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ORIGINAL ARTICLE

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Mechanical bending-induced tension wood formation with reduced lignin biosynthesis in *Liriodendron tulipifera*

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Abstract Mechanical bending treatment was employed to cause tension wood formation in the stem of the fast-growing yellow poplar. The tension wood induced by mechanical bending had many characteristic features of tension wood, i.e., eccentric growth toward tension wood, increased frequency of fiber cell types, vessels with reduced size, and reduced lignin content in the developed xylem. The significant reduction of the guaiacyl (G) and *p*-hydroxyphenyl (H) units of lignin in the tension wood was clearly visualized in samples that had been subjected to bending for 7 and 14 days when analyzed by lignin histochemical analysis using phloroglucinol-HCl. The syringyl (S) unit in the tension wood lignin was also significantly decreased in the developed xylem of the 14-day bending treatment sample, as indicated by Mäule's staining. Expression analysis of several representative genes in the lignin biosynthetic pathway clearly demonstrated that the overall phenylpropanoid pathway toward biosynthesis for both the lignin monomers and the flavonoids was greatly downregulated on bending treatment. In addition, the genes encoding laccases, which have been reported to be involved in the polymerization of monolignols to produce lignin macromolecules, were significantly downregulated in the tension wood. Despite the very limited sequence information for the genes/enzymes in the phenylpropanoid pathway in yellow poplar, histochemical staining and expression analysis using quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) successfully demonstrated the anatomical and chemical characteristics associated with mechanical bending-induced tension wood formation in the yellow poplar.

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Introduction

Woody plants develop a specialized tissue known as reaction wood when their stem is perturbed from an upright position in response to gravitational stimuli.[1] In general, gymnosperms form reaction wood in the lower side of leaning stems and branches where the compression forces are generated, whereas angiosperms develop specialized tissues in the upper side of leaning stems and branches where the tensile forces are generated.[2] The formation of reaction wood is considered to be an effective means for assisting displaced stems and branches recover their upright position and determines the overall shape of a tree by controlling the angles of stems and branches.[1,3]

Reaction wood in angiosperms, called tension wood, is characterized by a modified secondary cell wall structure with a reduction in the size and frequency of vessels and an increase in the proportion of fiber-type cells with different structure and composition from those of normal wood.[4] Tension wood fiber is often characterized by the presence of an innermost gelatinous layer in most angiosperms.[5] Based on the patterns of occurrence of the gelatinous layer, tension wood fibers have been classified into the following groups: S1 + S2 ± S3 + G and S1+G.[4] The gelatinous layer is composed of a high proportion of cellulose with increased deposition of the syringyl (S) unit of lignin.[6] In addition, the cellulose microfibrils in the gelatinous layer are oriented parallel or nearly parallel to the longitudinal axis and have a high degree of crystallinity.[7] Although the presence of tension wood in timber is a detrimental feature (in terms of the texture and properties of the timber for its application in the field of furniture and building construction), its chemical characteristics, such as reduced lignin content and increased cellulose content, are good genetic traits for the genetic engineering of wood species for making highly effi-

cient energy crops with increased levels of fermentable sugar with easy removal of lignin.[8,9]

Liriodendron tulipifera, L., a member of the Magnoliaceae family with many common names such as tulip tree, tulip poplar, yellow poplar, white poplar, and whitewood,[10] is a fast-growing tree species found in many countries, including the eastern United States[10] and South Korea. Because of its relatively high stress resistance to both abiotic and biotic stresses and its relatively low growing cost, it has been chosen as an important angiosperm species for bioplantation in Korea. In addition to its practical applications, yellow poplar is also regarded as a very important tree species that has often been used in studies of the evolution of flowering plants and of specific gene families since it occupies an important phylogenetic position as a basal angiosperm that has retained numerous putatively ancestral morphological characters.[10,11] However, the mechanism underlying the growth and development of tension wood formation in the yellow poplar is largely unknown at the molecular level, although it is known not to form gelatinous layers in the tension wood fiber. This study was therefore performed as the first step in gaining an understanding of the mechanism for the secondary cell wall assembly of the fast-growing yellow poplar by focusing on the lignin biosynthesis caused by the mechanical bending. Light microscopic and histochemical analyses clearly demonstrated that mechanical bending of 2-year-old yellow poplar plants induced morphological changes in the tension wood with the downregulation of lignin deposition. Quantitative real-time RT-PCR analysis for the lignin biosynthetic genes suggested that the reduced amount of lignin deposition in the tension wood was regulated not only by the biosynthesis of lignin monomers but also by the polymerization step of lignin monomers on bending treatment.

Materials and methods

Plant materials and bending treatment

Two-year-old yellow poplar plants (seven plants per treatment) obtained from Bokfarm Nursery (Korea) were planted on April 5, 2008, and were maintained in the experimental field of Kookmin University at Pyungchang-dong until needed. The straight stems were bent to maintain a 45° angle to the vertical on August 20, 2008, as shown in Fig. 1d. After 1, 7, and 14 days of bending, stem tissues were carefully harvested and immediately processed for light microscopic analysis. For quantitative real time RT-PCR analysis, the tension wood and wood from the opposite side of the stem (opposite wood), including phloem tissues, were harvested after 6 h of bending treatment, frozen separately in liquid nitrogen, and stored at –80°C until needed.

Light microscopic analysis

Stems in the middle of the bent position were harvested from the yellow poplar after 1, 7, and 14 days of bending, as well as from the control plant that had been maintained in the upright position. The harvested tissues were immediately fixed overnight at 4°C in formaldehyde acetic acid alcohol (FAA) solution containing 10% (v/v) formaldehyde, 5% (v/v) glacial acetic acid, and 50% (v/v) ethanol. The tissues were then dehydrated gradually in 50%, 70%, 80%, 95%, and 100% ethanol solutions and were finally washed three times with 100% ethanol for 20 min each time. After infiltration and embedding with paraffin, thick sections (12 μm) were obtained using a rotary microtome (Leica RM2245, USA) and were mounted on microscopic

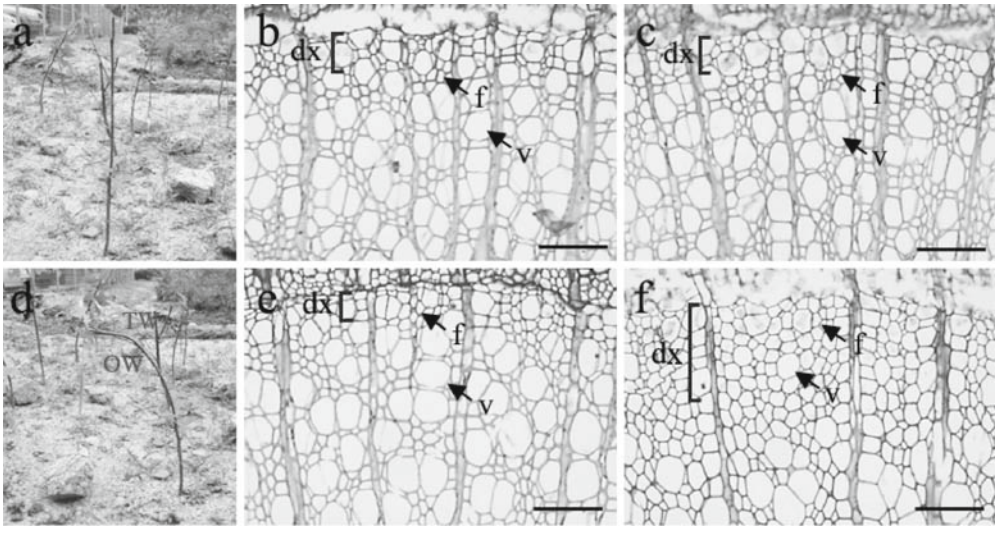

Fig. 1a–f. Photographs of the bending treatment and cross-sectional views of the developed xylem in 2-year-old yellow poplars after mechanical bending treatment in an experimental field located at Pyungchang-Dong. **a** Upright control; **b,c** light photomicrographs of both sides of the stem after 0 days of mechanical bending treatment; **d** yellow poplar with 45° bending treatment configuration; **e** light micrograph for the opposite wood (*OW*) stem after 14 days of bending in the 45° configuration; **f** light photomicrograph of the tension wood (*TW*) after 14 days of bending in the 45° configuration. *dx*, newly developed xylem; *v*, vessel; *f*, fiber. *Bars* 200 μm

Table 1. Lignin biosynthetic genes available in the National Center for Biotechnology Information database

Enzyme	Accession number	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
PAL	EU190449	acgatggctccttattgttc	cacatggctcactgcgtt
4CL	DQ223433	agggttatggaatgaccgaa	atgtgagtcatccaagtgac
CAD	DQ223432	catgaagttgtaggggaagt	ttggtccacaatcatggaag
LAC2-1	U73103	tttcatcgacaaagctccagat	cgaacgagatgttattcatgga
LAC2-2	U73104	tccatccaacttcaatctcg	tctctccatacaacgtccaa
LAC2-3	U73105	tgcgtctatcaacaacgtctc	ccccttgaatgcttgtgtc
LAC2-4	U73106	gccgcatctgtcaacaatg	gccccttgaatgcttgtgt
ACT	GQ246181	tgaagatcaaggttgttgcc	cacaggcgcacaatcttctt

PAL, phenylalanine ammonia-lyase; 4CL, 4-coumarated: CoA ligase; CAD, cinnamyl alcohol dehydrogenase; LAC, laccase; ACT, actin

slides (Fisher Scientific, USA). For morphological observations, the thick sections were stained with toluidine blue and cross-sectional views were taken under the light microscope with a digital camera (Olympus BX 51, Japan).

Lignin histochemical analysis

After paraffin was removed by immersion of the slides in xylene (Fisher Scientific), sections were subsequently rehydrated in 100%, 95%, 80%, 50%, and 30% ethanol followed by distilled water; the sections were finally air dried. For histochemical analysis of the lignin, the sections were stained using phloroglucinol-HCl according to the method described by Krishnamurthy.[12] All photographs were taken after 2–3 min of staining. For staining S units in the lignin macromolecule using Mäule's method, deparaffinized cross sections were immersed in 1 % (w/v) potassium permanganate solution for 5–10 min followed by washing in water twice before adding 3% (v/v) HCl. After 2 min of treatment with ammonia, photographs were taken under the light microscope (Olympus BX 51, Japan).

RNA purification and quantitative real-time RT-PCR

Fresh tissues (3 g, fresh weight) from the tension wood and the opposite of three replications pulverized in liquid nitrogen in a mortar with a pestle. After homogenization, total RNA was purified according to the method described by Chang et al.[13] The quality and quantity of purified total RNA were confirmed by spectrophotometric analysis and gel electrophoresis. Total RNA (5 µg) was used to synthesize first-strand cDNAs by using superscript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, USA). Briefly, total RNA was heat denatured at 70°C for 10 min and used for the reverse transcription reaction in buffer containing 50 mM Tris (hydroxymethyl) aminomethane hydrochloride (Tris–HCl) (pH 8.3), 75 mM potassium chloride (KCl), 3 mM magnesium chloride ($MgCl_2$), and 10 mM dithiothreitol (DTT), 0.5 mM deoxyribonucleotide triphosphate (dNTP), 1 mM random hexamer (Amersham Pharmacia Biotech), and 200 units of Superscript II (Life Technologies, Rockville, MD, USA) in a total volume of 20 µl for 50 min at 42°C. Following incubation, the resulting first-strand cDNAs were diluted with sterile distilled water and 2 µl of diluted cDNA was used for quantitative RT-PCR analysis in 20 µl of ready-to-use, hot-start PCR reaction mixture containing LightCycler FastStart DNA Master SYBR Green I (Roche, Germany). For each reaction, the reaction mixture without cDNA was used as a negative control. PCR was performed using a LightCycler 1.5 instrument system (Roche, Germany) and the amplification condition for PCR consisted of 45 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 45 s with addtional extension of 72°C for 10 min, according to the manufacturer's instructions. The primer sequences are listed in Table 1. As a control, actin was amplified using the primers (forward, 5′-tgaagatcaaggttgttgcc-3′; reverse, 5′-cacaggcgcacaatcttctt-3′) designed by the sequence information obtained previously in our laboratory (GQ246181). The fold changes of gene expression in the tension wood compared to those of the opposite wood were analyzed by the delta–delta Threshold Cycle (CT) method.[14]

Results and discussion

Characterization of tension wood formation on mechanical bending in yellow poplar

Tension wood formation has been reported to be a process inducible by external factors such as altered gravitational stimulation,[4,15,16] mechanical stress,[17] and the application of growth hormones such as gibberellins.[18] In order to induce tension wood formation in two-year-old yellow poplar plants, upright stems were mechanically bent to maintain a 45° angle to the vertical for different periods (Fig. 1); this procedure causes mechanical stress with altered gravitational stimulation in the stem. As previously reported, where tensile stress is generated, secondary xylem develops more cell layers adjacent to the vascular cambium than those of the opposite wood (Fig. 1); this indicates the action of growth promotion in the secondary xylem where tensile stress is generated on bending (Fig. 1f). As shown in Fig. 1f, the amount of newly developed xylem tissues in the tension wood from the 14-day bending treatment sample was almost three times that of the opposite wood (Fig. 1e), whereas the amount of newly developed xylem in the control plant was very similar on both sides around the

vascular cambium (Figs. 1b,c). In addition to the growth promotion of the tension wood, morphological changes and the frequency of vessel- and fiber-type cells were altered on bending treatment. For example, the occurrence of vessel-type cells and the vessel diameter were both significantly reduced in the tension wood (Fig. 1f) compared to those of the opposite wood (Fig. 1e). On the other hand, the frequency of fibers was significantly increased in the newly formed xylem of the tension wood compared to that of the opposite wood (Fig. 1e). Although the size and frequencies of vessel- and fiber-type cells were altered in the tension wood of the yellow poplar, no gelatinous layer was detected in the fiber, as has been previously reported in the tension wood of labour poplar brander.[11] Therefore, our experimental conditions successfully induced tension wood formation in the stems of yellow poplar plants with characteristic anatomical features of tension wood including growth promotion, a reduction of vessel size and frequency, and an increase of fiber-type cells that do not develop a gelatinous layer as the innermost cell wall layer.

Lignin histochemical analysis

In general, angiosperm lignins mainly contain coniferyl alcohol-derived guaiacyl (G) units and sinapyl alcohol-derived S units in roughly equal proportions, with a small number of *p*-coumaryl alcohol-derived *p*-hydroxyphenyl (H) units (Fig. 2b).[15] However, the lignin content of the tension wood was significantly decreased by about 20% of the cell wall dry weight with an increased S/G ratio compared with the content of the normal and the opposite wood. Because mechanical bending induced many anatomical features that are characteristic of tension wood in the stem of the yellow poplar, apart from the G layer formation in the fiber, histochemical analysis was performed using phloroglucinol-HCl and Mäule's reaction for staining H/G units and S units, respectively, to determine whether the mechanical bending affected the lignin deposition and lignification. In order to get a precise result using the constant thickness of the xylem tissues, harvested xylem was embedded with paraffin and transverse sections with a thickness of 12 μm were obtained using a microtome; these sections were used for histochemical analysis after deparaffinization (see Materials and methods). Deparaffinized sections obtained from the upright control and from trees bent for 1, 7, or 14 days were mounted on the same microscope slide to ensure that exactly the same staining conditions were applied to each so as to minimize the experimental variation. As shown in Fig. 3, lignin staining of the upper and lower sides of the xylem tissues from the control tree did not display any differences of staining intensity in the newly

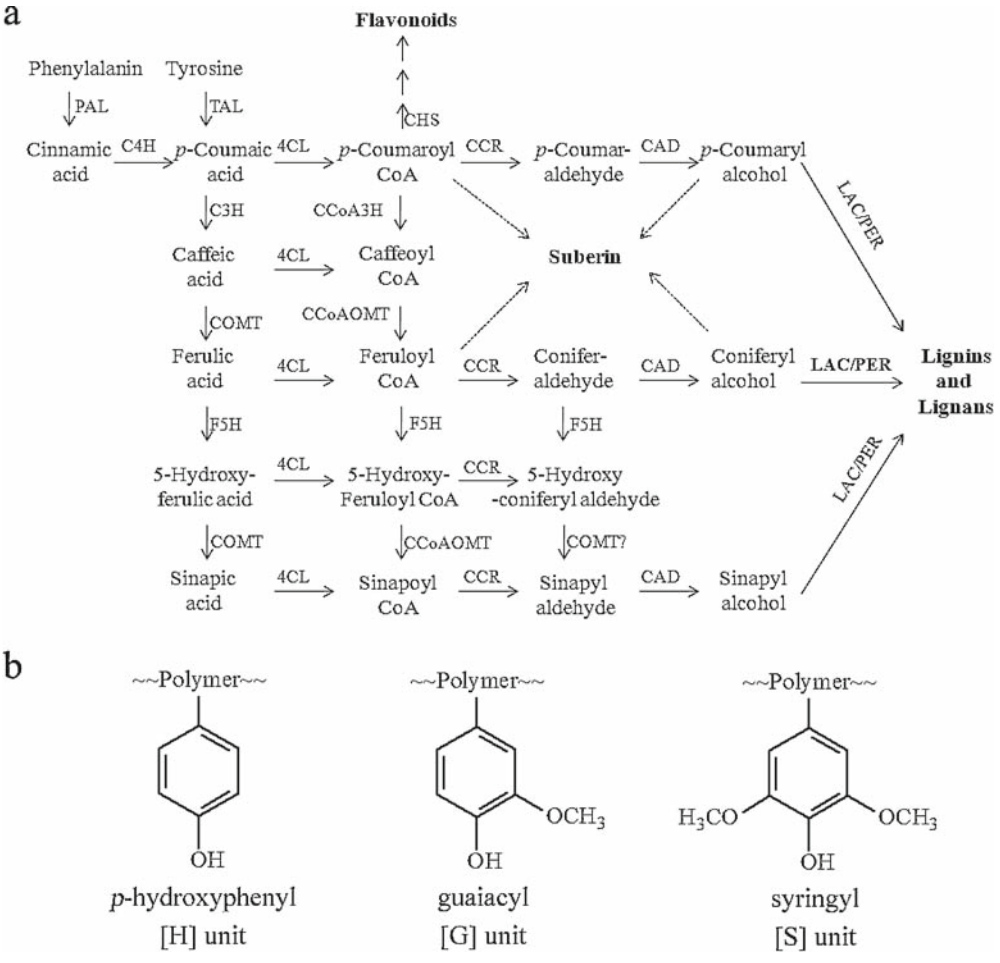

Fig. 2a,b. Overview of the phenylpropanoid pathway leading to the biosynthesis of three monomers and the lignin subunits. **a** Phenylpropanoid pathway showing various routes to the three monolignols, precursors of both lignins and lignans; **b** structure of the three subunits in lignin macromolecules. *PAL*, phenylalanine ammonia-lyase, *TAL*, tyrosine ammonia-lyase; *C4H*, cinnamate 4-hydroxylase; *C3H*, *p*-coumarate 3-hydroxylase; *CCoA3H*, *p*-coumaryl CoA 3-hydroxylase; *F5H*, ferulate 5-hydroxylase; *OMT*, hydroxycinnamoyl *O*-methyltransferase, CCoAOMT, *p*-coumaryl CoA *O*-methyltransferase; *4CL*, hydroxycinnamoyl CoA ligase; *CHS*, chalcone synthase; *CCR*, cinnamoyl-CoA oxidoreductase; *CAD*, cinnamyl alcohol dehydrogenase; *LAC*, laccase; and *PER*, peroxidase (redrawn from Anterola and Lewis, 2002)[25]

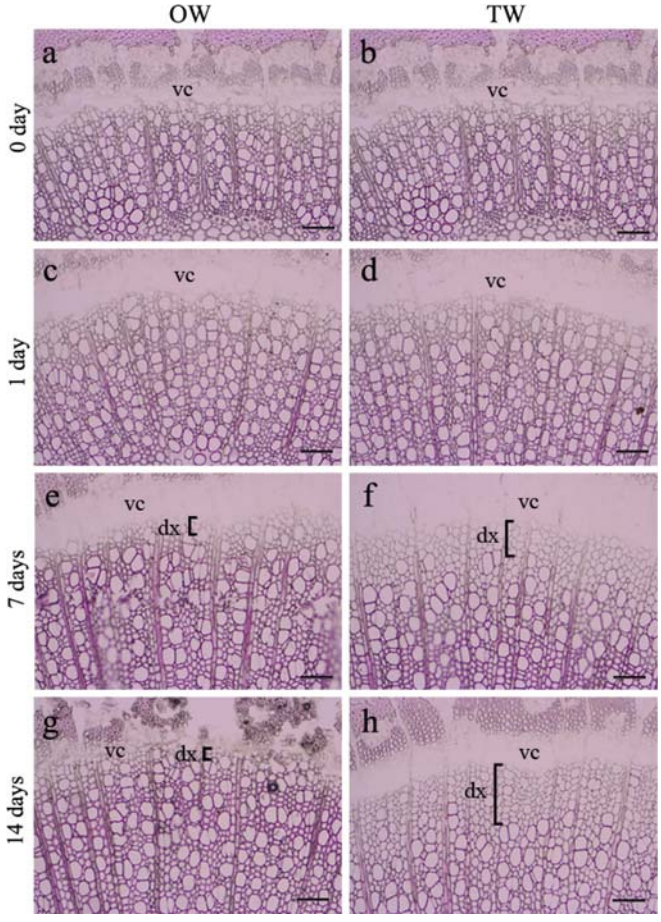

Fig. 3a–h. Histochemical analysis of lignin deposition using phloroglucinol-HCl reagent. **a,b** Newly developed xylem of yellow poplar with an upright stem; **c,d** newly developed xylem of the opposite (*left*) and tension (*right*) wood with mechanical bending for 1 day; **e,f** newly developed xylem of opposite (*left*) and tension (*right*) wood with mechanical bending for 7 days, showing different intensities of the lignin deposition; **g,h** newly developed xylem of opposite (*left*) and tension (*right*) wood with mechanical bending for 14 days, displaying significant reduction of lignin in the tension wood compared to that of the opposite wood. *vc*, vascular cambium; *Bar* 200 μm

developed xylem adjacent to the vascular cambium (Figs. 3a,b). The tension wood of the 1-day bending treatment samples did not display any differences when compared with the opposite wood (Figs. 3c,d) or with the control (Figs. 3a,b), in which the staining intensity might have been very low in the differentiating region. After 7 days of bending treatment, however, lignin staining was significantly reduced in the newly developed xylem tissues adjacent to the vascular cambium of bent stems (Fig. 3f) compared to that of the opposite wood (Fig. 3e) and upright control (Fig. 3a,b). The differences in lignin deposition between the tension wood and opposite wood were more clearly detected in the 14-day bending treatment samples (Figs. 3g,h). This indicated that the bending treatment of yellow poplar in a 45° configuration induced an overall reduction of lignin deposition in the tension wood, which is one of the most characteristic chemical features found in tension wood.

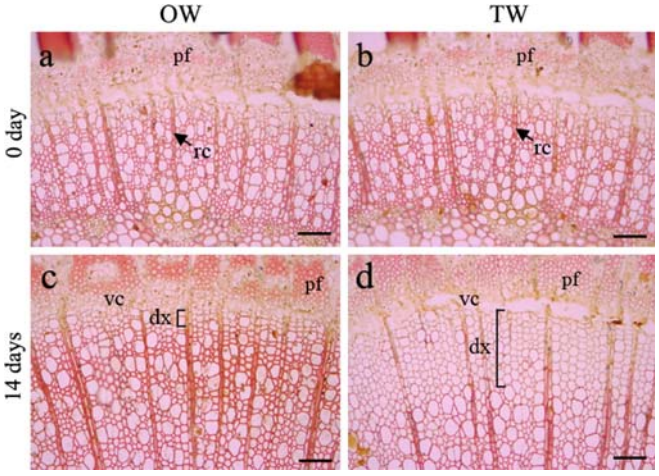

Fig. 4a–d. Histochemical staining of the S unit of lignin macromolecules in the newly developed xylem of yellow poplar using Mäule's method. **a,b** Developed xylem in the upright control; **c** developed xylem of the opposite wood after 14 days of mechanical bending; **d** developed xylem of the tension wood after 14 days of mechanical bending, showing significant reduction of the *reddish-brown* color (indicative of the S unit) in the newly developed tension wood. *rc*, ray cell; *pf*, phloem fiber; *Bar* 200 μm

Although the overall lignin content in the tension wood was lower than that of the normal and opposite wood, the relative proportion of S units in the tension lignin was reported to be increased, thereby increasing the S/G ratio, compared to the normal and opposite wood,[19] even in the yellow poplar that does not form a gelatinous layer in the tension wood fiber.[11] Our histochemical staining using Mäule's staining reagent clearly demonstrated the reduced amount of the S unit in the tension wood (Fig. 4d) compared to that in the opposite wood (Fig. 4c) and in the upright control (Figs. 4a,b). As shown in Fig. 4, the reddish-brown colored stains indicating the S unit of lignin were significantly reduced in the young xylem of the tension wood (Fig. 4d) compared to that of the opposite wood (Fig. 4c) after 14 days of bending treatment. The S unit was previously demonstrated to be mainly deposited in the gelatinous layer in the tension wood fiber by immunolocalization analysis using an antiserum specific to the S unit.[6] Therefore, the absence of a gelatinous layer in the fiber of the tension wood might have contributed at least partially to the significant reduction of S unit deposition in the tension wood of the yellow poplar. Interestingly, the S unit in the radial parenchyma cells was also significantly down-regulated in the tension wood (Fig. 4d) compared to that of the opposite wood (Fig. 4c), as were the H and G units in the radial parenchyma cells in the xylem (Fig. 3). Since the genes and enzymes involved in the biosynthesis of other phenolic compounds such as lignans were shown to be localized in these cells in the secondary xylem,[6] the reduced number of S units in the rays of the yellow poplar tension wood might suggest a reduction in various monolignol-derived compounds other than lignin, i.e., lignans.[20] Although the histochemical analysis did not determine the exact S/G ratio in the yellow poplar tension wood, it successfully visualized the reduced level of lignin in the newly

developed xylem of the tension wood formed on mechanical bending. Further experimentation involving precise chemical analysis of the lignin composition together with the analysis of gene expression and the activity of enzymes related to S unit biosynthesis might provide better insight into the control of monolignol biosynthesis during tension wood formation in the yellow poplar.

Expression analysis of lignin biosynthetic genes

Because the lignin staining method clearly demonstrated the reduced lignin deposition in the tension wood of the yellow poplar on mechanical bending (Fig. 3), the lignin biosynthetic genes were subjected to expression analysis using quantitative real-time RT-PCR (Fig. 5). Unlike for other economically important fast-growing trees such as *Eucalyptus* species and poplar, such sequence information is scant in the NCBI database (as of February 2009). Recent research on the development of the BAC library for yellow poplar provides partial sequence information for several genes in phenylpropanoid pathways of yellow poplar.[10] These partial sequences include 124 bp of phenylalanine ammonia lyase (PAL; DQ223434), 1966 bp of 4-coumarate: CoA ligase (4CL; DQ223433), and 1755 bp of cinnamyl alcohol dehydrogenase (CAD; DQ223432). Phenylalanine ammonia-lyase (PAL), the enzyme catalyzing the deamination of phenylalanine to form cinnamic acid (Fig. 2), is an entry point enzyme to the phenylpropanoid pathway leading to lignin, lignan, and flavonoids. Cinnamate 4-hydroxylase (4CL) is a cytochrome P450[21] hydroxylase that introduces a hydroxyl group to cinnamic acid regiospecifically in an NADPH-dependent manner. PAL and 4CL are enzymes in the phenylpropanoid pathway[21] leading to the formation of various secondary metabolites including lignin, lignan, suberin, isoflavonoids, and flavonoids (Fig. 2). In contrast, CAD is an enzyme involved in the biosynthesis of monolignols, which are precursors of lignin and lignan.[21,22] Using the partial sequence information for PAL (EU190449), 4CL (DQ223433), and CAD (DQ223432), gene-specific primers were designed (Table 1) and used for quantitative real-time RT-PCR (Fig. 5). For the expression analysis of lignin biosynthesis in the tension wood of yellow poplar, the transcript levels of PAL, 4CL, and CAD were analyzed after 6 h of bending treatment in order to understand the induction of tension wood formation on mechanical bending. As shown in Fig. 5a, all the tested genes were significantly downregulated at the transcription level in the tension wood compared to those in the opposite wood. Interestingly, the fold-decreases of the early stages of the phenylpropanoid pathway enzymes, i.e., PAL and 4CL, were considerably greater compared to that of CAD (Fig. 5b). Since the expression of chalcone synthase, which is the entry point enzyme to flavonoid biosynthesis, was also significantly reduced in the tension wood compared to that of the opposite wood (data not shown), the downregulation of lignin biosynthesis was attributed to the overall reduction of the phenylpropanoid pathway on mechanical bending. Despite the limited amount of sequence information for lignin biosynthesis genes, the decreased lignin deposition in the tension wood of yellow poplar was demonstrated to be controlled by mechanical bending at the transcription level. However, further analysis will be required to determine the mechanism of transcriptional control of each monolignol biosynthetic pathway on mechanical bending in yellow poplar, and thereby provide insight into the regulation of lignin monomeric composition on mechanical bending.

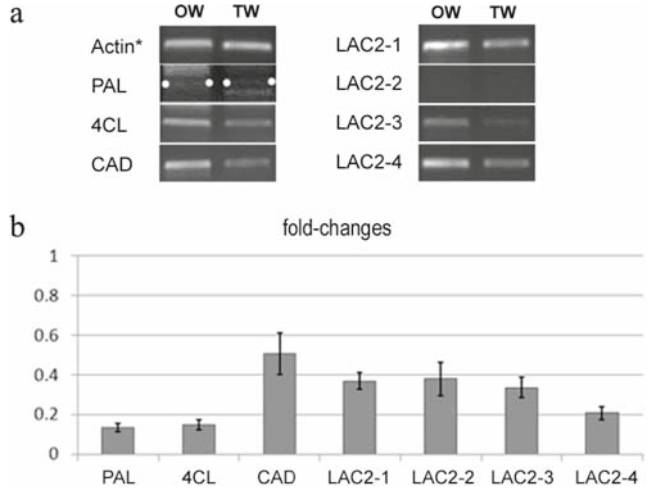

Fig. 5a,b. Expression analysis of lignin biosynthetic genes in the tension wood and opposite wood in yellow poplar. **a** Gel electrophoresis of quantitative real-time reverse transcription-polymerase chain reaction product; **b** fold-changes of the expression levels of the lignin biosynthetic gene in the tension wood compared to that of the opposite wood. The experiments were repeated three times with three duplicates. Normalization was carried out using actin as an internal control

Expression of laccases

Laccases, in conjunction with peroxidase, are postulated to be involved in lignin polymerization and cell wall lignification by catalyzing the one-electron oxidation of monolignols.[24] Laccases were also suggested to be involved in regiospecific and stereospecific monolignol coupling to form 8′-8′ pinoresinol in the presence of dirigent proteins in *Forsythia intermedia*.[23] The cDNAs encoding yellow poplar laccases (LAC2-1/LAC2-4) were cloned and their activities were demonstrated to be correlated with monolignol oxidation in the xylem cells undergoing active lignification.[24] Based on the possible involvement of laccases in lignin polymerization, the expression levels of the four laccases were analyzed in tension wood and opposite wood by quantitative real-time RT-PCR (Fig. 5). Since these four laccases of yellow poplar share about 70%–80% sequence identities at the nucleotide level, the gene-specific primers (Table 1) were designed from the 3′ untranslated region and nonhomologous cDNA regions of each laccase. As shown in Fig. 5a, the expression level of each laccase was significantly differed in the tension wood and the opposite wood of yellow poplar, as previously reported in the normal second-

ary xylem of the yellow poplar.[24] LAC2-3 and LAC2-4 displayed a relatively higher expression level than LAC2-2 at transcription level (Fig. 5a). The medium level of LAC2-3 mRNA expression and high expression level of LAC2-4 in the xylem were consistent with a previously reported result obtained from northern analysis.[24] However, the expression of LAC2-1 mRNA, which was previously reported to be lower than both LAC2-3 and LAC2-4 in xylem, displayed a similar expression level with that of LAC2-3 and LAC2-4 (Fig. 5a). The discrepancy may have be arisen from the sources of xylem tissues for the RNA isolation, i.e., the whole stem, including phloem, of the two-year-old yellow poplar in this study versus the xylem tissue of the 17-year-old yellow poplar of the previous study.[24] Therefore, the discrepancy in the overall expression level of LAC2-1 mRNA was probably caused by the expression of LAC2-1 mRNA in the phloem tissues in the tension wood or by the expression differences caused by the different developmental stages. In either case, the expression levels of the four laccases were significantly downregulated in the tension wood compared to those of the opposite wood (Fig. 5b), which was consistent with the result obtained from histochemical analysis (Fig. 4). Taken together with the downregulation of PAL, 4CL, and CAD, these results strongly suggest that the reduced lignin deposition in the tension wood induced by mechanical bending treatment was caused by both the downregulation of biosynthesis and the laccases-mediated polymerization of monolignols.

Although the currently available sequence and protein information for yellow poplar are very limited, the use of histochemical staining and expression analysis using quantitative real-time RT-PCR was successful in demonstrating the anatomical and chemical characteristics associated with the formation of tension wood in the yellow poplar on mechanical bending. However, the mechanism for the downregulation of lignin and the altered monomeric composition of lignin could not be determined because of the limited information on the lignin biosynthetic genes and enzymes in yellow poplar. Further identification of inducible genes in the tension wood of yellow poplar might facilitate an understanding of the mechanism of tension wood formation in this species, in comparison with other species that form a gelatinous layer in the tension wood fiber.

Conclusions

Mechanical bending was used to induce tension wood formation in the stem of two-year-old yellow poplar plants. The associated anatomical and histochemical features were analyzed using light microscopy. Although the information and genetic resources for the lignin biosynthetic genes are very limited for the yellow poplar, expression analysis using quantitative real-time RT-PCR was successful in demonstrating the downregulation of lignin monomer biosynthesis and of the possible polymerization in the tension wood of yellow poplar formed on mechanical bending. However, the increased S/G ratio that has been reported in the tension wood of angiosperms could not be explained in the yellow poplar tension wood due to the lack of information on lignin biosynthetic genes and enzymes, together with the technical limitations of the histochemical analysis method. Because the yellow poplar does not form a gelatinous layer as part of the tension wood, the induced formation of tension wood in yellow poplar may be utilized as a tool to understand the possible correlation of the presence of a G layer in the tension wood fiber with the monomeric composition in the tension wood lignin. Such an understanding will help to elucidate the mechanism underlying the control of cell wall lignification on mechanical bending. Therefore, further analysis should be conducted to determine the transcriptional control of monolignol biosynthesis on mechanical bending in yellow poplar and thereby provide better insight into the control of lignin monomeric composition on mechanical stress with altered gravitational stimulation.

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