The Expression Analysis of Genes in Fatty Acid Biosynthesis Pathway during the Seed Development of Tung Tree

Guixiong Jiang[1,2,a], Hao Chen[1,a], Xiaofeng Tan[1*]

[1]*The Key Lab of Non-wood Forest Products of State Forestry Administration*
College of Forestry
Central South University of Forestry and Technology
Changsha 410004, Hunan, China
E-mails: jgxpaul@163.com, chenhao198223@gmail.com, tanxiaofengcn@126.com

[2]*Forestry Department of Guangxi Zhuang Autonomous Region*
Nanning 530022, Guangxi, China
E-mail: jgxpaul@163.com

[a]*These authors contributed equally to this work.*

[*]*Corresponding author*

Received: March 21, 2013 **Accepted: June 14, 2013**

Published: July 29, 2013

Abstract: *Tung tree (Vernicia fordii) is one of the important oil plants in China. The current researches on tung tree are mainly focused on the cultivation and breeding while the molecular mechanisms hidden in the back of tung tree's phenotypes are still uncovered. This research compared the transcriptome of three different stages during tung tree's seed development using RNA-Seq and obtained a lot of differentially expressed Unigenes. Through GO classification and pathway enrichment analysis, all of these differentially expressed Unigenes were classified into 128 signaling pathways including fatty acid biosynthesis. Fourteen homologous proteins were obtained when the sequences of 54 Unigenes within fatty acid biosynthesis pathway were aligned against KEGG database and the expression profiles of the genes encoding these proteins during seed development were analyzed. This research provides necessary data platform to elucidate the molecular mechanism underlies fatty acid biosynthesis of tung tree's seeds and theoretical guidance of tung tree varieties' improvement to increase output of tung oil.*

Keywords: *Tung tree, Seed development, Transcriptome, Fatty acid biosynthesis, Differentially expressed genes, Expression profiles.*

Introduction

Tung tree (*Vernicia fordii*) originating in China is a world-renowned industrial oil tree and has some excellent features such as a long history of cultivation, a wide range of distribution and rapid growth [1]. Tung oil extracted from tung tree's seeds has a high economic value and can be used to make industrial materials such as coating and paint. However, researches on tung tree so far have focused on cultivation and breeding and there are no in-depth molecular studies. Existing molecular biology researches were only limited to homologous gene's clone and have yet to involve identification of gene's function and analysis of its role in signal network [2-6]. The main reason for this phenomenon is caused by the lack of useful reference information. The next-generation sequencing technologies provide necessary technical approaches to solve this problem. Until now there are a lot of successful researches in the field of plant molecular biology based on next-generation sequencing technologies [7-10].

Transcriptome sequencing also known as RNA-Seq which is one of the next-generation sequencing technologies has many advances such as high-throughput, low-cost, high sensitivity, good reproducibility and un-necessity of a known reference sequence compared with gene chip and gradually replaces the gene chip as the primary approach of transcriptome research [11-14]. RNA-Seq detected more differentially expressed genes than gene chip under the same FDR (false discovery rate) value [15]. Moreover, it can carry out transcriptome analysis without reference genome sequence.

Tung oil is made up of many kinds of fatty acids which are synthesized through fatty acid biosynthesis metabolic pathway [16]. The expressive regulation researches of genes involved in fatty acid biosynthesis contribute to better understanding in tung oil synthesis and lay the theoretical foundation for tung tree's improvement using genetic engineering methods. This research provides the necessary data to further explore the molecular mechanism of the fatty acid synthesis of tung tree and theoretical guidance for improving tung tree's varieties.

Materials and methods

Plant materials and total RNA isolation

Due to the rapid oil accumulation during their development, tung tree's seeds were sampled as research material at three different stages (June, August, and October) which represents the seed's typical developmental state. Total RNA was isolated from the seeds with RNeasy Plant Mini Kit (Qiagen). The remaining potential genomic DNA contamination in the total RNA sample was eliminated by RNase-free DNase I (Takara).

Sequencing library preparation and transcriptome sequencing

First-strand cDNA synthesis was performed by SuperScript™ II RT (Invitrogen) using random hexamer primers. Second-strand cDNA was synthesized using *E. coli* DNA polymerase I (Invitrogen). The paired-end cDNA library used for RNA sequencing was prepared according to Illumina's protocols. RNA sequencing was performed on a HiSeq 2000 platform.

Original data processing, sequence assembly and screening of differentially expressed Unigenes

Image data acquired from transcriptome sequencing was transformed into short nucleotide sequences (raw reads) and each of these sequences was called "reads". Clean reads were obtained while low quality reads, such as reads contain adaptor sequence, with unknown nucleotide ratio greater than 5%, were eliminated from raw reads. In view of no reference genome sequence, tung tree's transcriptome sequencing data was *de novo* assembled by Trinity software [17]. Reads with overlapping sequence were assembled into long sequence fragments called contigs and then these contigs were subsequently assembled into no longer extended sequences called Unigenes. The expression levels of all Unigenes were calculated with FPKM (fragments per kb per million fragments) algorithm in order to find out differentially expressed Unigenes [18]. The genes with Benjamini-Hochberg corrected *p*-values less than 0.05 were defined as differentially expressed.

Functional annotation, GO classification and pathway enrichment analysis of Unigenes

In order to obtain protein annotation information, Unigene sequences were aligned against online protein database such as nr, Swiss-Prot, KEGG and COG. Unigenes were then categorized into gene ontologies using Blast2GO software after alignments [19].

Gene overrepresentations in the GO (gene ontology) categories were calculated using a fisher test with the criterion that the FDR *p*-values < 0.05. Pathway enrichment analyses of Unigenes were performed by making use of KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

Results

RNA quality supervision

RNA quality supervisions were performed on Agilent 2100 Bioanalyzer. The results showed that the quality of total RNA has reached sequencing standard with sufficient amount and concentration, RIN (RNA integrity number) value greater than 7 and 28S:18S ratio greater than 1.5. The results of capillary electrophoresis showed that RNA samples had no degradation (Fig. 1). Sample 1, Sample 2, Sample 3 in Fig. 1(A) represent the total RNA from tung tree's seeds in June, August and October, respectively. Vertical axis and abscissa in Fig. 1(B) indicate the UV absorption value and the nucleotide numbers of RNA electrophoretic bands, respectively.

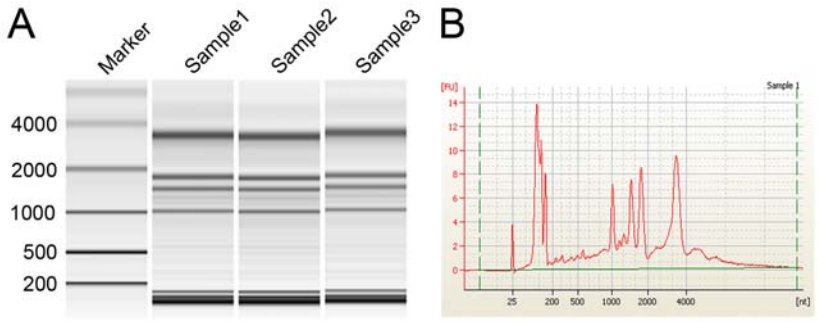


Fig. 1 The results of total RNA quality test for RNA-Seq experiment. (A) Capillary electrophoresis of the total RNA from tung tree's seeds at three different developmental stages. (B) UV absorption peaks of the total RNA bands in capillary electrophoresis.

Statistics of transcriptome sequencing data

Transcriptome sequencing of tung tree's seeds at three developmental stages (June, August and October corresponded to I, II and III stage, respectively) totally obtained 58439 non-redundant Unigenes in which I, II and III stage possessed 61001, 54679 and 44495 Unigenes, respectively. Sequence length of these Unigenes mainly distributed around 200-3000 nucleotides (Fig. 2). There were 1749 Unigenes with sequence length more than 3000 nucleotides accounting for 0.03 percent of total Unigenes. With the increase of the sequence length, the Unigene number was progressively decreasing without obvious discontinuity. These results indicate that transcriptome sequencing completed with high quality. All non-redundant Unigenes can be classified into three GO (gene ontology) categories including molecular function, cellular component and biological process (Fig. 3). Numbers (1-61) of abscissa in Fig. 3 represent GO terms of biological adhesion, biological regulation, carbon utilization, cell killing, cell proliferation, cellular component organization or biogenesis, cellular process, death, developmental process, establishment of localization, growth, immune system process, localization, locomotion, metabolic process, multi-organism process, multicellular organismal process, negative regulation of biological process, nitrogen utilization, pigmentation, positive regulation of biological process, regulation of biological process, reproduction, reproductive process, response to stimulus, rhythmic process, signaling, sulfur utilization, viral reproduction, cell, cell junction, cell part, extracellular matrix, extracellular matrix part, extracellular region, extracellular region part, macromolecular

complex, membrane, membrane part, membrane-enclosed lumen, organelle, organelle part, symplast, virion, virion part, antioxidant activity, binding, catalytic activity, channel regulator activity, electron carrier activity, enzyme regulator activity, metallochaperone activity, molecular transducer activity, nucleic acid binding transcription factor activity, nutrient reservoir activity, protein binding transcription factor activity, protein tag, receptor activity, structural molecule activity, translation regulator activity and transporter activity, respectively. Unigenes associated with two GO terms, catalytic activity and metabolic process, were significantly enriched maybe due to strong metabolism during seed development (Fig. 3). Pathway enrichment analysis classified all non-redundant Unigenes into 128 signal pathway. Metabolic pathway had the most number of Unigenes (4736) accounting for 21.4 percent of all Unigenes whereas betalain biosynthesis pathway had only one Unigene.

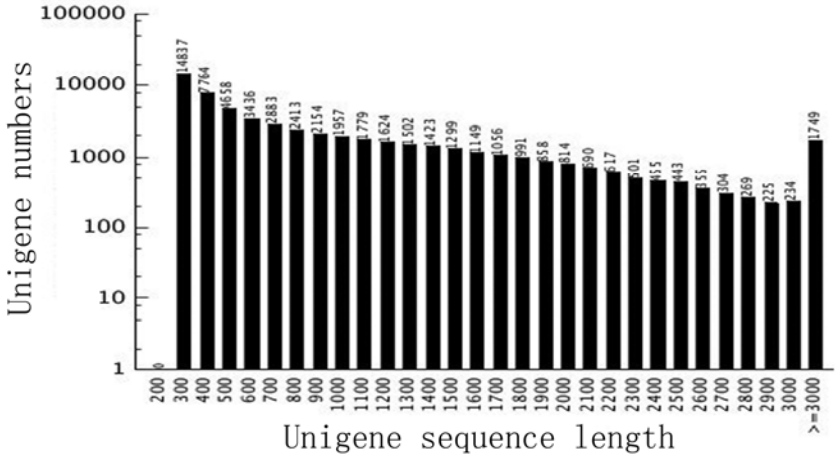


Fig. 2 Length distribution of Unigene sequences

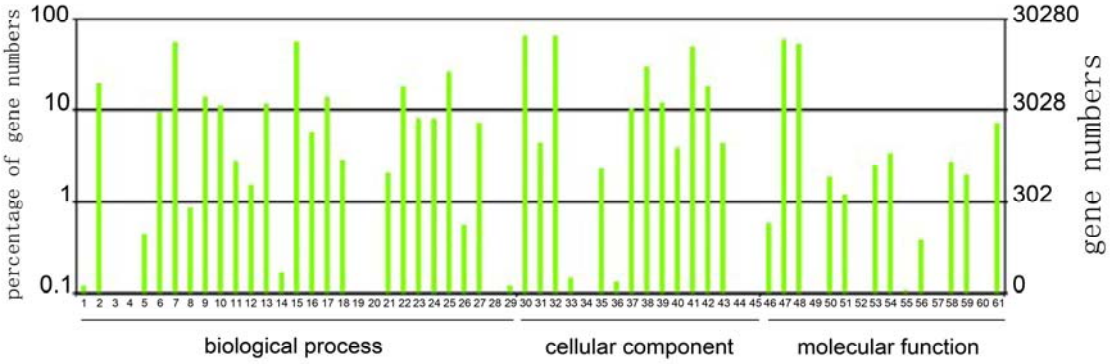


Fig. 3 GO classification of Unigenes

Expression analysis of critical genes in fatty acid biosynthesis during seed development

After pairwise comparisons among three seed development stages, expression analysis showed that more and more Unigenes exhibited up-regulated trend along with the seed development whereas the numbers of down-regulated genes were much lower than up-regulated genes and their changes were irregular throughout three stages (Fig. 4). I, II and III period in Fig. 4 represent the periods of fruit development in June, August and October, respectively. Accounting for 0.24 percent of all non-redundant Unigenes, a total of 54 Unigenes were enriched in fatty acid biosynthesis pathway. Fourteen homologous proteins

related to fatty acid synthesis were identified through aligning 54 Unigene sequences against KEGG database (Table 1). Some of the differentially expressed genes obtained from pairwise comparisons also can be enriched in fatty acid biosynthesis pathway after alignment against KEGG database (Fig. 5). The red rectangles in Fig. 5 indicate genes differentially expressed among pairwise comparisons of transcriptome. Functions and genes' expression levels of some critical enzymes in fatty acid biosynthesis will be especially described in the following paragraphs.

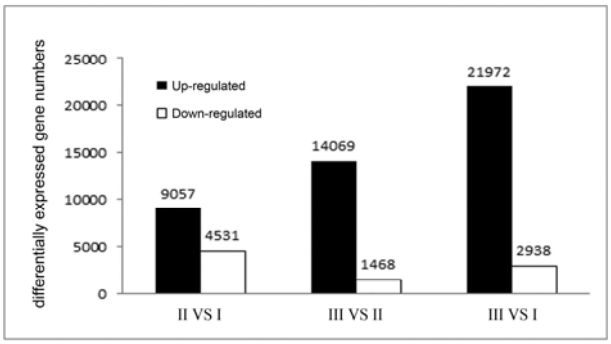


Fig. 4 The numbers of differentially expressed genes at three different stages during the development of tung tree seeds

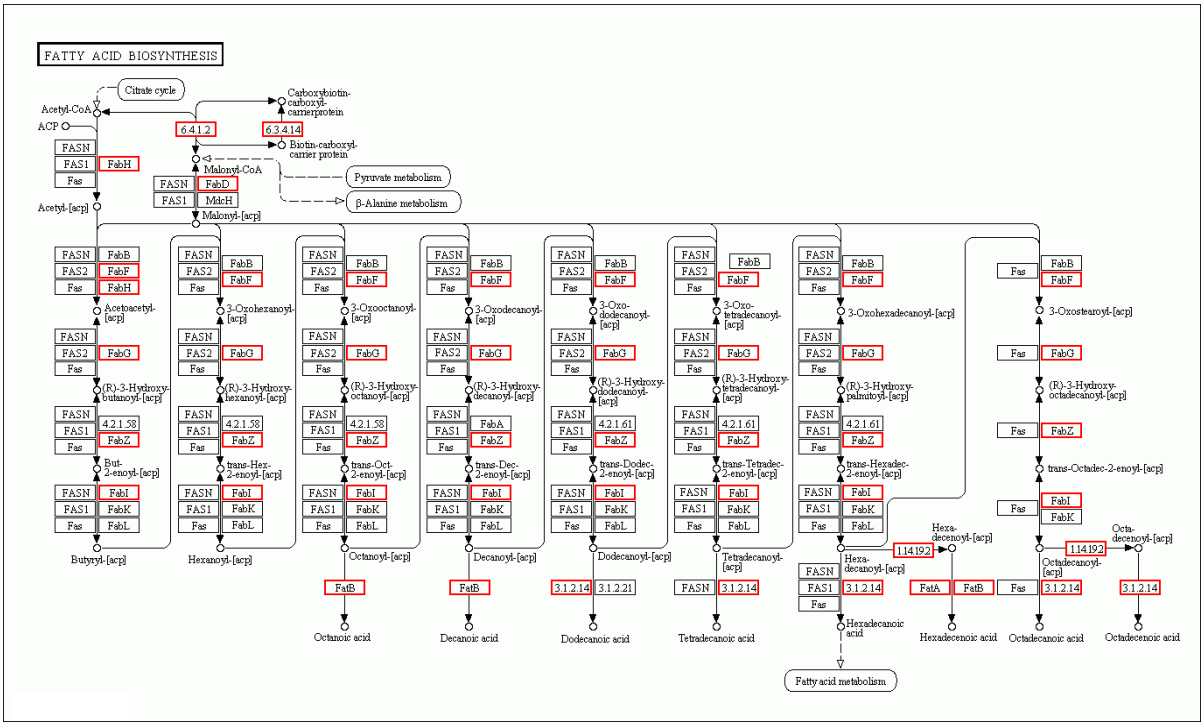


Fig. 5 Diagram of fatty acid biosynthesis pathway

Acetyl-CoA carboxylase

Acetyl-CoA carboxylase (ACCase) catalyzes the original reaction through which acetyl-CoA is transformed into malonyl-CoA [20]. There are two types of ACCase, homomeric and heteromeric ACCase, existed in higher plants. Carboxylation reaction is catalyzed by heteromeric ACCase existed in higher plants' plastid [20]. Heteromeric ACCase consists of four subunits which are BCCP (biotin carboxyl carrier protein), BC (biotin carboxylase), α-CT (carboxyl transferase) and β-CT. *accB* also known as *bccP* (encoding BCCP) was consistently up-regulated whereas *ACAC* (encoding BC) and *accD* (encoding β-CT) were

down-regulated throughout the three tung tree seed development stages. The expression level of *accA* (encoding α-CT) first rose until II stage then gradually declined, moreover, its expression level at III stage was lower than at I stage (Table 1).

Table 1. Differentially expressed genes in fatty acid biosynthesis obtained from pairwise comparisons of tung tree seeds' transcriptome at different developmental stages

Comparative group	KEGG orthology	Gene name	Protein definition	Expression profile
II vs I	K00059	*fabG*	3-oxoacyl-ACP reductase	Up-regulated
	K10782	*FATA*	fatty acyl-ACP thioesterase A	Up-regulated
	K11262	*ACAC*	acetyl-CoA carboxylase / biotin carboxylase	Down-regulated
	K01963	*accD*	acetyl-CoA carboxylase carboxyl transferase subunit beta	Down-regulated
	K00208	*fabI*	enoyl-ACP reductase I	Up-regulated
	K01961	*accC*	acetyl-CoA carboxylase, biotin carboxylase subunit	Up-regulated
	K01962	*accA*	acetyl-CoA carboxylase carboxyl transferase subunit alpha	Up-regulated
	K01716	*fabA*	β-hydroxyacyl-ACP dehydratase	Up-regulated
	K00645	*fabD*	ACP S-malonyltransferase	Up-regulated
	K09458	*fabF*	β-Ketoacyl-ACP synthase II	Up-regulated
	K02160	*accB, bccP*	acetyl-CoA carboxylase biotin carboxyl carrier protein	Up-regulated
	K02372	*fabZ*	β-hydroxyacyl-ACP dehydratases	Up-regulated
	K00648	*fabH*	β-Ketoacyl-ACP synthase III	Up-regulated
III vs II	K00059	*fabG*	3-oxoacyl-ACP reductase	Down-regulated
	K11262	*ACAC*	acetyl-CoA carboxylase / biotin carboxylase	Down-regulated
	K02160	*accB, bccP*	acetyl-CoA carboxylase biotin carboxyl carrier protein	Up-regulated
	K01962	*accA*	acetyl-CoA carboxylase carboxyl transferase subunit alpha	Down-regulated
	K01716	*fabA*	β-hydroxyacyl-ACP dehydratase	Up-regulated
	K00648	*fabH*	β-Ketoacyl-ACP synthase III	Down-regulated
	K01963	*accD*	acetyl-CoA carboxylase carboxyl transferase subunit beta	Down-regulated
	K02372	*fabZ*	β-hydroxyacyl-ACP dehydratases	Down-regulated
III vs I	K00059	*fabG*	3-oxoacyl-ACP reductase	Up-regulated
	K10782	*FATA*	fatty acyl-ACP thioesterase A	Up-regulated
	K11262	*ACAC*	acetyl-CoA carboxylase / biotin carboxylase	Down-regulated
	K02160	*accB, bccP*	acetyl-CoA carboxylase biotin carboxyl carrier protein	Up-regulated
	K01963	*accD*	acetyl-CoA carboxylase carboxyl transferase subunit beta	Down-regulated
	K00208	*fabI*	enoyl-ACP reductase I	Up-regulated
	K01961	*accC*	acetyl-CoA carboxylase, biotin carboxylase subunit	Up-regulated
	K01962	*accA*	acetyl-CoA carboxylase carboxyl transferase subunit alpha	Down-regulated
	K01716	*fabA*	β-hydroxyacyl-ACP dehydratase	Up-regulated
	K00645	*fabD*	ACP S-malonyltransferase	Up-regulated
	K10781	*FATB*	fatty acyl-ACP thioesterase B	Down-regulated
	K09458	*fabF*	β-Ketoacyl-ACP synthase II	Up-regulated

ACP-malonyltransferase

ACP-malonyltransferase catalyzes the reaction that malonyl-CoA transfer its malony to acyl-carrier-protein (ACP) in order to form malonyl-ACP as the substrate of consequent condensation reaction [21]. *fabD* (encoding ACP-malonyltransferase) was up-regulated until II stage then remained unchanged to III stage during tung tree seed development (Table 1).

β-Ketoacyl-ACP synthase

Type II fatty acid synthesis pathway (FAS II) in plants is one of the two known fatty acid metabolic pathways in organism [22]. Three kinds of β-Ketoacyl-ACP synthase (KAS) (KAS I, II and III) in plants corresponded to their bacterial counterpart (*fabB*, *fabF* and *fabH*) function in FAS II. KAS III catalyzes the condensation reaction between acetyl-ACP and malonyl-ACP whereas KAS I and KAS II catalyze the condensation reaction that leads to the extension of acyl chain [23]. During seed development, *KAS II* was strongly induced until II stage then remained unchanged to III stage. *KAS III* first exhibited up-regulated expression profile until II stage then began decreasing the expression level and restored it to the level at I stage (Table 1). *KAS I* showed no detectable expression changes throughout three stages.

β-Ketoacyl-ACP reductase
β-Ketoacyl-ACP reductase can transform β-Ketoacyl-ACP to produce β-hydroxyacyl-ACP through reducing action in the presence of NADPH [24]. The expression level of *fabG* (encoding β-Ketoacyl-ACP reductase) was first strongly induced until II stage then gradually decreased, nevertheless, it still higher than the level at I stage (Table 1).

β-hydroxyacyl-ACP dehydratase
β-hydroxyacyl-ACP dehydratase catalyzes β-hydroxyacyl-ACP to dehydrate one H_2O molecule to generate enoyl-ACP [25]. The expression level of *fabA* (encoding β-hydroxyacyl-ACP dehydratase) was consistently up-regulated throughout three seed development stages (Table 1).

Enoyl-ACP reductase
Enoyl-ACP reductase transforms enoyl-ACP into acyl-ACP in the presence of NADPH, which is the last step of first cycle in fatty acid biosynthesis [26]. The expression level of *fabI* (encoding enoyl-ACP reductase) was up-regulated until II stage then remained unchanged to III stage (Table 1).

Fatty acyl-ACP thioesterase
Fatty acyl-ACP thioesterase catalyzes dissociation of acyl chain from ACP while the acyl chain extends to specific length after a series of cyclic reactions [27, 28]. The expression level of *FATA* (encoding Fatty acyl-ACP thioesterase) was up-regulated until II stage then remained unchanged to III stage (Table 1).

Discussion

ACCase which catalyzes transformation of acetyl-CoA into malonyl-CoA is the rate-limiting enzyme of *de novo* fatty acid synthesis [29]. ACCase consists of four subunits (BCCP, BC, α-CT and β-CT) which are encoded by *accB*, *ACAC*, *accA* and *accD*, respectively. The expression profiles of these four genes were not exactly the same. These results imply that the content of four subunits of ACCase protein *in vivo* is different. ACP has no enzyme activity and functions as a carrier in fatty acid biosynthesis. *acpP* encoding ACP had no detectable changes of expression level during seed development. This phenomenon might be the result of the massive ACP accumulation caused by negative feedback regulation of fatty acid biosynthesis [30]. The expression level of all critical enzyme genes exhibited up-regulated profiles between I and II stage of seed development. However, up-regulated, down-regulated and unchanged expression levels of these genes were observed between II and III stages but all of these expression levels at III stage were not lower than their counterpart at I stage. These results indicate that there is a positive correlation between massive accumulation of oil and expression level of critical genes in fatty acid biosynthesis. There are few researches on the expression profiles of genes in fatty acid biosynthesis so lots of hard works in future will be done to elucidate the molecular mechanism of fatty acid biosynthesis.

Conclusion

This research analyzed the transcriptome of tung tree's seeds in different development stages through RNA-Seq technology. Genes differentially expressed among three different development stages were classified into and enriched in related GO categories and metabolic pathways, respectively. Moreover, the results supplied preliminary elucidation of the functions and expression profiles of critical genes in fatty acid biosynthesis. This research provides necessary database which will be used to elucidate the molecular mechanism of fatty acid biosynthesis of plants in future. Furthermore, tung tree will probably increase the

production of tung oil if plant breeders apply the achievements in this research to tung tree's breeding.

Acknowledgements

This work was supported by the key projects of state forestry research and public service industry (201204403).

References

1. Tan X. F. (2006). Status and Suggestion on Development of *Vernicia fordii*, Nonwood Forest Research, 24(3), 62-64, (in Chinese with English abstract).
2. Shockey J. M., S. K. Gidda, D. C. Chapital, J. C. Kuan, P. K. Dhanoa, J. M. Bland, S. J. Rothstein, R. T. Mullen, J. M. Dyer (2006). Tung Tree DGAT1 and DGAT2 Have Nonredundant Functions in Triacylglycerol Biosynthesis and Are Localized to Different Subdomains of the Endoplasmic Reticulum, The Plant Cell, 2006, 18(9), 2294-2313.
3. Wang D. Y., Y. Li, P. Li (2007). Cloning and Sequence Analysis of *FADX* Gene from *Vernicia fordii*, Journal of Zhejiang Forestry Science and Technology, 27(2), 1-5, (in Chinese with English abstract).
4. Li J. A., Y. Sun, H. Chen, L. Liu, W. Guo (2008). Cloning and Analysis of LEAFY Homologous Gene Fragment from Tung Oil Tree, Journal of Central South University of Forestry and Technology, 28(4), 21-26, (in Chinese with English abstract).
5. Li Y., Y. Wang, P. Li, J. Wei (2008). Study on the Cloning and Sequence Analysis of FADX Gene from *Vernicia fordii*, Journal of Anhui Agriculture Science, 36(11), 4753-4755, (in Chinese with English abstract).
6. Long H., X. F. Tan, L. Zhang, L. Xie, H. Chen (2011). Cloning and Sequence Analysis of Full-length cDNA Encoding Cyclophilin in *Vernicia fordii*, Journal of Central South University of Forestry and Technology, 31(12), 111-117, (in Chinese with English abstract).
7. Alagna F., N. D'Agostino, L. Torchia, M. Servili, R. Rao, M. Pietrella, G. Giuliano, M. L. Chiusano, L. Baldoni, G. Perrotta (2009). Comparative 454 Pyrosequencing of Transcripts from Two Olive Genotypes during Fruit Development, BMC Genomics, 10, 399.
8. Zenoni S., A. Ferrarini, E. Giacomelli, L. Xumerle, M. Fasoli, G. Malerba, D. Bellin, M. Pezzotti, M. Delledonne (2010). Characterization of Transcriptional Complexity during Berry Development in *Vitis vinifera* using RNA-Seq, Plant Physiology, 152(4), 1787-1795.
9. González-Ballester D., D. Casero, S. Cokus, M. Pellegrini, S. S. Merchant, A. R. Grossman (2010). RNA-seq Analysis of Sulfur-deprived Chlamydomonas Cells Reveals Aspects of Acclimation Critical for Cell Survival, The Plant Cell, 22(6), 2058-2084.
10. Bennetzen J. L., J. Schmutz, H. Wang, R. Percifield, J. Hawkins, A. C. Pontaroli, M. Estep, L. Feng, J. N. Vaughn, J. Grimwood, J. Jenkins, K. Barry, E. Lindquist, U. Hellsten, S. Deshpande, X. Wang, X. Wu, T. Mitros, J. Triplett, X. Yang, C. Y. Ye, M. Mauro-Herrera, L. Wang, P. Li, M. Sharma, R. Sharma, P. C. Ronald, O. Panaud, E. A. Kellogg, T. P. Brutnell, A. N. Doust, G. A. Tuskan, D. Rokhsar, K. M. Devos (2012). Reference Genome Sequence of the Model Plant Setaria, Nature Biotechnology, 30(6), 555-561.
11. O'Loughlin A., D. J. Lynn, M. McGee, S. Doyle, M. McCabe, B. Earley (2012). Transcriptomic Analysis of the Stress Response to Weaning at Housing in Bovine Leukocytes Using RNA-seq Technology, BMC Genomics, 13(1), 250.
12. Shao W., Q. Y. Zhao, X. Y. Wang, X. Y. Xu, Q. Tang, M. Li, X. Li, Y. Z. Xu (2012).

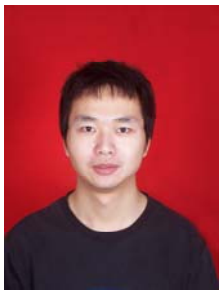
Alternative Splicing and Trans-splicing Events Revealed by Analysis of the *Bombyx mori* Transcriptome, RNA, 18(7), 1395-1407.
13. Yang D., Q. Liu, M. Yang, H. Wu, Q. Wang, J. Xiao, Y. Zhang (2012). RNA-seq Liver Transcriptome Analysis Reveals an Activated MHC-I Pathway and an Inhibited MHC-II Pathway at the Early Stage of Vaccine Immunization in Zebrafish, BMC Genomics, 13(1), 319.
14. Torales S. L., M. Rivarola, M. F. Pomponio, P. Fernández, C. V. Acuña, P. Marchelli, S. Gonzalez, M. M. Azpilicueta, H. E. Hopp, L. A. Gallo, N. B. Paniego, S. N. Marcucci Poltri (2012). Transcriptome Survey of Patagonian Southern Beech *Nothofagus nervosa* (= *N. alpina*): Assembly, Annotation and Molecular Marker Discovery, BMC Genomics, 13(1), 291.
15. Marioni J. C., C. E. Mason, S. M. Mane, M. Stephens, Y. Gilad (2008). RNA-seq: An Assessment of Technical Reproducibility and Comparison with Gene Expression Arrays, Genome Research, 18(9), 1509-1517.
16. Fu W., X. Gu, G. Tao, J. Tang, Z. Jiang (2008). Analysis of Fatty Acids and the Structure Identification of Triacylglycerols in Tung Oil, Natural Product Research and Development, 20, 964-968, (in Chinese with English abstract).
17. Grabherr M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, A. Regev (2011). Full-length Transcriptome Assembly from RNA-seq Data without a Reference Genome, Nature Biotechnology, 29(7), 644-652.
18. Mortazavi A., B. A. Williams, K. McCue, L. Schaeffer, B. Wold (2008). Mapping and Quantifying Mammalian Transcriptomes by RNA-seq, Nature Methods, 5(7), 621-628.
19. Conesa A., S. Götz, J. M. García-Gómez, J. Terol, M. Talón, M. Robles (2005). Blast2GO: A Universal Tool for Annotation, Visualization and Analysis in Functional Genomics Research, Bioinformatics, 21(18), 3674-3676.
20. Konishi T., K. Shinohara, K. Yamada, Y. Sasaki (1996). Acetyl-CoA Carboxylase in Higher Plants: Most Plants Other Than Gramineae Have Both the Prokaryotic and the Eukaryotic Forms of This Enzyme, Plant and Cell Physiology, 37(2), 117-122.
21. Ruch F. E., P. R. Vagelos (1973). The Isolation and General Properties of *Escherichia coli* Malonyl Coenzyme A-Acyl Carrier Protein Transacylase, Journal of Biological Chemistry, 248(23), 8086-8094.
22. Rock C. O., J. E. Cronan (1996). *Escherichia coli* as a Model for the Regulation of Dissociable (Type II) Fatty Acid Biosynthesis, Biochimica et Biophysica Acta, 1302(1), 1-16.
23. White S. W., J. Zheng, Y. M. Zhang, C. O. Rock (2005). The Structural Biology of Type II Fatty Acid Biosynthesis, Annual Review of Biochemistry, 74, 791-831.
24. Fisher M., J. T. Kroon, W. Martindale, A. R. Stuitje, A. R. Slabas, J. B. Rafferty (2000). The X-ray Structure of *Brassica napus* Beta-keto Acyl Carrier Protein Reductase and Its Implications for Substrate Binding and Catalysis, Structure, 8(4), 339-347.
25. Heath R. J., C. O. Rock (1996). Roles of the *fabA* and *fabZ* Beta-hydroxyacyl-acyl Carrier Protein Dehydratases in *Escherichia coli* Fatty Acid Biosynthesis, Journal of Biological Chemistry, 271(44), 27795-27801.
26. Heath R. J., C. O. Rock (1995). Enoyl-acyl Carrier Protein Reductase (fabI) Plays a Determinant Role in Completing Cycles of Fatty Acid Elongation in *Escherichia coli*, Journal of Biological Chemistry, 270(44), 26538-26542.
27. Voelker T. (1996). Plant Acyl-ACP Thioesterases: Chain-length Determining Enzymes in Plant Fatty Acid Biosynthesis, Genetic Engineering, 18, 111-133.
28. Yuan L., T. A. Voelker, D. J. Hawkins (1995). Modification of the Substrate Specificity of

an Acyl-acyl Carrier Protein Thioesterase by Protein Engineering, Proceedings of the National Academy of Sciences of the United States of America, 92(23), 10639-10643.
29. Ohlrogge J. B., J. G. Jaworski (1997). Regulation of Fatty Acid Synthesis, Annual Review of Plant Physiology and Plant Molecular Biology, 48, 109-136.
30. Andre C., R. P. Haslam, J. Shanklin (2012). Feedback Regulation of Plastidic Acetyl-CoA Carboxylase by 18:1-acyl Carrier Protein in *Brassica napus*, Proceedings of the National Academy of Sciences of the United States of America, 109(25), 10107-10112.

Guixiong Jiang, Ph.D. Student
E-mail: jgxpaul@163.com

Guixiong Jiang (1966-, senior engineer) is a Ph.D. candidate in Central South University of Forestry and Technology of China. He has worked as a senior engineer in Forestry Department of Guangxi Zhuang autonomous region so he has a lot of practical experience in forest breeding and protection. At present, he is interested in the study of molecular biology and forest cultivation.

Hao Chen, Ph.D.
E-mail: chenhao198223@gmail.com



Hao Chen (1982-, Ph.D.) acquired his Ph.D. degree in Hunan Normal University of China at the year 2012. At present, he is doing post-doctoral research in the key lab of non-wood forest products of state forestry administration belonged to central south university of forestry and technology. Hao Chen has focus on molecular biology for several years. At present, he is mainly engaged in the study of plant molecular biology.

Prof. Xiaofeng Tan, Ph.D.
E-mail: tanxiaofengcn@126.com

Xiaofeng Tan (1956-, Ph.D., professor) acquired his Ph.D. degree in Central South University of Forestry and Technology. Now he is the PI of the key lab of non-wood forest products of state forestry administration. He has been engaged in molecular biology research and breeding of forest for a long time since he graduated from college. At present, the main directions of his scientific researches are focused on molecular biology, forest cultivation and plant protection.