cv. Samsun) lines with overexpression constructs of LeT6 (Janssen et al., 1998a,b) and Kn1 (Sinha et al., 1993), the wild-type tomato (Solanum lycopersicum cv. Rutgers), and the mutant plant Mose ears (Me; Parish et al., 1997; Cardenas et al., 2012) were also used. Tobacco and tomato seeds were planted directly onto soil and grown in the greenhouse under long day (18 h day: 6 h night) conditions. All plant materials were harvested when they had achieved the onset of reproductive maturity.

HORMONE TREATMENTS

Maize (Z. mays) and tobacco (Nicotiana tabacum) seedlings were grown and treated with external sprays of GA3 (Sigma, MO, USA) and uniconazole (Uni; Sumagic, Valens, CA, USA). Treatments continued twice a week until the earliest evidence of initiation of flowering. GA3 and Uni were diluted into 250 ml water with five drops Tween-20 to aid in leaf surface adhesion. GA treatment was at 100 μM GA3 (Sigma, MO, USA). Uniconazole-P (Sumagic, Valens, CA, USA) treatment was at 50 μM. Control was water with Tween-20 and no additive.

PHYLOGENETIC ANALYSIS

All maize [Liguleless (Lg) (NM_001112088), (Lg4β) (AF457118), Lg1 (AF457119), Knotted1 (Kn1) (NP_001108436), Gnt1 (Gnt1) (AAP75320), Roughsheath1 (Rsh1) (NP_001103311), Arabidopsis (Arabidopsis knotted1-like (KNOT1)) (NM_105719), KNOT1) (NM_102187), SHOOT MERISTEMLESS (STM) (NM_104916), BREVIPEDICELLUS (BPE)/KNOT1 (NM_116884)] class I KNOX genes, as well as the tomato LeT6 (AF000141) gene, were placed into an unrooted cladogram using unambiguously aligned portions of the amino acid sequences and analyzed in Paup (Phylogenetic analysis using parsimony; Sinauer Associates, Inc. Publishing, Sunderland, MA, USA) using neighbor joining with 1000 replications.

HISTOLOGY

Mature stem tissues were hand sectioned with a razor blade; cross sections were stained, and mounted in phenol-lignin-HCL, a stain specific for lignin (Ruzin, 1999). Bright field images were taken on a Zeiss Discovery V8 microscope, digital pictures taken on an AxioCam MRc, and images were saved using an AxioVision Ac camera (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). All photographs were processed in Adobe Photoshop CS3 (Adobe Systems Inc., USA).

LIGNIN QUANTIFICATION

Lignin was quantified using a thiglycollic acid protocol as described by Hatfield and Fukusima (2005). Measurements of absorption at 280 nm were made on a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, DE, USA). Tissues were used were leaf midrib for maize and stem for tobacco. To ensure consistency among experiments, fresh tissue was placed in 100% methanol with daily changes for 5 days. Contents were dried overnight under vacuum at 37°C to remove the methanol for dry weights. Absorption 280 measurements were made on NANODROP. Comparisons of lignin content were expressed as Absorption 280 divided by dry weight for each sample and relative amounts being proportional to wild-type.

CINNAMYL ALDEHYDE MEASUREMENTS

One to two grams of tobacco and tomato stems were frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The powder was added to 50 ml of 100% methanol for 30 min and four changes were made to remove soluble components. Contents were dried overnight under vacuum at 37°C to remove the methanol for dry weight determination. Cinnamoyl moity content in lignin was measured as described by Ralph et al. (1998)
and MacKay et al. (1999) for each of the four biological replicates per treatment. Assay conditions (545 nm) were taken on a Beckman DU® 640 spectrophotometer (Beckman Coulter, Fullerton, CA, USA) using Ascoryl Cuvette 67-740 (Sartorit, Newton, NC, USA).

DATA COLLECTION AND ANALYSIS
Data for total lignin content in maize and tobacco and cinnamyl aldehyde content in tobacco were assessed using one-way analysis of variance (ANOVA) with a Dunnett comparison test to assess differences between the control (wild-type) versus the other genotypes. Data presented are means of total samples (±SE, N = 3) from QRT experiments. These analyses were performed using SigmaPlot12.5® software (SPSS Science, IL, USA).

HOMOLOG IDENTIFICATION
Putative tobacco orthologs for lignin biosynthetic genes were obtained by using the NCBI blastn utility using the Arabidopsis protein sequence query to search translated nucleotide databases: At4CL1 (At1g51680), AtPRXR9GE (At3g21770), AtCAD1 (At1g39330), AtCCR1 (At1g15950), LeCAD-F (accession DV999468). For tomato, the following primer sequences were used: for peroxidase (NtPRXR-F 5'-GATGG-3', NtPRXR-R 5'-CCTGACTGCTTATCTC-3'), cinnamoyl CoA reductase (LeCAD-F 5'-CCACCACTACTAC-3', LeCAD-R 5'-GACCAAGACCCAAGAAACAC-3') (accession AM851933); for 4-coumarate:CoA ligase 1 (LeT6-F 5'-GGTGCCAAGAAGGTTGTG-3', LeT6-R 5'-ACCATTAGTGCCAGTCTTAA-3') (accession AB044153); for CAD1 (NtCAD1-F 5'-GGAAGCTGCACCATTGTTATG-3', NtCAD1-R 5'-AACACTGGCATTCACATTCTG-3') (accession DV999468). Absorption 545 nm readings were taken on a Beckman Du® 640 spectrophotometer (Beckman Coulter, Fullerton, CA, USA).

RESULTS
Kn1, Gn1, and Rs1 are Putative Orthologs of BP in Maize
A cladogram generated with maize and Arabidopsis class 1 KNOX genes was used to determine which genes in maize are orthologs of BP. Amino acid sequences for class 1 KNOX genes from maize [Liguleless (Lg3), (Lg4a, Lg4b), Knotted1 (Kn1); Gnreetlay (Gn1); Roughsheath1 (Rs1)], Arabidopsis [Arabidopsis Knotted-like (KNAT2), (KNAT6); SHOOT MERISTEMLESS (STM); BREVIPEDICELLUS (BP)/KNAT5], and tomato [Lycopersicon esculentum 76 (LeT6)] were aligned. The three maize genes, Kn1, Gn1, and Rs1, formed a clade with Arabidopsis BP with 93% bootstrap support (Figure 1A, circled gray area). Based on their phylogenetic relatedness to BP, the three maize KNOX genes (Kn1, Gn1, and Rs1) were further investigated to determine their effect on lignin deposition.

Kn1 is a Modulator of Lignin Deposition in Maize
Relative lignin content was quantified using a thioglycolate acid protocol (Hatfield and Fukushima, 2005) on wild-type and maize mutant lines overexpressing Kn1, Gn1, and Rs1. We found that all mutant lines were significantly different when compared to wild-type. However, in Kn1 mutant plants lignin levels were much lower than wild-type (Figure 1B) showing that Kn1 can strongly affect lignin deposition in the stem. To confirm that Kn1 lignin reduction was not an allele specific effect, a segregating population of Kn1-N2, another Kn1 allele, was selected for analysis. Plants homozygous for Kn1-N2 showed reduced lignin content when compared to segregating wild-types confirming that Kn1 lignin reduction is not allele specific (Figure 1C).

Maize Kn1 Reduces Lignin Content in Tobacco
Since lignin content was significantly lower in Kn1 overexpressing maize plants (Figure 1B), transgenic tobacco lines overexpressing Kn1 and LeT6, a non-BP-type KNOX gene from tomato orthologous to Arabidopsis STM, were analyzed for lignin content and cellular localization to ascertain whether maize Kn1 could regulate lignin deposition in a rudicnt. First, gross morphology of wild-type and the transgenic lines overexpressing Kn1 or LeT6 were observed. In wild-type tobacco, leaves are ovate and arranged in a spiral phyllotaxy (Figure 2A). While leaves of both transgenic tobacco lines are round and curled with short petioles (Figures 2B, 2C). Notably, only wild-type and 35S:LeT6 showed normal erect stem growth (Figures 2A, B) while 35S:Kn1 plants were less rigid (Figure 2C). To correlate whether the stem weakness observed in 35S::Kn1 plants might due to a decrease in lignin, lignin content was measured in the tobacco lines. Lignin content was not significantly altered in 35S:LeT6 but was significantly lower in 35S::Kn1 plants (Figure 2D). Phloroglucinol stained...
sections of the tobacco stems confirmed these results showing that maize Kn1 (Figure 2G), but not LeT6 (Figure 2F), reduces lignin content in the eudicot tobacco (Figures 2E–G).

Gibberellic acid is a known regulator of lignification in flowering plants (Wang et al., 2011a,b) while KNOX genes are known regulators of GA biosynthesis and degradation (Mun et al., 2010; Parkin et al., 2010; Navabi et al., 2011). To ascertain whether KNOX proteins modulate lignin content via attenuation of GA biosynthesis or perception, we treated wild-type and transgenic tobacco with GA3 and Uni, a GA biosynthesis inhibitor. Treatment with GA3 resulted in increased internode lengths in wild-type and transgenic lines (Figures 3A–C) as well as a visible increase in lignin deposition (Figure 4). Measurements of lignin content in stems from wild-type and transgenic lines treated with GA3 or water, each showed a similar increase in lignin upon treatment with GA3 (Figure 3D). While treatment with GA3 caused similar growth stimulation (increased stem elongation) in all tobacco lines (Figures 3A–C), treatment with Uni reduced all treated plants to rosettes yielding insufficient stem material to allow for lignin quantification (Figures 3A–C). Examination of phloroglucinol–HCl stained stem sections showed additional reduction in lignification in Uni treated wild-type and transgenic lines (Figure 4).

This reduction in lignification, however, may also be a result of the decreased number of xylem elements that are formed in the Uni treated plants (Figure 4). The additive effects of GA3 or Uni treatments on transgenic lines suggests that lignin regulation by KNOX genes is not saturated and can be influenced in either direction (increased or decreased lignification) by the addition of GA3 or GA biosynthesis inhibitors (Figures 3 and 4). To establish that GA is a regulator of lignification independent of secondary growth, maize plants were treated with GA3 and uniconizole. Maize It was selected because these plants do not undergo secondary growth and reductions in lignin from Kn1 overexpression is a result of reduced levels of lignin in secondary walls of xylem elements and other primary tissues in the vascular bundle. We found that GA3 treated maize tissue did not show a significant increase in lignin, however, Uni treated plants did show a significant decrease in lignin content (P < 0.050; Figures 5A, B).

**PEROXIDASE AND CAD TRANSCRIPT LEVELS ARE ALTERED IN TOBACCO**

Since our results indicated that lignin content was lower in 35S::Kn1 than in 35S::LeT6 (Figure 2D), transcript levels of key lignin biosynthetic genes were evaluated for possible alterations in these overexpressing plants. Using QRT-PCR, we analyzed the relative expression levels of the four key dedicated lignin biosynthetic genes common to all lignin subunits: 4CL1, CCR, CAD, and PRX (peroxidase; Figure 6A, pathway). We found that in 35S::Kn1 only the PRX gene was significantly increased (P < 0.001) while a significant decrease (P = 0.009) in CAD was observed in both 35S::Kn1 and 35S::LeT6, with respect to wild-type transcript levels (Figure 6A, asterisks) suggesting that KNOX genes regulate at least two crucial steps (CAD and PRX) in the lignin biosynthetic pathway. Although 35S::LeT6 did not show a change in lignin quantity they suggest possible changes to lignin composition, based on these results, cinnamyl aldehyde (the monomeric
FIGURE 2 | Comparison of (A) wild-type (Wt), (B) 35S:LeT6, and (C) 35S:Kn1 tobacco plants. (D) Relative lignin content in Wt and transgenic 35S:LeT6 and 35S:Kn1 tobacco plants. Error bars represent standard error (±SE; N = 4) where P < 0.001. Phloroglucinol stained cross sections of (E) Wt, (F) 35S:LeT6, and (G) 35S:Kn1 tobacco stems. Scale bars = 5 cm (A–C); 0.1 mm (B–G).

FIGURE 3 | Effects of gibberellic acid (GA3) and GA biosynthesis inhibitor uniconazole (Uni; left), water (H2O) control (middle), and gibberellic acid3 (GA3; right) on (A) wild-type (Wt), (B) 35S:Kn1, and (C) 35S:LeT6 tobacco plants. (D) Total lignin content in water control and GA3 treated on Wt and transgenic 35S:LeT6 and 35S:Kn1 tobacco plants. Error bars represent standard error (±SE; N = 4). Scale bars = 5 cm (A–C).
FIGURE 4 | Effects of gibberellic acid (GA3) and uniconazole (Uni) treatment on stems of wild-type (Wt) and transgenic 35S::LeT6 and 35S::Kn1 tobacco plants. Stem cross sections were stained with phloroglucinol. Scale bar = 0.5 mm.

precursor of lignin; Figure 6A) moiety levels were analyzed to determine if lignin composition was altered in the tobacco lines. We found that cinnamyl aldehyde moieties in 35S::LeT6 was not significantly different ($P = 0.064$) when compared to wild-type (Figure 6B). Cinnamyl aldehyde moieties in 35S::Kn1 stems, however, were significantly lower ($P = 0.023$) with respect to wild-type (Figure 6B), likely as a result of the overall reduced lignin content. These results suggest that Kn1 may suppress lignification through changes in levels of biosynthetic steps upstream of 4CL and that CAD levels are not limiting with this reduced flux through the pathway.

NATURAL LeT6 OVEREXPRESSION MUTANTS IN TOMATO SHOW REDUCED LeCAD TRANSCRIPTION

The classical tomato mutant Mo is a naturally arising mutant in which the expression of the transcription factor LeT6 is under control of the promoter of the housekeeping gene pyrophosphatase (Chen et al., 1997; Parnis et al., 1997; Janssen et al., 1998a). To determine if CAD is a normal target of LeT6 regulation, stems of the tomato Mo mutant were analyzed. First, LeT6 expression levels in wild-type and Mo were determined. QRT-PCR showed a 7.5-fold increase in the expression of LeT6 in the stems of Mo plants when compared to wild-type (Figure 7A). Transcript levels of LeCAD was then determined for wild-type and Mo. We found that transcript levels of LeCAD were significantly lower ($P = 0.033$) in the Mo plants compared to wild-type tomato (Figure 7B) confirming that transcriptional regulation of the CAD gene is conserved between tomato and tobacco.

DISCUSSION

We show that the maize class I KNOX gene, Kn1, is a strong regulator of lignification both in a selected monocot and a eudicot. Although three co-orthologs of BP exist in maize, only the Kn1 overexpression mutants seem to negatively regulate lignin, implying sub-functionalization of this role in the maize BP-like KNOX1 genes. This function is retained even when expressed in the heterologous eudicot system, tobacco. Maize Kn1 regulates lignin biosynthetic genes differently than LeT6 and suppresses lignification in maize more efficiently than Gnt or Rs1, suggesting the possibility of a specialized role for this transcription factor in lignin regulation. Despite the large differences in cell wall composition between monocots and eudicots (Shedletzky et al., 1992), the maize Kn1 gene has the ability to negatively regulate lignin in both of these groups of plants. Additionally, maize Kn1 is able to modulate lignin deposition in vascular tissue during secondary growth. All tissues in grasses are primary but the majority of lignin...
Knotted1 regulates lignin deposition

in the majority of older eudicots is derived from secondary tissues in the stem. This would allow the maize Kn1 gene to be used as a general biotechnological tool in modifying lignin content to improve industrial or forage characteristics in plants. In addition, its efficacy at reducing total lignin across divergent species may allow for many crop species to be optimized for utilization of post-harvest crop residues. We found that LeT6 can regulate lignin composition and based on QRT-PCR results, we further showed that overexpression of LeT6 reduces NtCAD transcript levels by ~2.5-fold. The increase in cinnamoyl aldehyde content in Me and 35S::LeT6 plants is consistent with previous studies.
It remains to be seen, however, by what specific mechanisms these transcription factors regulate genes in the lignin biosynthetic pathway.

We propose that different KNOX1 genes transgenically expressed in tobacco independently modulate different aspects of lignin biosynthesis and affect GA-related developmental phenotypes by acting on different portions of the GA biosynthetic and catabolic pathways. (Figure 8). KNOX1 gene expression is coupled to GA regulation (Parkin and Lydiate, 1997), although the mode of this regulation, via alteration of biosynthesis or degradation of bioactive GAs, can vary depending on the individual KNOX1 gene and the species in which it originates (Vicente et al., 2002; Mayerhofer et al., 2005; Palatnik et al., 2007; Hegedus et al., 2008; Mateos et al., 2010). It has been demonstrated in maize that overexpression of Kn1 results in an increase in GA2-oxidase transcript abundance (Palatnik et al., 2007; Navabi et al., 2010) by a direct interaction of KN1 protein with sequences in a GA2-oxidase intron (Himmelsblau et al., 2009). Tobacco NTH15, Arabidopsis STM, and potato PotH1 directly reduce transcript of GA20-oxidase in these species (Vicente et al., 2002; Mayerhofer et al., 2005; Hegedus et al., 2008). STM can, via activation of isopentenyl transferases (IPTs; Parkin et al., 2005), lead to GA catabolism by up-regulating GA2-oxidase (Doughty et al., 1998).

We showed that the effects of expression of a maize and tomato gene in tobacco can parallel the effects of these genes in their native species. This supports our hypotheses that expression of specific KNOX genes and their effects on lignin levels and composition are transferable across plant species. Additionally, we found that the large reduction in lignin content in Kn1 overexpressing plants can be explained by reduction of endogenous GA. Since stem elongation in LeT6 overexpressing plants was not as severely affected as in Kn1 overexpressing plants, it is likely that GA concentrations are still high enough for lignification to occur somewhat normally in these plants (Figure 2). The lignin reduction and dwarfing phenotype of the plants overexpressing Kn1 are much more severe than the plants overexpressing LeT6 (Figure 2).

Since GA2-oxidase not only leads to deactivation of bioactive GAs, but also acts on precursors of bioactive GAs (Figure 8), regulation of GA by GA2-oxidase expression would be greater than down-regulation of GA20-oxidase. Additionally, inability of GA3 treatment to fully restore lignin content in Kn1 overexpressing plants would be expected since the exogenous GA would be subject to degradation by GA2-oxidase. We found parallel effects of KNOX1 expression and GA regulation of lignification in grass and eudicot species. Here, a monocot species proves a very useful model for the study of secondary cell wall regulation. In dicot plants there can be simultaneous effects of hormonal alterations on both the modulation of secondary cell wall deposition and on secondary growth (xylem development), whereas typical monocot plants lack secondary growth and thus, provides a valuable comparison.

The KNOX1 genes function beyond regulation of cell wall properties. Additional traits under regulation of KNOX1 genes are potentially useful for crop improvement. For instance, alteration of crop architecture and drought resistance could also be targets for crop improvement using KNOX1 genes as regulators of growth processes. Cytokinin biosynthetic genes are up-regulated...
by expression of KNOX and the overexpression phenotypes of IPT and KNOX genes are distinctly similar. Increased levels of cytokinin leads to bud breaking and increased branching (Hagedus et al., 2003; Osborn et al., 2003; Ekuere et al., 2004). These traits (e.g., increased branching resulting in increased leaf production) may be desirable in many circumstances so that light capture can be maximized and photosynthetic density increased as is the case in cereal crops such as wheat (Parkin et al., 1995). Expression of KN1 genes under the control of a senescence-activated promoter in tobacco has been shown to delay onset of senescence in leaves (Lukens et al., 2003). Similarly, increasing levels of IPT in stressed tissues has been demonstrated to lead to significant improvement in survival and recovery from drought in tobacco (Parkin et al., 2003) and in rice (Gao et al., 2003), resulting in significant yield improvement when compared to wild-type plants. KNOX genes have an indispensable role in compound leaf development (Parkin et al., 2002), with leaf shape being an important determinant of many physiological parameters (Sillito et al., 2000). In tomato interspecies introgression lines, the degree of leaf complexity correlates with brix and sugar levels, suggesting KNOX genes could also be utilized to alter morphological and nutritional characteristics of crop species (Chithwood and Sinha, unpublished). Use of transcription factors offers the potential to coordinately regulate many genes relating to complex developmental and metabolic processes which in turn could simplify engineering of desirable traits and broaden the range of plants that can be successfully modified when compared to gene specific methods such as RNAi (RNA interference).

ACKNOWLEDGMENT

This work was supported by a National Science Foundation grant (0820854) to Neelima R. Sinha.

REFERENCES

Baucher, M., Bernard-Vailhe, M. A., Chabbert, B., Boiz, J. M., Opron, C., Van Montagu, M., et al. (1999). Down-regulation of cinnamyl alco- hol dehydrogenase in transgenic alfalfa (Medicago sativa L.) and the effect on lignin composition and digestibility. Plant Mol. Biol. 39, 457–467.

Cardoso, P. D., Gajardo, H. A., Huibert, V., Parkin, I. A., Iniguez-Luy, F. L., and Federico, M. L. (2012). Retention of triplicated phytoene synthase (PSY) genes in Brassica napus L. and its diploid progenitors during the evo- lution of the Brassicaeae. Theor. Appl. Genet. 124, 1215–1228.

Cavall, A. C., Lykine, D. I., Parkin, I. A., Dean, C., and Trick, M. (1998). Collinearity between a 50- centimorgan segment of Arabidopsis thaliana chromosome 4 and dupli- cated regions within the Brassica napus genome. Genome 41, 65–69.

Chen, J. J., Jasmon, B. J., Williams, A., and Sinha, N. (1997): A gene fusion at a homeobox locus: alterations in leaf shape and implications for mor- phological evolution. Plant Cell 9, 1299–1304.

Deyou, J., Schrammkep, M., Senn, A., and Akho, R. (2010). Enhancing plant growth and fiber production by silencing GA 2-oxinase. Plant Biotech J 8, 425–435.

Doughty, J., Dian, S., Hincov, S. J., Wilks, A. C., Parkin, I. A., and Dickinson, H. G. (1998). PGP-1A, a defensin-like Brassica pollen coat protein that binds the S locus gly- coprotein, is the product of gameto- phytic gene expression. Plant Cell 10, 1355–1367.

Douglas, S. J., Chuck, G., Douglas, R. E., Polesard, L., and Riggs, C. D. (2012). KNAT1 and ERECTA regulate inflo- rescence architecture in Arabidopsis. Plant Cell 14, 547–558.

Douglas, S. J., and Riggs, C. D. (2003). Pedicel development in Ara- bidopsis: contribution of the BREVIPEDICELLUS and ERECTA genes. Dev. Biol. 264, 452–467.

Du, I., Murra, E., Robuchon, M., Martin, C., and Grosser, A. (2011). The Populus Class III HD ZIP transcription factor PPOCORONA affects cell differentiation during sec- ondary growth of woody stems. PLoS ONE 6:e17458. doi: 10.1371/jour- nal.pone.0017458.

Ekuere, U. U., Parkin, I. A., Brownson, C., Marshall, D., and Lydiate, D. J. (2004). Latent S alleles are widespread in culivated self-compatible Brassica napus. Genome 47, 257–263.

Esaai, K. (1985). Vascular Differentiation in Plants. New York: Holt, Rindust and Winston.
T ownesley et al.

Gao, M. J., Schafer, U. A., Parkin, I. A., Hegeden, D. D., Lydiate, D. J., and Hammon, A. D. (2001). A novel protein from Brussaisia napus has a putative KID domain and responds to low temperature. Plant J. 25, 1073–1084.

Gilliland, E. M., and Foster, A. S. (1998). Morphology and Evolution of Vascular Plants. New York: W. H. Freeman and Co.

Grossner, A. T., Manfredi, S. D., DiPari, S. P., Strippoli, G., Fontana, J. R., Miller, B., et al. (2008). The Popu-

lar homoeologous gene ALTERNATIVE REVEALS overlapping mechanisms reg-

ulat ing the shoot apical meristem and the vascular cambium. Plant Mol. Biol. 61, 917–932.

Herbst, R., and Fuchssteiner, R. (2005). Can lignin be accurately measured? Crop Sci. 45, 452–459.

Hugo, D., Yu, M., Baldwin, D., Gruber, M., Sharp, A., Parkin, I., et al. (2003). Molecular characteriza-

tion of Brussaisia napus NAC domain transcriptional activators induced in response to biotic and abiotic stresses. Plant Mol. Biol. 53, 383–397.

Hugo, D. D., H. S., Buschwald, L., Parkin, L., Whitehill, S., Gao, M., et al. (2008). Brussaisia napus possesses an expanded set of poly- 
galacturonase inhibitor protein genes that are differentially regulated in response to Sclerotinia sclerotiorum infection, wound ing and defense hormone treatments. Plant Sci. 176, 241–253.

Hill, J., Takaki, H., Shibata, D., and Hiugha, T. (1995). Increase of cinnamyl alcohol dehydrogenase. Biochem. Biophys. Acta 118, 118, 121–125.

Hillman, E., Gahler, E., E. A., Bueno, K., Barcelo, C., Meinerz, C., et al. (2008). Forward and reverse genetic approaches for rapid-cycling. Brussaisia oleracea. Theor. Appl. Genet. 118, 933–942.

Jannen, B. J., Land, L. and Sinha, N. H. (1998a). Overexpression of a homoeologous gene converts tepidite 

features in the tomato vascular cambium. Plant Physiol. 117, 771–786.

Jannen, B. J., Williams, A., Chen, J., Meinerz, H., Hiller, S. and Sinha, N. H. (1998b). Isolation and charac terization of two knotted-homologous genes from tomato. Plant Mol. Biol. 36, 407–422.

Kim, H., Ralph, J., Lu, F., Ralph, S. A., Boulet, A., Maclay, J. R., et al. (2005). NMR analysis of lignin in 

CAD-deficient plants. Part I: Incorporation of hydroxybenzyl alcohols and hydroxybenzyl aldehydes into lignins. Org. Biomol. Chem. 1, 268–284.

Kimura, S., Koenig, D., Kang, J., Yong, F. X. and Sinha, N. (2008). Natural variation in leaf morphology results from mutation of a novel KNOX gene. Curr. Biol. 18, 672–677.

Larka, N. J., Lydiate, D. J., Parkin, I. A., Nelson, M. E., Epp, D. J., Cowd- 

ing, W. A., et al. (2015). The Brussaisia napus blocking resistance gene LepikP encodes a receptor-like protein ty- 

phosphatase involving the Lepitaphylla maculata effector. ARLML. Plant Mol. Biol. 197, 595–608.

Lu, L., Bruggemann, A., Wang, X., Fried- 

man, M., Fornara, N., Sardage, R. A., et al. (2012). The Class II KNOX gene KNAT7 negatively reg- 

ulates secondary wall formation in Arabidopsis and is functionally con- 

served in Populus. New Phytol. 194, 102–113.

Long, J. A., Mean, E. I., milkwood, J. I. and Burton, M. K. (1996). A member of the KNOLLED class of homeodomain proteins encoded by the STM gene of Arabidopsis. Nature 379, 66–69.

Luken, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Luzatto, A., Uhl, I., Justin, M. J., Gen-

narelli, A., Ulyanov, R., Pala- 

mien, S., et al. (2008). Arelxhoxy cinnamate aggregation in arachnothorax: cell signaling and factor- 

Locus gene. Curr. Pharm. Bioch. 41, 49–56.

Mayerhofer, M., Lydiate, D., Bansal, V. K., 

Nature 496, 504–511.

Muir, I., Mean, E. I., Milkwood, J. I. and Burton, M. K. (1996). A member of the KNOLLED class of homeodomain proteins encoded by the STM gene of Arabidopsis thaliana. Nature 379, 66–69.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Mayerhofer, M., Lydiate, D., Bansal, V. K., 

Nature 496, 504–511.

Muir, I., Mean, E. I., Milkwood, J. I. and Burton, M. K. (1996). A member of the KNOLLED class of homeodomain proteins encoded by the STM gene of Arabidopsis thaliana. Nature 379, 66–69.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.

Muller, L., Zien, F., Lydiate, D., Parkin, I., and Osborn, T. (2015). Compari-

sion of a Brussaisia oleracea genetic map with the genome of Arabidopsis thaliana. Genome 58, 518–572.
Townsley, E., Shumak, M., Trainin, T., Kalman, S., and Delmer, D. (1992). Cell wall structure in cells adapted to growth on the cellulose-synthesis inhibitor 2,6-dichlorobenzonitrile: a comparison between two dicotyledonous plants and a graminaceous monosperm. Plant Physiol. 100, 120–130.

Silfver, D., Perkins, A. I., Maruyama, R., Lydiate, D. J., and Good, A. G. (2000). Arabidopsis enhancer: a source of candidate disease-resistance genes for Brassica napus. Genome 43, 452–460.

Sinha, N., Williams, R. E., and Hake, S. (1993). Overexpression of the maize homeobox gene, KNOTTED-1, cause a switch from determinate to indeterminate cell fates. Genes Dev. 7, 787–795.

Spinelli, S. V., Martin, A. P., Viola, I. L., Gonzalez, D. H., and Palatnik, J. F. (2011). A mechanistic link between STM and CUC1 during Arabidopsis development. Plant Physiol. 156, 1894–1904.

Testone, G., Condello, E., Verde, I., Nicolodi, C., Caboni, E., Dettori, M. T., et al. (2012). The peach (Prunus persica L. Batsch) genome harbours 10 KNOX genes, which are differentially expressed in stem development, and the class 1 KNOPE1 regulates elongation and lignification during primary growth. J. Exp. Bot. 63, 5417–5435.

Vuille, M. A., Provam, G. J., Sohlibc, L., Chevasson, A., Marlin, M. P., Cermu, A., et al. (2000). Effect of phenolic structures on the degradability of cell walls isolated from newly extended apical internodes of tall fescue (Festuca arundinacea Schreb.). J. Agric. Food Chem. 48, 618–623.

Wang, J., Lydiate, D. J., Parkein, I. A., Falamin, C., Debrarme, R., Carion, P. W., et al. (2011a). Integration of linkage maps for the Amphiploid (Brassica napus) and comparative mapping with Arabidopsis and Brassica rapa. BMC Genomics 12:101. doi: 10.1186/1471-2164-12-101

Wang, X., Wang, H., Wang, J., Sun, R., Wu, J., Liu, S., et al. (2011b). The genome of the mesopolyploid crop species Brassica rapa. Nat. Genet. 43, 1035–1039.

Xu, B., Sathitsuksanoh, N., Tang, Y., Udvardi, M. K., Zhang, J. Y., Shen, Z., et al. (2012). Overexpression of AtLOV1 in Switchgrass alters plant architecture, lignin content, and flowering time. PLoS ONE 7:e47399. doi: 10.1371/journal.pone.0047399

Yu, B., Gruber, M. Y., Khachatourians, G. G., Zhou, R., Epp, D. J., Hegedus, D. H., et al. (2012). Arabidopsis cisSRP4 regulates carotenoid accumulation in Arabidopsis and Brassica napus. J. Exp. Bot. 63, 5409–5422.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 February 2013; accepted: 16 April 2013; published online: 05 May 2013.

Citation: Townsley BT, Sinha NR and Kang J (2013) KNOX1 genes regulate lignin deposition and composition in monocots and dicots. Front. Plant Sci. 4:121. doi: 10.3389/fpls.2013.00121

This article was submitted to Frontiers in Plant Evolution and Development, a specialty of Frontiers in Plant Science.

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