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# COMPARATIVE TRANSCRIPTOME ANALYSIS OF *PTEROCELTIS TATARINOWII* MAXIM., AN ENDEMIC FIBER TREE

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## Abstract

The bark of *Pteroceltis tatarinowii* Maxim., an endemic tree in Ulmaceae, is the main raw material for manufacturing Xuan Paper which is widely used in calligraphy and painting field. The characteristics of *P. tatarinowii* bark is the main limiting factor for the quality of Xuan Paper specially the content of cellulose and lignin. The molecular basis related to cellulose and lignin synthesis in *P. tatarinowii* would be helpful to understand and seek higher quality raw materials for Xuan Paper. RNA-seq was utilized to reveal transcriptome differences in *P. tatarinowii* from three far isolated localities (AL, JX and XA) under different climate environments. A total of 290 million reads were generated for further analysis in three libraries. In total, 2,850, 2,038 and 1,986 DEGs were identified in XA, JX and AL, respectively. Compared with the sample from XA, there were 822 up-regulated and 1706 down-regulated in AL sample. AL sample has 611 up-regulated genes and 647 down-regulated genes in comparison with JX sample. Comparing XA and JX samples, 443 were up-regulated and 1,783 were down-regulated in XA. Three samples had similar GO enrichment patterns. There were 19 and 9 genes identified as *CESA* and *CSL* (E-value less than 1.0E-20), respectively. Although no significant expression differences were found in three samples, *KOB1*, *GPI-anchored protein gene* and *CTL1* were differently expressed, and *KOB1* and *GPI-anchored protein gene* were up-regulated in JX. A number of the unigenes (474) that were involved in 'phenylpropanoid biosynthesis', were mostly not differently expressed. Only a few genes annotated as *PAL*, *4CL*, *C4H* and *CAD* were significantly different in expression. In AL, 3 *CAD* and 1 *PAL* were up-regulated, whereas 6 *CAD*, 3 *4CL* and 1 *HCT* were up-regulated in XA, and 1 *PAL*, 2 *4CL*, 2 *C4H* in JX. JX sample had the highest cellulose content and XA sample had the highest lignin content, which being consistent with the hierarchical cluster analysis of differently expressed genes. Differences in the expression of these genes might influence the cellulose and lignin content.

## Introduction

*Pteroceltis tatarinowii* Maxim., belonging to Ulmaceae, is widely distributed covering 19 provinces in the mainland of China. *P. tatarinowii* phloem fiber is the main raw material for Xuan Paper which is precious and vital in calligraphy and painting field. Several studies have shown that the quality of Xuan Paper such as the ink embellish, durability and paper whiteness is closely related to the characteristics of *P. tatarinowii* bark (Liu *et al*. 1985, 1986, Fang *et al*. 2008, Wu 2008). In all of these *P. tatarinowii* bark features, the bark fiber morphology, cellulose and lignin content are the most important influencing factors and are also important indexes to evaluate economical values and practicability of *P. tatarinowii* (Cui 2006). Therefore, increasing the bark cellulose content and the bark production has always been the concerned goal of *P. tatarinowii* breeders (Li *et al*. 2001, Fang *et al*. 2002).

The present studies mainly focused on physiology, morphology, ecology and genetic structure of *P. tatarinowii* (Song *et al*. 2006, Zhang *et al*. 2007, Fang *et al*. 2007 and Wei *et al*. 2007, Li *et al*. 2012, Zhang *et al*. 2012a,b) who revealed that the bark quality of *P. tatarinowii* growing in different soil forming rocks has notable differences. In previous research, through quantitative comparison

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of bark indexes including cellulose content, lignin content and fiber length in *P. tatarinowii*, a conclusion was drawn that the population with the highest cellulose content was in Jingxian county (JX), Anhui province. The population with the longest fiber length was in the Anlong county (AL), Guizhou province, and the individuals in Xian (XA), Shanxi province, harbored the lowest lignin content compared with other populations (Liu *et al*. 2015). It is noticed that the previous result of *P. tatarinowii* was in accordance with the haplotype pattern of this species using chloroplast markers (Li *et al*. 2012). The haplotype composition and distance from the out group (*Celtis sinensis* Pers.) were both different, which might show light on their different evolutionary history. In addition, few data were documented targeting functional genes of *P. tatarinowii* bark and differential expression. Therefore, it is worthy to further investigate whether there is a difference in the bark functional genes of *P. tatarinowii* among different habitats.

A lot of information related to cellulose synthesis has been obtained using modern technical methods in some model plant. The cellulose synthesis is a complicated process, which involves many aspects, such as catalysis, localization, adjustment and coordinated expression. Proteins and enzymes associated with cellulose are also very diverse, such as cellulose synthase complex (*CesA*) (Pear *et al*. 1996, Endler and Persson 2011), microtubules (Gu and Somerville 2010), sucrose synthase (*SuSy*) (Barratt *et al*. 2009), UDP-glucose pyrophosphorylase (*UGPase*) (Fujii *et al*. 2010), *KORRIGAN* (Szyjanowicz *et al*. 2004). Lignin monomer synthesis via phenylpropanoid biosynthesis which starts from phenylalanine deamination, afterwards takes hydroxyl, methylation and oxidation reduction reaction, and finally generates three monomers (Humphreys *et al*. 2002). The pathway also involves many enzymes including phenylalanine ammonialyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), coumarate 3-hydroxylase (*C3H*), ferulate 5-hydroxylase (*F5H*), caffeic acid-3-O-methyltransferase (*COMT*), caffeoyl-CoA 3-O-methyltransferase (*CCoAOMT*), cinnamoyl-CoA reductase (*CCR*), and cinnamoyl alcohol dehydrogenase (*CAD*). Among them, *PAL* is the key enzyme of the pathway and *F5H* is necessary for the S-lignin synthesis, *COMT* catalyzes coffee acid into ferulic acid (Humphreys *et al*. 2002).

High-throughput mRNA sequencing technology is especially suitable for gene expression profiling in non-model organisms that lack genomic sequence data and was widely applied (Lorkowski and Cullen 2003). To provide accurate and genome-wide insights into the molecular mechanisms involved in *P. tatarinowii* cellulose and lignin synthesis, RNA-seq was utilized to reveal transcriptome differences in *P. tatarinowii* from three isolated populations (Guizhou: AL; Anhui: JX; Shanxi: XA) with different climate conditions. The present study was aimed at accumulating molecular information data for elucidating the differences in enzyme units, co-expression networks and regulatory network related to cellulose synthesis in *P. tatarinowii* phloem.

**Materials and Methods**

In March and April 2015, three mixed *P. tatarinowii* phloem samples (XA, AL, JX) were obtained from three populations (Xian in Shanxi Province: E108°12′, N34°10′12″, Jingxian in Anhui Province: E118°24′, N30°42′ , and Anlong in Guizhou Province: E105°31′24″, N25°18′36″), coded as ‘XA’, ‘AL’ and ‘JX’, respectively. The samples were collected from stem with the diameter of 2.5 ~ 3 cm. Twenty random individuals in each population were sampled, mixed up and frozen in drikold (liquid nitrogen) to extract RNA.

Three samples were performed transcriptome sequencing at Personal Biotechnology Company, Shanghai, China (http://www.personalbio.cn/) using Illumina Nextseq500 platform.

The assembled unigenes were compared with sequences in the National Center for Biotechnology Information (NCBI) non-redundant (Nr) protein and nucleotide (Nt) databases, the Swiss-Prot protein database. The Blast2GO program was used to obtain GO annotation of the

unigenes. The WEGO software was then used to perform GO functional classification of all unigenes to view the distribution of gene functions. KEGG was used to summarize the pathway information involved in *P. tatarinowii* phloem. The putative sequences related to cellulose biosynthesis pathway were identified according to previous studies. Then, CDS of *Arabidopsis* cellulose biosynthesis pathway were aligned *to P. tatarinowii* homologs using DNAMAN6.0 and unigenes with identity larger than 60% were selected.

Differential gene expression was identified through the R package DESeq. The unigenes were mapped to calculate the number of reads in three samples. The DESeq was performed to normalize signal of the unigenes. Differential expression was reported according to the fold change of unigene expression values and p-values. The p-value < 0.05 was identified as a significant differential expression.

The phloem of *P. tatarinowii* was dried to constant weight, and the indexes were measured. The cellulose content was measured by using an ethanol nitrate method. The acid insoluble lignin content was measured using a sulfuric acid method.

**Results and Discussion**

In order to compare transcriptome of *P. tatarinowii* phloem in three samples (Anhui, Guizhou and Shanxi), Illumina paired-end sequencing technology was used to yield a total of 295 million 150 bp raw reads with an average of 98 million reads per sample. After stringent quality checking and data cleaning, a total of 290 million high quality reads were obtained with useful data percentage ranging from 98.50 to 98.59 (Table 1).

**Table 1. Summary statistics for *P. tatarinowii* genes based on the RNA-Seq data.**

| Summary | | AL | XA | JX |
|---|---|---|---|---|
| Raw reads | Total | 86,328,484 | 105,837,100 | 103,005,822 |
| | Raw data (bp) | 12,710,626,744 | 15,642,196,601 | 15,258,092,786 |
| | Q20 (%) | 91.85 | 89.03 | 89.1 |
| | GC (%) | 53.63 | 51.87 | 52.98 |
| | Reads length | 150 | 150 | 150 |
| Clean reads | Total | 85,090,654 | 104,347,726 | 101,447,800 |
| | Clean data (bp) | 12,526,178,760 | 15,422,349,250 | 15,029,302,788 |
| | Useful reads (%) | 98.57 | 98.59 | 98.49 |
| | Useful data (%) | 98.55 | 98.59 | 98.5 |

Trinity was used to employ for *de novo* assembly, which divided the splicing process into three parts, Inchworm, Chrysalis and Butterfly (Table 2). Inchworm was used to build high quality reading into the k-mer library to form 3,996,276 contigs with the mean sizes of 275 bp and N50 of 319 bp. The contigs with the length of more than 500 bp accounted for about 9.99%. The chrysalis was used to build contigs into a component to generate 1,957,032 transcripts with an average length of 460 bp and N50 of 509 bp. Then, all the transcripts were blasted against the reference protein library, and unigenes were obtained according to tophithit results. The reference protein library is generally preferred nonredundant and with the high-quality database so that the results are more reliable. Finally, 238,210 unigenes were generated, with an average length of 740 bp size ranging from 200 to 11,045 bp (Fig. 1).

Of all the genes obtained, 60,926 (25.57%) matched with the strong homologs in databases with known function (<1.0e-50) (Fig. 2). Of these genes, 46,043 (JX), 37,581 (XA) and 42,307(AL) were identified. Examination of the transcript levels (by $log_2$RPKM) showed that most of the mRNAs occurred at extremely low levels, with a very small proportion of highly expressed mRNAs in all three samples (Table 3). Gene transcriptional level showed similar patterns of expression in the three libraries. For example, 2525 unigenes distributed more than 5 ($log_2$RPKM), and only 49 unigenes showed high expression of more than 10 ($log_2$RPKM) in AL sample. In this study, experiments were transcriptome profiling of *P. tatarinowii* phloem in three far apart habitats to enrich the transcriptomics data and improve the understanding of the molecular basis of *P. tatarinowii* phloem development performed. Compared with previous transcriptomic studies, herein report the small proportion of transcripts was significantly matched to known isogenesis genes (Chen *et al.* 2015, Upadhyaya *et al.* 2015), which is a good complement to the functional gene data of *P. tatarinowii* were reported.

**Table 2. Summary statistics for *P. tatarinowii* genes based on the RNA-Seq data.**

| Components | Total length (bp) | Sequence No. | Max length (bp) | Average length (bp) | N50 | >N50 Reads No. | GC (%) |
|---|---|---|---|---|---|---|---|
| Contigs | 1,100,133,747 | 3,996,276 | 19,569 | 275.29 | 319 | 882,909 | 53.82 |
| Transcripts | 901,039,505 | 1,957,032 | 11,045 | 460 | 509 | 483,734 | 53.52 |
| Unigenes | 176,200,509 | 238,210 | 11,045 | 740 | 1,029 | 51,853 | 51.72 |

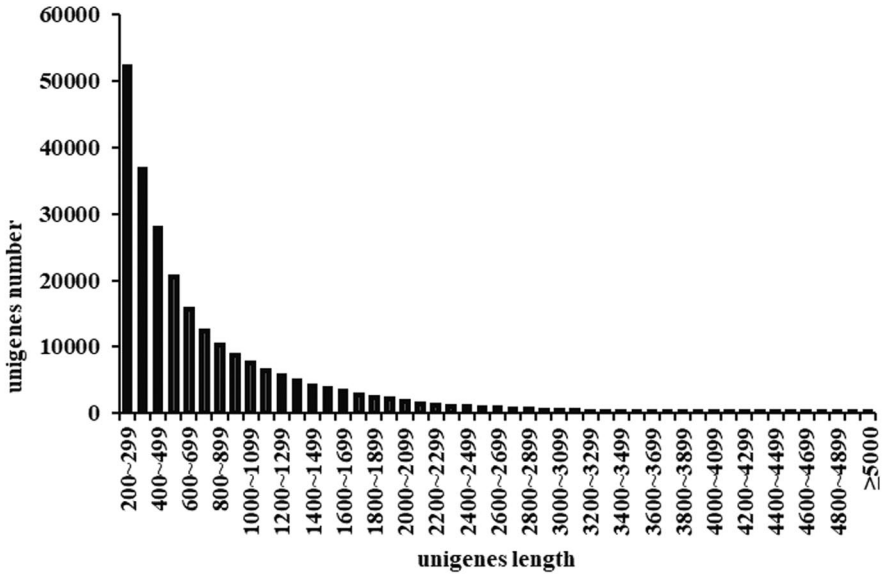


Fig. 1. The unigenes length distribution of *P. tatarinowii*.

To measure the changes in gene expression and find the key genes, the significantly differently expressed genes (DEGs) using $log_2FC \geq 1$ or $\leq -1$ (p-value < 0.05)were further selected. In total, 2,850 2,038 and 1,986 DEGs were identified in XA, JX and AL, respectively (Fig. 3). Among them, 2,528 (AL vs XA), 1,258 (AL vs JX) and 2,223 (XA vs JX) genes were either up- or down-

regulated. Compared with the sample of XA, there were 2,528 different expression genes in AL sample, with 822 up-regulated and 1706 down-regulated. Of the 1,258 different expression genes in AL sample vs JX sample, 611 were up-regulated and 647 were down-regulated. Finally, of the 2,226 different expressions of genes in XA sample vs JX sample, 443 were up-regulated and 1,783 were down-regulated. The size distribution of contigs, transcripts and unigenes were compiled. The sequencing data are deposited into a NCBI gene expression omnibus (GEO).

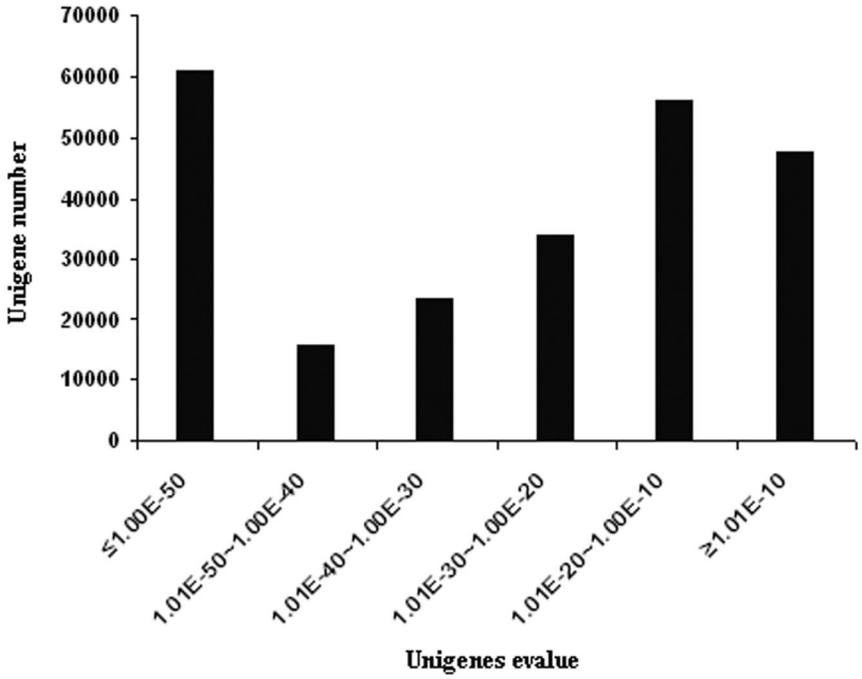


Fig. 2. Characteristics of similarity search of unigenes against the databases.

**Table 3. Expression abundance in three samples.**

| $Log_2$RPKM | ≥10 | 5~10 | ≤5 |
|---|---|---|---|
| Unigene number in AL | 49 | 2476 | 39782 |
| Unigene number in JX | 55 | 2451 | 43538 |
| Unigene number in XA | 48 | 3052 | 34481 |

Earlier studies showed that the bark quality of *P. tatarinowii* growing in different soil forming rocks were significantly different (Fang *et al*. 2007), and the bark quality was influenced by environmental factors. These studies revealed important information but involved no detailed molecular basis. In this paper, significantly enriched GO terms were detected and unigenes in three samples were found to have similar enrichment patterns, which would illustrate similar growth and development mechanism overall in the present three samples. But there were still differences in the amount of GO enrichment among the three samples. These differences might be related to the previously reported bark quality difference in the different environments.

Gene ontology (GO) classification and the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to annotate the functions of the expressed genes.

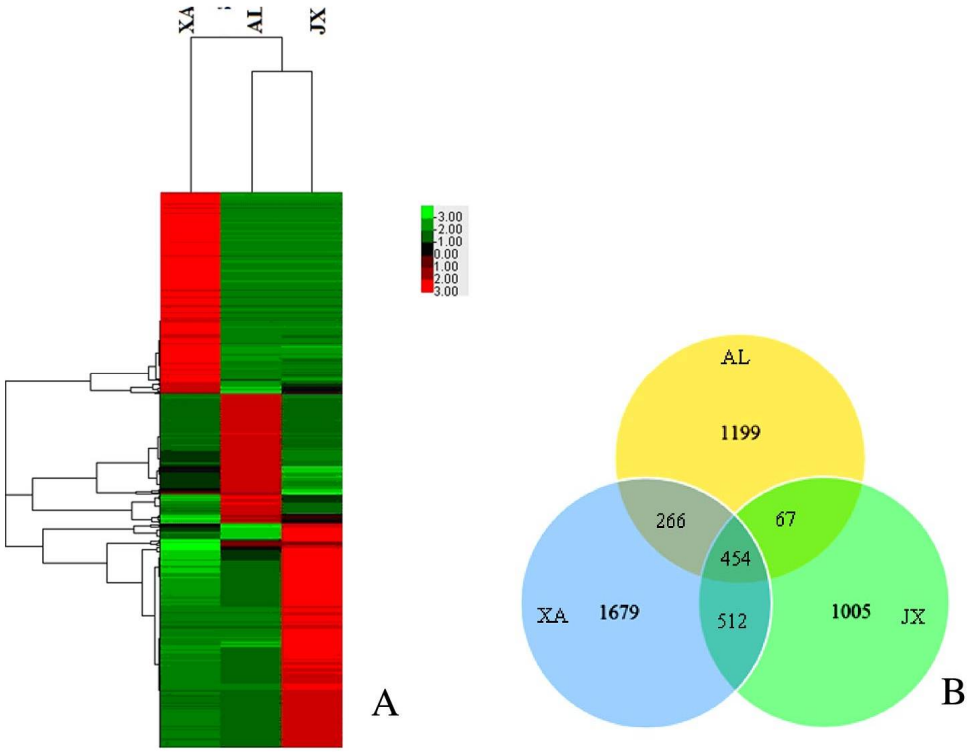


Fig. 3. Analysis of differential expression genes among three samples. A. Hierarchical cluster analysis of differently expressed genes based on the log ratio fold change data. The column and row indicate the sample and gene, respectively; the color scale indicates the gene expression level: green color represents decreased transcript abundance and red color represents increased transcript abundance. AL, XA and JX represent the sample in the Anlong, Xian and Jingxian, respectively and B. Venn diagram displays the number of specific genes in the three samples.

To reveal the functions of the significantly different expressed genes (DEGs), the enriched GO categories were checked using p-value $\leq 0.05$ as the cutoff for significant GO categories (Fig. 4). There were 15,110 unigenes assigned into three main GO functional categories including biological process (44.63%), cellular component (40.57%) and molecular function (14.79%). In general, three samples have similar GO enrichment. For “biological process” term, terms related to “cellular process” (GO:0016043) and “metabolic process” (GO:0040007) were highly enriched in DEGs in three samples. In “cellular component”, “cell” (GO:0005575), “cytoplasm” (GO:0005623), “intracellular” (GO:0005794) and membrane(GO:0005622) could be abundant as GO terms, while within “molecular function”, “binding” (GO:0003674) was a larger cluster.

To validate and annotate the assembled unigenes, all the unigenes were searched against the NCBI nucleotide sequence database (NT) and SwissProt protein database using BlastN and BlastX. The differential unigenes were screened by DESeq analysis according to expression multiplier and p-value (≤0.05).

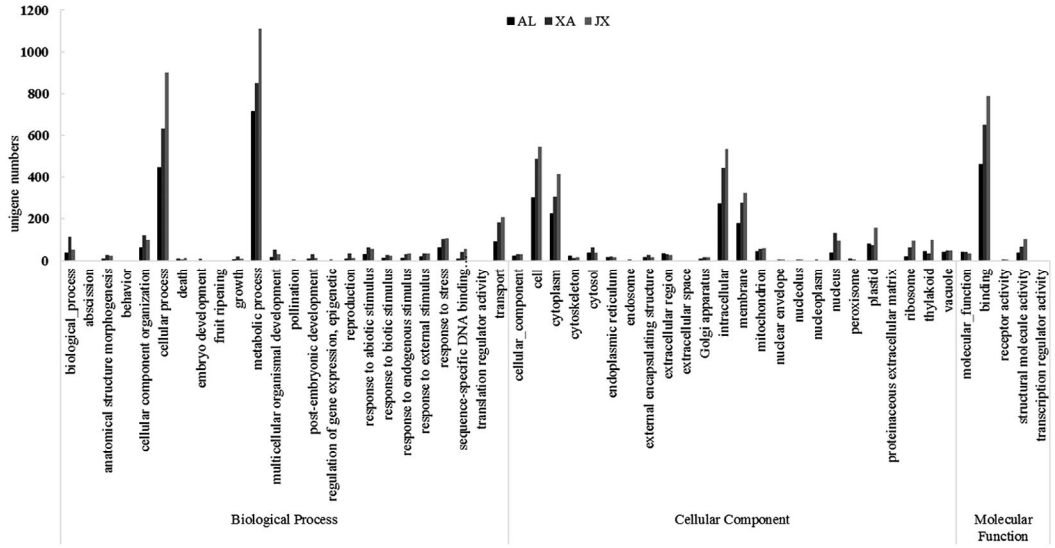


Fig. 4. GO Enrichment analysis of the expressed genes.

To explore the synthesis process of *P. tatarinowii* phloem cellulose, present authors blasted the genes related to cellulose biosynthetic process. In total 19 and 9 genes were identified as "cellulose synthase A catalytic subunit" (*CESA*) and cellulose synthase-like (*CSL*) (E-value less than 1.0E-20), respectively. Although these genes were not a significant expression difference in these three samples, there were 10 *CESA* with highest RPKM in AL sample, 7 in XA sample and 2 in JX sample. A number of *CSL* genes with maximum RPKM were the highest still in AL (Table 4). Other genes related to cellulose biosynthetic process were identified including sucrose synthase (*SuSy*), KORRIGAN (*KOR*), KOBITO1 (*KOB1*), COBRA (*COB*), *GPI-anchored protein gene* and chitinase-like proteins (*CTL*). The expression level of the *SUS*, *KOR*, *COB* had no significant difference. The 2 *KOB1* genes were expressed differently in three samples, and one was up-regulated in JX, the other was up-regulated in AL. The 3 *GPI-anchored protein genes* were up-regulated in JX. 2 *CTL1* was up-regulated in XA compared with AL and JX (Table 4).

Despite cellulose content was different in the three samples (JX > AL > XA, $p < 0.05$), the great majority of the genes involved in cellulose synthesis had no significant expression difference except that *KOB1*, *GPI-anchored protein gene* and *CTL1*. *KOB1* was up-regulated in JX and AL compared with XA, which is a protein localized to the endomembrane system and/or plasma membrane as well as the apoplast (Pagant *et al*. 2002). The localization of *KOB1* suggested that it might be involved in cellulose synthase complex (CSC) trafficking to the plasma membrane (Gavlighi *et al.* 2013). Some previous studies found mutation of *KOB1* which could cause disruption of cellulose (Wang *et al*. 2015). *GPI-anchor protein*, including *COB*, is localized primarily to the cell wall and is inferred to have a profound effect on CSC activity (Roudier *et al*. 2005). A research indicated that the cellulose content of mutant cob in root was lower than that in wild type (Roudier *et al*. 2005). Two genes in the present study were up-regulated in JX, which encoded LysM domain-containing GPI anchored protein and could influence cellulose synthesis. *CTL1* could bind xyloglucans and cellulose with *CTL2*. Mutants defective in these proteins resulted in a reduced ratio of crystalline to amorphous cellulose (Sampathkumar *et al*. 2013). This differential expression might influence cellulose content of *P. tatarinowii*, the detailed regulatory mechanism needs to be further investigated through replication experiments.

There were 474 unigenes involved in 'phenylpropanoid biosynthesis', and encoding 10 enzymes including 'phenylalanine ammonia-lyase' (*PAL*), 'cinnamate 4-hydroxylase'(*C4H*), 'p-coumarate 3-hydroxylase' (*C3H*), 'caffeic acid 3-methyl transferase' (*COMT*), 'ferulate-5-hydroxylase' (*F5H*), '4-coumarate-CoA ligase' (*4CL*), 'shikimate O-hydroxycinnamoyl transferase' (*HCT*), 'Caffeoyl-CoA O-methyltransferase' (*CCoAMT*), 'cinnamoyl-CoA reductase' (*CCR*), 'cinnamyl alcohol dehydrogenase' (*CAD*) (Yoon *et al*. 2015). Of these genes, most were not differentially expressed. Only a few genes annotated as *PAL*, *4CL*, *C4H* and *CAD* showed significant differences in expression. In AL, 3 *CAD* and 1 *PAL* were up-regulated. 6 *CAD*, 3 *4CL* and 1 *HCT* were up-regulated in XA and 1*PAL*, 2 *4CL*, 2 *C4H* were also up-regulated in JX (Table 4). Lignin is an important secondary metabolite possessing a great effect on plant growth and product quality (Feng *et al*. 2017). There were 474 unigenes involved in 'phenylpropanoid biosynthesis', which had a higher expression level compared with other studies (Feng *et al*. 2017). These might suggest the harvest period (April) was a vigorous period of lignin synthesis for *P. tatarinowii*.

*PAL* was the first rate-limiting enzyme in the phenylpropanoid pathway (Olsen *et al*. 2008), and down-regulation of *PAL* can lead to a reduction of lignin content. *4CL* and *C4H* also participated in this pathway (Koutaniemi *et al*. 2007). When the antisense *4CL* gene was integrated into the genome DNA of alfalfa, the lignin content was reduced compared to the wild-type plants (Meng *et al*. 2017). The results *HCT* is the key enzymes in lignin biosynthesis and affected lignin content (Sykes *et al*. 2015). In *Arabidopsis* mutants, deficiency of *HCT* or *C3H* led to the decrease of lignin content and the relatively higher proportion of H-units than G-lignin and S-lignin (Feng *et al*. 2017). The reduced expression of *CAD* resulted in less lignin content (Tamasloukht *et al*. 2011). The study revealed that the lignin content was consistent with up-regulated genes in the phenylpropanoid biosynthesis pathway, implying that might be involved in lignification and some enzymes might play a major role in lignin synthesis.

**Table 4. Identification of DEG involved in cellulose and lignin monolignol biosynthesis.**

| Pathway | Enzyme | Name | No. of unigene | Unigene ID |
|---|---|---|---|---|
| Cellulose biosynthesis | *KOB1* | KOBITO1 | 2 | c603263_g5_i1; c648227_g1_i1 |
| | *GPI* | GPI-anchored protein | 3 | c642941_g1_i1; c642941_g1_i1; c75774_g1_i1 |
| | *CTL* | Chitinase-like proteins | 2 | c650541_g1_i1; c612753_g1_i2 |
| Lignin monolignol biosynthesis | *PAL* | Phenylalanine ammonia-lyase | 2 | c628172_g1_i1; c649134_g1_i1 |
| | *C4H* | Cinammate 4-hydroxylase | 2 | c648512_g1_i2; c595114_g1_i1 |
| | *4CL* | 4-coumarate--CoA ligase | 5 | c650453_g1_i1; c578103_g1_i1; c625465_g2_i1; c565218_g1_i1; c643853_g2_i1 |
| | *HCT* | Shikimate O-hydroxycinnamoyl transferase | 1 | c642625_g2_i1 |
| | *CAD* | Cinnamyl alcohol dehydrogenase | 9 | c632553_g2_i2; c639605_g3_i2; c632629_g1_i4; c597569_g1_i1; c598607_g1_i1; c635999_g1_i2; c610352_g1_i1; c616799_g1_i1; c596647_g2_i1 |

The content of lignin and cellulose of *P. tatarinowii* bark in three samples were measured and analyzed with analysis of variance (ANOVA, p - value < 0.05). It showed that the bark cellulose content in JX was the highest and the lignin content in XA was the maximum (Table 5).

**Table 5. The content of cellulose and lignin, and related gene expression.**

| Sample | Cellulose content (%) | Lignin content (%) | Up-regulated genes in cellulose biosynthesis | Up-regulated genes in lignin biosynthesis |
|---|---|---|---|---|
| AL | 38.41 ± 2.92b | 11.59 ± 1.94a | 1 | 4 |
| JX | 40.69 ± 1.38b | 12.48 ± 1.00a | 3 | 5 |
| XA | 28.76 ± 1.87a | 16.75 ± 1.33b | 1 | 10 |

The same letters are not significant differences and different letters represent significant differences in the table.

In summary, three samples had similar enrichment patterns, which had subtle differences in gene number. The genes involved in lignin biosynthesis showed significant differential expression, which might be related to the difference in lignin content in three samples. *KOB* and *GPI-anchored protein* might influence the cellulose content.

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## References

Barratt DHP, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ and Smith AM 2009. Normal growth of *Arabidopsis* requires cytosolic invertase but not sucrose synthase. Proc. Natl. Acad. Sci. USA **106**(31): 13124-13129.

Chen JH, Chen BB and Zhang DQ 2015. Transcript profiling of *Populus tomentosa* genes in normal, tension, and opposite wood by RNA-seq. BMC Genomics **16**: 164.

Cui TL 2006. Effects of site conditions and management practices on bark quality of Wingceltis and property of Xuan Paper. Nanjing Forestry University Master's thesis 8.

Endler A and Person S 2011. Cellulose synthases and synthesis in Arabidopsis. Mol. Plant **4**(2): 199-211.

Fang SZ, Li GY and Fu XX 2002. Effects of site conditions on mineral element contents in the bark of wingceltis (*Pteroceltis tatarinowii*). Scientia Silvae Sinicae **38**(1): 8-14.

Fang SZ, Cui TL and Yu MK 2007. Effects of soil-forming rocks and sprout ages on the bark quality of *Pteroceltis tatarinowii*. Journal of Beijing Forestry University **29**(2): 122-127.

Fang XY, Wu DT and Lu YK 2008. Research and investigation about qiannianguxuan Xuan Paper at Anhui Province Jing County. J. Beijing Institute Graphic Communication **16**(6): 1-8

Feng H, Xu L, Wang Y, Tang M, Zhu X, Zhang W, Sun X, Nie S, Muleke EM and Liu L 2017. Identification of critical genes associated with lignin biosynthesis in radish (*Raphanus sativus* L.) by de novo transcriptome sequencing. Mol. Genet. Genomics **292**: 1151-1163.

Fujii S, Hayashi T and Mizuno K 2010. Sucrose synthase is an integral component of the cellulose synthesis machinery. Plant Cell Physiol. **51**(2): 294-301.

Gavlighi HA, Meyer AS, Mikkelsen JD 2013. Enhanced enzymatic cellulose degradation by cellobiohydrolases via product removal. Biotechnology Letters **35**(2): 205-212.

Gu Y and Somerville C 2010. Cellulose synthase interacting protein: A new factor in cellulose synthesis. Plant Signal Behav. **5**(12): 1571-1574.

Humphreys JM and Chapple C 2002. Rewriting the lignin roadmap. Curr. Opin. Plant Biol. **5**(3): 224-229.

Koutaniemi S, Warinowski T, Kärkönen A, Alatalo E, Fossdal CG, Saranpää P, Laakso T, Fagerstedt KV, Simola LK, Paulin L, Rudd S and Teeri TH 2007. Expression profling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. Plant Mol. Biol. **65**(3): 311-328.

Li GY, Fang SZ, Lv JJ and Wang XS 2001. The effects of sites conditions on above-ground biomass production and bark yield of *Pteroceltis tatarinowii* plantations. Journal of Nanjing Forestry University (Natural sciences edition) **25**(4): 49-53.

Li XH, Shao JW, Lu C, Zhang XP and Qiu YX 2012. Chloroplast phylogeography of a temperate tree *Pteroceltis tatarinowii* (Ulmaceae) in China. J. Systematics Evolution **50** (4): 325-333.

Liu RQ and Hu YX 1985. A study of the wetting property of Chinese ink on Xuan Paper. China pulp paper **2**: 3-29.

Liu RQ and Qu YL 1986. Permanency of Xuan Paper. China Pulp paper **6**: 32-37.

Liu HJ, Zhang L, Wang F, Jiang X and Zhang XP 2015. Variation in bark quality among eleven populations of *Pteroceltis tatarinowii* Maxim. and their relationship with environmental factors. J. Nanjing Forestry University (Natural Science Edition) **39**(3): 78-84.

Lorkowski S and Cullen P 2003. Chapter 4. High-Throughput and Industrial Methods for mRNA Expression Analysis. Analysis Gene Expression: A Handbook of Methods: Possibilities and Pitfalls, Print ISBN: 9783527304882.

Meng J, Li C, Zhao M, Wang C, Ru Y, Cui Z and Han Y 2017. Lignin biosynthesis regulated by the antisense 4cl gene in alfalfa. Czech J. Genet. Plant Breed **54**(1): 26-29.

Olsen KM, Lea US, Slimestad R, Verheul M and Lillo C 2008. Differential expression of four Arabidopsis PAL genes; PAL1 and PAL2 have functional specialization in abiotic environmental-triggered flavonoid synthesis. J. Plant Physiol. **165**(14): 1491-1499.

Pagant S, Bichet A, Sugimoto K, Lerouxel O, Desprez T, McCann M, Lerouge P, Vernhettes S and Höfte H 2002. KOBITO1 encodes a novel plasma membrane protein necessary for normal synthesis of cellulose during cell expansion in Arabidopsis. Plant Cell **14**(9): 2001-2013.

Pear JR, Kawagoe Y, Schreckengost WE, Delmer DP and Stalker DM 1996. Higher plants contain homologs of the bacterial celA genes encoding the catalytic subunit of cellulose synthase. Proc. Natl. Acad. Sci. USA **93**(22): 12637-12642.

Roudier F, Fernandez AG, Fujita M, Himmelspach R, Borner GHH, Schindelman G, Song S, Baskin TI, Dupree P, Wasteneys GO, Benfey PN 2005. COBRA, an Arabidopsis extracellular glycosyl-phosphatidyl inositol-anchored protein, specifically controls highly anisotropic expansion through its involvement in cellulose microfibril orientation. Plant Cell **17**(6): 1749-1763.

Sampathkumar A, Gutierrez R, McFarlane HE, Bringmann M, Lindeboom J, Emons AM, Samuels L, Ketelaar T, Ehrhardt DW and Persson S 2013. Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in Arabidopsis interphase cells. Plant Physiol. **162**(2): 675-688.

Song LY and Fang SZ 2006. Physiological responses of *Pteroceltis tatarinowii* seedlings under hydroponic culture to NaCl stress. Journal of Nanjing Forestry University (Natural sciences edition) **30**(2): 94-98.

Sykes RW, Gjersing EL, Foutz K, Rottmann WH, Kuhn SA, Foster CE, Ziebell A, Turner GB, Decker SR, Hinchee MAW and Davis MF 2015. Down-regulation of p-coumaroyl quinate/shikimate 3′-hydroxylase (C3′H) and cinnamate 4-hydroxylase (C4H) genes in the lignin biosynthetic pathway of Eucalyptus urophylla × E. grandis leads to improved sugar release. Biotechnol Biofuels. **8**: 128.

Szyjanowicz PM, McKinnon I, Taylor NG, Gardiner J, Jarvis MC and Turner SR 2004. The irregular xylem 2 mutant is an allele of korrigan that affects the secondary cell wall of Arabidopsis thaliana. Plant J. **37**(5): 730-740.

Tamasloukht B, Wong Quai Lam MS, Martinez Y, Tozo K, Barbier O, Jourda C, Jauneau A, Borderies G, Balzergue S, Renou JP, Huguet S, Martinant JP, Tatout C, Lapierre C, Barrière Y, Goffner D and Pichon M 2011. Characterization of a cinnamoyl-CoA reductase 1 (CCR1) mutant in maize: effects on lignifcation, fbre development, and global gene expression. J. Exp. Bot. **62**(11): 3837-3848.

Upadhyaya NM, Garnica DP, Karaoglu H, Sperschneider J, Nemri A, Xu B, Mago R, Cuomo CA, Rathjen JP, Park RF, Ellis JG and Dodds PN 2015. Comparative genomics of Australian isolates of the wheat stem rust pathogen *Puccinia graminis f.* sp. *tritici* reveals extensive polymorphism in candidate effector genes. Front Plant Sci. **5**: 759.

Wang X, Jing Y, Zhang B, Zhou Y and Lin R 2015. Glycosyltransferase-like protein ABI8/ELD1/KOB1 promotes Arabidopsis hypocotyl elongation through regulating cellulose biosynthesis. Plant Cell Environ. **38**(3): 411-422.

Wei XL, Yu LF, Zhu SQ and Xu XZ 2007. Effect of soil drying-wetting alternation on physiology and growth of *Pteroceltis tatarinowii* seedlings. Scientia Silvae Sinicae, **43**(8): 23-28.

Wu SX 2008. Studies on producing technique and performance of ink embellishment for Chinese Xuan Paper. China Pulp Paper Industry **29**(7): 64-67.

Yoon J, Choi H and An G 2015. Roles of lignin biosynthesis and regulatory genes in plant development. J. Integr. Plant Biolo. **57**(11): 902-912.

Zhang XW, Zhang XP, Yang KJ and Zhang ZX 2007. Study on anatomical structure of leaf and ecological adaptability of *Pteroceltis tartarinowii* Maxim. Bulletin of botanical research **27**(1): 38-42.

Zhang L, Lu C, Li XH, Wang L and Zhang XP 2012a. Age structure and inter- and intra-species competition of *Pteroceltis tatarinowii* in Huangcangyu Natural Reserve. Journal of Shanghai Jiaotong University (Agricultural science) **30**(1): 34-40.

Zhang L, Zhang XP, Lu C and Li XH 2012b. Spatial pattern of *Pteroceltis tatarinowii* populations in Langya Moutain of Anhui Province. Scientia Silvae Sinicae **48**(2): 9-15.

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