METABOLITES AND CHEMICAL GROUP CHANGES IN THE WOOD-FORMING TISSUE OF *PINUS KORAIENSIS* UNDER INCLINED CONDITIONS

Jiangtao Shi and Jian Li*

The differential expression of metabolites in wood-forming tissue in response to abiotic stresses might regulate or decide the cell wall contents and architecture via multiple pathways or networks. In order to determine whether such chemical modifications were associated with compression wood formation in a *Pinus koraiensis* sapling stem, polar metabolites were identified by a gas chromatography-mass spectrometer (GC-MS) and their major chemical components were measured. Varieties and abundances of metabolites were significantly affected by the number of days during which the saplings were bent into an inclined position. Polysaccharides like glucose, fructose, and glucopyranoside sharply decreased in treated stems, and simultaneously, some compounds related to biological resistance increased. This indicated that the monomer content showed changes in polymer synthesis. However, major metabolites clearly showed changes at an stage of stress application but were not obvious at later stages. Fourier Transform Infrared Spectroscopy (FTIR), cellulose crystallinity, and quantitative analysis of lignin showed similar variation patterns at different inclined times, but no consistent relationship

Keywords: Compression Wood; GC-MS; Wood-forming Tissue; Polar Metabolite

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INTRODUCTION

Wood is an important regenerative resource that is produced by the secondary growth of trees and plays a pivotal role in an ecosystem's carbon cycle. Variations in the anatomy, morphology, and chemical constitution of wood are induced by the habitat of trees and affect the end utility properties. Trees usually develop reaction wood in response to the perception of gravity and/or to mechanical stimuli (Timell 1969, 1986; Du and Yamamoto 2007). In conifers, reaction wood is referred to as compression wood and develops at the lower side of leaning stems or branches. However, it is named tension wood and is formed on the upper side of leaning angiosperm trees (Timell 1969; Scurfield 1973; Timell 1986). In comparison to vertical wood, reaction wood is often characterized by a modified secondary cell wall structure, broader rings, rounder cells in cross section, and is composed of different polymers (Kollmann and Côté 1968; Yeh *et al.* 2005; Yamashita *et al.* 2009; Jin and Kwon 2009; Moon *et al.* 2011).

In general, reaction wood is known as a serious defect for wood utility, but it provides a valuable experimental system for the understanding of the molecular mechan-

isms in wood formation. In recent years, many reports have revealed preferential expression of some genes and proteins in reaction wood formation, which were key regulators involved in macromolecule polymer biosynthesis and cell wall architecture (Allona *et al.* 1998; Zhang *et al.* 2000; Plomion *et al.* 2000; Gion *et al.* 2005; Paux *et al.* 2005; Jin and Kwon 2009; Mast *et al.* 2010; Jin *et al.* 2011). Yamashita (2008) found that 24 fragments showed reproducible expression patterns through fluorescent differential display (FDD) screened from wood-forming tissue of *Chamaecyparis obtusa*, indicating that these cDNA changed their expression during compression wood formation (Yamashita *et al.* 2008). Furthermore, kinds and expression abundances of transcripts are considerably different between mild and severe compression wood, demonstrating that some genes participated in the development of compression wood (Yamashita *et al.* 2009). The metabolic pathways of cell wall compositions are also regulated by these genes or proteins, and the metabonomics are regarded as a vital part of functional genomics and integral biology. Metabolic profiling is a chromatographic technique that allows the study of changes in metabolite pools during development or in response to habitat or chemical stresses (Fiehn *et al.* 2000; Fiehn 2002; Goodacre 2004; Morris *et al.* 2004). Yeh showed that compression wood and normal wood of loblolly pine can indeed be distinguished metabolically (Yeh *et al.* 2006).

In this paper, the metabolic profiling of a 4-year-old sapling stem of *Pinus koraiensis* at different incline intervals is reported. Chemical modification was also measured by FTIR. Molecular level results combined with phenotype properties suggested that changes in the metabolites produced during compression wood formation were related to the number of inclined days.

EXPERIMENTAL

Plant Materials

This experiment was conducted from April to September, 2010 in a plot at Flower Biological Engineering Institution of Northeast Forestry University. Four-year-old *Pinus koraiensis* saplings (about 25 cm in height and 7 mm in diameter) were planted in plastic pots filled with a mixture of black soil and compost. The straight stems of sapling (three for each treatment) were successively bent at an angle of 40° to the south (Fig.1).

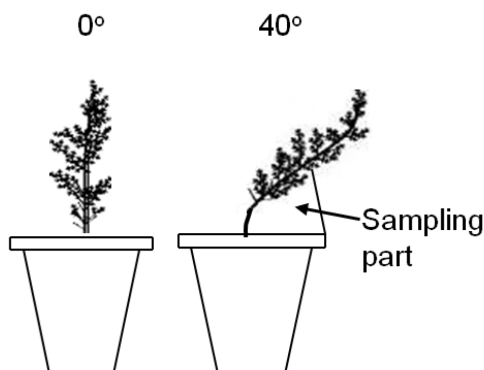

Fig. 1. Schematic diagram of mechanical bending

These trees were inclined for 60 days (July 5), 30 days (August 4), 15 days (August 20), 7 days (August 27), and one day (September 3) before harvest on September 4. Immature xylem tissue was obtained by first peeling a rectangular section of bark (outer cambium) from about 10 cm above the ground on the underside of both the bent stems and straight stems, and then scraping the immature xylem with a fresh razor blade. Samples were kept frozen at all times at -80 °C until being ground. The samples were quickly ground to a fine powder using a liquid N_2-chilled mortar and pestle. Equivalent tissue from three duplicates was mixed for testing.

Fourier Transform Infrared Spectroscopy (FT-IR)

Samples were dried at -60 °C in a freeze vacuum concentrator. The FT-IR spectra were measured by direct transmittance using the KBr pellet technique. Spectra were recorded using a Nicolet Magna-IR 560 E.S.P. spectrometer. Spectral data between 400 and 4,000 cm^{-1} were collected, averaging 40 scans at a resolution of 4 cm^{-1}. Peak heights of absorption bands were measured by OMNIC software (Version 8.0, Nicolet Instruments Corporation, USA) according to previous methods (Pandey and Pitman 2003). Since the location of the characteristic peak maximum varied somewhat between the samples, the height was determined at a slightly different wave number. For H_{1429} the maximum varied between 1424 and 1427 cm^{-1}, for H_{897} it varied between 895 and 900 cm^{-1}, and for H_{2900} it varied between 2925 and 2928 cm^{-1}.

Lignin Content Determination

Lignin content was determined via the acetyl bromide method (Iiyama and Wallis 1988; Foster *et al.* 2010) using 1.5 to 2.5 mg prepared cell wall tissue in 2.0 mL centrifuge tubes, with one tube empty for a blank. 100 μL of freshly made acetyl bromide solution (25% v/v acetyl bromide in glacial acetic acid) was added to 4 μL 70% perchloric acid, then the cap tube was heated at 70 °C in a thermomixer at 300 rpm for 30 minutes. The tube was then cooled on ice to room temperature. 200 μL of 2 M sodium hydroxide was added and put on ice for 10 minutes. It was filled up to exactly 4 mL with glacial acetic acid, capped, and inverted several times to mix. The percentage of acetyl bromide soluble lignin (%ABSL) was determined with absorbance at 280 nm.

Metabolites Extraction and Derivatization

Metabolites from wood-forming tissue were extracted according to the protocol of Lisec (Lisec *et al.* 2006). The frozen (-80 °C) and ground developing xylem tissue (50 mg±2 mg) was first extracted with 1 mL of 100% methanol (pre-chilled at -20 °C) and 45 μL of Ribitol (CAS No.488-81-3, 2 mg mL^{-1} stock in dH_2O) as an internal quantitative standard (IS) and vortex for 10 s. The mixture was mixed for 15 min at 70 °C in a thermomixer at 950 rpm and centrifuged for 10 min at 12000 g. 500 μL of supernatant was transferred to a new 1.5 mL tube, then 500 μL of chloroform (pre-chilled at -20 °C) was added and vortexed for 10 s. The mixture was in the thermomixer for 5 min at 37 °C at 950 rpm. 500 μL of dH_2O (4 °C) was added and vortexed for 15 s, centrifuged 15 min at 4000 g, and 200 μL was transferred from the upper phase (methanol/water, polar phase) into a new tube and stored at -80 °C.

A 200 μL sample was dried for 4 hours at -60 °C in a freeze vacuum concentrator and redissolved in 50 μL of methoxyamine hydrochloride (CAS No.593-56-6, 20 mg mL^{-1} in Pyridine, CAS No.110-86-1) with shaking for 2 hours at 37 °C. The samples were derivatized with 100 μL of N-methyl-N-trimethlsilyl-trifluoroacetamide (MSTFA) (CAS No.24589-78-4) and mixed for 30 min at 37 °C in the thermomixer at 260 rpm. The samples stood overnight at room temperature to ensure the reaction completed.

GC-MS Analysis

The samples were analyzed on a Varian450GC-240MS system (USA). A VF-5ms 30m×0.25mm×0.25μm column was used for the analysis with a helium (99.99%) carrier gas flow of 2 mL min^{-1}. The injection temperature was 250 °C. The GC oven was held at 70 °C for 1 min and then ramped at 5 °C min^{-1} to 300 °C where it was held for 5 min and cooled to 70 °C. It took about 52 min per sample. 1 μL injection with a 20:1 split and flow of 1 mL min^{-1} was used throughout. A threshold cutoff for metabolite analysis was set at 2% of the peak area of the internal standard.

Metabolites were identified with an online 240 EI-Mass Spectra (Varian, USA) device and MS Workstation software (version 6.9.3). The ion source was 300 °C and mass spectra were acquired over the range m/z 50 to 1000 with an electron ionization of 70 eV. The mass ions were scanned at the rate of 2 spectum s^{-1}.

Metabolites Identification and Quantification

The metabolites were identified using NIST Mass Spectral Search Program (National Institute of Standard and Technology, version, USA) based on comparison to authentic compounds with retention time and mass spectra. Data were analyzed and mapped by Origin Pro.8.0 and SPSS (Version 18.0). Formula (1) was employed to calculate the relative abundance of a single compound.

$$\text{Relative content } (\mu g \bullet g^{-1}) = [(PA_x / PA_0) \times C_0] / W \quad (1)$$

Where PA_x and PA_0 are peak areas of unknown compound and IS, respectively. C_0 is concentration of IS ($\mu g \bullet \mu L^{-1}$), and W is weight of wood-forming tissue (g).

RESULTS AND DISCUSSION

FT-IR Analysis

Compression wood formation is reported to be a process inducible by external factors such as mechanical stress or the application of growth hormones such as gibberellins (Timell 1986; Zhang *et al.* 2000; Plomion *et al.* 2001; Funada *et al.* 2008). In order to induce compression wood formation in 4-year-old *Pinus koraiensis* saplings, upright stems were mechanically bent to maintain a 40° angle for different periods of time. As is previously reported, the chemical composition and structure were changed in secondary xylem under mechanical stress. FTIR has been widely used to detect and quantify the chemical composition of wood, and the FTIR spectra of inclined wood forming-tissue changed considerably from that of upright stems (Fig. 2). As shown in

Fig. 1, the intensity of the O-H absorption band (around 3400 cm^{-1}) and C-H absorption band (around 2927 cm^{-1}) sharply decreased in the early stages (1-day, 7-day, and 15-day) of bent samples, but in later stages (30-day and 60-day), it hardly displayed any further differences.

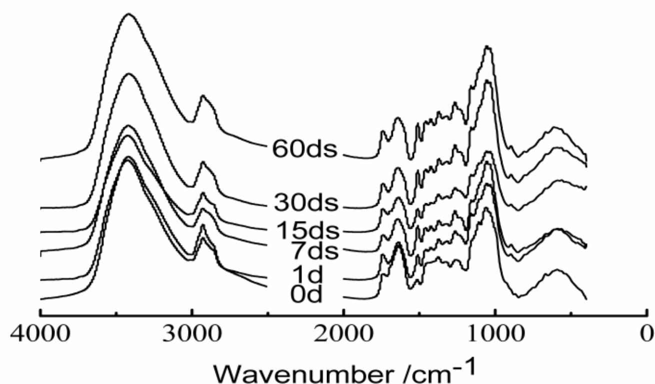

Fig. 2. FT-IR spectra of wood forming tissue from sapling under various inclination days

The decrease of the intensity of the C-H absorption band can be explained by the reduction of cellulose content. The aromatic skeletal vibration at 1510 cm^{-1} significantly increased, revealing that the content of aromatic compounds in bending wood cell walls increased when compared to the normal stem. As lignin is the main aromatic compound in a wood cell wall, so it can experience higher lignin deposition in the bending stem, which is one of the most chemical features found in compression wood. In addition, the peak at around 1265 cm^{-1} resulting from syringyl ring breathing and C-O stretching vibration in lignin and xylan may also account for the increase of lignin deposition in wood cell wall of bent stem. Both intensity of absorption peaks at 1510 and 1265 cm^{-1} displayed a slight increase in the early bent periods (1-day, 7-day, and 15-day) but remarkably increased in the later stages (30-day and 60-day). Detailed peak positions and assignments (Pandey 1999; Li 2003; Vu Manh Tuong and Li 2010) of untreated and treated wood-forming tissue samples are listed in Table 1.

Inclined Treatment Affects Cellulose Crystallinity in the Secondary Cell Wall

The physical and mechanical properties of polymers are profoundly dependent on the degree of crystallinity (Mo *et al.* 1994). The ratio of peak intensity at 1425 and 897 cm^{-1} (I_{1425}/I_{897}) in FT-IR spectra of wood samples was used as an approach to the determination of crystallinity of celluloses in wood samples (Åkerholm *et al.* 2004). The results show that all the relative crystallinity index values of the stem subjected to mechanical bending treatment decreased compared to the control sample (Fig. 3). The decrease in crystallinity may be explained as a decrease in cellulose content. The cellulose crystallinity shows variance in different bending periods but no strict variation patterns. These results correspond to the changing chemical constitutions through FTIR analyses.

Table 1. Assignment of Absorption IR Spectra Peaks in Wood-Forming Tissue

Wave number (cm^{-1})						Band assignment
d	1d	7d	15d	30d	60d	
3421	3424	3421	3423	3415	3419	O-H stretching in hydroxyl groups
2927	2926	2928	2926	2926	2927	C-H stretching in methyl and methylene groups
1738	1741	1739	1740	1739	1740	C=O stretching in unconjugated ketone
1636	1637	1634	1637	1641	1641	C=O stretching vibration in conjugated carbonyl of lignin
1514	1510	1510	1511	1510	1510	Aromatic skeletal vibrations
1457	1457	1457	1457	1460	1457	CH2 deformation stretching in lignin and xylan
1425	1425	1425	1425	1424	1424	Aromatic skeletal combined with C-H in-plane deforming and stretching
1377	1373	1372	1376	1371	1372	Aliphatic C-H stretching in methyl and phenol OH
1320	1320	1320	1320	1317	1320	C1-O vibrations in S derivatives, CH in-plane bending in cellulose I and cellulose II
1259	1265	1266	1265	1265	1266	Syringyl ring breathing and C-O stretching in lignin and xylan
1160	1162	1162	1162	1162	1162	C-O-C asymmetric stretching in cellulose I and cellulose II
1061	1057	1056	1057	1057	1057	C-O stretching vibration (cellulose and hemicellulose)
1034	1034	1034	1034	1034	1034	C-O stretching vibration (cellulose , hemicellulose and lignin)
895	900	894	897	900	897	C1 vibration

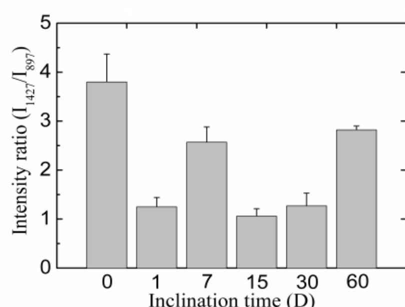

Fig. 3. Cellulose crystallinity in wood-forming tissue at tissue at different bending times

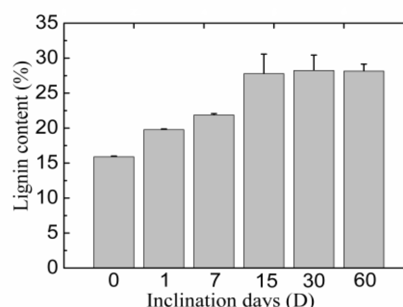

Fig. 4. Lignin content in wood-forming different bending times

Normally, compression wood contains higher lignin content than normal wood. The tracheid cell wall lacks an S3 layer and deposition of an extra lignin layer between the S1 and S2 layers in compression wood (Kollmann and Côté 1968). Acetyl bromide lignin content was measured at the different bending times (Fig.4), and on the whole, lignin content increased during bending treatment when compared to an upright stem. The lignin content shows a slight increase in the early bent stages (1-day and 7-day), a significant increase after 15-day bending treatment, and then it tended to be stable. However, in the fast-growing tree yellow poplar, the wood's anatomical structure and

lignin content in 1-day bending treatment did not show any differences compared with the control tree. Rather significant changes appeared during the 7-day to 15-day treatments (Jin and Kwon 2009; Moon *et al.* 2011). This indicated that characteristic chemical features often emerge in later stages of reaction wood formation.

Mechanical Bending Treatment Affects Metabolites in the Wood-Forming Tissue

The polar metabolites from wood-forming tissue of normal and inclined wood were identified. These identified metabolites include polysaccharides, lipids, alcohol, organic acid, and *N*-compound (Schauer *et al.* 2005). The relative content of polysaccharides is the highest, with lipids, and *N*-compound following. The results indicated a clear difference between metabolites and their relative abundance in inclined and normal saplings (Table 2). These differences presented themselves in the various inclination times. 1-cyclohexene-1-carboxylic acid, 2H-1-benzopyran,3,4-dihydro-2-[3,4-bis(trimethylsilyl)oxy]phenyl]-3,5,7-tri(trimethylsilyl)oxy]-, 2 hydroxymandelic acid, and hexadecanoic acid were detected in bent samples but not in control saplings. On the other hand, cyclodecasiloxane eicosamethyl and tetracosamethyl-cyclodedecasiloxane were detected only in the upright saplings. The foregoing compounds were identified by means of a GC-MS database. The change of metabolites indicates that carbon flow storage and allocation also changed in wood formation under bending treatment. However, further research is required to confirm whether these specific expression compounds are concerned with compression wood cell wall formation. The relative content of polysaccharides was significantly lower in the bending treatment samples, especially the relative content of glucose,2,3,4,5,6-pentakis-O-(trimethylsilyl) and alpha,D-glucopyranoside,1,3,4,6-trimethylsilyl. In the wood-forming tissue from upright wood samples, their content was almost four times that of the bending treatment samples. The content of fructose,1,3,4,5,6-pentakis-O-(trimethylsilyl) in vertical wood was higher than bent wood. The reduction in the relative abundance of polysaccharides in the bent stem is in accordance with the decrease of cellulose content in compression wood (34%), compared to normal wood (44%) (Yeh *et al.* 2005). Cellulose synthesis in wood cell walls is a complex polymerization process performed by substrate, precursor, and many relevant enzymes. According to current studies, UDP-glucose is regarded as the immediate substrate for cellulose synthesis (Kqczkowshi 2003; Coleman *et al.* 2007), but UDP-glucose can be formed by two metabolic pathways. First, it changes glucose to glucose-6-phosphate, glucose-1-phosphate, and at last via the conversion to UDP-glucose by pyrophosphorylase. Secondly, sucrose can be converted into UDP-glucose and fructose by sucrose synthase (Babb and Haigler 2001; Delmer and Haigler 2002). The reduction of glucose in bent stems could result in a decrease of UDP-glucose and the decrease of fructose could reduce the conversion of sucrose to UDP-glucose. All of these effects cause the lower cellulose content in the bent stem and are in accordance with the results from FTIR and chemical constitution analyses. Furthermore, a higher abundance of inositol was detected in disease-stressed plants (Nelson *et al.* 1998) and also increased in the bent stem compared to the upright tree (Fig 4). However, some metabolites that participate in the lignin synthesis pathway, e.g. shikimic acid, coniferin, and *p*-glucocoumaryl alcohol,

which were identified in previous research (Yeh *et al.* 2006), were not found in this experiment.

Table 2. Relative Abundance of Significant Metabolites from Different Wood-forming Tissues

Metabolites	Relative Content (μg·g^{-1})					
	0d	1d	7d	15d	30d	60d
Lactic acid TMS	0.037±0.004	0.029±0.006	0.030±0.002	0.023±0.002	0.031±0.001	0.028±0.003
Ethanedioic acid TMS	0.068±0.007	0.060±0.006	0.061±0.006	0.047±0.005	0.045±0.003	0.060±0.005
Glycerol TMS	0.063±0.005	0.035±0.014	0.073±0.034	0.043±0.003	0.196±0.001	0.074±0.004
1-Cyclohexene -1-carboxylic acid TMS	-	0.065±0.003	0.056±0.011	0.082±0.007	0.041±0.003	0.111±0.005
Octanedioic acid TMS	0.042±0.006	0.096±0.012	0.102±0.018	0.204±0.009	0.121±0.015	0.148±0.003
Fructose,1,3,4,5,6-pentakis-O-(trimethylsilyl)	0.290±0.021	0.169±0.017	0.207±0.018	0.225±0.021	0.216±0.017	0.193±0.006
Glucose,2,3,4,5,6-pentakis-O-(trimethylsilyl)	0.379±0.063	0.039±0.004	0.075±0.007	0.083±0.007	0.062±0.010	0.050±0.002
Cyclodecasiloxane, eicosamethyl-	0.133±0.026	-	-	-	-	-
Tetracosamethyl-cyclododecasiloxane	0.331±0.031	-	-	-	-	-
Myo-inositol	0.025±0.004	0.031±0.006	0.166±0.013	0.113±0.015	0.153±0.012	0.070±0.003
Hexadecanoic acid TMS	-	0.231±0.025	0.299±0.023	0.272±0.039	0.265±0.033	0.410±0.041
Androst-2-en-17-amine,4,4-dimethyl-N-(2-)	0.142±0.035	0.100±0.016	0.120±0.004	0.109±0.007	0.100±0.005	0.098±0.004
Octadecanoic acid TMS	0.343±0.023	0.177±0.019	0.228±0.021	0.198±0.006	0.195±0.006	0.289±0.032
Cyclodecasiloxane eicosamethyl-	0.407±0.050	-	-	-	-	-
Tetracosamethyl-cyclododecasiloxane	0.540±0.086	-	-	-	-	-
3Trifluoromethylbenzylamine, N,N-diundecyl	0.012±0.001	0.040±0.010	0.053±0.002	0.047±0.007	0.041±0.003	0.070±0.005
4-Methylthio-N-pheny-1,2-carbazoledicarboximide	0.009±0.004	0.041±0.002	0.049±0.005	0.045±0.005	0.041±0.002	0.066±0.003
Silane,dimethylpentyloxy -hexadecyloxy-	0.160±0.017	0.088±0.005	0.112±0.006	0.106±0.008	0.102±0.007	0.112±0.007
alpha,D-Glucopyranoside, 1,3,4,6-trimethylsilyl	0.878±0.058	0.229±0.006	0.246±0.013	0.433±0.044	0.245±0.022	0.316±0.013
Cyclodecasiloxane eicosamethyl-	0.515±0.053	-	-	-	-	-
Bis(trimethylsilyl) monostearin	0.152±0.030	0.128±0.004	0.147±0.014	0.142±0.013	0.118±0.015	0.193±0.009
2-Hydroxymandelic acid TMS	-	-	-	-	0.017±0.006	0.135±0.011
2H-1-Benzopyran,3,4-dihydro-2-[3,4-bis (trimethylsilyl)oxy]phenyl]-3,5,7-tri(trimethylsilyl)oxy]-	-	-	-	0.072±0.012	0.026±0.015	0.090±0.008
alpha,D-Glucopyranoside,1,3,4,6-trimethylsilyl	-	-	0.124±0.013	0.244±0.024	0.065±0.004	-

TMS= trimethylsilyl ester

Note: Results are the mean ± standard deviation; "- "Not detected.

Comparing metabolite patterns after different bending times, the content of polysaccharide sharply decreased after 1-day of bending treatment, and the discrepancy was an appropriate shrink at 7-day and 15-day bending. However, there was a significant decrease at later bending stages (30-day and 60-day) (Fig. 5). Similarly, the relative

abundance of other metabolites also shows a turning point at early bending stages (7-day and 15-day). Generally, reaction wood formation was produced after 1 to 2 weeks of the bending treatment. Interestingly, trees can transiently make changes at a molecular level in response to stress stimuli. A majority of metabolites were much different after the 1-day bending treatment. Expressed sequence tag results show that COMTs and a majority of auxin-related genes were down-regulated significantly in response to 6 hours of bending treatment in a 2-year-old yellow poplar sapling (Jin *et al.* 2011). COMTs catalyze the methylation of caffeate and 5-hydroxyferulate during monolignol biosynthesis (Anterola *et al.* 2002). The variation of compression wood cell wall composition was regulated by differential expression at the integral molecular level of wood-forming tissue.

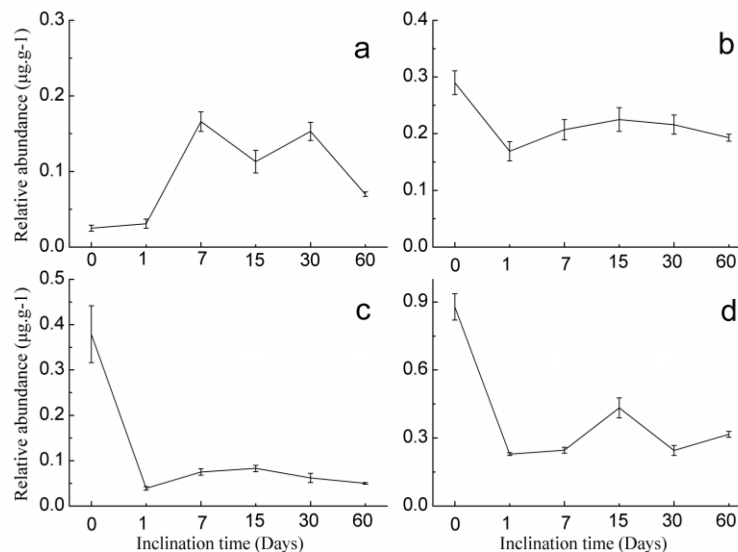

Fig. 5. Significant metabolite changes in wood forming tissue of different bend times (data points were the mean ± standard deviation)
a: Myo-inositol, b: Fructose,1,3,4,5,6-pentakis-O-(trimethylsilyl), c: Glucose,2,3,4,5,6-pentakis-O-(trimethylsilyl), d: alpha,D-Glucopyranoside,1,3,4,6-trimethylsilyl

Currently, wood formation is considered to be the development of secondary xylem in a woody plant. The cell walls of a woody plant exhibit great variation in morphology and composition in different species, individual trees, or even within a single tree (Li 2002). The trees' cell walls are strongly influenced by genes and proteins expressed during the formation of the primary and secondary walls (Zhang *et al.* 2000). However, there is little known about the molecular mechanisms of wood formation. Reaction wood is an ideal system to explore the mechanism of secondary cell wall formation. In recent decades, many genes and proteins were identified that were specifically expressed in reaction wood cell wall formation. Taken together with the up-regulation of several representative genes in the lignin and monolignol biosynthetic pathway, this evidence strongly suggests that the increased lignin deposition in the compression wood that was induced by the mechanical bending treatment was caused by

both the up-regulation of biosynthesis and the laccases-mediated polymerization of monolignols (Jin and Kwon 2009). The finding that compression wood cell walls had higher lignin content and that *PtaPRP1* was expressed more often in compression wood than in the vertical stem might reflect a relationship between some proline-rich proteins and lignin formation (Zhang *et al.* 2000).

Qualitative or quantitative analysis of the changes in metabolites in trees resulting from changes of ambient conditions could imply relevant metabolic pathways and networks through the variation of corresponding metabolites. Subsequently, it could reveal key regulatory factors and molecular mechanisms in metabolic pathway through describing a series of substrates, products, intermediates, and key enzymes in these pathways. Metabolite analysis is timesaving and an accurate way to estimate an expression level of a gene and conclude its function. Therefore, this method is suitable for wood formation research because gene functional verification requires a long period of time in the case of transgenic plants. Identification and comparison of metabolites related to cell polymers, biosynthesis, and important wood properties from wood-forming tissue contributes to finding different genes. As a result of a multiplicity of regulatory interactions at all levels in xylem cells, a particular change in wood function or phenotype occurs. However, single point mutations might often lead to complex responses at the level of the whole organism. Therefore, metabolic profiling results combined with genetic and protein results can better reveal the molecular mechanisms of cell wall development and wood formation.

CONCLUSIONS

1. Metabolites and chemical contents changed their abundance when pine saplings were bent into an inclined position for different periods of time.
2. Metabolites in wood-forming tissue display significant differences in bent stems compared to vertical samples. The expression abundance of carbohydrates sharply decreased and some substances related to stress increased in bent stems. However, no strict correlation was shown between the changes in metabolite expression abundance and the number of inclined days.
3. The intensity of the absorption peak at about 2900 cm^{-1} and 1740 cm^{-1} clearly decreased in wood-forming tissue from bent stems. Simultaneously, the intensity of the peak at 1510 cm^{-1} significantly increased and had a positive correlation to inclination days. Cellulose crystallinity was reduced in bent stems and reached its minimum at 15-days bending. Acetyl bromide lignin content increased in bent stems and reached its maximum at 15-days bending.
4. Compression wood tissue can be induced by inclining stems for a 15-day period in *Pinus koraiensis* saplings. The changes of metabolites associated with chemical results in inclined wood forming tissue suggest that wood cell wall-related monomers were changed in the biosynthesis network.

ACKNOWLEDGMENTS

The authors are grateful for the support of the Programme of Introducing Talents of Discipline to Universities of China No. B08016.

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Article submitted: November 20, 2011; Peer review completed: April 16, 2012; Revised version received: May 14, 2012; Further revisions: May 22, 2012; Accepted: June 14, 2012; Published: June 19, 2012.