

Transcriptome profiling of *Antrodia cinnamomea* fruiting bodies grown on *Cinnamomum kanehirae* and *C. camphora* wood substrates

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Abstract

Antrodia cinnamomea is a rare medicinal fungus endemic to Taiwan, China. It has a high medicinal value, being rich in bioactive components such as triterpenes and polysaccharides. However, only a few studies have been conducted on *A. cinnamomea* fruiting bodies grown on *Cinnamomum camphora* wood culture. In this study, we performed transcriptome sequencing of *A. cinnamomea* fruiting bodies grown on *C. kanehirae* (NZF) and *C. camphora* (XZF) wood substrates. 18391 unigenes were annotated from the obtained reads, including 592 differentially expressed genes (170 up-regulated and 422 down-regulated) mainly enriched in the carbohydrate metabolism, lipid metabolism, and amino acid metabolism pathways. Comparing the transcriptome of NZF and XZF, genes encoding acetyl CoA synthetase and acetyl CoA C-acetyltransferase, involved in the terpenoid backbone biosynthesis, were highly expressed in NZF, while genes encoding glycosyltransferases and cytochrome P450s were highly expressed in XZF. There were no significant differences in the expression of the genes encoding farnesyl diphosphate farnesyltransferase, isopentenylidiphosphate isomerase, 2,3-oxidosqualene cyclase, and terpenoid synthase between NZF and XZF. The transcriptome analysis further supports that *C. camphora* is feasible as an alternative host of *A. cinnamomea*.

Introduction

Antrodia camphorata is a fungus of the Basidiomycota division, the Agaricomycetes class, and the Polyporales order, and it is a valuable medicinal fungus unique to Taiwan¹. In Taiwan, it is often used by the local population as folk medicine to treat food poisoning, diarrhea, and high blood pressure and to enhance liver function². Several studies have demonstrated the antioxidant, anticancer, anti-inflammatory, hepatoprotective, and immunomodulatory effects of *A. cinnamomea*^{3,4}. It produces various bioactive metabolites, including triterpenes, polysaccharides, benzenes, polyphenols, maleic acid, and succinic acid derivatives^{5,6}. Among these, triterpenes are considered to be the most biologically active components due to their potent medicinal and therapeutic properties⁷.

Triterpenoids are a kind of secondary metabolites with important physiological activities, which exist in large quantities in medicinal fungi⁸. The triterpene biosynthetic pathway in *A. cinnamomea*, includes the mevalonic pathway (MVA), which uses acetyl coenzyme A to synthesize mevalonic acid through a series of reactions for the production of various terpenoids⁹. In the MVA triterpenoid synthesis pathway of *Ganoderma lucidum*, several key genes and enzymes have been identified, such as Acetyl-CoA acetyltransferase (AACT), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), Mevalonate pyrophosphate decarboxylase (MVD), farnesyl diphosphate synthase (FPS), squalene synthase (SQS) and lanosterol synthase (LS)^{10–15}. Using genomic and transcriptomic analyses, Lu et al. (2014) found that the *A. cinnamomea* triterpenoid biosynthetic pathway is similar to that of *Ganoderma lucidum*¹⁶. In the *A. cinnamomea* triterpene synthesis pathway, Geranyl diphosphate synthase (GPP) and Farnesyl diphosphate synthase (FPP) are important enzymes for terpenoid biosynthesis. Squalene synthase (SE)

is the last key rate-limiting enzyme of the *A. cinnamomea* triterpene pathway, catalyzing the 2,3-oxido squalene biosynthesis from squalene, leading to the synthesis of lanosterol and other compounds¹⁷.

The potential medicinal properties of triterpenes in the *A. cinnamomea* fruit bodies have attracted strong research interest in identifying their pharmacological mechanisms. For example, ergosterane-type triterpenoids, a specific camphor component, play an important role in cancer treatment. Antcin K, Antcin H, and methyl antcinates A, B, and G have significant cytotoxic activity against colon, liver, and breast cancer cells¹⁸. The antiproliferative activity and molecular mechanisms of antcin K were studied in the fruit bodies of basswood-cultivated *A. cinnamomea*. Antcin K was shown to induce apoptosis mediated by mitochondrial and endoplasmic reticulum stress in human liver cancer cells and could effectively reduce the Hep 3B cells activity within 48 hours, indicating that antcin K may be medically exploited for liver cancer treatment¹⁹.

At present, the slow *A. cinnamomea* fruiting bodies' growth rate and the difficulty of artificial cultivation make them very expensive to grow²⁰. Under artificial cultivation, wood substrate cultivation results in the highest triterpene content, followed by a solid-substrate culture and, finally, liquid fermentation, which results in almost no triterpene production¹⁹. Therefore, it is important to establish artificial cultivation protocols for *A. cinnamomea*. In previous research, *A. cinnamomea* fruiting bodies have been successfully developed and grown on different wood substrates, including wood from two broad-leaf trees, *Cinnamomum kanehirai* (the original host) and *C. camphora*, as well as the conifer, *Cunninghamia konishi*²¹. In this study, we obtained the transcriptome of *A. cinnamomea* fruiting bodies cultivated in *C. kanehirae* and *C. camphora* wood substrates using next-generation sequencing. We assessed differences in their development and expression and identified candidate genes involved in terpenoid biosynthesis-related pathways, further confirming the feasibility of *C. camphora* as an alternative host of *A. cinnamomea*.

Materials And Methods

***A. cinnamomea* growth conditions.** The *A. cinnamomea* fruiting bodies were cultivated under sterile conditions in the laboratory of Green Development of Southwest Forestry University. The original strain was isolated from *A. cinnamomea* (BSNZZ) in Baoshan, provided by the key laboratory of Yunnan Forestry and Grassland Science Research Institute. *C. kanehirae* and *C. camphora* wood substrates were obtained from forestry center of Southwest Forestry University. The plant collection and use were in accordance with all the relevant guidelines. The first stage was the *A. cinnamomea* liquid culture under the following conditions: *A. cinnamomea* mycelia were inoculated into 500 mL volumetric triangular flasks containing 300 mL of growth medium and were incubated in a shaker for 14 d (25°C, 150 rpm). A PDA liquid growth medium was used (200 g potato boiled and filtered, 1 g potassium dihydrogen phosphate, 0.5 g magnesium sulfate, 20 g glucose, 5 g yeast powder, 0.1 g VB₁). The second stage included the treatment of wood substrates: *C. kanehirae* and *C. camphora* wood substrates were soaked in tap water for 7 d, changing the water 2–3 times during this period. After the 7-day period, the logs were

sterilized at high temperatures (121 °C, 120 min). The third stage included the wood substrates inoculation: The mycelia obtained in the first stage were inoculated into the bags containing *C. kanehirae* and *C. camphora* wood substrates at 28 °C. After 15 days of growth, they were transferred to a sterilized glass tank to be sealed, and the growth of *A. cinnamomea* fruiting bodies was observed and recorded. *A. cinnamomea* fruiting bodies grown *C. kanehirae* and *C. camphora* segmental wood cultures (Fig. 1), were collected in three biological replicates from each sample, labeled (NZF and XZF), rapidly frozen in liquid nitrogen and then sent to Personalbio (Shanghai, China) for transcriptome sequencing.

Total RNA extraction and transcriptome sequencing. Total RNA from the NZF and XZF samples was extracted using the Trizal reagent. RNA degradation and contamination were monitored by agarose gel electrophoresis, and RNA integrity was assessed using an Agilent 2000 Bioanalyzer (Agilent Technologies, CA, USA). The mRNA with a polyA structure was enriched by Oligo (dT) magnetic beads and was fragmented to 300 bp fragments by ion interruption. Using RNA as a template, first-strand cDNA synthesis was performed with a 6-base random primer and reverse transcriptase. Second-strand cDNA synthesis was performed with the first strand of cDNA as a template. Once the library was constructed, PCR amplification was carried out to enrich the library fragments, selecting cDNAs of 450 bp in size. Finally, the obtained libraries were paired-end (PE) sequenced using Next Generation Sequencing (NGS), based on the Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) sequencing platform.

De novo Transcriptome Assembly and Annotation. The fastp software was used to filter the original sequence data, and remove the reads with a length less than 50 bp, and an average sequence quality below Q20. The high-quality sequences were used to obtain the transcript sequences and to cluster the transcripts from a single gene. The longest transcript was selected as the unigene, and its gene function was annotated. The databases used for gene function annotation include NR (NCBI non-redundant protein sequences), GO (Gene Ontology, KEGG (Kyoto Encyclopedia of Genes and Genome, eggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups), Swiss-Prot and Pfam.

Differential expression and functional enrichment analyses. Each transcript's expression level was calculated using the fragments per kilobase exon per million mapped reads (FPKM) method²² to identify the DEGs between two samples. Single Gene Abundance was estimated using the RSEM Software²³. The differential analysis of gene expression was conducted by DESeq. The conditions for screening differentially expressed genes were: expression difference multiple $|\log_2\text{FoldChange}| > 1$, significant P-value < 0.05 . In addition, a functional enrichment analysis of GO and KEGG was performed using the topGO and clusterprofiler software. Significant enrichment (defined at P-value < 0.05) was calculated by the hypergeometric distribution method to identify GO terms and KEGG pathways that were significantly enriched for differential genes compared to the whole genomic context to identify the main biological functions performed by the differentially expressed genes.

Real-time quantitative (qRT) PCR analysis. To verify the RNA-Seq data accuracy, 9 DEGs were randomly selected for real-time fluorescent quantitative reverse transcription PCR (qRT-PCR). RNA from samples stored at -80°C was extracted, and primers for qRT-PCR were designed using Primer Premier 5.0

software(Table 1). The Real-Time RT-PCR amplification conditions were as follows: denaturation at 94°C for 2 min; 40 cycles of 94°C for 15 s, 65°C for 15 s, and 72°C for 45 s, with a final step of 72°C for 10 min. All quantitative PCRs were repeated three times. Expression was calculated using the $2^{-\Delta\Delta Ct}$ method²⁴.

Table 1
Primer sequences

primer name	sequence(5'-3')
AC602_c0_g1F	GAGAATCAACTAGGTGTCAA
AC602_c0_g1R	TTCGGCTTGACAGTGACATT
AC49_c4_g1F	CAGCGGAAGATGCTGAATCC
AC49_c4_g1R	GTCGGTAACAAGTCCCTAAG
AC2999_c0_g1F	ATAGCGAGACTCGTTCAGGA
AC2999_c0_g1R	ATACTCGCGCTCAACAGCCA
AC1121_c0_g1F	CACACACGGACTGTCTATAT
AC1121_c0_g1R	TATGAGGTGCATATAGATCC
AC1645_c0_g1F	AGACACACTAGCATATCTGG
AC1645_c0_g1R	TTGAGGACGAGGTCCATGTT
AC1303_c1_g1F	GACTACGACCTGTACTGCCA
AC1303_c1_g1R	CATCTGCTCCATCTTGGTGA
AC1738_c1_g1F	TCCACTTATGAGCGCGATTA
AC1738_c1_g1R	GTAAATTCGCCGAGGATGTT
AC2021_c0_g1F	GTCTCGAGAACTGAATACA
AC2021_c0_g1R	CCGAACAATGGCATCGACAG
AC2124_c0_g1F	TTGGATGACAAGCACAAGAT
AC2124_c0_g1R	AAGCACACCGCCTACAGTAC

Results

De novo transcriptomic sequencing and reads assembly. In order to compare the gene expression differences between the NZF and XZF cultured fruit bodies, we constructed a cDNA library, and we sequenced and assembled the transcriptome. As shown in Table 2, the total number of reads generated

by s NZF and XZF were 49,596,004 and 54,986,028, respectively. By removing the reads with lengths less than 50 bp and average sequence quality less than Q20, the final clean read numbers were 46,582,176 and 51,766,838, respectively. The Q20 and Q30 values of each sample were higher than 97% and 93%, respectively, indicative of high-quality sequencing data that could be analyzed in the next step.

Table 2

Statistical results Sample: sample name; Reads No: total number of Reads; Clean Reads No: high-quality serial reads; N (%): percentage of fuzzy bases; Q20(%): the percentage of bases whose base recognition accuracy was more than 99%; Q30(%): the percentage of bases whose base recognition accuracy was above 99.9%; Clean Reads %: the percentage of high-quality sequence reads compared to the total sequenced reads

Sample	Reads No	Clean Reads No	N(%)	Q20(%)	Q30(%)	Clean Reads(%)
NZF1	49,596,004	46,582,176	0.000544	97.62	93.92	93.92
XZF	54,986,028	51,766,838	0.00058	97.77	94.27	94.14

Function annotation classification. All single genes were compared to sequences from the NR, GO, KEGG, Pfam, eggNOG, and Swissprot databases using the BLASTx algorithm ($E\text{-value} \leq 1.0 \times 10^{-5}$), and annotations were obtained (Table 3). A total of 18,391 genes were effectively annotated, of which 11,435 were successfully annotated using the NR database, accounting for 62.18% of the unigene number, followed by Swissprot (11,155, 60.65%), eggNOG (10,539, 57.31%), Pfam (8,136, 44.24%), GO (6,947, 37.77%), and KEGG (5,994, 32.59%). The species distribution of the annotations was analyzed (Fig. 2). In the predicted protein homology distribution, most of the proteins (37.88%) had a 60–80% homology with proteins from other fungi in the NR database, and only a few (9.95%) had < 40% homology. According to the NR database, the gene sequences of *A. cinnamomea* had the largest number of matches with *Fibropolia radiosa* (14.54%) and *Laetiporus subphureus* 93 – 53 (13.17%), followed by *Daedalea quercina* L – 15889 (5.53%), *Gelatoporia subpopura* B (4.78%).

Table 3
Summary of annotation results. Database: type of database; Number: the number of unigenes successfully annotated in the respective database; Percentage (%): the proportion of unigenes successfully annotated in the database to the total unigenes; In all database: The number of unigenes annotated in all databases

Database	Number	Percentage (%)
NR	11,435	62.18
GO	6,947	37.77
KEGG	5,994	32.59
Pfam	8,136	44.24
eggNOG	10,539	57.31
Swissprot	11,155	60.65
In all database	3,661	19.91

Differentially expressed genes (DEGs). The differentially expressed genes were assessed using DESeq and volcano mapping with the R language ggplots2 package (Fig. 3 and Table S1). 592 DEGs were identified between NZF and XZF (170 were up-regulated, 422 were down-regulated). Among the up-regulated genes were two genes encoding cytochrome P450s (*AC263_c0_g1*, *AC3065_c0_g1*), one terpenoid synthase (*AC3097_c0_g1*), and one GST (*AC3305_c0_g1*). Among the down-regulated genes, 11 genes were found encoding for cytochrome P450s (*AC49_c4_g1*, *AC218_c0_g1*, *AC245_c2ug1*, *AC363_c0_g1*, *AC588_c0_g1*, *AC1102_c1ug3*, *AC1414_c0_g1*, *AC1641_c0_g3*, *AC1685_c0_g1*, *AC2006_c0_g1*, *AC2915_c0_g1*), 1 TPS (*AC2874_c0_g1*) and 1 acetyl CoA synthase (*AC57_c0_g1*). These findings indicate that several cytochrome P450 genes play an important role in triterpene synthesis.

GO functional enrichment analysis of DEGs. To explore the function of NZF and XZF unigenes, we performed GO enrichment analysis (Fig. 4 and Table S2). According to the international standardized gene function classification system²⁵, a 8,679 unigenes were divided into 3 main functions (biological process, cell component, and molecular function) and 30 functional subgroups. 2,010 DEGs were enriched in the molecular function category (487 were up-regulated and 1,523 were down-regulated), and the dominant subgroups were "structural construction of ribosome" and "structural molecule activity". 4,573 DEGs were enriched in the biological process category, (1,037 were up-regulated, and 3,536 were down-regulated). The dominant subgroups were "amide biological process" and "translation." 2,096 DEGs were enriched in the cellular component category (301 were up-regulated, and 1,795 were down-regulated). Notably, except for one up-regulated gene enriched in the amide biological process, 261 enriched in the six dominant subgroups were down-regulated genes. And their expression in NZF was

much higher than that in XZF. Most were genes encoding for ribosomal proteins, indicating that they play an important role in protein biosynthesis in the *A. cinnamomea* fruit body.

KEGG enrichment analysis of the DEGs. To better understand the biological function of the unigenes, we compared 4,959 unigene sequences with the reference KEGG database pathways. A total of 216 DEGs were assigned to 71 KEGG pathways (Fig. 5 and Table S3). In addition, the unigenes were classified into six pathway categories: Genetic Information Processing, Metabolism, Environmental Information Processing, Cellular Processes, Organismal Systems, and Human Disease. Many unigenes in the *A. cinnamomea* fruiting bodies were enriched in Metabolism, including Carbohydrate metabolism, Lipid metabolism, Amino acid metabolism, Glycan biosynthesis and metabolism, Xenobiotics biodegradation and metabolism, and Metabolism of cofactors and vitamins. In the top 10 pathways with significant enrichment, 28 differentially expressed genes (3 up-regulated and 25 down-regulated) had a much higher expression in XZF, except for one gene encoding for endoglucanase. These results provide important insights for further research into the development and function pathways that are specific to NZF and XZF.

Differentially expressed terpenoid biosynthesis genes between NZF and XZF. The FPKM values were for gene expression quantification, and 44 genes were found to be differentially expressed between NZF and XZF (Fig. 6 and Table S4). Among the terpenoid backbone biosynthesis genes, ACS1, ACS2, and ACS5 were significantly highly expressed in NZF. Among them, ACS1 expression in NZF was two-fold higher compared to XZF. On the other hand, ACS3 and ACS4 expression was higher in XZF; however, the expression difference was not significant. The OSC, FPS, IPP, TPS, MPD, HMGCS, and XI-FIT genes were all highly expressed in XZF; however, the difference in expression was not significant. All 14 genes encoding glycosyltransferases (GT) were highly expressed in XZF; however, GT1, GT2, GT3, GT5, GT6, GT7, GT9, GT11, GT12, and GT13 exhibited non-significant differences in expression between NZF and XZF. The difference in expression of GT4, GT8, GT9, GT10, and GST1 was significant, and the expression of GT10 was 1.5-fold higher in XZF than that in NZF. CYP4501, CYP4502, CYP4503, CYP4504, CYP4507, CYP4512, and CYP4516 encoding cytochrome P450s were highly expressed in XZF, albeit with non-significant differences in expression. CYP61, CYP51, CYP4505, CYP4506, CYP4508 CYP4509, CYP4510, CYP4511, CYP4513, CYP4514, CYP4515 were significantly differentially expressed. CYP61 and CYP51 exhibited a two-fold higher expression in XZF compared to NZF, while CYP4506 and CYP4515 exhibited a five-fold higher expression in NZF compared to XZF. The above data indicate that the culture substrate influences the synthesis of terpene metabolites and suggests that *C. camphora* is viable as an alternative host of *A. cinnamomea*.

Validation of DEGs by qRT-PCR. To further validate the RNA-Seq data accuracy, we randomly selected 9 genes of the triterpene synthesis pathway and performed qRT-PCR to validate their expression in NZF and XZF. qRT-PCR results (Fig. 7) showed confirmed the differential expression patterns of these genes. With NZF as the control, the expression of IPS, CYP51, 2,3-OSC, TPS, and GT1, encoding the terpenoid

backbone biosynthesis genes, were up-regulated in XZF, while genes encoding for CYP450, ACS, SQS, and GT2 were down-regulated, respectively. The above data further indicated that the culture substrate affected terpene metabolites synthesis, with their expression between NZF and XZF being highly variable.

Discussion

Using the Illumina deep sequencing technology, we provide, for the first time, the transcriptome dynamics of *A. cinnamomea* fruiting bodies cultured in different wood substrates (*C. kanehirae* and *C. camphora*). Our data analysis highlights specific genes and processes associated with the molecular mechanisms that differ between NZF and XZF. It further aids in the identification of metabolic pathways involved in the biosynthesis of multiple bioactive secondary metabolites such as triterpenes and polysaccharides.

Triterpenes are secondary metabolites synthesized via the isoprenepathway. It is the most diverse metabolic pathway in all prokaryotes and eukaryotes, leading to the biosynthesis of many primary and secondary metabolites. In fungi, isoprene-like compounds are synthesized via the classical MVA pathway²⁶. For example, shu et al. (2016) revealed, through de novo sequencing and transcriptome analysis, that the triterpenoids of *Wolfiporia cocos* originated only from the mevalonate pathway. Diphosphomevalonate decarboxylase (MVD), farnesyl diphosphate synthase (FDPS), hydroxymethyl glutaryl coenzyme A reductase (HMGR), and lanosterol synthase (ERG7) were up-regulated in the hyphal stage, indicating their potential involvement in triterpenoid biosynthesis in the hyphal stage²⁷. Wang et al. (2021) found that *Sanghuangporus Baumii* triterpenoids were synthesized through the MVA pathway and identified a lanosterol synthase gene²⁸.

In the MVA pathway, the acetyl-CoA C-acetyltransferase (ACCT) is the first enzyme that catalyzes the conversion of acetyl-CoA into acetoacetyl-CoA²⁹. Our results revealed 5 genes encoding acetyl-CoA synthetase and 2 genes encoding ACCT. Among them, 3 genes encoding for acetyl-CoA synthases were highly expressed in NZF, and 2 genes were highly expressed in XZF. Both genes encoding for ACCT were up-regulated in NZF compared to XZF. In a study of *Ganoderma lucidum*, an increase in triterpene production was observed when AACT was overexpressed, suggesting that AACT plays an important role in triterpene biosynthesis³⁰. Squalene synthase (SQS) is a membrane-bound enzyme. It catalyzes the conversion of two farnesyl pyrophosphates to squalene, an important intermediate between isoprene and downstream triterpenes³¹. In *Ganoderma lucidum*, SQS is oxidized to 2,3-oxidosqualene cyclase via oxidosqualene cyclases¹⁰. 2,3-Oxidosqualene cyclase (OSC) is a key enzyme in triterpenoid biosynthesis, converting 2,3-oxidosqualene (OS) into polycyclic triterpenoids. In our study, the genes encoding SQS and OSC were both significantly expressed on NZF and XZF, consistent with the findings of Lin et al. (2015) on AcOSC expression in the wild-type *A. cinnamomea* fruiting body under culture conditions³². In particular, the gene encoding for SQS was more highly expressed in NZF than in XZF. In contrast, the gene encoding for OSC was downregulated in NZF compared to XZF, indicating that the expression patterns of the two enzymes differed between the two substrate species.

Farnesyl diphosphate synthase (FPS) is a key enzyme in terpene biosynthesis, located in the first branching point of the isoprenoid biosynthesis pathway. It catalyzes the chain elongation of dimethyldiphosphate (DMAPP) or geranyl diphosphate (GPP) and isopentenyl pyrophosphate (IPP) to form farnesyl pyrophosphate (FPP)²⁶. Thus, alterations in FPS activity may alter the flux of isoprene-like compounds in the different pathways, thus playing a central role in regulating several important cellular functions¹². In eukaryotes, HMGR is an integral membrane glycoprotein located in the endoplasmic reticulum, catalyzing the NADP-dependent reduction of HMG-CoA to form MVA, and is a commonly regarded key regulatory enzyme of the sterol biosynthetic pathway in higher eukaryotes¹¹. In our study, the FPS and IPP gene expression was higher in XZF than in NZF, and HMGR expression was higher in NZF than in XZF. Notably, the accumulation of triterpenes in *Ganoderma lucidum* was increased by overexpression of the FPS, SQS, HMGR, and OSC genes, shown to play an important role in the control of triterpene biosynthesis in *Ganoderma lucidum*¹⁰⁻¹²

Triterpene biosynthesis also requires cytochrome P450s and glycosyltransferases. Although cytochrome P450s comprise a large gene family, their most common function is to catalyze monooxygenation. P450 monooxygenases decorate basic triterpene skeletons by the introduction of hydroxyl, ketone, aldehyde, carboxyl, or epoxy groups³³. Previous analyses of specific gene expression of basidiomatal formation in *A. cinnamomea* revealed that several cytochrome P450 genes are significantly expressed in natural basidiomes, such as the lanosterol 14- α -demethylase (CYP51). CYP51 is an essential enzyme for sterol biosynthesis and is considered a unique P450 with lanosterol and 24-methylene-dihydro lanosterol as its preferred substrates that is functionally conserved in most organisms,³⁴. In many studies, cytochrome P450 enzymes are considered to be involved in the fruiting body development. For example, one OSC and numerous cytochrome P450 genes were found in the *Ganoderma lucidum* genome. Almost half of the cytochrome P450 genes were up-regulated during the transition from the primordium to the fruiting body stage and were associated with the expression of lanosterol and triterpenoid production³⁵. *Pleurotus ostreatus* encodes for a cytochrome P450 gene, with a high and specific expression during fruit body formation³⁶. Lu et al. (2014) found that the *A. cinnamomea* 14- α - Demethylase was highly expressed in binuclear mycelia and fruiting bodies, indicating its role in matrix formation and was also associated with the high content of ergot estroditerpene in fruiting bodies. Uniquely, the five genes encoding for CYP512 and the three genes encoding CYP5140 were the most highly expressed in the *C. kanehirae* (NZF) and *C. camphora* (XZF) wood substrates and may be involved in ergotane compounds modification¹⁶. CYP61 is known to be a c-22 sterol desaturase that participates in the later ergosterol biosynthesis pathway; it is, therefore, necessary for membrane structural features and is an efficacious target of antifungal compounds. CYP61 was stably expressed in the *A. cinnamomea* AC-9 but strongly expressed in natural fruit bodies³⁷. In our study, the genes encoding for CYP51 and CYP61 were significantly differentially expressed between the substrates. Both were much more highly expressed in XZF than in NZF. Glycosyltransferases (GTs) are another large multigene family in plants. In general, glycosylation is the final step in secondary metabolite biosynthesis, and to date, almost all functionally

characterized glycosyltransferases involved belong to UGTs³⁸. In our study, a total of 13 genes encoding GTs were found to be expressed, with most being highly expressed in XZF compared to NZF.

Conclusions

In this study, transcriptome analysis indicated that the MVA pathway is mainly responsible for triterpene biosynthesis in *A. cinnamomea* fruiting bodies cultivated in *C. kanehirae* (NZF) and *C. camphora* (XZF) wood substrates. In the MVA pathway, most genes encoding for ACS were highly expressed in NZF, while GTs and cytochrome P450s were highly expressed in XZF. Genes encoding for FPP, IPP, 2,3-OSC, and TPS exhibited no significant difference in expression between NZF and XZF. The above data showed that *C. camphora* could be used as an alternative host of *A. cinnamomea*. These results will further help to optimize the production of *A. cinnamomea*.

Declarations

Data availability

The data that support the findings of this study are openly available in NCBI GenBank database at (<https://www.ncbi.nlm.nih.gov>), the associated BioProject, SRA, and Bio-Sample numbers of the raw sequence data are PRJNA923826 and SRX19035503, SAMN32737829 respectively. which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. <https://www.ncbi.nlm.nih.gov/sra/SRX19035503>.

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Author contributions

Yi Wang and Yuan Zheng designed the project, conducted writing review and editing, Yi Zhang and Yi Wang wrote the main manuscript text, Yi Zhang and Xiaolong Yuan analyzed data, Yuming Yang and Juan Wang provide resources and prepared figures 1 for this project. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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Figures

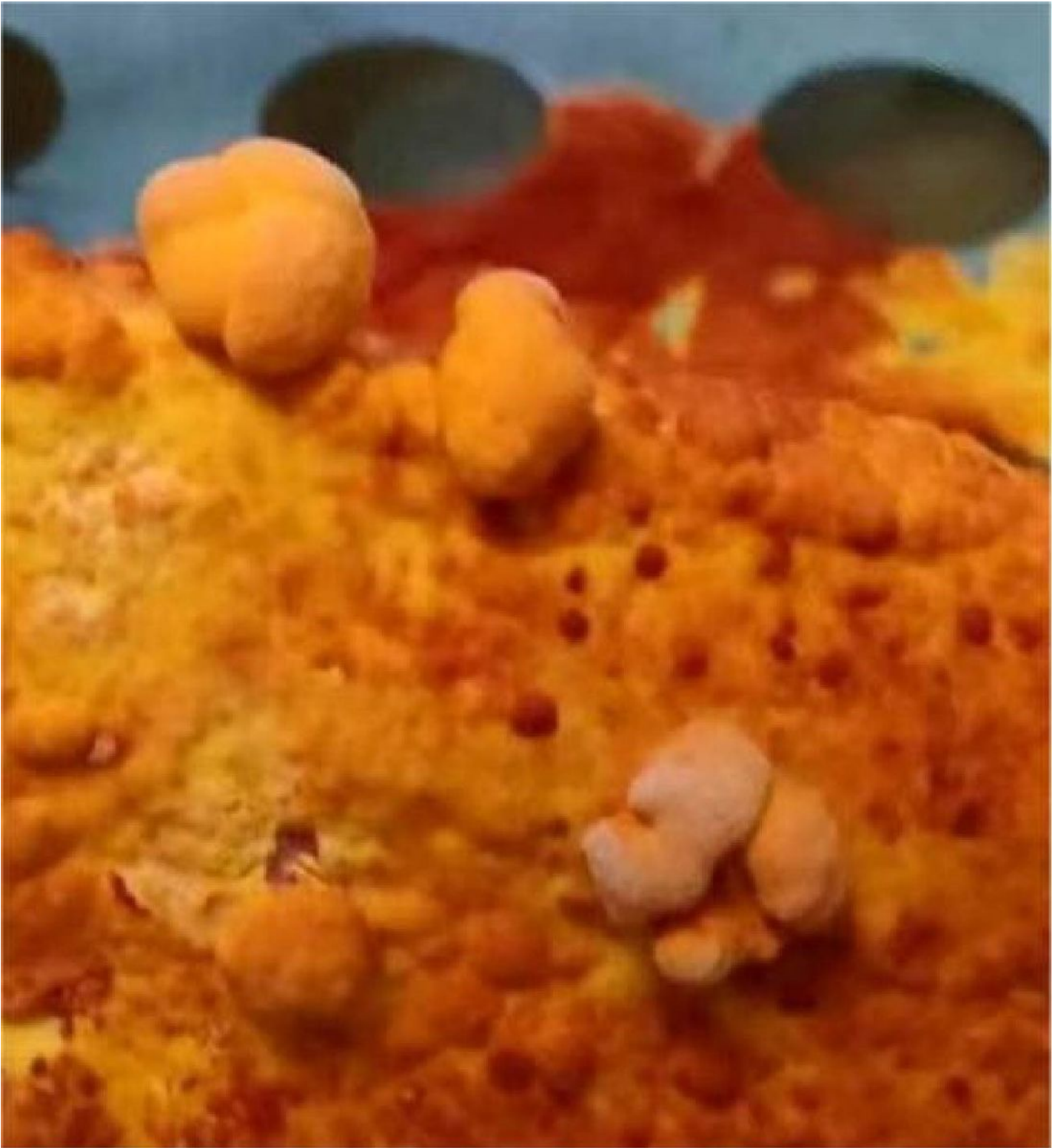


Figure 1

Morphological observations of *A. cinnamomea* fruiting bodies obtained in this study. (a): Fruiting Bodies of *C. kanehirae* segmental wood Cultivated *A. cinnamomea* (NZF). (b): Fruiting Bodies of *C. camphora* segmental wood Cultivated *A. cinnamomea* (XZF)

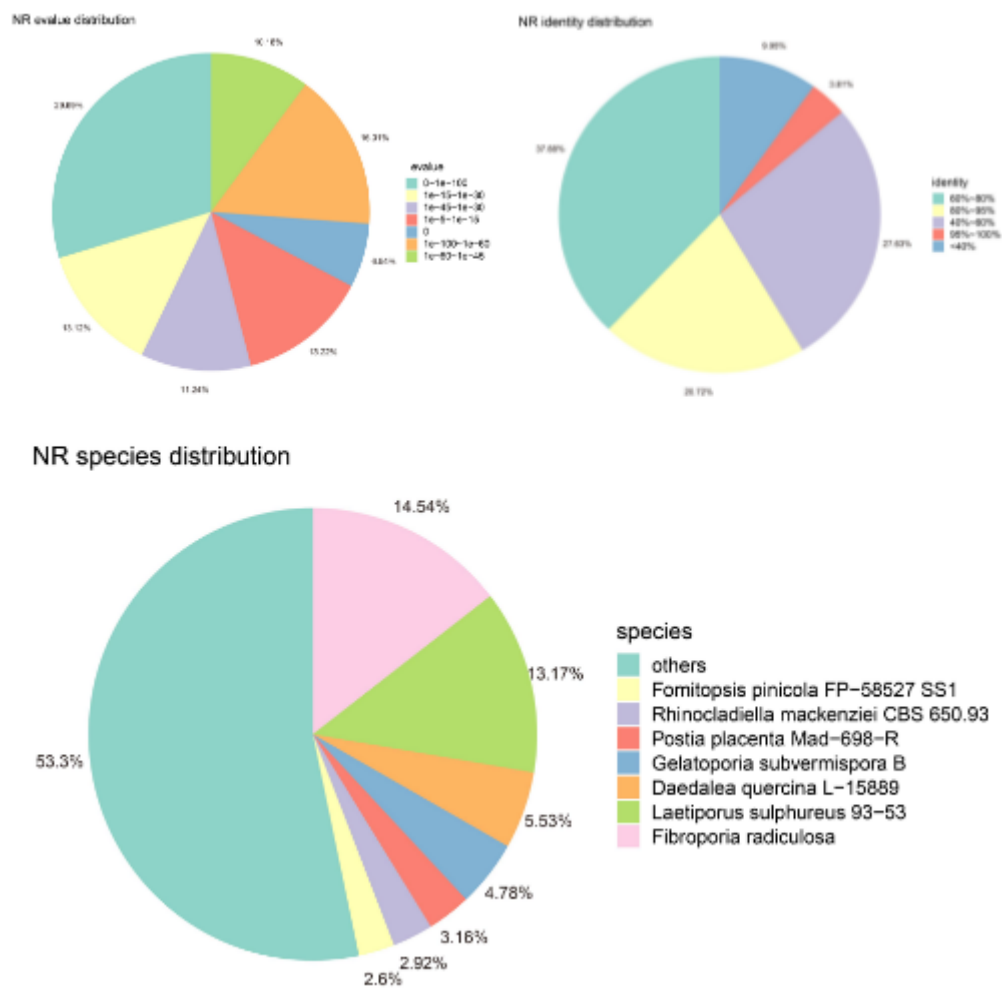


Figure 2

Species distribution results of the expressed sequence tags homology search against the nr database.

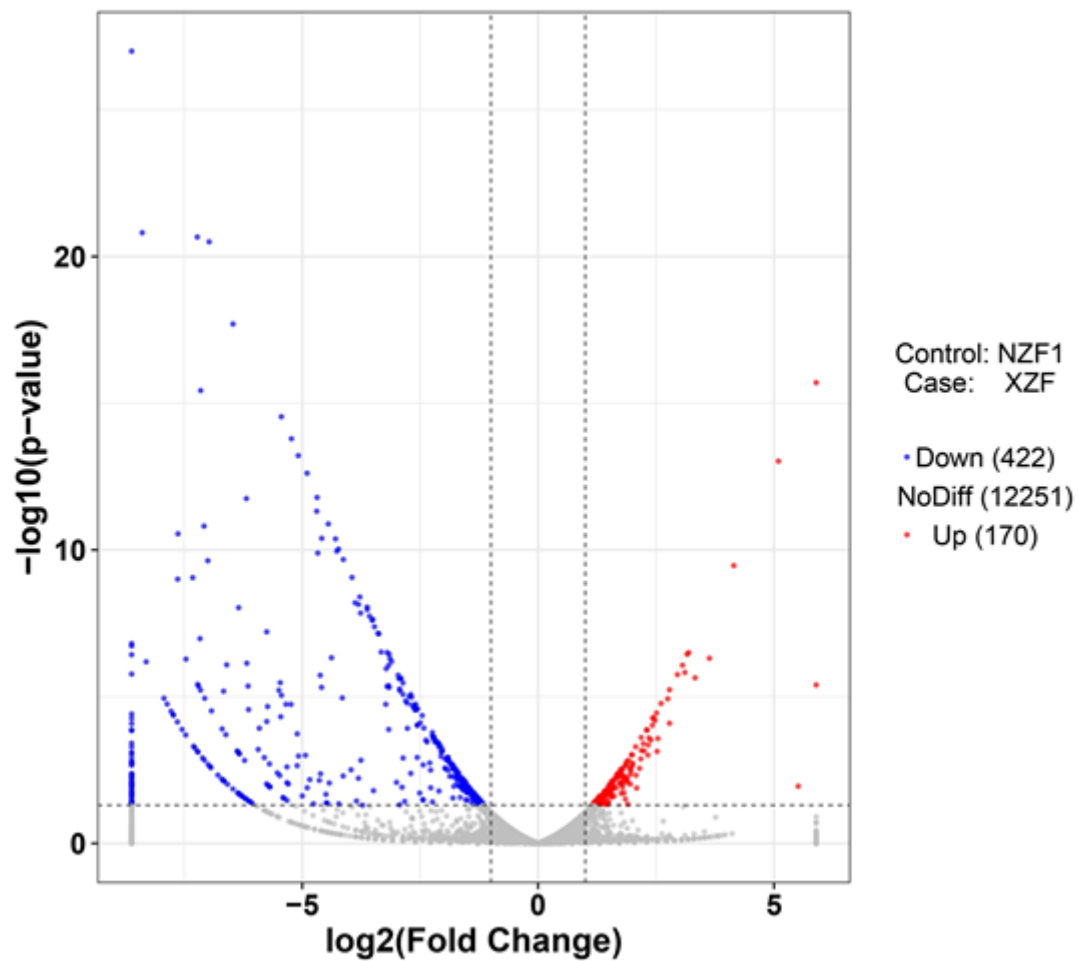


Figure 3

Volcano map: the abscissa corresponds to $\log_2$ Fold Change, and the ordinate to $-\log_{10}(\text{p-value})$. The two vertical dotted lines indicate the two-fold expression difference threshold; The horizontal dotted line indicates the $P\text{-value}=0.05$ threshold. The red dots represent the up-regulated genes, the blue dot represents the down-regulated genes, and the gray dot represents the non-significant differentially expressed gene.

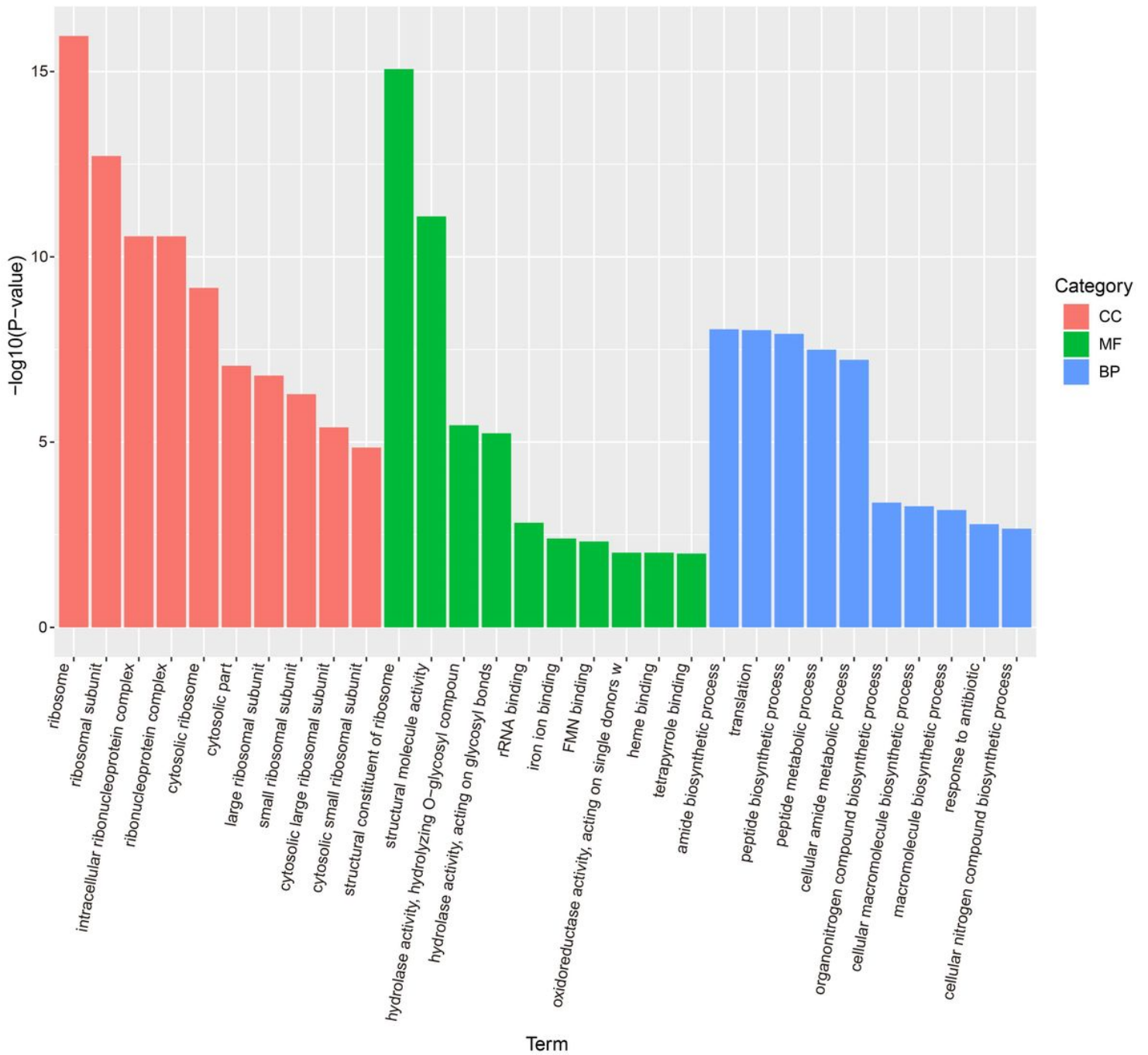


Figure 4

GO enrichment of the DEGs. The x-coordinate represents the GO Terms, and the ordinate is $-\log_{10}$ (p-value) of the enriched GO Terms

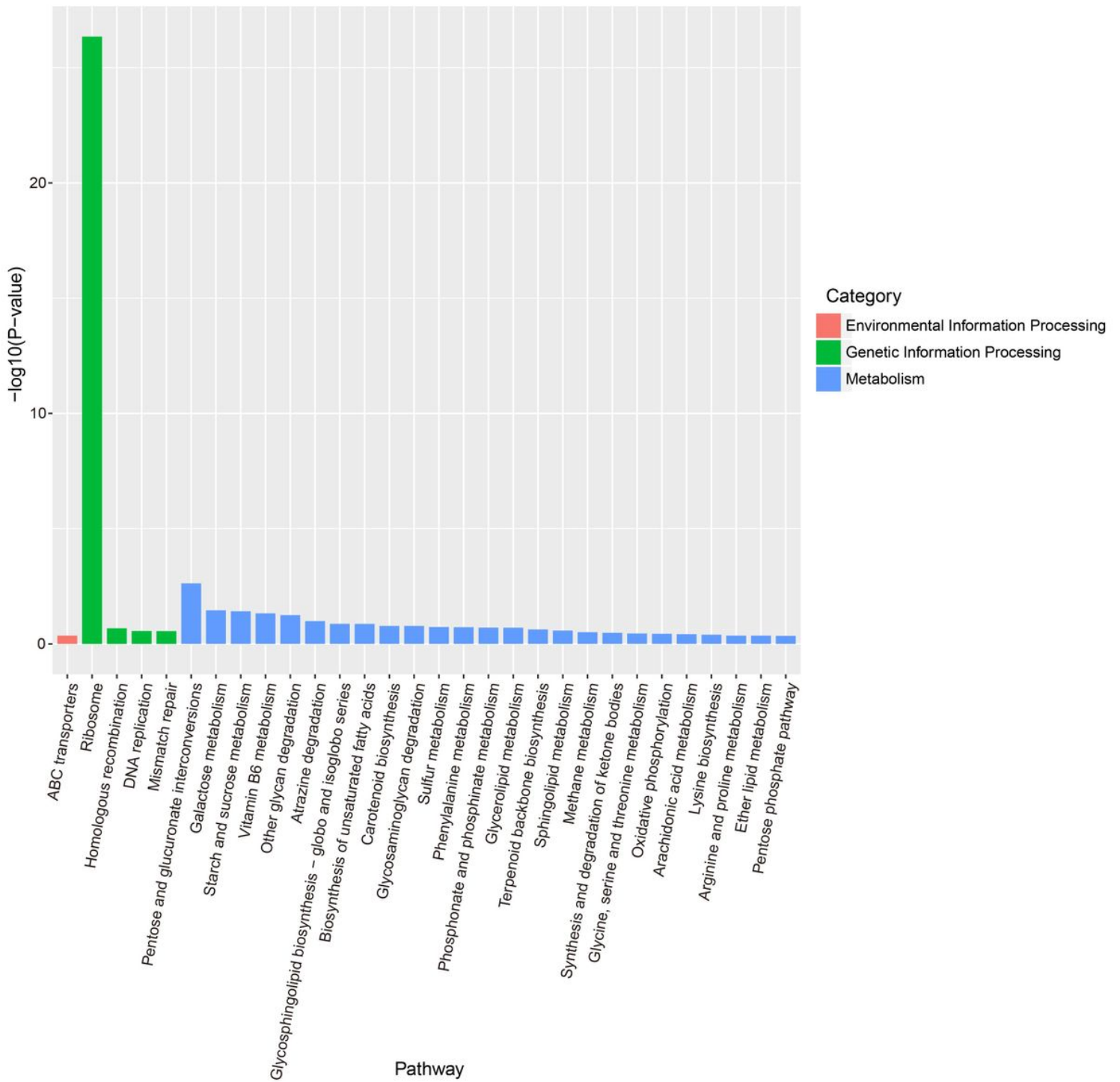


Figure 5

KEGG enrichment of DEGs. The x-coordinate represents the KEGG Pathways, and the ordinate is $-\log_{10}(\text{p-value})$ of the enriched KEGG terms.

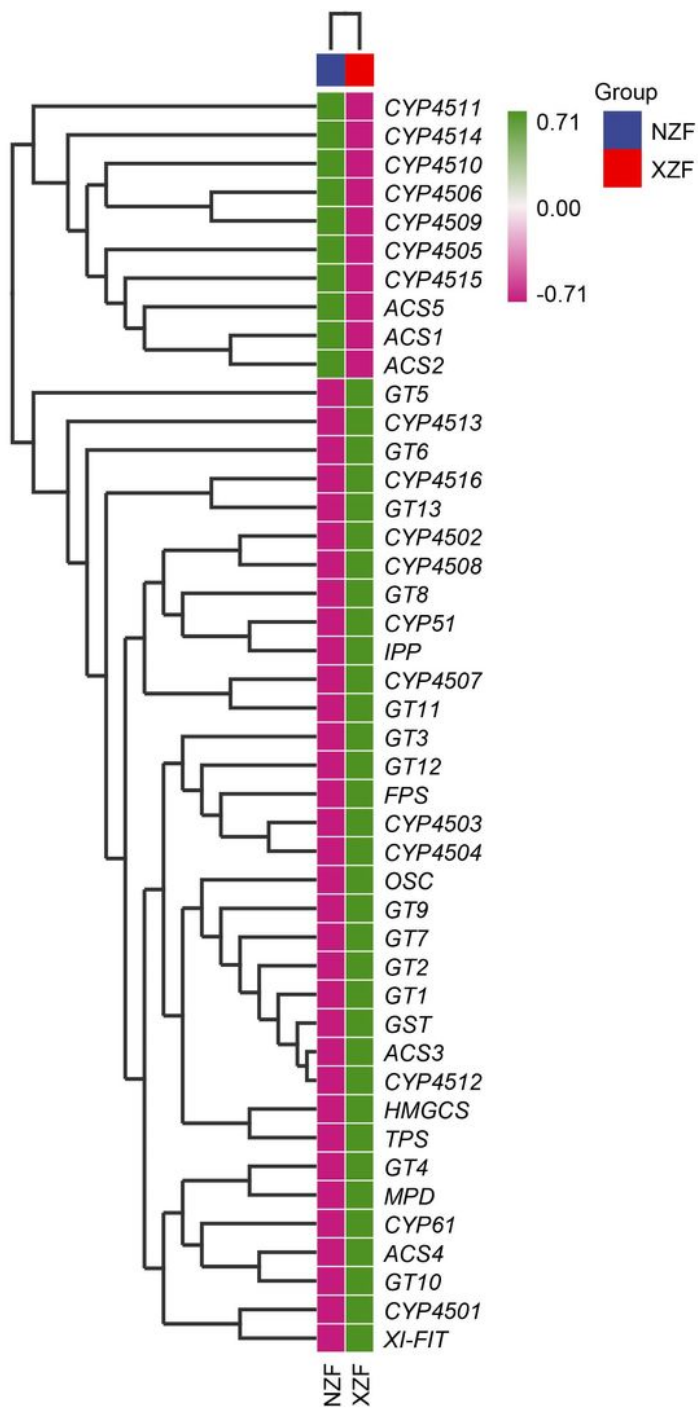


Figure 6

Heat map of the 44 differentially expressed genes. The genes are listed horizontally, and each column represents a substrate. Red represents highly expressed genes, blue represents low expressed genes. ACS: acetyl-CoA synthetase; OSC: 2,3-oxidosqualene cyclase; FPS: farnesyl-diphosphate farnesyltransferase; IPP: Isopentenylidiphosphate isomerase; TPS: terpenoid synthase; MPD: mevalonate pyrophosphate decarboxylase; HMGCS: hydroxymethylglutaryl-CoA synthase; XI-FIT: protoheme IX farnesyltransferase;

GT: glycosyltransferase; GST: glutathione-S-transferase; CYP51: lanosterol 14-alpha-demethylase; CYP450: cytochrome P450

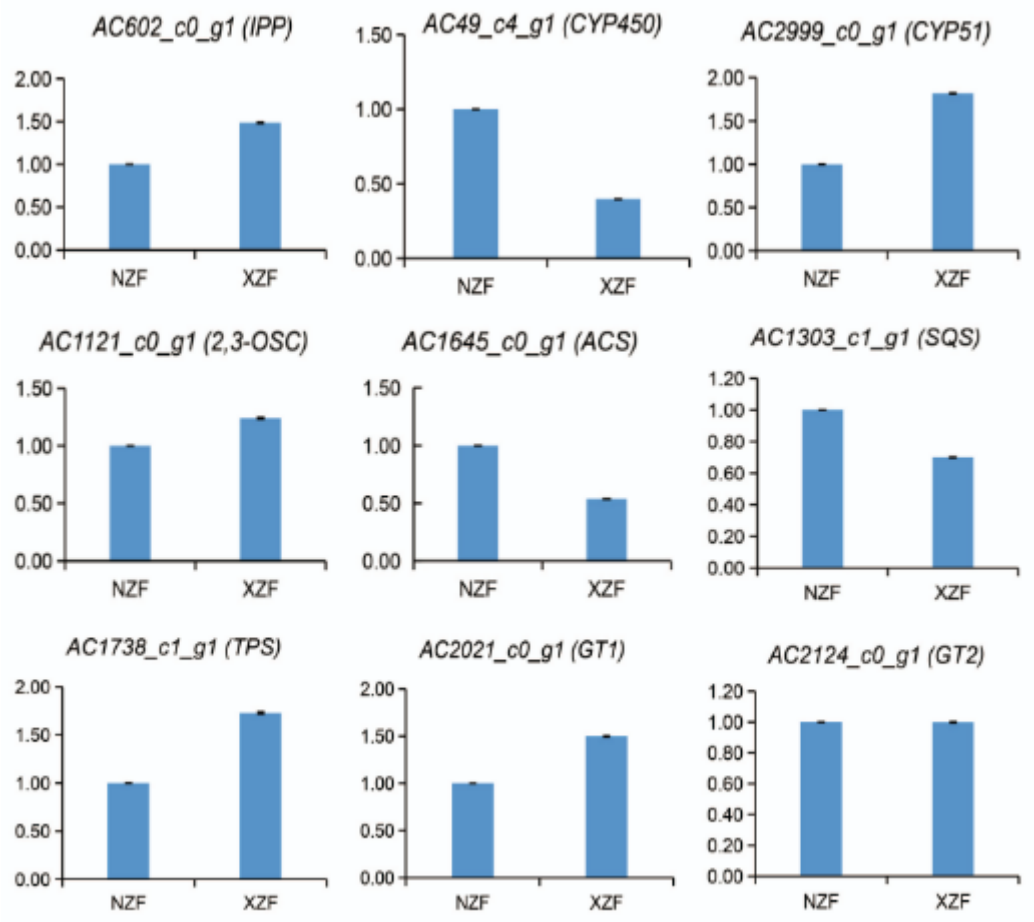


Figure 7

The expression profiles of nine transcripts in NZF and XZF obtained by qRT-PCR. The x-coordinate corresponds to the different substrates, and the ordinate indicates the DEG expression changes. The *AC602_c0_g1* is annotated as Isopentenylidiphosphate isomerase (IPP), *AC49_c4_g1* is annotated as cytochrome P450 monooxygenase (CYP450), *AC2999_c0_g1* is annotated as lanosterol 14-alpha-demethylase (CYP51), *AC1121_c0_g1* as 2,3-oxidosqualene cyclase (2,3-OSC), *AC1645_c0_g1* as acetyl-CoA synthetase (ACS), *AC1303_c1_g1* as squalene synthase (SQS), *AC1738_c1_g1* as terpenoid synthase (TPS), *AC2021_c0_g1* as glycosyltransferase (GT1), and *AC2124_c0_g1* as glycosyltransferase (GT2), respectively.

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