Sequencing and analysis of full-length transcriptome functional genes for dormancy release of Eucommia ulmoides axillary buds

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Abstract

Eucommia ulmoides Oliv contains many kinds of active and nutritional components, which have important medicinal and nutritional value. In order to reveal the cultivation technology of *Eucommia ulmoides* and molecular regulation mechanism of axillary bud development, illumina hiseq high-throughput sequencing technology was used to extract mRNA from the top and basal dormant buds, and axillary buds of the plant after stubble, then the transcriptome sequencing analysis was conducted. The results showed that 29353 UniGenes were annotated, and the annotation rate was 30.04%, the remaining 68367 UniGenes were not annotated, which may be new genes; The number of genes differentially expressed (DEGs) in Eucommia ulmoides axillary bud was the highest after stubble treatment, a total of 5384 genes were detected, which 3137 genes were up-regulated and 2247 genes were down regulated. The number of differentially expressed genes was the least in the top and basal dormant buds. The functional analysis of COG, GO and KEGG genes showed that axillary bud differential genes were enriched in the biological processes and metabolic pathways related to primary metabolism, secondary metabolism, and plant hormone signal transduction pathway. The key genes of flavonoid and phenylpropane synthesis and the expression level of abscisic acid (ABA) regulating the dormancy of axillary buds were down-regulated, which promoted the release of the dormancy of axillary buds. At the same time, auxin response genes such as AUX / IAA, GH3, ARF and SAUR and primary cytokinin response genes such as AHP and A-ARR were up-regulated, which promoted the synthesis of auxin and cytokinin in axillary buds, marking the beginning of axillary bud development. The gene expression patterns of the two dormant buds were consistent, mainly concentrated in photosynthesis related pathways. Real time PCR showed that the expression profile of DEGs was consistent with that of RNA sequencing. This study provides basic data for the cultivation and molecular breeding of Eucommia ulmoides.

1. Introduction

Axillary bud is one of the important links in plant morphogenesis. It is common in the axil of the leaves of seed plants and has the potential to develop into a complete branch like the terminal bud. Meanwhile, the axillary bud structure, as the main source of plant regeneration branch, its generation and development ability determine the integrity of plant regeneration structure and its importance to plant survival and reproduction. The function of axillary bud is mainly reflected in the branching of plant stem and the production of various plant forms. At the same time, based on the evolution of plant adaptation to the environment and its own genetic information, from dormancy release to growth of component branches, axillary buds are more important as an important protective mechanism for plants to avoid the loss of terminal buds and other injuries, and an important aspect of plants' adaptation to the environment in the whole life cycle (Domagalska and Leyser, 2011). In recent years, With the rapid development of modern high-throughput transcriptomic sequencing technology, the mechanisms of axillary bud generation, dormancy and induction have been widely reported, and some important functional genes have been discovered and used. For example, the dormancy related transcription factors *DAM* and *GH17* gene family have been identified (Wu et al., 2012). Wang (Wang et al., 2020) found that the growth of rice

axillary buds under nitrogen stress is mainly due to cell division and elongation, which are regulated by genes related to nitrogen, phosphorus and sugar metabolism, hormone signaling, primary and secondary metabolism and photosynthesis. MADS-box transcription factor can participate in differentiation of apical meristem, regulation of photosynthesis and nutrition metabolism, and hormone signal transduction (Yamane et al., 2008). Thus, high-throughput transcriptome sequencing has become an efficient method for functional gene research.

Eucommia ulmoides Oliv, also known as bakelite, belongs to *Eucommia* genus of *Eucommia* family. It is a unique multi-purpose economic tree species in China. *Eucommia bark*, as a traditional Chinese medicine, mainly contains lignans, iridoids and phenylpropanoids. It has many health care functions such as improving hypoxia tolerance, immune function, and blood pressure (Zhang et al., 2002). China's wild *Eucommia* is mainly distributed in Shanxi, Hunan, Anhui and other southern provinces and regions. Now it is widely planted in 27 provinces and cities in China, with an area of 360000 hectares. Due to its wide adaptability, *Eucommia* has been introduced to Asia, Europe, and North America (Wang, 2017). The cultivation habitat of *Eucommia ulmoides* is quite different, and the growth potential of introduced *Eucommia ulmoides* from different places is significantly different, so it is of great practical significance to study the cultivation technology and cultivation physiology of *Eucommia ulmoides*. However, the existing studies on axillary buds of *Eucommia ulmoides* mainly focus on tissue culture (Li et al, 2019), cutting (Shi et al, 1994) and other issues. The development process and molecular regulation mechanism of axillary buds after dormancy release are still lack of systematic research. Therefore, our study used three generations of the transcriptome high-throughput sequencing technologies, the development of axillary and dormant buds of *Eucommia ulmoides* was analyzed by transcriptional histochemistry, the obtained data were filtered, assembled, and annotated, preliminary analysis on the molecular regulation mechanism of dormancy release and development of axillary buds. The research can be used for further study and cultivation of the regulation mechanism of axillary bud development of *Eucommia ulmoides* physiology lays a theoretical foundation.

2. Materials And Methods

2.1. Test materials and design

The experiment was conducted in the experimental park of the key laboratory of forest plant ecology, ministry of education, Northeast Forestry University from June 2019 to August 2019. The experiment materials are three-year-old seedlings of *Eucommia ulmoides* in pot. The seedlings of three-year-old *Eucommia ulmoides* were cut to the top, The top dormant bud (CK), the basal dormant bud (T2) and axillary bud of the first armpit in bottom of stubble (T1) as the research objects. When the axillary buds were developed to the stage of Fig. 1, the samples were taken. Each sample of axillary buds and dormant buds was taken more than 1g, store all the materials in − 80℃ refrigerator. The samples covering the main development period were selected for mixing, with 3 replicates for each sample. The samples were sent to Beijing Baimai biotechnology company for transcriptome sequencing.

2.2. Transcriptome sequencing and assembly

Based on sequencing by synthesis (SBS) technology, using Illumina Hiseq high-throughput sequencing platform sequenced the cDNA library. Trinity software first broke the sequenced reads into shorter segments (K-mer), then extended these small segments into longer segments (Contig), and used the overlap between these segments to get the fragment set (Component). Finally, used the method of De Bruijn map and the sequencing of read information are used to recognize the transcript sequence in each fragment set (Fig. 2). To estimate the expression levels of genes in samples, FPKM (fragmentsper kilobase of transcript per million reads) were calculated using FeatureCounts.

2.3. Sequence annotation and functional classification

Using BLAST software to compare the UniGenes sequence with Swiss-Prot, GO, COG, KOG, eggNOG 4.5, KEGG, Pfam database, using KOBAS2.0 to get the KEGG ontology result of UniGene in KEGG, after the prediction of the amino acid sequence of UniGene, using HMMER software to compare with Pfam database, to get the annotation information of UniGene.

2.4. RNA extraction and construction of cDNA library

A total amount of 1g RNA per sample was used as input material for the RNA sample preparations. Total RNA was extracted from the same axillary bud and dormant bud samples used in transcriptome analysis by Trizol method. The purity, concentration and integrity of RNA samples were detected by ultramicro spectrophotometer (nanodrop). The first strand synthesis kit of BeyoRT™ Ⅱ cDNA was used (Nanjing biyuntian biological company, Jiangsu). The specific steps were: 3 μl RNA (100–500 ng/μl), 1 μl Oligo (dT) (0.5 μg/μl) and 8 μl ddH_2O were mixed, incubated at 65 ℃ for 5 min, cooled on ice, and then added 4 μl buffer, 1 μl RNase inhibitor (20 U/μl), 2 μl dNTP, 1 μl RNase inhibitor reverse transcriptase (RT-MLV, 200 U/μl), 20 μl in total, reacts at 42 ℃ for 1 h, reacts at 80 ℃ for 10 min, stops the reaction on ice, and obtains single strand cDNA.

2.5. Validation of RNA-seq with quantitative real-time PCR (qRT-PCR)

To verify the RNA-seq data, nine DEGs were randomly selected from transcripts for qRT-PCR. Primers for qRT-PCR were designed by NCBI and synthesized by Shenggong Bioengineering (Shanghai) Co., Ltd. 40S gene was used as internal reference gene. All gene specific primers are listed in Table 1.

Table 1
Primers used in quantitative real-time PCR

Gene name	Sequence (5′-3′)	
40s	F	GGTTGTCAATGTTCCTTCCTTC
	R	TCTTCATCCTCTTCATCTCCATC
c-57051-c2	F	AATGCCAAACCCACGTTCCT
	R	TTCTGCTCACGTTGCCCTAC
c-56074-c0	F	AAACCTCCGACGGCTTACAG
	R	GATATCCGAACCGGAACGCT
c-54871-c1	F	GCATTGTCTCAAGCTTCGCC
	R	CTTCGATGCCGGTTTTGGTG
c-28669-c1	F	TCCCGGGATTGTTTTCGTGT
	R	ACAGGCTTCCATAACTCGGC
c-47907-c0	F	CCTGGGATGCACATTGACCT
	R	TTCAGGAAGAGCACACTCGC
c-43400-c0	F	GCGCTTGAGAAACTCTTCGG
	R	AAGGTACATCTCCCACGAGC
c-50756-c0	F	TAGGCAAGATGTTCCGCTCC
	R	ATCGCCAACGAGCATCCAAT
c-57107-c1	F	TAGCGAACACAAGACCGAGC
	R	GGACTTGGGTTGTCCCCATT

The PCR was performed in a 20 μL reaction mixture containing 10 μL of SybrGreen qPCR Master Mix (2×), 1.5 μL of forward primer, 1.5 μL of reverse primer, 2 μL reverse-transcribed cDNA, and 5 μL of ddH_2O. The PCR conditions were as follows: 2 min at 95°C; 40 cycles of 95°C for 15 s, 52°C for 30 s, and 72°C for 60 s; followed by a melt curve analysis from 60°C to 95°C. Each reaction was repeated three times, and the expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Finally, RNA-seq-derived expression levels were compared with those derived from qRT-PCR.

3. Results And Analysis

3.1.1. Sequencing data output statistics

71.10 GB of clean data were obtained from the quality control of Illumina Hiseq sequencing of axillary buds and dormant buds of *Eucommia ulmoides* Oliv. The most output of reads was axillary buds (T1) of *Eucommia ulmoides*, and the average output of reads reached 29821904; the second was top of plant dormant buds (CK), the average output of reads was 27832335; the least output of reads was basal dormant buds (T2), the average output of reads was 21581032. The results show that the GC contents of 9 samples' reads is between 46.39% and 48.21%, and the ratio of the quality value of each sample's sequencing data greater than or equal to 30 base (Q30) is over 91.11% (Table 2). These data indicate that the sequencing quality of 9 samples (including 3 replicates) is high, and the transcriptome data obtained can meet the needs of subsequent analysis.

Table 2
Evaluation statistics of sample sequencing data

Sample	Read number	Base number (bp)	GC content (%)	% ≥ Q30
CK-1	21 090 187	6 312 392 186	46.44%	93.13%
CK-2	42 863 152	12 828 259 144	46.39%	92.71%
CK-3	19 543 667	5 851 331 164	46.60%	93.02%
T1-1	21 905 904	6 562 650 396	46.46%	91.11%
T1-2	29 965 914	8 962 682 466	47.39%	93.04%
T1-3	37 593 894	11 245 628 838	47.12%	92.97%
T2-1	21 323 173	6 372 668 956	47.17%	91.49%
T2-2	21 976 083	6 559 496 494	48.21%	92.68%
T2-3	21 443 839	6 403 513 572	47.87%	91.71%

Note: CK: dormant buds on the top of plant before top cutting; T1: the axillary buds on the first short branch at the lower end of the amputationdormant; T2: dormant bud of plant root and stem.

3.1.2. Sequencing data and assembly results

The primer joints of all the obtained reads were intercepted and the low-quality data is filtered. The sequence of the obtained clean reads was compared with the assembled transcript or UniGene library, and the mapped reads will be used for subsequent analysis. The results showed that the lowest comparison efficiency was 78.81%, and the highest was 82.13%. The average comparison efficiency of *Eucommia* axillary buds sequencing data was 81.73%, and the comparison efficiency of dormant buds of the two treatments was lower than that of axillary buds (Table 3).

Table 3
Comparison statistics of sequencing data and assembly results

Sample	Clean reads number	Mapped reads number	Mapped ratio (%)
CK-1	21 090 187	16 622 007	78.81%
CK-2	42 863 152	34 186 250	79.16%
CK-3	19 543 667	15 452 823	79.07%
T1-1	21 905 904	17 739 521	80.98%
T1-2	29 965 914	24 595 359	82.08%
T1-3	37 593 894	30 876 786	82.13%
T2-1	21 323 173	16 947 499	79.48%
T2-2	21 976 083	17 881 395	81.37%
T2-3	21 443 839	17 003 703	79.29%

Note: CK: dormant buds on the top of plant before top cutting; T1: the axillary buds on the first short branch at the lower end of the amputationdormant; T2: dormant bud of plant root and stem.

After the raw data correction and deredundancy analysis, the length frequency distribution of the third-generation full-length transcripts was shown in Table 4, and a total of 336932 transcripts and 97720 UniGene sequences were obtained. Most of the transcripts were between 1–2 kbp and larger than 2 kbp, with 79277 and 101211, respectively, accounting for 23.53% and 30.04% of the total transcripts. The length of the UniGene sequence was mostly between 200–500 bp, with 61628, accounting for 60.07% of the total.

Table 4
Distribution of length and frequency of Transcripts and UniGene sequences after deredundancy

Length Range	Transcript	UniGene
200–300	47612(14.13%)	34933(35.75%)
300–500	47288(14.03%)	26695(27.32%)
500–1000	61544(18.27%)	18326(18.75%)
1000–2000	79277(23.53%)	9950(10.18%)
> 2000	101211(30.04%)	7816(8.00%)
Total Number	336932	97720
Total Length	533878536	71072285

Note: length range: indicates different length ranges of UniGene; the number in the table indicates the number of UniGene in the corresponding range, and the percentage in brackets indicates the proportion of UniGene in the corresponding length range; total number: indicates the total number of UniGene assembled; total length: indicates the total length of UniGene assembled.

3.1.3. UniGene feature notes

Select blast parameter E-value not greater than 1e-5 and HM-MER parameter E-value not greater than 1e-10 for comparative analysis, a total of 29353 UniGenes were annotated, with the annotation rate of 30.04% (Fig. 3). Results 7362 UniGenes were successfully annotated in COG database, the annotation rate was 25.08%; 15914 UniGenes were annotated in GO database, the annotation rate was 54.22%; 8665 UniGenes were annotated in KEGG database, the annotation rate was 29.52%; 15891 UniGenes were annotated in KOG database, the annotation rate was 54.14%. In the Pfam database, 15730 UniGenes were annotated successfully, the annotation rate was 53.59%; in the eggNOG database, 15891 UniGenes were annotated successfully, the annotation rate was 85.81%. The number of UniGene with 300 < = length < 1000 and length > = 1000 was the most annotated in the eggNOG database, and the annotation rate is 80.63% and 94.85% respectively. The results showed that the annotation rate of full-length transcriptome sequence of *Eucommia ulmoides* was higher.

3.1.4. Differentially expressed genes in germination and dormancy buds of *Eucommia ulmoides*

To study the expression characteristics of transcriptome in the process of dormant bud release and axillary bud development, the transcriptome information of axillary bud and dormant bud was analyzed. A total of 12113 differentially expressed genes were detected in the axillary buds of *Eucommia ulmoides* Oliv. Compared with CK, 5384 differentially expressed genes were detected in the axillary buds of T1 treatment group, of which 3137 genes were up-regulated and 2247 genes were down regulated (Fig. 4a); 1962 differentially expressed genes were detected in dormant buds of *Eucommia ulmoides* Oliv in T2 treatment, 1255 genes were up-regulated and 707 genes were down regulated. Compared with T1 (Fig. 4b). In conclusion, the number of differentially expressed genes in axillary buds in T1 treatment

group was the most abundant, which indicated that the gene expression was the most abundant in all the ways of axillary bud development after topping. The dormancy of axillary buds was released after topping, and the morphological and structural changes of axillary buds were involved in many molecular activities.

3.1.5. Correlation evaluation of differentially expressed genes among samples

Pearson correlation coefficient r was used as the evaluation index of correlation between samples. The closer r^2 is to 1, The stronger the correlation between the two samples. There are three treatment groups in this experiment, each treatment group was set with three replicates to correlate the gene expression of each group. The correlation diagram is shown in Fig. 5. It can be seen from the figure that the similarity between CK-1, CK-2 and CK-3 was high, and the correlation coefficient was above 0.978; the similarity between T1-1 and T1-2 was the lowest, and the correlation coefficient was 0.883, and the correlation coefficient between T1-1 and T1-3, T1-2 and T1-3 were 0.929 and 0.992, respectively; there was also significant correlation between T2-1, T2-2 and T2-3, and the correlation coefficient is above 0.988; The correlation between T1-1 and T2 was the weakest, the coefficient was 0.82, the results showed that the most differentially expressed genes were between T1 and T2 treatment. The correlation between T1 and CK was the second, while that between T2 and CK was the strongest. In conclusion, there was a significant correlation between the biological repeats of each treatment group, the differential expression genes were reliable, the correlation order of differentially expressed genes was T1 > T2 > CK.

3.2. Enrichment analysis of differentially expressed genes

3.2.1 GO enrichment of differentially expressed genes

Through the analysis of significant difference enrichment of GO, we can know the main biological functions of the differentially expressed genes in the process of dormant bud release and axillary bud development of *Eucommia ulmoides* Oliv. The analysis of GO differential enrichment in this study is shown in Fig. 6. Compared with CK, there were 2810 and 969 differentially expressed genes annotated in GO database in T1 and T2 treatment groups, including 16 and 18 biological functional pathways. In the cell components, the differentially expressed genes in T1 treatment group were mainly concentrated in cell (1294) and cell parts (1283). T2 treatment group was mainly enriched in cell (359), membrane structure (401), and cell part (354). T1 treatment group had two more pathways than T2 treatment group, namely virion and virion part. In the molecular function, T1 and T2 treatment groups were all enriched in the catalytic activity and binding activity pathways, and T1 had one more nutrient reservior activity pathway than T2 treatment group, which indicating that axillary buds need a large amount of nutrients and metabolites in the process of germination to meet the requirements of exogenous. In biological process, T1 treatment group was mainly enriched in metabolic process (1499) and cell process (1358). The T2 treatment group was mainly enriched in metabolic process (467), cellular process (423), single organic process (324), emergency response (141), localization (111), and biological regulation (160). The T2 treatment group had two more pathways than the T1 treatment group, namely, locomotion and cell

killing pathways. The number of differentially expressed genes involved in the up-regulation and down-regulation of axillary buds was the highest in cell components, molecular functions, and biological processes, indicating that these processes had a significant impact on the development of eucommia ulmoides axillary buds, especially the metabolic process.

3.2.2. COG enrichment of differentially expressed genes

Through the analysis of the significant difference enrichment of COG, we can know which life activities are regulated by differentially expressed genes during the development and dormancy of *Eucommia ulmoides* axillary buds. Through COG database of axillary bud and dormant bud raised difference analysis found that the gene enrichment mainly in the amino acids transport and metabolism, carbohydrate transport and metabolism, translation, ribosomal structure and biogenesis, post translation modification, protein folding and molecular chaperone, general function prediction only the five pathways, which rise in axillary bud gene numbers above the sleeping bud. This indicates that the growth of axillary buds after plain stubble was mainly concentrated in the primary metabolism, and some sugars, amino acids and proteins were synthesized to meet the needs of germination (Fig. 7).

3.2.3. Analysis of metabolic pathway of differential expression gene KEGG

The analysis of the significant enrichment of KEGG can further determine the signal transduction pathway and the main metabolic pathway involved in the differentially expressed genes. Through the enrichment analysis of KEGG pathway in axillary buds and dormant buds, it was found that the number of up-regulated genes in axillary buds was significantly lower than that in dormant buds in plant hormone signal transduction and plant pathogen interaction, but most genes were highly expressed in ribosome synthesis of gene information process. It was suggested that hormones in dormant buds are rarely used. The reason of hormone decrease in axillary buds may be due to the synthesis of many primary compounds to maintain their own growth in the late exogenous process. Dormant buds mainly play a role in maintaining the plant from being destroyed and maintaining the autoimmune function. In addition, the number of up-regulated genes in amino acid biosynthesis, carbohydrate metabolism, starch and sucrose metabolism, amino sugar nucleotide metabolism in axillary buds was significantly higher than that in dormant buds. The results showed that the axillary buds mainly absorbed and transported carbohydrates to promote the growth of axillary buds. The results of enrichment analysis of GO and COG databases also verified that there were genes affecting axillary bud germination in primary metabolic pathway.

3.2. Alidation of RNA-seq data with qRT-PCR

The reliability of RNA-seq data was assessed with qRT-PCR using nine DEGs that were randomly selected (Fig. 9). However, the expression trends of all 9 genes were highly consistent between the two methods. Thus, qRT-PCR results validated the reliability of RNA-seq data.

4. Discuss

Transcriptome represents the collection of all transcriptional RNA in cells or tissues, which reflects genes expressed in different life stages, different tissue types, different physiological states, and different environmental conditions (Song et al., 2020). The study of transcriptome can reflect the expression and regulation of genes in the whole cell. In the study of axillary buds and dormant buds of *Eucommia ulmoides* Oliv., transcriptome sequencing was undoubtedly the most effective way to explore and analyze the mechanism of dormancy release and development of axillary buds. This study uses the third-generation sequencing technology represented by Illumina hiseq can obtain high-quality full-length transcripts information by virtue of its super long reading advantage, and then carry out gene annotation and functional classification on the sequencing results to obtain the transcriptome information of the axillary bud development process of *Eucommia ulmoides* Oliv. At the same time, it can also detect and annotate or unknown functional genes and genomic sites, providing convenience for the mining of new genes (He et al., 2019). Based on the analysis of full-length transcriptome sequencing technology and bioinformatics, the Q30 obtained in this study has reached over 91.11%; the sequence comparison efficiency between the obtained clean reads and the assembled transcription or UniGene library is 78.81% – 82.13%. Zu (Zu et al., 2020) and others sequenced the transcriptome of 6 male flower buds of *Eucommia ulmoides*, and sequenced the clean reads of each sample with the genome of *Eucommia ulmoides*. The percentage of Q30 base was 90.75% and above, and the efficiency was 90.56% – 93.01%. It was speculated that the reason for this result may be that the genetic difference between the axillary buds of *Eucommia ulmoides* Oliv. Sequenced in this study and the *Eucommia* varieties sequenced by Zu (Zu et al., 2020) was quite large. Therefore, this study carried out the nonparticipation transcriptome to analyze the specificity of axillary buds of *Eucommia ulmoides* Oliv. A total of 97720 UniGene sequences were obtained after the sequence was spliced and assembled. The length of the UniGene sequences was mostly between 200–500 bp, with a total of 61628, accounting for 60.07% of the total UniGene sequences. It shows that the sequencing quality is high and meets the quality requirements of subsequent analysis.

It was the initial stage for the axillary buds to develop into short branches from dormancy to release after cutting the top of *Eucommia ulmoides* Oliv. In this study, by comparing the gene expression difference and the change of expression quantity between the axillary buds and dormant buds of *Eucommia ulmoides* Oliv, we found that the most differentially expressed genes were found in the process of dormancy release of axillary buds after the top cutting treatment from the sequenced transcriptome data, and the key genes controlling the dormancy and release of axillary buds could be found in these differentially expressed genes. The differentially expressed genes were enriched with COG, GO and KEGG. The differentially expressed genes in axillary buds are mainly concentrated in carbohydrate transport and metabolism, starch and sucrose metabolism, and amino acid biosynthesis. During the growth of axillary buds, sugar is needed to provide energy and carbon skeleton for RNA and protein biosynthesis. Carbohydrate plays an important role in regulating the growth and development of axillary buds, which was like many studies. Kircher (Kircher and Schopfer) found that endogenous sucrose promoted the growth of Arabidopsis taproot and lateral root. Koch suggested that sucrose and starch had a positive effect on tillering or branching, while glucose and fructose had no similar effect. Carbohydrate was the

initial direct regulator of apical dominance, and this early regulation is independent of auxin. When the apical buds were removed, the change of auxin in the lateral buds was earlier than that in the adjacent trunk, and then promoted the redistribution of carbohydrate substances, resulting in the rapid germination of axillary buds. The results showed that the number of upregulation genes in the plant hormone signal transduction pathway in axillary buds was less than that in dormant buds, while the number of saccharide related genes was increased. Liu (Liu et al., 2011) found that the main reason for breaking dormancy of axillary buds of Pinus brevifolia might be oxidative stress, and the obvious interaction among plant hormones, carbohydrates and other signal transduction factors also led to the synergetic promotion of sprouting after plant apex. It has been reported that the down regulation of sl-ARF4 gene in tomato can cause the up regulation of starch biosynthesis related genes and enzyme activities, and increase sugar content. Therefore, sl-ARF4 plays an important role in auxin regulation of fruit sugar metabolism (Sagar et al., 2013). Carbohydrate not only directly regulates auxin metabolism, but also regulates auxin transport and signal transduction. Endogenous carbon sensing pathway in plants can promote auxin transport and lateral bud growth. Overexpression of rice sugar transporter protein OsSWEET5 not only affects sugar metabolism and transportation, but also leads to the decrease of auxin level (Zhou et al., 2014). Therefore, the results of this study suggest that the decrease of up-regulated genes in plant hormone signal transduction pathway during axillary bud growth may be due to the direct involvement of endogenous hormones in the regulation of sucrose and starch accumulation, through the synthesis of many primary metabolites, strengthening the cell wall toughness and promoting axillary bud growth. The increase of up-regulated genes related to plant hormones in dormant buds is since there are few substances mobilized in dormant buds, which do not participate in compounds and synthesis, but participate in resistance to invasion of pathogenic organisms by activating plant immune response. Therefore, differential expression genes of dormant buds are significantly expressed in plant pathogen interaction pathway.

5. Conclusion

In this study, we used Illumina hiseq high-throughput sequencing platform to analyze the transcriptome of axillary buds and dormant buds of *Eucommia ulmoides* Oliv. The results showed that the GC content of reads in each sample was between 46.39% and 48.21%, and the percentage of Q30 base was over 91.11%. We intercepted primer splices and filtered the low-quality data of all the reads, and got clean the efficiency of sequence alignment between reads and the assembled transcript or UniGene library was 78.81% − 82.13%, and the efficiency of sequencing data comparison of axillary buds is higher than that of dormant buds. After the second-generation data was corrected and de redundant analysis, 336932 transcripts and 97720 UniGene sequences were obtained. The most differentially expressed genes are found in axillary bud development of *Eucommia ulmoides* after the top cutting treatment. Through the analysis of COG, GO function enrichment and KEGG metabolic pathway enrichment, it was found that the up-regulated genes in axillary buds were mainly concentrated in carbohydrate, sucrose, starch metabolism and transportation pathways, while those in dormant buds were mainly concentrated in plant hormone signal transduction and pathogen interaction pathways. The results of qRT-PCR of nine randomly selected genes were consistent with the results of transgenic group, which verified the reliability

of transcriptome. This study provides a theoretical basis for *Eucommia* resource cultivation and molecular breeding.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All the data and plant material are available with the corresponding author (AKT).

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

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Authors' contributions

Dewen Li: Visualization, Investigation, Methodology, Data curation. Wenling Zhou and Dandan Du: Writing - Review & Editing, Visualization, Supervision, Writing- Original draft preparation. Zhonghua Tang and Yusen Zhao: Funding acquisition, Conceptualization, Methodology, Conceptualization, Investigation. Supervision, Project administration. Hongling Wei. Xucheng Tian. Shennan Xie: Investigation, Resources. Ying Liu1and Xueying Yu: Methodology, Conceptualization, Visualization.

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Authors' information (optional)

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Figures

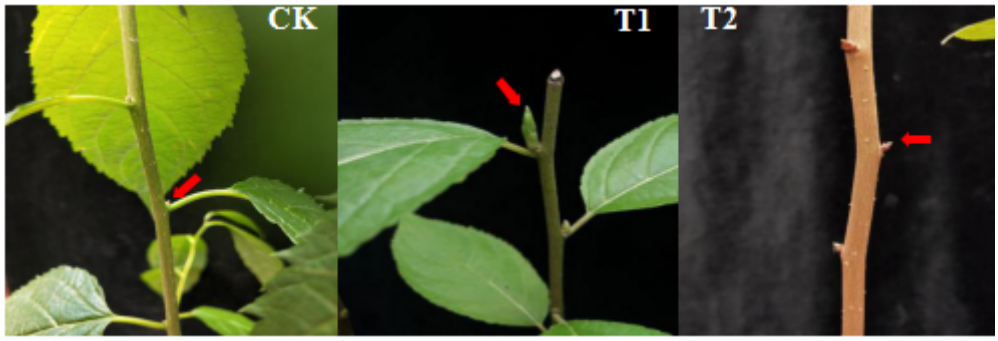

Figure 1

Morphology of axillary buds and dormant buds of *Eucommia ulmoides* Oliv

(CK: dormant buds on the top of plant before top cutting; T1: the axillary buds on the first short branch at the lower end of the amputationdormant; T2: dormant bud of plant root and stem)

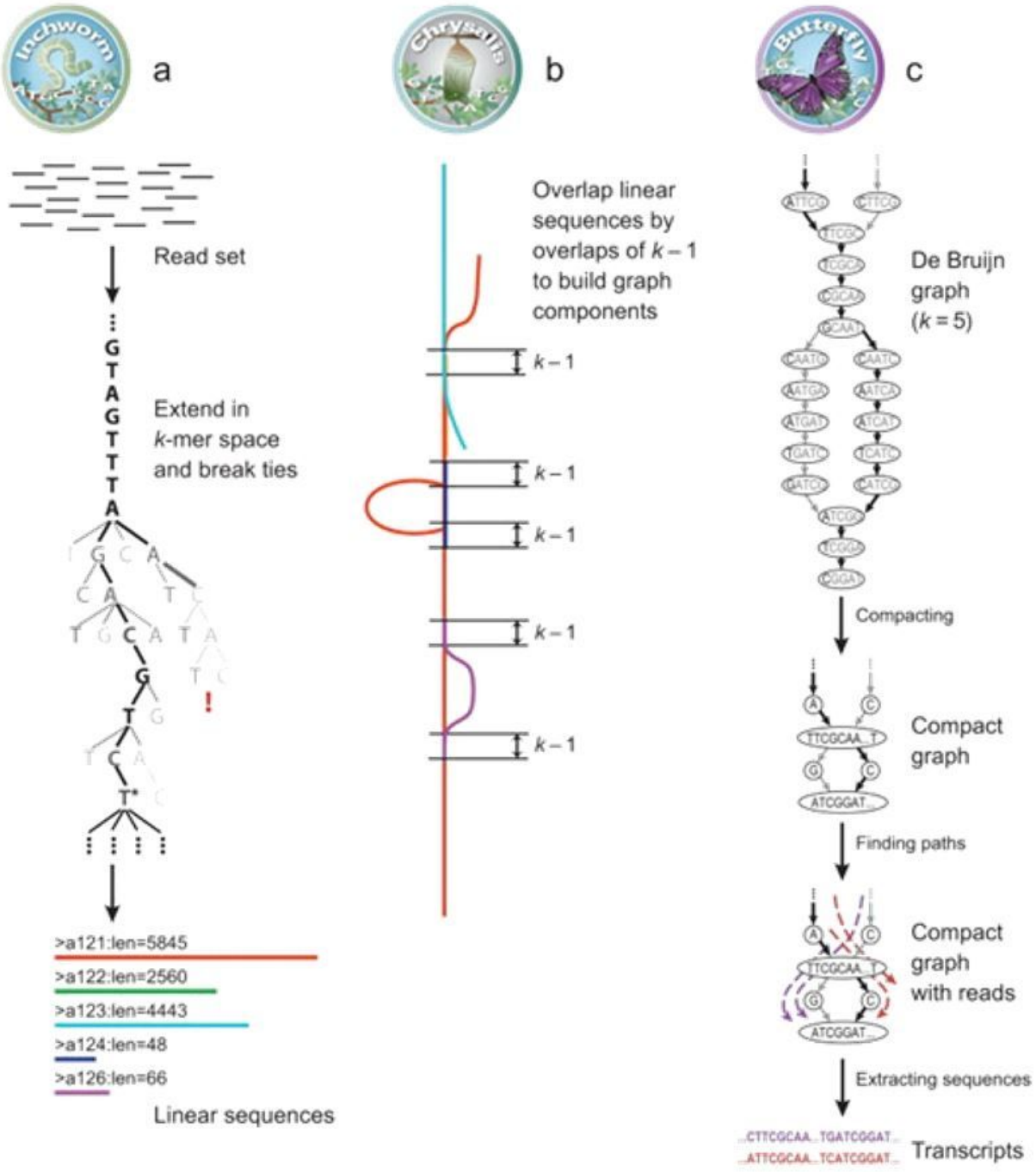

Figure 2

Trinity assembly program schematic

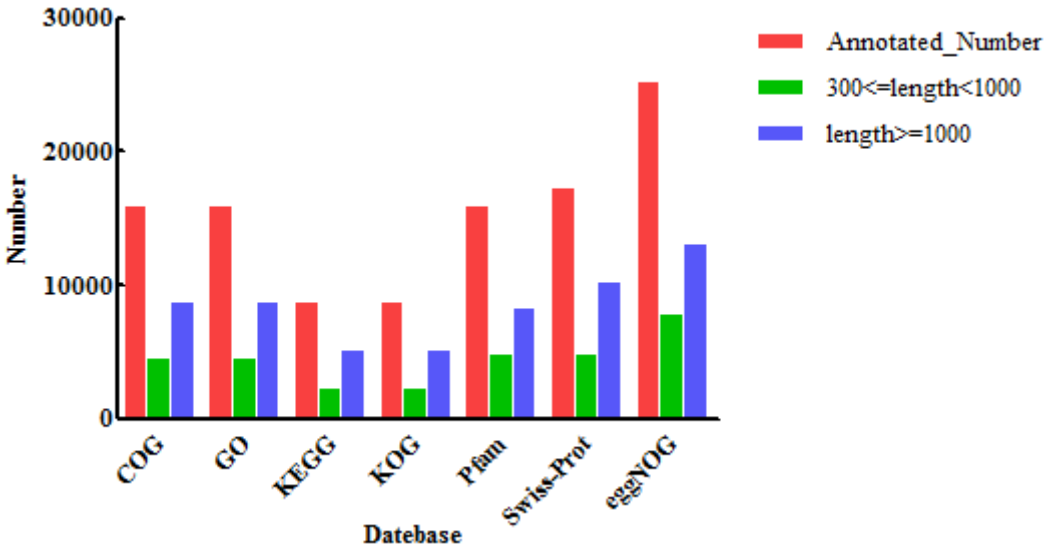

Figure 3

Statistical chart of annotation results of seven databases.

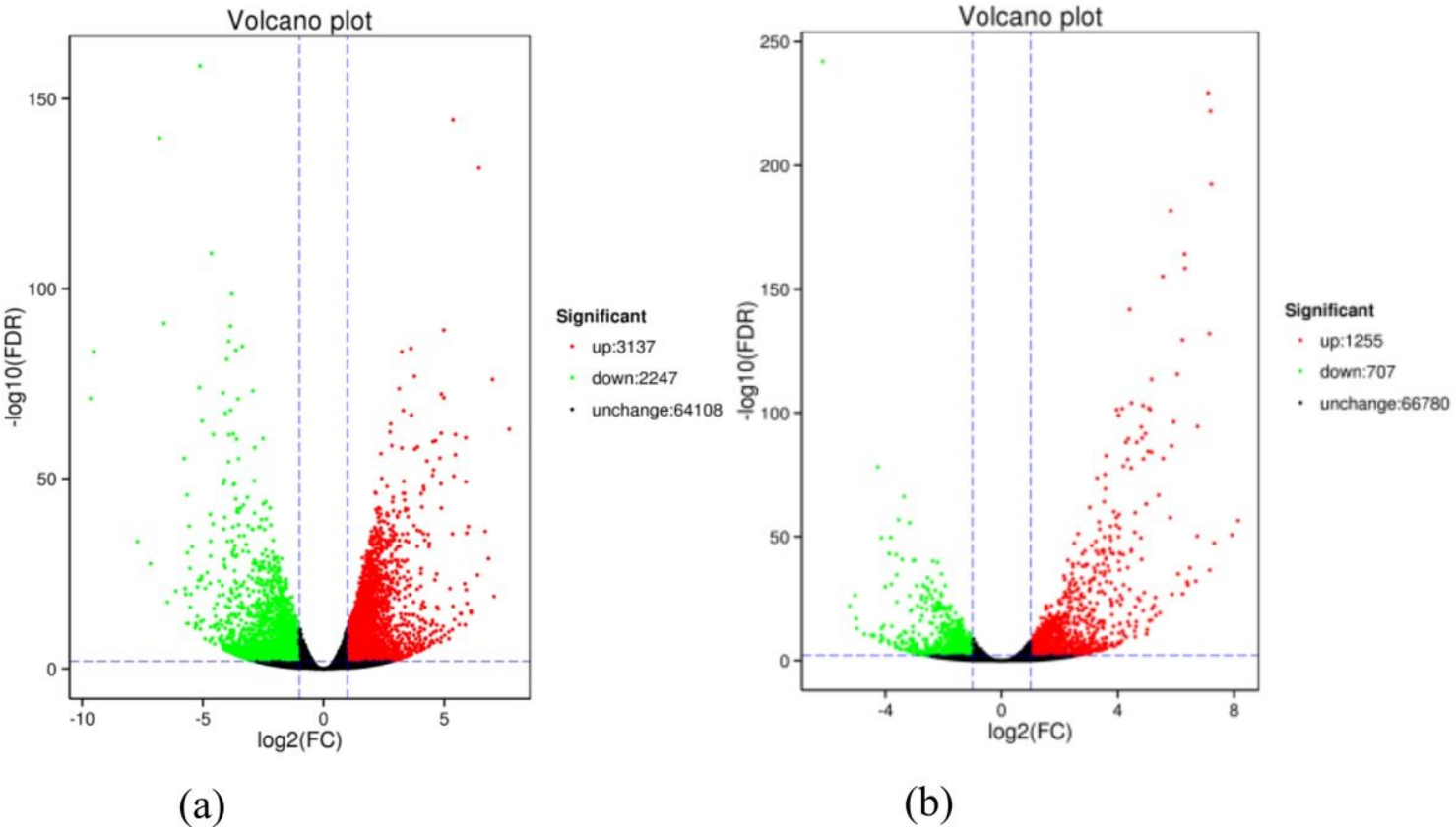

Figure 4

Volcanic map of differentially expressed genes between axillary buds and dormant buds of *Eucommia ulmoides.*

(Note: Figure (a): between CK and T1; Figure (b): between CK and T2. Each point in the differential expression volcanic map represents a gene, the abscissa represents the logarithm of the multiple of the expression amount of a gene in two samples, and the ordinate represents the negative logarithm of the error detection rate. In the figure, green and red dots represent genes with significant expression differences, green represents genes with down-regulated expression, red represents genes with up-regulated expression, and black dots represent genes without significant expression differences.)

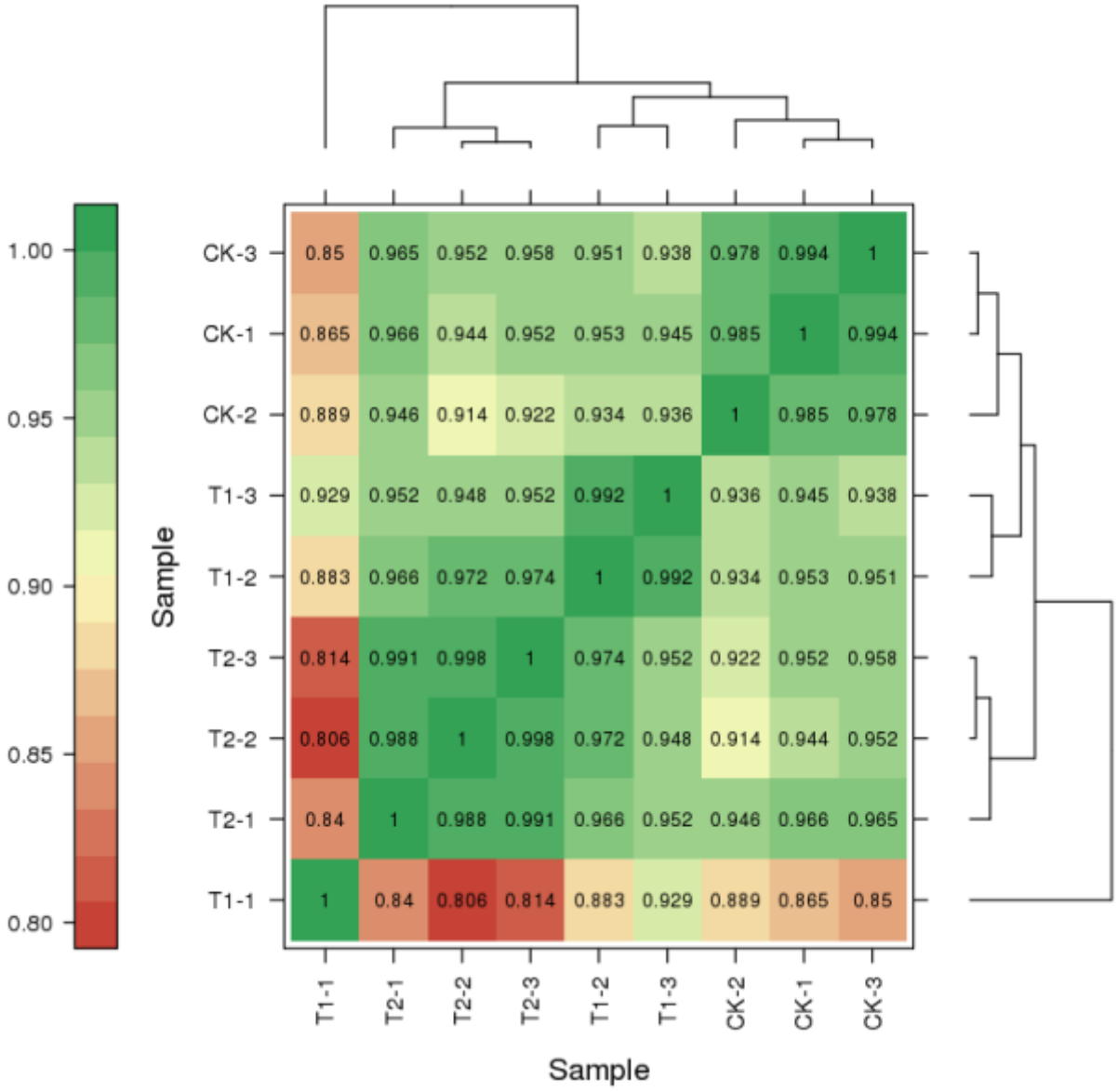

Figure 5

Heat map of correlation between axillary buds and dormant buds of *Eucommia ulmoides.*

(Note: The color from red to green indicates that the correlation between samples is from weak to strong)

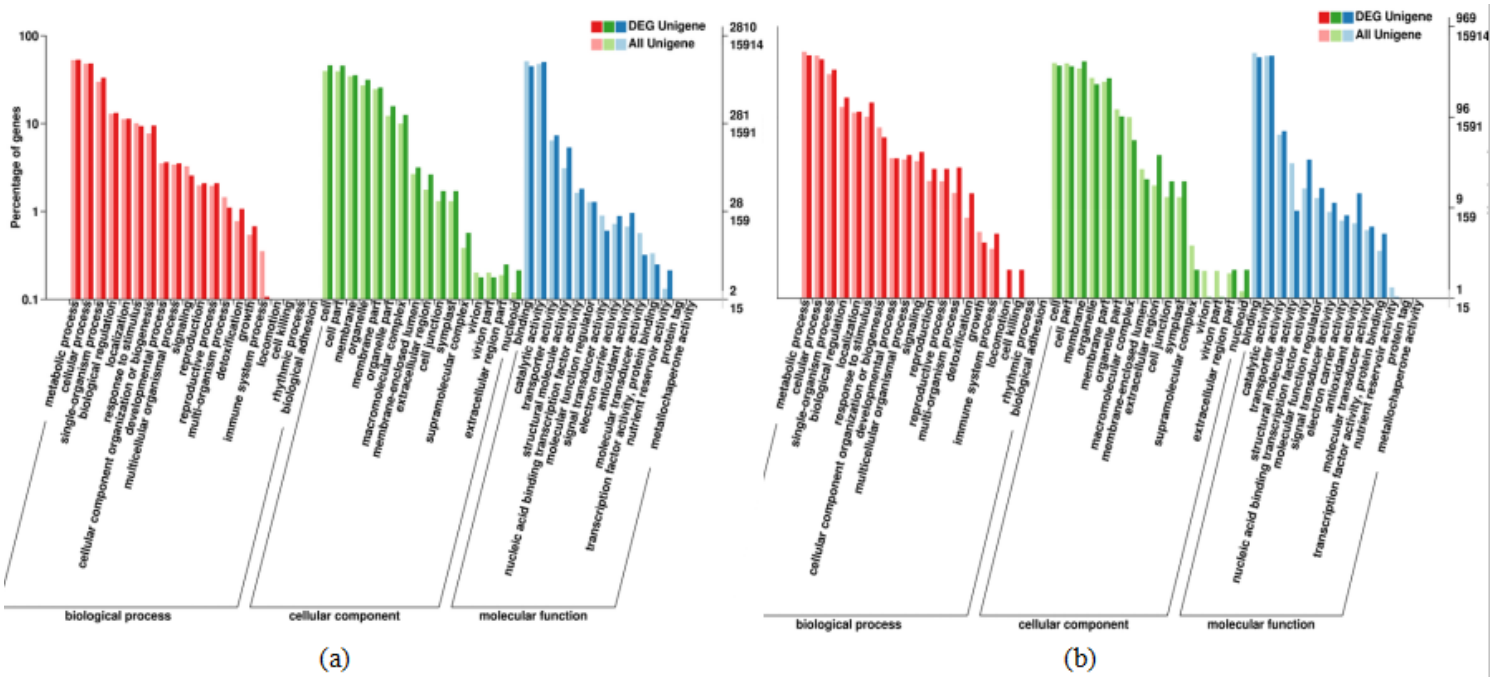

Figure 6

Statistical map of GO annotation of differentially expressed genes between axillary buds and dormant buds of *Eucommia ulmoides* Oliv.

(Note: Figure (a): between CK and T1; Figure (b): between CK and T2. The abscissa is the GO classification, the left side of the ordinate is the percentage of gene number, and the right side is the gene number.)

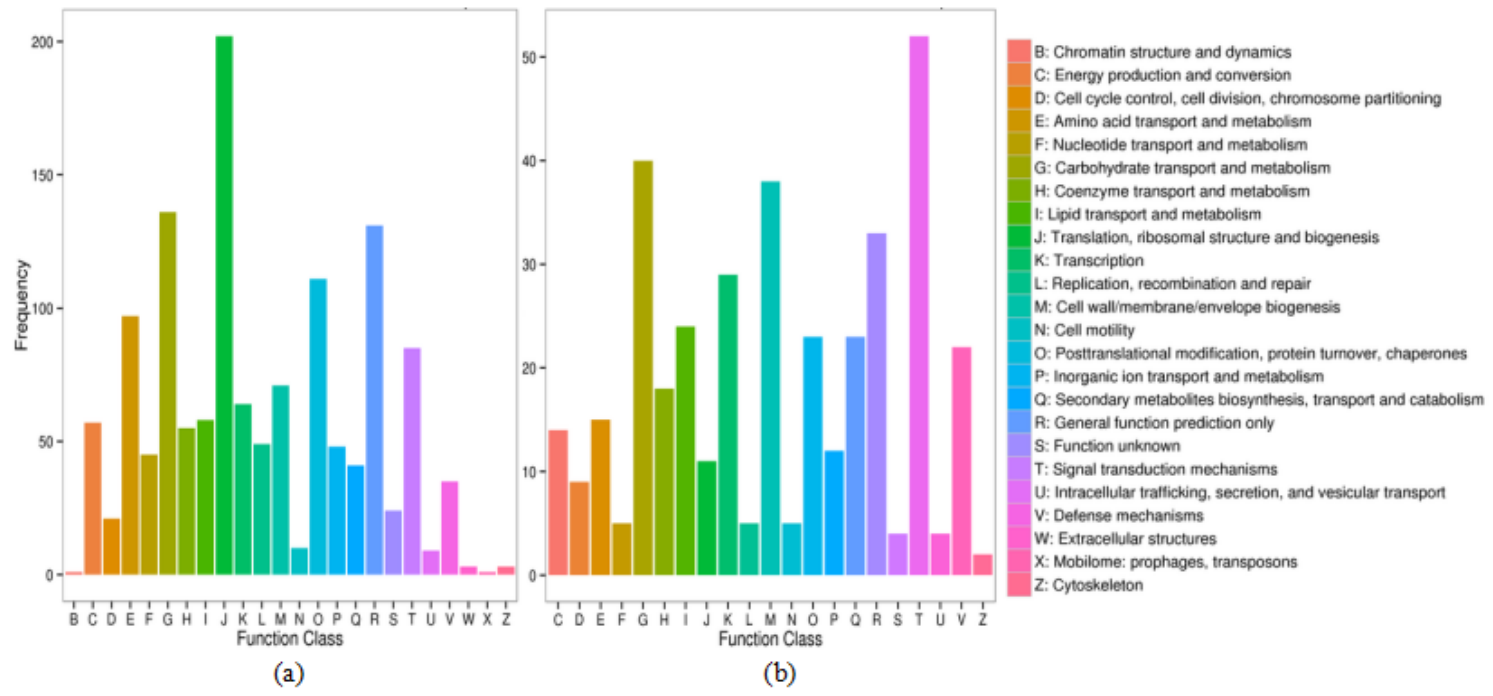

Figure 7

Statistical map of COG annotation of differentially expressed up regulation genes between axillary buds and dormant buds of *Eucommia ulmoides* Oliv.

(Note: Figure (a): between CK and T1; Figure (b): between CK and T2. The abscissa is COG classification and the ordinate is gene number.)

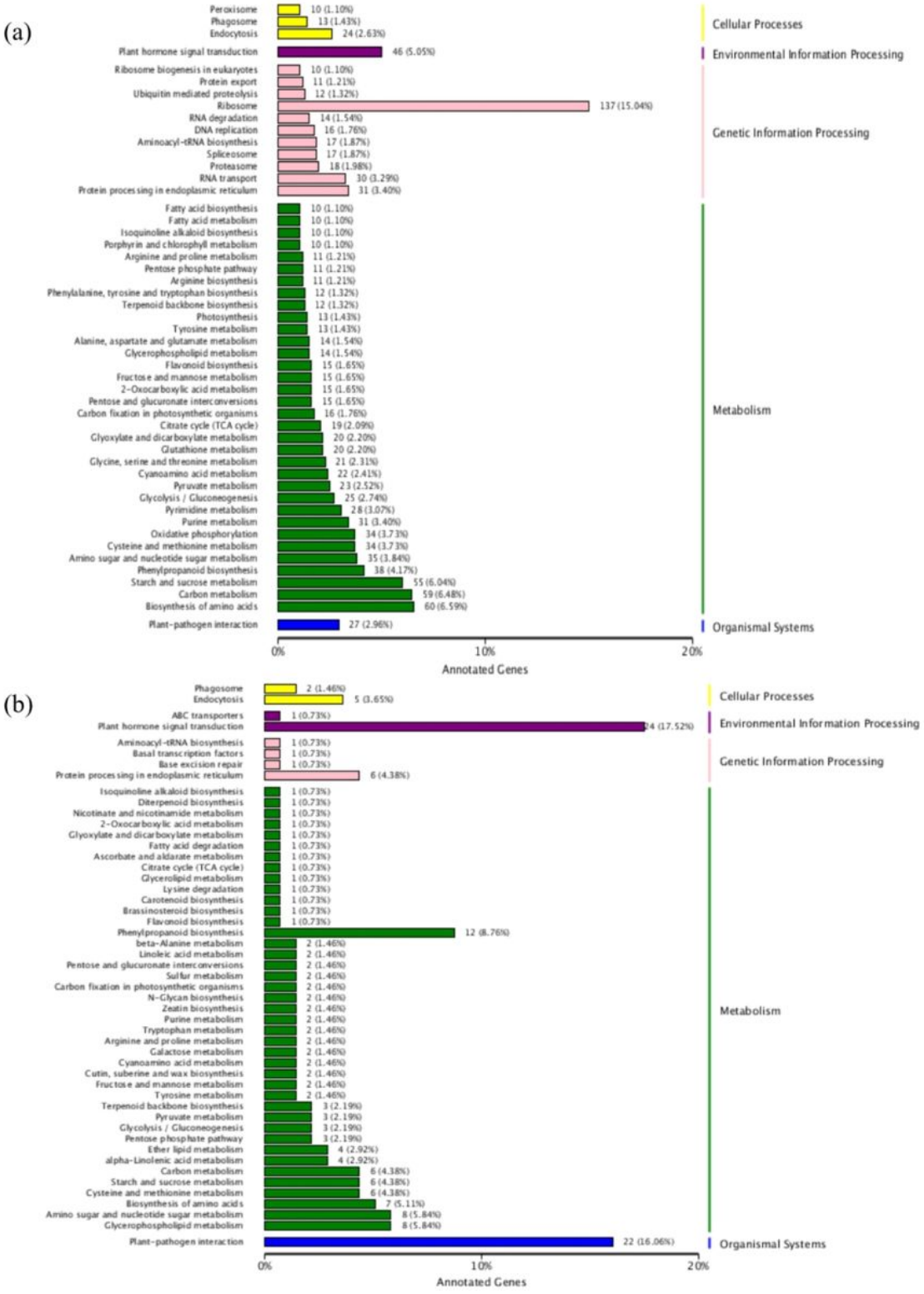

Figure 8

Statistical map of KEGG annotation of differentially expressed up regulation genes between axillary buds and dormant buds of *Eucommia ulmoides* Oliv.

(Note: Figure (a): between CK and T1; Figure (b): between CK and T2. The ordinate is the name of KEGG metabolic pathway, and the abscissa is the number of genes annotated to the pathway and the proportion of the number of genes annotated to the total number of genes annotated.)

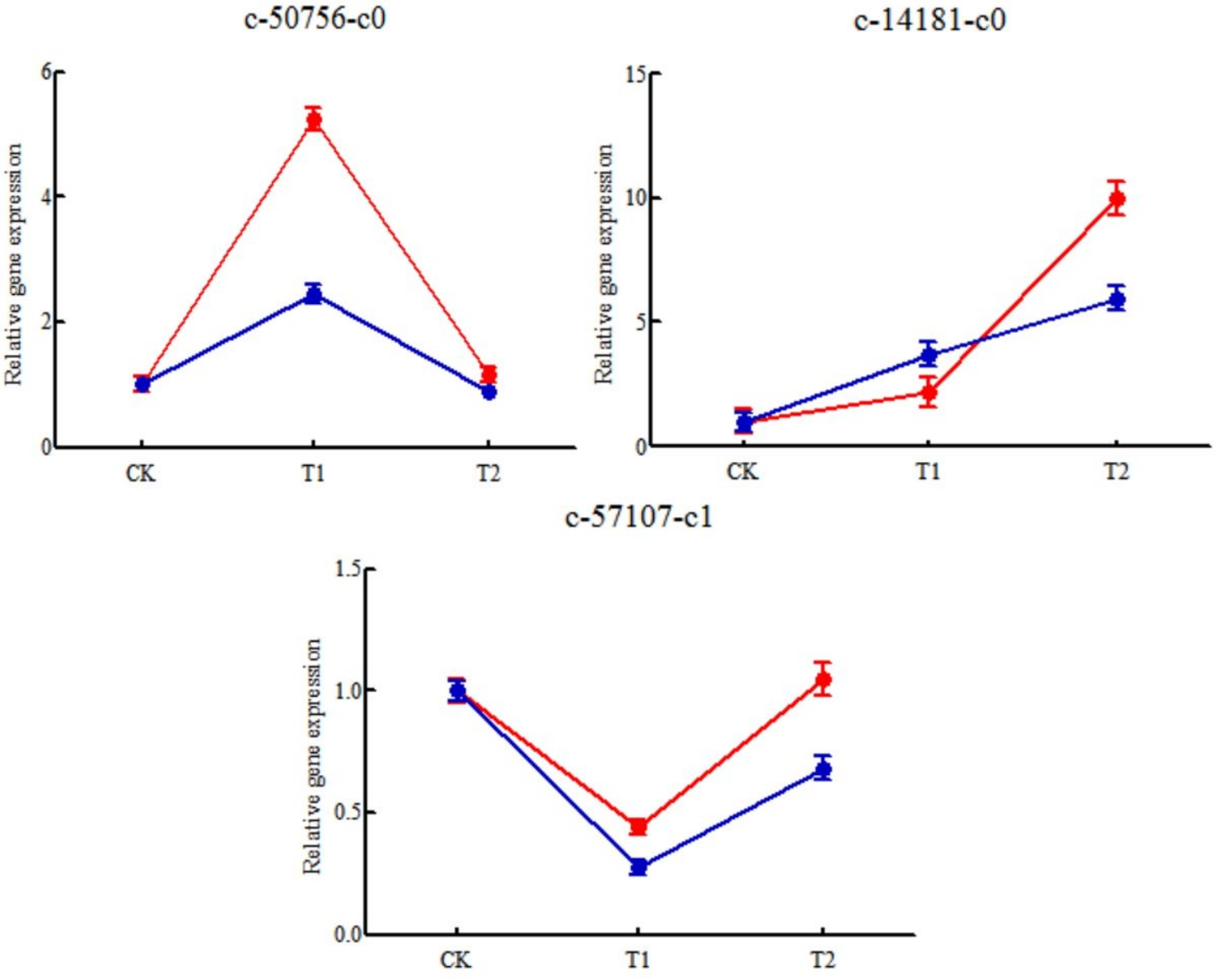

Figure 9

Quantitative real-time PCR validation of transcript levels evaluated with RNA sequencing of *Eucommia ulmoides*