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A COMPARISON OF METABOLITES IN WOOD-FORMING TISSUES FROM EIGHT COMMERCIAL TIMBER TREE SPECIES OF HEILONGJIANG PROVINCE IN CHINA

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ABSTRACT

Four coniferous and four deciduous commercial tree species from Northeastern of China were selected to investigate the differences of metabolites in wood-forming tissues by gas chromatography-mass spectrometry. The results showed that the identified metabolites mainly consisted of neutral sugars, lipids, and organic acids. The mean contents of both arabinofuranose and 1-cyclohexene-1-carboxylic acid were higher in coniferous trees than in deciduous ones. Similarly, the D-fructose and D-glucose content was significantly higher in coniferous trees than deciduous trees, but the total contents of these two sugars was roughly equal among most tree species. The mean content of lactic acid, glycerol and malic acid was lower in coniferous trees than deciduous trees. The malic acid content decreased in later-stages of wood formation than in early-stage for all tree species. The content of L-proline and myo-inositol was greater in later-stage of wood formation than early-stage. The content of octadecanoic acid, D-fructose and D-glucose decreased in later-stage of wood formation for most tree species. All of this suggested that the metabolites in wood-forming tissues showed the significance of species-specific and seasonal dynamic differences among the eight tree species.

KEYWORDS: Metabolic profiling, early wood, latewood, wood formation, sugars.

INTRODUCTION

Wood formation is controlled by both endogenous and exogenous factors. Endogenous factors such as tree genotype or physiological processes (Schrader et al. 2004), can be termed as

different genus and different tree species, e.g. coniferous trees and deciduous trees. Generally Coniferous trees produce softwood where hardwoods come from deciduous trees. It is known that softwoods mainly consist of uniform wood cells (tracheids), whereas multitudinous cells (vessels, fibers, rays) make up the structure of hardwoods. Furthermore, softwoods possess a higher lignin content than hardwoods, whereas hardwoods contain a greater concentrations of hemicellulose. It is due to these differences and variation in the anatomy and chemistry of woods that result in the difficulties that softwoods and hardwoods face for many applications. On the other hand, temperature, light and water availability (Deslauriers and Morin 2005) also serve as important exogenous factors that affect the development of wood characteristics, like early-wood, later-wood, and growth rings. From a biochemistry perspective, wood formation is the results of a tree converting free carbon dioxide to organic carbon via photosynthesis and through the complex and efficient biosynthesis, which is a process of energy metabolic and substance transformation. During wood forming there are there are many precursors, intermediates and enzymes, which influence the metabolites present and participate in the physiological activity of the wood forming. The metabolization of wood formation is a dynamic life function. However, the knowledge of the metabolites present during wood formation and their difference within tree species is limited.

Because of the large variation and differences in metabolites, the metabolism was employed as the basic analytical method to understand the wood forming. By focusing on the metabolites, molecular weight less than 1000 Da, all participated in organism or physiology histocyte. Morris et al found that the relationship between the cellulose content and metabolites in the wood forming tissues from various genotypes of Loblolly pine, as well as showing that the metabolic level can be used as tool for the study of wood formation (Morris et al. 2004). Robinson reported that the metabolites in wood-forming tissues of *Pseudotsuga menziesii* showed a strong dependence on phenotypic characteristic and growing location, rather than hereditary constitution (Robinson et al. 2007). Compared to normal wood, the relative contents of shikimic acid and coniferin, which serve as important intermediates in lignin biosynthesis, significantly increased in accordance with the higher lignin content present in compression wood (Yeh et al. 2006). Andersson-Gunneras et al. (2006) measured metabolites present in wood-forming tissues of *Populus davidiana* Dode using GC-MS combined with the gene expression analysis, the results suggested that the cell wall polymer biosynthesis undergoes de novo programmed regulation. Saccharides and lipids content was greater in *Pinus koraiensis* compression wood-forming tissues compared to normal wood tissues and the changes were related to inclination angle and degree (Shi and Li 2012). However, the analysis of the metabolic process of wood formation and simultaneous comparison among several tree species has never been reported or found in any literature review.

Herein, eight tree species, *Pinus koraiensis*, *Pinus sylvestris*, *Abies nephrolepis*, *Larix gmelini*, *Betula platyphylla*, *Fraxinus mandshurica*, *Populus simonii* and *Ulmus propinqua* were selected. These tree species are economically and ecologically important species in the timberline of northeastern of China, where they play an important role in forestry ecology and wood supply. In our previous studies (Shi and Li 2016), the early-stage

wood-forming tissues were analyzed by Fourier Transformed Infrared spectroscopy method to distinguish the differences among tree species. Thus, the objective of this work aimed to understand: *(1)* the differences between the metabolites in wood-forming tissue of coniferous and deciduous trees species, *(2)* the changes of metabolites in early-stages and later-stages of wood formation in one growth season. The differences in the metabolites present in wood-forming tissue of the different tree species were determined and compared based on the differences between coniferous and deciduous trees, as well as early-stage and late-stage wood formation.

MATERIAL AND METHODS

Plant materials and collection

Eight important coniferous and deciduous trees growing under the same climatic conditions, which were healthy stands with three replicates for each tree species, were selected from the forestry land, Harbin, China. The ages and diameters of the trees are presented in Tab. 1. The wood forming-tissues were collected in June and September 2016 according to the former publishes (Shi and Li 2012, Paiva et al. 2008). To collect the wood-forming tissues, the bark of each tree (three trees each species) was removed at chest height (above ground 1.25 m) and the differentiating xylem tissues were scraped with a razor blade from the outermost side of the exposing stem. The harvested tissues were frozen in the liquid N_2 immediately. Wood samples were well grinded in the liquid nitrogen and stored at -80°C.

Tab. 1: Information of sampling trees.

Species	Average diameter at breast high (cm)	Average tree age (year)
Pinus sylvestris	26.8	55
Pinus koraiensis	16.5	52
Abies nephrolepis	25.3	58
Larix gmelini	21.6	46
Fraxinus mandshurica	21.3	59
Populus simonii	13.5	9
Ulmus propinqua	13.5	28
Betula platyphylla	14.5	35

Metabolites extraction and GC-MS examination

The metabolites of wood-forming tissues were extracted according to our previous reports (Shi and Li 2012, Lisec et al. 2006). In brief, about 50 mg of frozen wood powder was placed in a 1.5 mL tube and mixed with 1 mL of precooled methanol and 45 µL Ribitol (Sigma, USA) (dissolution in ddH_2O and 2 $mg \cdot mL^{-1}$, according to an internal standard). The mixture was incubated at 70°C and 950 rpm for 15 min then for centrifuged 10 min at 12000 rpm. The supernatant was then mixed with equal parts volume of precooled chloroform and incubated at 37°C and centrifuged 950 rpm for 5 min. Then 500 µL of ddH_2O at 4°C was added and centrifuged at 4000 rpm for 15 min. The 200 µL supernatant was vacuum-dried at -60°C for 4 hours and then re-dissolved in 50 µL Solution A [20 $mg \cdot mL^{-1}$ Methoxyamine hydrochloride

(Sigma, USA) dissolved in Pyridine (Sigma, USA)]. The mixture was incubated at 37°C for 2 hours and then 100 μL of Solution B [20 $\mu L \cdot ml^{-1}$ Alkanes (Sigma, USA) dissolved in MSTFA (Sigma, USA)] was added at the same temperature and incubated for 30 min. After 24 hours at room temperature, one micro liter extraction was injected into the GC-MS (Varian450GC-240MS, USA), which has a VF-5 ms column (30 m × 0.25 mm × 0.25 μm). Injection port temperature was 250°C, with a flow rate of 1.0 $ml \cdot min^{-1}$, initial temperature of 70°C and heating rate of 5°C$\cdot min^{-1}$ until a 300°C and cooled for 5 min until 70°C was reached. Helium (99.99%) was used as a carrier gas and a flow rate of 2 $ml \cdot min^{-1}$ was maintained with a split-flow of 50 : 1000. The electron bombardment of the MS ionization was at 70eV with an ion source temperature of 300°C and mass range of 50-1000 $m \cdot z^{-1}$.

Data acquisition and analyze

The gas chromatogram was observed using MS Workstation version 6.9.3 and the online results were searched with the NIST (National Institute of Standard and Technology, USA) mass spectrum data with a similarity ≥ 75%, and referred to related literature for metabolites identification and classification (Yeh et al. 2006, Shi and Li 2012, 2015). The metabolites relative contents were expressed by the equation of:

$$C_x = [(A_x/A_i) \times 0.045 \times 2 \text{ mg mL}^{-1}] / m_0 \ (\text{mg} \cdot \text{g}^{-1}) \quad (1)$$

where: C_x is the relative content of identified metabolite; A_x is the peak area of identified metabolite; A_i is the peak area of internal standard; and m_0 is the dry weight of wood-forming tissue (Shi and Li 2012).

RESULTS AND DISCUSSION

Overall of metabolites in wood forming-tissue of coniferous and deciduous trees

The comprehensive comparison of chromatograms in the eight tree species and 45 peaks were selected. Based on the mass spectrum database reported by (Yeh et al. 2006, Shi and Li 2012), 34 peaks were identified and 11 peaks were unknown. Figs. 1 and 2 show the categories and varieties of metabolites in wood-forming tissue from the eight-tree species. In these figures, it can be observed that metabolites were different between tree species. The identified metabolites were mainly classified into neutral sugars, lipids, organic acid, amino acid, *N*-compounds. Neutral sugars, which are the foundation of energy and substance metabolism in trees, are the most predominant metabolites. D-fructose, D-glucose and a-d-glucopyranoside were the most abundant sugars in the wood-forming tissue, but their contents varied by tree species and the growth season (Tabs. 2 and 3). The percentage of total sugars in coniferous tree metabolites was usually from 43.74% - 84.04%, but was 31.22% - 87.95% in deciduous trees (Figs. 1 and 2). The total percentage of sugars present was lowest at 31.22% and highest at 87.95% in *F. mandshurica* early-stage and *P. simonii* late-stage of the growth season, respectively. In all eight species, the total sugar concentration increased in

the later-stages compared to the early-stages of the growth season. This seasonal trend of total sugar content was consistent with the former reports (Budzinski et al. 2016, Gruber et al. 2013, Dietze et al. 2014), which suggested that, during later-stages of wood formation, low temperature and water availably caused a reduction of wood cell wall biosynthesis. More carbohydrates were able to be mobilized for the next growing season when there was more supply respiration during low photosynthesis (Ögren 2000), better response to cold acclimation (Welling and Palva 2006, Bonhomme et al. 2009, Turhan and Ergin 2012) and early spring growth (Barbaroux and Breda 2002). D-xylose was only detected in deciduous species, which corresponded to higher xylose content in hardwood hemicellulose.

The lipids were the second most abundant metabolites and were comprised of hexadecanoic acid, octadecanoic acid and monostearin. Similar to sugar metabolites, the percentage of lipids was commonly lower in coniferous woods (0.92%-7.14%) than in deciduous woods (1.19%-12.52%). However, the lipids concentration in all tree species sharply decreased in later-stage of the growth season than in the early-stage. Obst (1998) defined hexadecanoic acid and octadecanoic acid as cutin, which plays an important role in protecting woods (Obst 1998).

The result indicated that when living at boreal and temperate regions, higher of levels of lipid present in the wood tissue is beneficial for trees during colder temperatures and trends to slow wood formation.

In addition, many organic acids, such as malic acid and lactic acid, were detected in wood-forming tissues of all the tree species. The organic acid was commonly intermediate of the tricarboxylic acid (TCA) cycle and incorporated in the TCA cycle to generate energy (Fernie et al. 2004, Hijza and Killiny 2017). The percentage of organic acid was much higher in deciduous trees (4.8% - 6.13%) than in coniferous trees (1.23% - 2.17%) at the early-stage of wood formation (Fig. 1). This was maybe due to greater photosynthesis effectiveness of deciduous trees over conifer trees. However, at late-stage wood formation the organic acid percentages was almost the same (Fig. 2). In *A. nephrolepis* and *L. gmelini,* organic acid concentration increased more in winter than in summer, whereas *P. koraiensis* and *P. sylvestris* showed the inverse change. Interestingly, the organic acid concentration in all deciduous woods significantly decreased in winter compared to the summer. The defoliation in deciduous trees is responsible for the decrease in organic acid concentrations in the winter.

Low quantities of alcohol metabolites were also detected, of which myo-inositol and glycerol were the most predominant in the wood-forming tissues. The percentage of alcohol was lower in coniferous trees than in deciduous trees (Figs.1 and 2). The variation of total alcohols during the change in seasons was different for each tree species. Contrary to the Budzinski's report (Budzinski et al. 2016), most amino acid metabolites were more abundant in the later-stage of wood formation for all tree species (Figs.1 and 2).

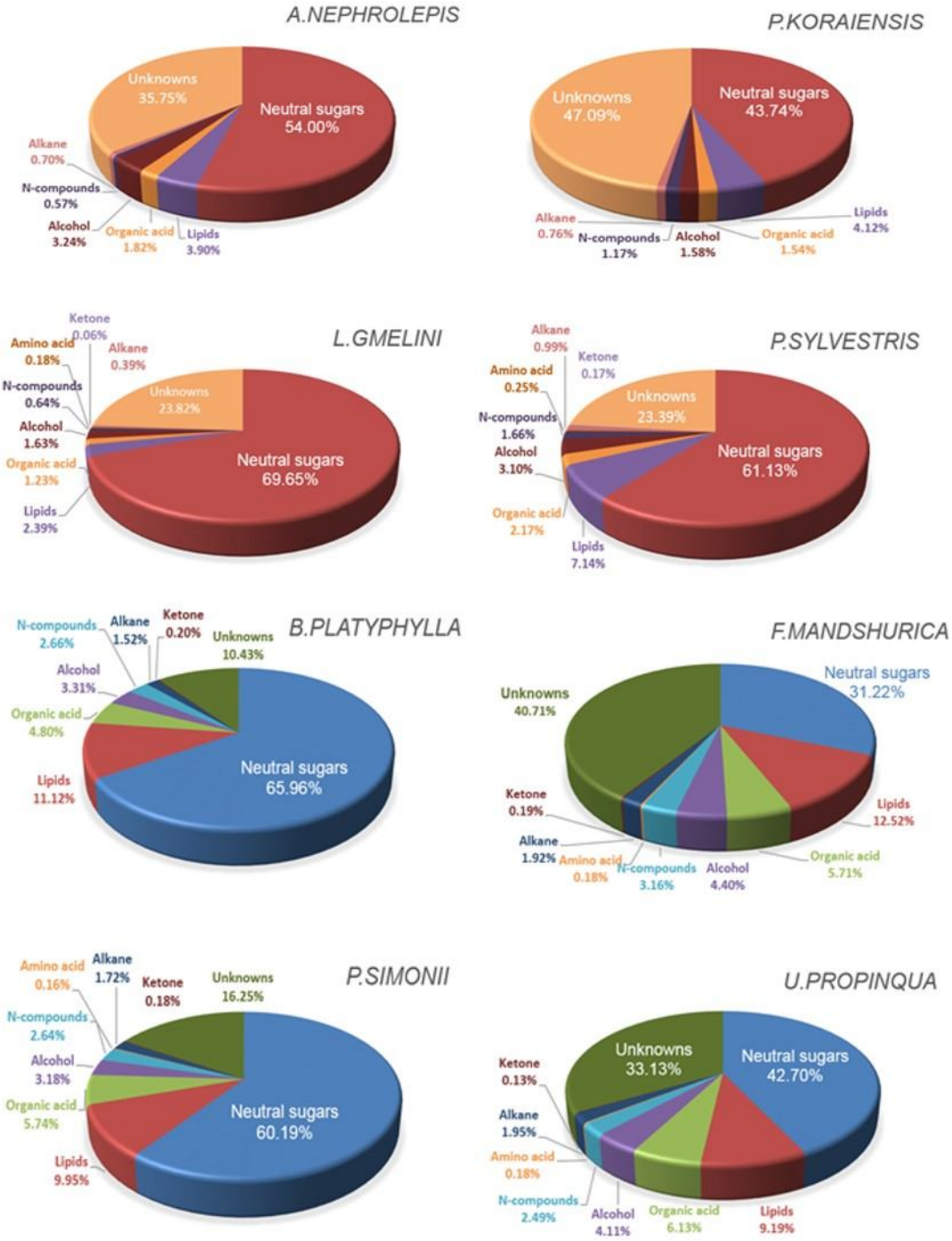

Fig. 1: Percentage composition of metabolites in the wood forming-tissue from 8 tree species at early-stage of growth season.

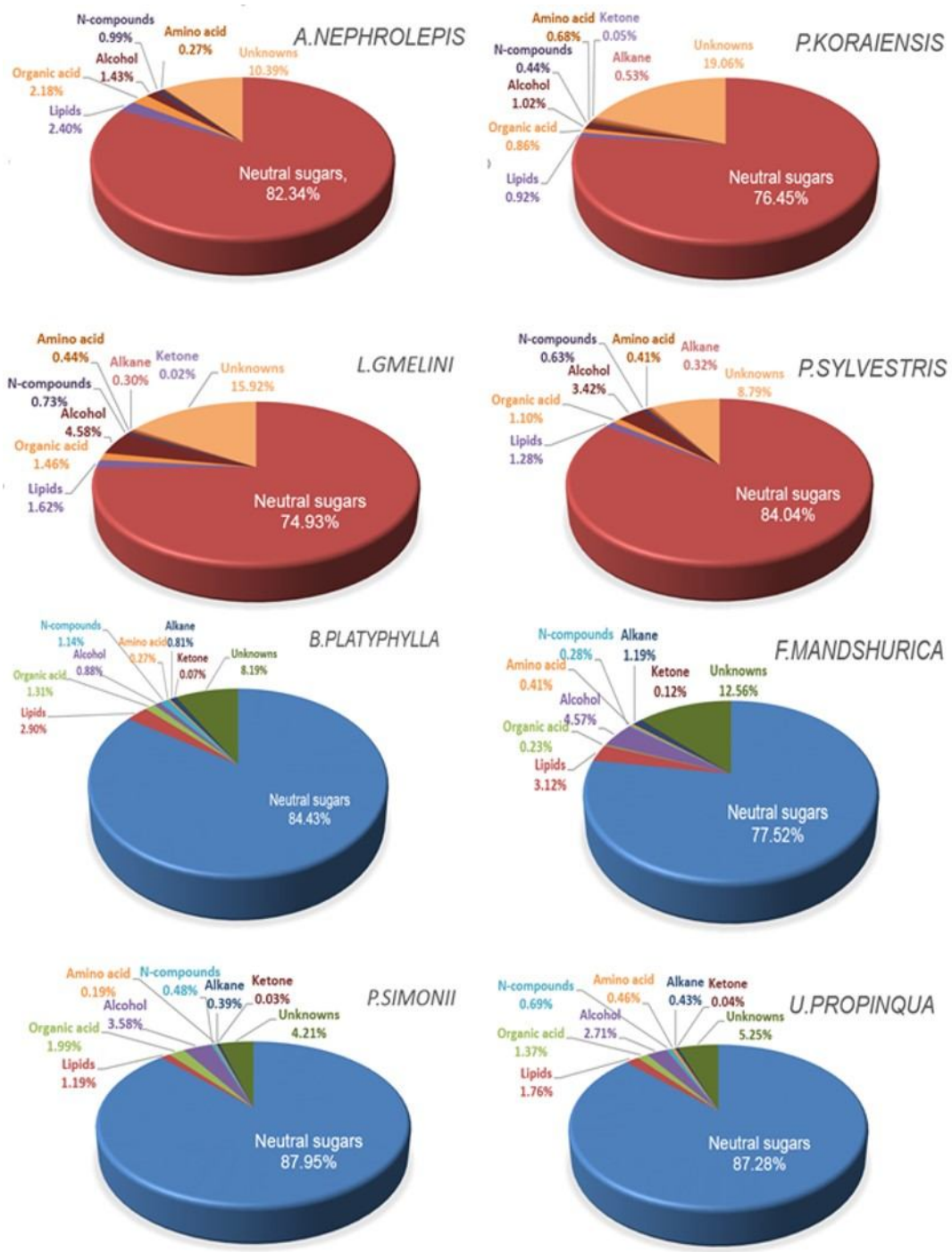

Fig. 2: Percentage composition of metabolites in the wood forming-tissue from 8 tree species at late-stage of growth season.

Metabolites changes in early-stage and late-stage of wood formation

The composition of most metabolites in the wood-forming tissues changed between the early-stage and late-stage of wood formation within tree species (Tabs. 2 and 3).

Sugars

Owing to the stability and reproducible derivatives for many sugars and organic acids,

trimethylsilyl (TMS) derivatization method was chosen to investigate the sugar content in wood-forming tissues. A-d-glucopyranoside was the most abundant sugar and its concentration notably increased in the late-stage of the growth season for all tree species. The greater increment of A-d-glucopyranoside content was present in *P. koraiensis* (from 11.591 $mg \cdot g^{-1}$ to 55.924 $mg \cdot g^{-1}$) and *U. propinqua* (from 9.474 $mg \cdot g^{-1}$ to 58.038 $mg \cdot g^{-1}$). D-fructose and D-glucose were the other most predominant sugars, but their concentrations decreased during late-stage wood formation in most trees besides *B. platyphylla* and *F. mandshurica*. In general, UDP-Glucose was regarded as the precursor for cellulose biosynthesis (Coleman et al. 2007, Kqczkowshi 2003). The relative glucose content decreased in the wood-forming tissues and lead to a lower yield of UDP-glucose. The fructose reduction also indicated that the conversion efficiency from sucrose to UDP-glucose declined. It was indicated that the cellulose synthesis efficiency decreased in latewood compared to early wood. In deciduous trees xylose content was higher during the summer, which may be caused by cell wall division and expansion during tree growth (Budzinski et al. 2016, Ko et al. 2011). **S**mall amounts of D-xylofuranose, arabinofuranose, D-turanose and lactose were detected, in most case the content increased or was only detected in later-stages of the growth season (Tabs. 2 and 3). It shows that the glucose and fructose could be converted to other monosaccharides and used as a source of energy in various biological processes. Although the composition of sugar metabolites varied in summer and winter seasons, the total sugar concentration was roughly equal among tree species. Budzinski et al (2016) investigated metabolites in Eucalyptus cambial tissue and reported that the total sugar concentration increased in the winter when identified by GC-MS; but their concentrations were similar in summer and winter seasons when measured by HPLC.

Organic acids

Organic acids served as important intermediates participant in several plant biochemical pathways, such as energy production, amino acid biosynthesis, and secondary metabolites biosynthesis (López-Bucio et al. 2000). The Malic acid concentration was more abundant during the early-stage of the growth season in all tree species (Tabs. 2 and 3), especially in deciduous trees (1.036 $mg \cdot g^{-1}$ to 2.246 $mg \cdot g^{-1}$). The acid content was reduced by 86.2% in *B. platyphylla* and not detected in *F. mandshurica* during the late-stage of the growth season. Apart from *A. nephrolepis* and *L. gmelini*, the other tree species showed lower lactic acid concentration in the late-stage of the growth season. Ethanedioic acid and 1-cyclohexene-1-carboxylic acid concentrations were higher in coniferous trees at the late-stage of the growth season, but it was the inverse in deciduous trees. Butanoic acid had a greater presence in coniferous trees (0.09 $mg \cdot g^{-1}$ to 0.345 $mg \cdot g^{-1}$) than in deciduous trees (0.017 $mg \cdot g^{-1}$ to 0.278 $mg \cdot g^{-1}$), however the content differs from species to species. Previous studies (Budzinski et al. 2016, Chia et al. 2000) showed that the more abundant organic compounds during summer was a result of vigorous activity of TCA cycle and metabolized to produce more other energy and carbon compounds.

Lipids and alcohols

The hexadecanoic acid, octadecanoic acid and monostearin were the most prominent lipids in the wood-forming tissues, and their concentrations were higher in deciduous trees. The hexadecanoic acid concentration ranged from 0.751 $mg \cdot g^{-1}$ to 1.46 $mg \cdot g^{-1}$ in coniferous trees, but up to 1.689 $mg \cdot g^{-1}$ to 2.844 $mg \cdot g^{-1}$ in deciduous trees during the early-stage of the growth season. The octadecanoic acid concentration was between 0.501 mg g^{-1} to 0.952 $mg \cdot g^{-1}$ in coniferous trees and was between 1.094 $mg \cdot g^{-1}$ to 1.900 $mg \cdot g^{-1}$ in deciduous trees during the early-stage of the growth season. Commonly, the lipid concentrations were more abundant during the early of growth season. Octadecanoic acid content was reduced by 98.5% in *P. simonii* and 97.1% in *P. koraiensis* during the late-stage of the growth season, respectively. According to former reports, the hexadecanoic acid and octadecanoic acid content significantly decreased in latewood and the former was the first production via fatty acid synthesis, which was benign from acetyl-CoA and then the latter was produced based on malonyl-CoA (Blerchert et al. 1995). The fatty acids served as the precursor for some of the secondary metabolite synthesis and its changes suggested the decline of the secondary metabolites accumulation rate in the later-stages of wood formation.

Myo-inositol is generally known as an important sugar alcohol in various cellular processes of plant physiology, such as stress response, cell wall biogenesis and growth regulation and so forth (Irvine and Schell 2001, Abid et al. 2009, Valluru and Van den Ende 2001). Myo-inositol total concentration was higher in coniferous than deciduous trees. Furthermore, myo-inositol concentration changed during the early and late growth seasons and was different in all eight tree species. At the late-stage of the growth season, the myo-inositol concentration decreased in *A. nephrolepis* (from 1.198 $mg \cdot g^{-1}$ dropped to 0.508 $mg \cdot g^{-1}$), *P. koraiensis* (from 0.567 $mg \cdot g^{-1}$ dropped to 0.441 $mg \cdot g^{-1}$) and *B. platyhylla* (from 0.409 $mg \cdot g^{-1}$ dropped to 0.074 $mg \cdot g^{-1}$). However, it increased in the other five tree species, especially in *F. mandshurica* (from 0.614 $mg \cdot g^{-1}$ to 4.418 $mg \cdot g^{-1}$) and *L. gmelini* (from 0.758 $mg \cdot g^{-1}$ to 3.434 $mg \cdot g^{-1}$). This finding means that the content of myo-inositol was tree species specific. Glucitol decreased in content from early-stage to late-stage in coniferous but it was the inverse in deciduous species. Another detected alcohol, glycerol, which is an intermediate of 3-phosphoglyceric acid metabolic, was more abundant in the early-stage of the growth season, especially in deciduous trees. This was a result of the reduction of glucose content during the late-stage of wood formation.

Amino acids and N-compounds

Small amounts of amino acids, such as L-proline, L-aspartic acid and serine were detected in the wood-forming tissues. Based on our previous results (Shi and Li 2012, 2015), amino acids were more abundant in the later-stage of the growth season and proline was the most prominent of the amino acids. The highest (0.486 $mg \cdot g^{-1}$) L-proline concentration was in late-stage *P. koraienis* wood-forming tissue. The proline primarily existed in cytoplasm solution and was produced from 2-oxoglutarate metabolism of the TCA. It was found that the Proline-rich protein not only participated in xylem cell differentiate but also being related to lignification, in which, the initial and reactive site for monolignol polymerization were likely provided (Zhang et al. 2000). Many reports showed that the content increased in compression wood formation

compared to the normal wood, indicating the proline responded to abiotic stress (Yeh et al. 2006, Shi and Li 2012, Kusano et al. 2008). The L-aspartic acid and serine were detected in the late-stage of all tree species and it had the highest concentration in *U. propinqua* (0.246 $mg \cdot g^{-1}$) and *F. mandshurica* (0.462 $mg \cdot g^{-1}$).

CONCLUSIONS

Metabolites in wood-forming tissues from eight important commercial tree species were extracted and 34 peaks were identified by GC-MS after being derivatized by TMS. The wood-forming tissues were rich in natural sugars, organic acids and lipids. Metabolites contents were determined to be tree species-specific and the changes in the early-stage and late-stage of growth the seasons. The percentage of natural sugars and lipids were higher in deciduous trees than in coniferous trees. D-xylose was detected only in deciduous trees. The total natural sugar content was more abundant in the late-stage than that in the early-stage and the a-d-glucopyranoside notably increased at the late-stage in all tree species. However, the lipids content sharply dropped in the late-stage of the growth season. The percentage of organic acid was much higher in deciduous trees than in coniferous trees at the early-stage of the growth season. The results suggested that the metabolic substance allocation in wood formation varied by tree species and growth seasons.

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Tab. 2: Metabolites in wood forming-tissue of four coniferous trees. Note: the values are given as mean ± SD (n=3). "---"in the table was not detected. EW: early wood; LW: late wood.

Retention time (min)	Metabolites	Relative amount ($mg \cdot g^{-1}$)							
		A.nephrolepis		*P. koraiensis*		*L. gmelini*		*P. sylvestris*	
		EW	LW	EW	LW	EW	LW	EW	LW
7.04-7.08	Lactic acid	0.121±0.012	0.210±0.008	0.136±0.007	0.064±0.009	0.057±0.013	0.141±0.031	0.133±0.027	0.096±0.015
9.141-9.181	Ethanedioic acid	0.090±0.005	0.358±0.037	0.103±0.003	0.168±0.011	0.06±0.003	0.237±0.032	0.053±0.008	0.117±0.012
12.590-12.613	Glycerol	0.598±0.081	0.488±0.091	0.431±0.053	0.348±0.045	0.298±0.071	0.474±0.039	0.411±0.068	0.349±0.055
14.778-14.892	Serine	---	---	0.031±0.011	---	---	---	---	
18.182-18.197	Malic acid	0.574±0.042	0.386±0.049	0.324±0.062	0.109±0.008	0.225±0.023	0.118±0.016	0.398±0.061	0.191±0.020
18.942-18.954	L-Aspartic acid	---	0.096±0.048	---	0.039±0.007	---	0.145±0.022	---	---
19.059-19.065	L-proline	---	0.118±0.019	---	0.486±0.047	0.118±0.021	0.232±0.026	0.102±0.033	0.262±0.056
19.166-19.176	Butanoic acid	---	0.224±0.068	0.132±0.006	0.116±0.017	0.158±0.030	0.345±0.056	---	0.09±0.011
19.457-19.468	Silane	0.306±0.061	0.368±0.064	0.412±0.046	0.175±0.041	0.193±0.012	0.301±0.033	0.330±0.052	0.226±0.036
21.188-21.207	D-Xylofuranose	---	0.174±0.012	---	0.129±0.035	---	0.141±0.068	0.046±0.012	0.124±0.027
24.355-24.362	Arabinofuranose	---	0.184±0.018	---	0.142±0.036	---	0.132±0.031	---	0.149±0.009
25.341-25.358	D-Fructose	0.176±0.033	---	0.348±0.079	---	0.103±0.026	---	---	---
25.454-25.493	1-Cyclohexene -1-carboxylic acid	0.302±0.022	0.482±0.094	0.192±0.021	0.211±0.029	0.13±0.026	0.285±0.039	0.188±0.028	0.157±0.016
26.526-26.555	D-Fructose	4.508±0.077	1.978±0.167	5.539±0.549	1.758±0.193	6.091±0.613	3.225±0.372	4.644±0.973	2.227±0.428
26.733-26.752	D-Fructose	3.792±0.051	1.414±0.831	4.366±0.336	1.324±0.118	4.631±0.728	2.387±0.269	3.406±0.521	2.227±0.351
26.898-26.912	Glucitol	0.142±0.007	0.07±0.042	0.122±0.033	0.026±0.006	---	---	---	0.034±0.005
27.038-27.053	D-Glucose	3.112±0.029	2.932±0.907	6.935±0.499	2.437±0.162	6.367±0.637	5.966±0.519	6.567±0.012	3.06±0.236
27.407-27.426	D-Glucose	0.790±0.016	0.648±0.051	1.932±0.088	0.574±0.035	1.835±0.232	1.471±0.662	1.538±0.189	0.742±0.048
28.283-28.296	Myo-Inositol	0.302±0.039	0.068±0.009	0.273±0.037	0.105±0.023	0.47±0.069	2.284±0.810	0.146±0.029	0.206±0.043
28.816-28.823	a-D-Glucopyranose	---	---	0.263±0.027	0.268±0.041	---	---	---	---
29.725-29.734	Gluconic acid	---	0.044±0.009	0.202±0.012	0.036±0.003	0.167±0.035	0.121±0.027	0.101±0.015	0.042±0.008
29.859-29.870	2-Ethoxyethanol	---	0.048±0.020	---	0.022±0.004	---	---	---	0.022±0.000
30.273-30.288	Hexadecanoic acid	1.124±0.062	1.272±0.139	1.346±0.102	0.514±0.035	0.751±0.067	1.005±0.093	1.460±0.112	0.548±0.039

30.742-30.752	Myo-Inositol	0.896±0.112	0.440±0.073	0.294±0.045	0.336±0.073	0.288±0.048	1.150±0.704	0.693±0.072	1.551±0.912
33.281-33.292	Androst-2-en-17 -amine	0.342±0.056	0.418±0.054	0.457±0.049	0.197±0.029	0.237±0.031	0.348±0.055	0.391±0.062	0.223±0.047
33.833-33.845	Octadecanoic acid	0.762±0.053	0.060±0.021	0.952±0.061	0.027±0.008	0.501±0.041	---	0.942±0.083	0.032±0.011
36.477-36.493	2-Trifluoromethyl-N,N-diundecylbenzylamine	---	0.184±0.033	0.185±0.026	0.092±0.012	0.09±0.016	0.149±0.042	0.143±0.020	0.098±0.018
37.519-37.538	4-Methylthio-N-phenyl-1,2-carbazoledicarboximide	---	0.166±0.028	0.185±0.039	0.074±0.017	0.088±0.022	0.127±0.035	0.135±0.016	0.079±0.025
39.313-39.332	Silane	0.112±0.026	0.134±0.037	0.124±0.022	0.092±0.013	0.058±0.017	0.095±0.024	0.069±0.014	0.047±0.009
39.643-39.658	a-D-Glucopyranoside	19.906±0.144	56.756±3.279	11.591±2.019	55.924±6.338	26.03±3.279	49.935±5.015	8.429±1.015	44.439±5.189
42.132-42.145	Monostearine	0.448±0.034	0.494±0.037	0.616±0.033	0.214±0.019	0.295±0.048	0.379±0.061	0.476±0.067	0.231±0.032

Tab. 3: Metabolites in wood forming-tissue of four deciduous trees. Note: the values are given as mean ± SD (n=3). "---"in the table was not detected. EW: early wood; LW: late wood.

Retention time (min)	Metabolites	Relative amount ($mg \cdot g^{-1}$)							
		B. platyphylla		*F. mandshurica*		*P. simonii*		*U. propinqua*	
		EW	LW	EW	LW	EW	LW	EW	LW
7.04-7.08	Lactic acid	0.141±0.021	0.189±0.045	0.314±0.044	0.138±0.026	0.146±0.037	0.056±0.008	0.139±0.012	0.122±0.006
9.141-9.181	Ethanedioic acid	0.215±0.047	0.273±0.050	0.14±0.029	---	0.165±0.033	0.112±0.024	0.135±0.041	0.142±0.029
12.590-12.613	Glycerol	0.490±0.077	0.321±0.059	1.202±0.118	0.712±0.083	0.684±0.075	0.858±0.082	0.849±0.099	0.432±0.048
14.778-14.892	Serine	---	0.013±0.005	---	0.462±0.047	---	0.013±0.002	---	0.024±0.007
18.182-18.197	Malic acid	1.036±0.128	0.234±0.036	2.246±0.215	---	1.735±0.139	0.716±0.067	1.689±0.151	0.542±0.062
18.942-18.954	L-Aspartic acid	---	0.077±0.015	---	---	---	0.043±0.009	---	0.246±0.069
19.059-19.065	L-Proline	---	0.08±0.012	0.09±0.010	0.182±0.023	0.065±0.015	0.039±0.021	0.067±0.024	0.076±0.033
19.166-19.176	Butanoic acid	---	0.028±0.006	---	---	0.075±0.011	0.047±0.005	0.278±0.047	0.121±0.035
19.457-19.468	Silane	0.101±0.042	0.116±0.057	0.146±0.022	0.35±0.036	0.162±0.035	0.075±0.018	0.100±0.011	0.052±0.006
21.188-21.207	D-Xylofuranose	0.049±0.012	0.136±0.037	---	---	0.041±0.008	0.115±0.025	0.041±0.011	0.158±0.042
23.288-23.3	D-Xylose	0.031±0.005	0.037±0.011	0.062±0.027	0.023±0.004	0.043±0.010	---	0.037±0.009	0.032±0.012
24.355-24.362	Arabinofuranose	---	0.126±0.033	---	0.072±0.016	---	0.124±0.025	---	0.171±0.031

25.341-25.358	D-Fructose	---	---	---	---	0.093±0.025	---	0.077±0.014	---
25.454-25.493	1-Cyclohexene-1-carboxylic acid	0.038±0.009	0.052±0.015	0.096±0.012	0.22±0.042	0.109±0.033	0.059±0.008	0.051±0.010	0.030±0.002
26.526-26.555	D-Fructose	0.917±0.102	1.626±0.236	0.402±0.087	2.136±0.318	3.222±0.525	1.033±0.272	1.835±0.321	1.686±0.442
26.733-26.752	D-Fructose	0.683±0.083	1.163±0.159	0.272±0.051	1.57±0.233	2.43±0.261	0.752±0.049	1.438±0.136	1.212±0.208
26.898-26.912	Glucitol	0.024±0.006	0.062±0.017	0.23±0.042	2.064±0.349	0.034±0.010	0.045±0.013	0.041±0.012	0.088±0.025
27.038-27.053	D-Glucose	1.374±0.182	2.420±0.219	2.034±0.256	10.942±2.014	5.322±1.389	1.719±0.306	2.667±0.285	2.698±0.312
27.407-27.426	D-Glucose	0.278±0.042	0.545±0.066	0.452±0.048	2.538±0.252	1.269±0.127	0.426±0.072	0.607±0.093	0.642±0.085
28.816-28.823	a-D-Glucopyranose	---	0.026±0.006	0.092±0.025	0.181±0.042	0.176±0.033	---	0.139±0.029	0.016±0.005
29.725-29.734	Gluconic acid	---	0.042±0.015	---	---	0.102±0.032	0.029±0.006	0.067±0.028	0.068±0.022
29.859-29.870	2-Ethoxyethanol	0.064±0.013	0.044±0.007	0.108±0.032	---	0.081±0.018	---	0.075±0.013	0.034±0.009
30.273-30.288	Hexadecanoic acid	1.763±0.134	1.207±0.099	2.844±0.253	0.8±0.059	1.883±0.193	0.414±0.052	1.689±0.207	0.876±0.085
30.742-30.752	Myo-Inositol	0.409±0.082	0.121±0.037	0.614±0.058	4.418±0.618	0.495±0.094	0.931±0.173	0.618±0.062	1.466±0.203
33.281-33.292	Androst-2-en-17-amine	0.477±0.078	0.397±0.045	0.868±0.093	---	0.606±0.061	0.138±0.027	0.54±0.082	0.288±0.042
33.833-33.845	Octadecanoic acid	1.094±0.179	0.059±0.006	1.900±0.215	0.41±0.056	1.330±0.168	0.019±0.003	1.172±0.123	0.038±0.007
36.477-36.493	2-Trifluoromethyl-N,N-diundecylbenzylamine	0.172±0.033	0.167±0.029	0.344±0.012	0.444±0.048	0.236±0.029	0.057±0.007	0.208±0.037	0.124±0.022
37.519-37.538	4-Methylthio-N-phenyl-1,2-carbazoledicarboximide	0.146±0.050	0.151±0.038	0.336±0.062	---	0.232±0.034	0.050±0.004	0.210±0.022	0.106±0.035
39.313-39.332	Silane	0.352±0.075	0.389±0.069	0.794±0.101	1.534±0.233	0.538±0.060	0.124±0.027	0.446±0.055	0.266±0.031
39.643-39.658	a-D-Glucopyranoside	16.222±2.025	46.582±5.317	11.758±1.843	48.435±6.336	11.698±2.119	39.695±4.627	9.474±1.501	58.038±7.910
41.612-41.639	2-Monostearine	0.035±0.008	0.054±0.011	0.122±0.024	0.402±0.051	0.061±0.012	---	0.116±0.018	0.03±0.004
42.132-42.145	Monostearine	0.427±0.057	0.492±0.081	1.266±0.103	3.318±0.318	0.776±0.067	0.173±0.032	0.624±0.063	0.372±0.039
42.918-42.923	D-Turanose	0.091±0.028	0.057±0.016	0.120±0.031	0.494±0.059	0.09±0.008	1.011±0.134	0.149±0.037	0.046±0.012
44.491-44.503	Lactose	---	0.109±0.012	---	5.368±0.318	---	0.093±0.017	---	0.342±0.041

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