

# Transcriptomic analyses reveal putative genes associated with bibenzyl biosynthesis in the traditional Chinese herb *Dendrobium officinale*

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## Research article

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# Abstract

**Background:** Dendrobium plants are well known for their uses in traditional Chinese herbal medicine. Bibenzyl compounds are the main active compounds in *Dendrobium officinale*. However, the physiological and molecular basis for the biosynthesis of bibenzyl compounds in *Dendrobium* plants remains underexplored.

**Results:** In this study, the accumulation of erianin and gigantol were studied as representative compounds of bibenzyl. Their presence in plant tissues were investigated. Our results show that root tissues contained the highest content of bibenzyl (erianin and gigantol). Based on the pre-experimental result that exogenous application of Methyl-Jasmonate promotes the biosynthesis of bibenzyl compounds in *D. officinale* root tissues, comparative transcriptomic analyses were conducted between the bibenzyl-accumulated root tissues and a control. In total, we identified 1,342 differentially expressed genes (DEGs) with 912 up-regulated and 430 down-regulated genes. Most of the identified DEGs are functionally involved in the JA signaling pathway and the biosynthesis of secondary metabolites. In particular, we identified 11 enzymatic genes functionally involved in bibenzyl biosynthesis.

**Conclusions:** Our study provide insights on the identification of putative genes associated with bibenzyl biosynthesis and accumulation in *Dendrobium* plants, and also paves the way for future research on dissecting the physiological and molecular mechanisms of bibenzyl synthesis in plants as well as on how to best utilize genetic engineering and molecular modification techniques to genetically improve *Dendrobium* varieties by increasing the content of bibenzyl for drug production and industrialization.  
**Keywords:** Bibenzyl, *D. officinale* , Differentially expressed genes, Secondary metabolites, Transcriptome analysis.

## Background

Plants produce vital secondary metabolites for growth and development and also in response to environmental stresses. These secondary metabolites (such as alkaloids, phenolics, flavonoid, and terpenoids) often accumulate within a specific group of plants or given tissues, which play crucial roles in helping plants in defense against various biotic and abiotic stresses [1–3]. In particular, these secondary metabolites provide essential resources for new drug innovations, insecticides, and flavors [4–6].

The *Dendrobium* plants (an herb in the family Orchidaceae) are known as traditional Chinese medicinal herbs (referred to as shihu in Mandarin). *Dendrobium* is widely distributed across Asia and the Pacific Islands [7]. Previous studies documented the health benefits (antipyretic, ophthalmic and regulative of immune system) of *Dendrobium* plants and their contribution to Chinese medicines [8]. In particular, owing to its wealth of active compounds with antitumor and antioxidants functions, *D. officinale* has received tremendous interest in Asian countries. In recent years it has been cultivated in many regions to provide feedstock for making cosmetic and medicinal products.

The active medicinal ingredients in *D. officinale* include: polysaccharides, alkaloids, phenols, terpenes, flavonoids and bibenzyl [9–10]. In *Dendrobium* plants, the content of sesquiterpene alkaloid is the main measure of its quality and medicinal efficacy [11–12]. Notably, previous studies have found that bibenzyl compounds (belonging to sesquiterpene alkaloids) might be the only bioactive ingredients in *D. officinale* [13–14]. Increasing evidence has shown that bibenzyl compounds are active antitumor agents, because of their antioxidant and cell-protective properties [15–19]. Bibenzyl compounds have been widely applied to produce several skincare products and medicinal drugs [20–21]. From *Dendrobium* species, previous studies have identified over 190 compounds including bibenzyl, with erianin and gigantol compounds as the main representative bio-active compounds in the genus *Dendrobium* [10,18]. Although the biosynthesis of bibenzyl compounds might be complex and conserved in plants, it generally requires the incorporation of dihydro-m-coumaroyl-CoA (1 mol.) and malonyl-CoA (3 mol.) along with the catalyzation of bibenzyl synthase. The initial biosynthesis of dihydro-m-coumaroyl-CoA may start with a molecule of phenylalanine to produce the cinnamate molecule with the catalyzation of ammonia lyase (PAL). The cinnamate is further incorporated into m-coumaric-CoA with the catalyzation of cinnamate 4-hydroxylase (C4H). Next, dihydro-p-coumaroyl-CoA is synthesized from p-coumaric-CoA with the catalyzation of 4-coumarate: CoA ligase (4CL) [22–26].

The biosynthesis pathway of secondary metabolites involves various physiological factors and regulatory modifications in different plants that have developed their strategies in response to environmental changes or stresses. Usually, the accumulation of secondary metabolites (such as bibenzyl compounds) is low among tissues [5]. However, market demands require a higher content of bibenzyl compounds in *D. officinale* tissues to meet the threshold for drug-making. There is, therefore, an immediate need to dissect the physiological and molecular mechanisms underlying how bibenzyl compounds are biosynthesized in *D. officinale* tissues. The identification of rate-limiting enzymes or regulatory factors, which are responsible for the biosynthesis of bibenzyl compounds, is thus also an area in need of further exploration. It is exceedingly helpful to use genetic engineering and molecular modification techniques to create improved varieties for commercial purposes. Investigating the physiological and molecular basis of the accumulation of bibenzyl compounds is an essential prerequisite to understanding molecular and genetic factors that regulate the biosynthesis of bibenzyl compounds in *D. officinale* tissues. Moreover, studies have found that jasmonate (JA), a plant-specific signaling molecule, is widely involved in the biosynthesis of diverse secondary metabolites. The exogenous application of methyl-jasmonate (MeJA) often results in the strong activation of secondary metabolites biosynthesis and has frequently been applied to induce the biosynthesis of secondary metabolites in plants [27–29].

With the rapid advancement in RNA-seq technology, transcriptomic data offers both a great opportunity and powerful tool for the discovery of crucial rate-limiting enzymes or regulators, which control the production of some secondary metabolites in plants under different conditions [5,30–31]. For instance, based on transcriptomic analysis, several putative rate-limiting enzyme genes responsible for the biosynthesis of terpenoids in *Eugenia uniflora* [32], lignin in *Apium graveolens* [33], and flavonoid in *Phyllanthus emblica*, *Dracaena cambodiana*, and *Solanum viarum* [34–36] have been identified. For

*Dendrobium* plants, several studies have reported on flavonoid biosynthetic pathway analysis and the gene mining of key enzymes [37] as well as alkaloid biosynthetic pathway analysis and the identification of key enzyme genes [12, 38–39]. However, the bibenzyl biosynthetic pathway and the potential genes responsible for regulating bibenzyl biosynthesis in *Dendrobium* plants remain underexplored.

In this study, we investigated the accumulation of bibenzyl in various tissues of *D. officinale* including in the leaf, root, basal stem and upper stem. We conducted comparative transcriptomic analyses to unravel the putative genes involved in the biosynthesis of bibenzyl in *D. officinale*. To our knowledge, this study is the first report that focus on the identification of putative genes associated with the bibenzyls biosynthesis in *Dendrobium* plants. This study will aid our understanding of unique genes involved in the synthesis of bibenzyl in *D. officinale* as well as provide new insights for future research into the molecular mechanisms of the genes involved in bibenzyl biosynthesis.

## Results

### ***Investigation of Bibenzyl Accumulation Among Tissues***

According to previous studies, bibenzyl compounds (mainly including erianin and gigantol) are the main bioactive ingredients in *D. officinale* for medicine production [15,18,40]. To investigate tissues with the highest accumulation of bibenzyl contents, we initially assessed the content of bibenzyl compounds in four different tissues (leaf, root, basal stem and upper stem tissues) of a three-year-old *D. officinale* plant. As shown in Table 1, the root tissues had the highest content of erianin and gigantol, which were  $2.63\pm 0.69$  and  $37.01\pm 2.16$   $\mu\text{g/g}$ , respectively, followed by the basal stem ( $0.61\pm 0.01$  and  $22.67\pm 0.15$   $\mu\text{g/g}$ ). Within the upper stem, erianin was not detectable while the content of gigantol was lower compared to the root and the basal stem tissues. However, we could detect neither erianin nor gigantol in the leaf tissues. These results indicate that bibenzyl biosynthesis and accumulation mostly occur in the root tissues.

A preliminary experiment conducted by us shows that the accumulation of bibenzyls may be induced by exogenous plant hormone jasmonate (JA). To test whether the accumulation of bibenzyls can be induced by exogenous JA, we treated the *D. officinale* with a methyl-jasmonate (MeJA) solution at different concentrations (0.2 mM, 0.5 mM, and 1.5 mM). We observed that the contents of bibenzyls (erianin and gigantol) in root tissues as showed in Fig 1a and b were significantly higher (13.01-fold and 8.43-fold increase, respectively) after being treated for 36 hours. However, the 0.5 mM concentration seemed to be the optimal accumulation for bibenzyl induction in root tissue. These results clearly show that the accumulation of bibenzyl was significantly induced after 24 hours by exogenous MeJA in root tissues.

### ***Transcriptome Sequencing Datasets***

To dissect the bibenzyl biosynthetic pathway and putative essential rate-limiting genes associated with bibenzyl biosynthesis in *D. officinale*, we carried out transcriptomic analysis using high-throughput RNA-

seq technology. Based on the above results, we treated the *D. officinale* with MeJA (0.5 mM) and reviewed the global transcriptomic changes after 24-hour treatment in comparison with the controls (CK), followed by the isolation of total RNA from the root tissues. With this, we constructed two cDNA libraries (control group [CK] and treatment group [MJ]) for transcriptome sequencing. The raw sequencing data are deposited in the NCBI Sequence Read Archive (SRA) database under the access numbers SRR9866323 and SRR9866324. In total, 83.21 and 82.62 million raw reads were generated from the CK and MJ libraries, and the Q30 percentages (sequencing error rate <0.1%) were 95.06 % and 95.40 %, respectively (Additional file 1). This confirms that the sequencing data were sufficient for further analyses. In total, 81.28 and 81.05 million high-quality clean reads were obtained from the two libraries, while 61.50 and 60.57 million reads were uniquely mapped to the reference genome, respectively (Additional file 1). Based on the two transcriptomic datasets, a total of 23,131 genes were annotated (Additional file 2).

## ***Identification of Differentially Expressed Genes***

To identify putative genes associated with bibenzyl biosynthesis in *D. officinale*, we analyzed the differentially expressed genes (DEGs) between the two sequenced datasets. Parameters of the False Discovery Rate (FDR) were set at < 0.05 and  $|\log_2 \text{Fold change}| > 2$  for identifying DEGs. In total, 1,324 DEGs, consisting of 912 up-regulated and 430 down-regulated DEGs, were identified compared to the CK (Fig. 2). To understand the possible functions of these DEGs, we performed Gene Ontology (GO) enrichment analysis for the identified DEGs. We found that these induced DEGs were significantly enriched in GO terms relating to the diverse processes of secondary metabolites, including sesquiterpene biosynthesis, response to wounding, flavonoid biosynthesis, regulation of defense response, and regulation of jasmonic acid-mediated signaling pathway (Fig. 3). Furthermore, all DEGs were mapped to terms in Kyoto Encyclopedia of Genes and Genomes (KEGG) database to search for enriched genes involved in secondary metabolic or signal transduction pathways. In total, 20 pathways with p-value < 0.05 were significantly enriched under MeJA treatment (Fig. 4). Notably, specific enriched DEGs were observed in the pathways of plant hormone signal transduction, phenylpropanoid biosynthesis, flavonoid biosynthesis and so on. Particularly, all genes involved in the JA signal pathway were up-regulated (Fig. 5a), including the LOC110098999 encoding phospholipase D (PLD), the LOC110097793 encoding phospholipase A1 (DAD1), the LOC110113631/LOC110094199 encoding lipoxygenase (LOX), and the LOC110100164/LOC110106376 encoding allene oxide synthase (AOS). Also, eight and four DEGs identified as JAZ and MYC2 in the JA signal pathway were significantly up-regulated under MeJA treatment.

A total of 59 transcription factors (TFs) of DEGs were identified among the CK and MeJA data. These include 45 up-regulated and 14 down-regulated TFs (Fig. 5b). The most abundant TF family was the AP2/ERF superfamily (20 TFs), followed by WRKY (11 TFs), MYB (4 TFs), MYC (4 TFs), NAC (4 TFs), BHLH (4 TFs), and another 12 TFs. Among the 20 AP2/ERF DEGs, 16 AP2/ERF TFs were up-regulated, and only 4 AP2/ERF TFs were down-regulated. All identified WRKY TFs

were up-regulated (Fig. 5b) under MeJA treatment. This indicates that the exogenous application of MeJA induced secondary metabolic biosynthesis by activating the JA signal pathway.

## ***Identification of Candidate Genes Involved in Bibenzyl Biosynthesis***

Our principal objective was to identify candidate genes involved in bibenzyl biosynthesis in this study. Bibenzyl, an alkaloid belonging to the group of sesquiterpene [14,41], is a downstream product of mevalonate (MVA) and methylerythritol 4-phosphate (MEP) pathway in plants [12,42]. In our dataset, most of the critical enzymes involved in these pathways such as hydroxymethylglutaryl-CoA synthase (HMGS), mevalonate kinase (MK), phosphomevalonate kinase (PMK), 1-deoxy-d-xylulose-5-phosphate synthase (DXS), 1-deoxy-d-xylulose-5-phosphate reductoisomerase (DXR), 4-diphosphocytidyl-2-C-methyl-d-erythritol kinase (CMK), 2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase (MDS), and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HDR) were identified. As shown in Fig. 6, bibenzyl compounds are usually synthesized using substrate (L-Phenylalanine) via cinnamic acid with phenylalanine ammonia-lyase (PAL) catalysis. The catalyzation of trans-cinnamate 4-monooxygenase (C4H) resulted in the two isomers of m-coumaric acid and p-coumaric acid. Along with the phenylpropanoid pathway using m-coumaric acid as substrate dihydro-m-Coumaric acid, dihydro-m-Coumaroyl-CoA and 3,3'5-Trihydrobibenzyl were subsequently synthesized with cytochrome P450 (CYP450), 4-coumarate-CoA ligase (4CL) and bibenzyl synthase (BBS) catalysis respectively. Our identified DEGs reveal that 11 genes, including two phenylalanine ammonia-lyase (PALs) (LOC110113904/LOC110115785), two trans-cinnamate 4-monooxygenase (C4H) (LOC110113575/LOC110101902), two cytochrome P450s (P450) [84A1 and 98A2] (LOC110097166/LOC110101632), two bibenzyl synthase and one bibenzyl synthase-like (BBS) (LOC110115249, LOC110105072, and LOC110105073) and two 4CL including one 4-coumarate-CoA ligase 1-like and one 4-coumarate-CoA ligase 2-like (4CL) (LOC110116024/LOC110107453) were significantly up-regulated via MeJA treatment. These genes play critical roles and are closely associated with the pathway of bibenzyl in *D. officinale*. Furthermore, genes potentially involved in flavonoid and phenylpropanoid biosynthesis were also identified.

To empirically validate the expression changes generated from high throughput RNA-seq, we randomly selected four critical genes involved in the bibenzyl biosynthesis pathway. These four are as follows: LOC110113575 (C4H), LOC110105072 (BBS), LOC110092466 Caffeoyl-CoA O-methyltransferase (CCoAOMT) and LOC110092996 Hydroxycinnamoyl-CoA shikimate/quinic acid hydroxycinnamoyltransferase (HCT). We examined changes in their expressions after MeJA treatment using the qRT-PCR technique. According to the transcriptomic data, the four genes exhibited significant differential expressions between the two libraries. As shown in Fig. 7, results from the qRT-PCR analysis show that the expression patterns of the four genes were highly consistent with our transcriptome sequencing data, i.e., the expressions of the four tested genes were significantly up-regulated under the

MeJA treatment. This experiment confirmed that the genes involved in bibenzyl biosynthesis were up-regulated and the pathway of bibenzyl biosynthesis was induced by MeJA treatment.

## Discussion

The *Dendrobium* plants are highly prized and have been used as traditional Chinese herbal medicine for many years, but the bioactive constituents of authenticating *Dendrobium* drugs are complex, including polysaccharides, alkaloids, flavonoids and bibenzyl compounds [43–44]. Polysaccharides perform immunomodulatory and hepatoprotective activities while bibenzyl compounds exhibit antioxidant, anticancer and immunomodulatory activities [18,19,45, 16, 46,47]. Several studies have been conducted on the identification of putative genes involved in polysaccharide biosynthesis [9,41,48]. However, little is known regarding the physiological and molecular bases of bibenzyl biosynthesis in planta. To our knowledge, this study is the first investigation into the biosynthesis of bibenzyl at the physiological and molecular levels. Bibenzyl compounds are composed of various formulas, with a pair of benzyl radicals. Of them, the erianin and gigantol are structurally similar, and they are represented as bibenzyl compounds [46,47,40,49]. As a result, in this study, the contents of erianin and gigantol were measured as representatives of bibenzyl compounds.

A recent study detected the total alkaloid content of *D. officinale* in the leaf and found a significant increase after exogenous MeJA treatment [50]; however, the contents of erianin and gigantol were not detectable in leaf tissues in our study. In our study, we found that bibenzyl compounds mainly accumulate in the roots and the basal parts of the stem tissues. This means that the roots of *Dendrobium* plants may be more critical in the extraction of bioactive ingredients of antioxidant and anticancer compounds than other tissues. Many studies have found that the biosynthesis of secondary metabolites (such as terpenoids, phenylpropanoids, flavonoids, and alkaloids) could be induced by hormone JA signaling [50–52]. As expected, the accumulation of bibenzyl was induced by the exogenous hormone MeJA in this study. The content of bibenzyl significantly increased between 24 to 36 hours after MeJA treatment, suggesting the rapid accumulation of bibenzyl during this timeframe. We reasonably assumed that most genes involved in bibenzyl biosynthesis were actively expressed. Thus, we compared global gene expressions between the root tissues at the highest accumulation of bibenzyl period and that of the control (untreated).

Although several generated transcriptomic data have been available for *Dendrobium* [9,48,53,50], in our study, based on the root tissues 23,131 unigenes were annotated, and this number was smaller when compared to previous data, but it is comparable to the identified unigenes using transcriptomic data of root tissues generated by Chen et al., (2017). In total, 1,324 DEGs, including 912 up-regulated and 430 down-regulated DEGs, were identified through the comparison of expression changes between our two libraries. Compared to a recent investigation of transcriptomic analyses using exogenous MeJA treatment in *D. officinale* leaves [50], we identified fewer DEGs. It is likely that the fewer identified unigenes and DEGs in our study resulted from the difference of tested tissues between these studies (only root tissues were investigated in this study, whereas other studies investigated either a leaf, stem or

mixed tissues). Most of the identified DEGs were mainly enriched in the JA signaling pathway and biosynthesis of secondary metabolites in this study. The induced DEGs were significantly enriched in the GO terms related to the diverse processes of secondary metabolites (including sesquiterpene and flavonoid biosynthesis) and in response to JA induction in the signaling pathway. As expected, many DEGs involved in the JA signal pathway were identified, such as PLD, DAD1, LOX, and AOS. In particular, JAZ and MYC are well-known responses to exogenous MeJA treatment in plants [54–59].

In *D. officinale*, several transcriptomic analyses have been performed, examining topics such as prediction of putative metabolic pathways [53], identification of functional genes in various metabolic pathways [9,48,50,60] and uncovering environmental responses [61]. In this study, our main objective was to identify potential candidate genes involved in the biosynthesis of bibenzyl (a group of sesquiterpenes) in *D. officinale*. Generally, sesquiterpenes are derived from farnesyl diphosphate (FPP), which is provided by the MVA and MEP pathways in the initial stage of sesquiterpenes biosynthesis in plants [42]. Recently, Chen et al. (2019) have identified several genes involved in FPP biosynthesis. These identified genes usually function at the initial stages of sesquiterpenes biosynthesis, and most of these identified genes such as HMGS, MK, PMK, DXS, DXR, CMK, MDS, and HDR appeared in our data, suggesting that these genes participated in regulating the initial biosynthesis of sesquiterpenes in *D. officinale* and also participated in the early biosynthesis of bibenzyl. In particular, we identified 11 enzyme genes (including two PALs, two C4Hs, two 4CLs, two P450s, and three BBSs) functionally involved in bibenzyl biosynthetic processes. Although it is well known that the enzyme genes CYP450, PAL, C4H and 4CLs with multi-members are functionally involved in various metabolic processes in plants [62–66], this study identified that these 11 enzyme genes may be especially active in regulating bibenzyl biosynthesis in *D. officinale*. It is possible that these genes (or some of them) may be rate-limiting for bibenzyl biosynthesis, and the expression levels of these genes may result in variation of bibenzyl content in different tissues. These identified enzyme genes could provide valuable genetic resources for future research toward increasing bibenzyl content by modifying their expression levels using genetic engineering techniques. Additionally, many differentially expressed TFs (such as bHLH, AP2, and WRKY) were identified in this study. Normally, their different expressions may result from responding to exogenous MeJA treatment. Previous studies have shown that these TF families, such as the bHLH, AP2, and WRKY families, are involved in different steps of the alkaloid biosynthesis pathways [50, 67]. However, whether their different expressions are directly associated with regulation of bibenzyl biosynthesis remains unknown. In addition, although the biosynthesis pathway of bibenzyls might be conserved in plants whether the identified putative genes associated with bibenzyl biosynthesis are species-specific in *D. officinale* remains uncertain in this study.

## Conclusions

In this study, we investigated the accumulation of bibenzyl in various tissues of *D. officinale*, including leaf, root, basal stem and upper stem. Our results reveal that bibenzyl compounds mostly accumulate in the root tissues. Based on the pre-experimental result that the exogenous application of JA promotes the biosynthesis of bibenzyls in *D. officinale* root tissues, comparative transcriptomic analyses of the root tissues between the treatment (MJ) and control (CK) generated 1,342 differentially expressed genes

(DEGs) in total, which consist of 912 up-regulated and 430 down-regulated genes. Most of these identified DEGs are functionally involved in the JA signaling pathway and the biosynthetic processes of secondary metabolites. In particular, we identified 11 enzyme genes in the route of bibenzyl biosynthesis that may play a critical role in regulating the bibenzyl biosynthesis of *D. officinale*. This study not only aids our understanding of unique genes involved in the synthesis of bibenzyl in *D. officinale*, but also provide insights on the identification of putative genes associated with bibenzyl biosynthesis and accumulation in *Dendrobium* plants, it also paves the way for future research on dissecting the physiological and molecular mechanisms of bibenzyl synthesis in plants as well as on how to best utilize genetic engineering and molecular modification techniques to genetically improve *Dendrobium* varieties by increasing the content of bibenzyl for drug production and industrialization.

## Methods

### Sample Collection

The plant materials used in this experiment were collected in July, 2017 from the Experimental Base of *Dendrobium* Breeding and Planting, located in San jia Cun, Simao town, Puer city, Yunnan Province (latitude 22°47'13"N; longitude 100°58'37"E; Altitude: 1342.2m above sea level). The collection site is a branch of *Dendrobium* domestication unit of the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China. The collected samples were formally identified by Professor Li Shu Yun, a taxonomist from Kunming Institute of Botany, Yunnan Province, China. The cultivated samples used in this study were collected in compliance with the institutional guidelines. No voucher specimens were collected and deposited during the sample collection.

### Extraction of Plant Materials and Analysis of Bibenzyl Content

The *D. officinale* specimens were grown in a greenhouse at the Kunming Institute of Botany Botanical Garden, Chinese Academy of Sciences, in Yunnan Province, China. To inspect the changes of bibenzyl compounds in different tissues, we investigated the contents of representative compounds of bibenzyl (erianin and gigantol) among leaf, root, basal stem and upper stem tissues from a three-year-old individuals. Fresh dissected samples were oven-dried at 55 °C until a consistent weigh was attained and then subsequently pulverized. The pulverized tissues (2 g) of each sample were accurately weighed and refluxed twice with 200 ml of 80 % ethanol in a water bath at 80 °C for 2 h. Extracts were concentrated and dried via evaporation; they were re-dissolved in a methanol-to-water ratio of 80:20 (v/v). The solutions were then subjected to MCI gel to determine the active compounds and eluted with 70 % ethanol. The eluted fractions were dried via evaporation and finally dissolved in 25 ml of absolute methanol. Before liquid chromatography-mass spectrometry (LC-MS), 1 ml of the solution was passed through a 0.22 µm microporous membrane. Extracts were analyzed with an Agilent ZORBAX SB-C18 column (4.6 × 50 mm, 2.7 µm) using Liquid Chromatography/Quadrupole Time-Of-Flight Mass

Spectrometry (Agilent 1290/6530). This mobile phase consisted of using a methanol-to-water ratio of 80:20 (v/v) at a flow rate of 500  $\mu\text{L}/\text{min}$ . The detection wavelength and column temperature were set at 230 nm and 30 °C. ESI/MS spectra in both positive and negative ion modes were also performed. The isolation width for isolating the precursor ion was 1 to 3  $m/z$ , and the collision energy was 25 to 45 %. Standard references of bibenzyl (erianin and gigantol) were prepared with an accurately known concentration of 0.005 mg/ml to help identify and quantitate the compounds.

## MeJA Treatment Experiment

At least three biological individuals of a three-year-old *D. officinale* plants were treated with or without exogenous MeJA using a handheld sprayer. Three *D. officinale* plants were sprayed with 0.2, 0.5 and 1.5 mM MeJA (dissolved in absolute ethanol solvent and water), respectively, as the treatment group and another three *D. officinale* plants were sprayed with only ethanol and water solution as the control group. After 3 h, 24 h, and 36 h of treatment, tissues including root, basal stem, upper stem and leaves were harvested. At least three biological replicates from three different individuals were sampled and immediately frozen in liquid nitrogen, before being stored at  $-80^{\circ}\text{C}$  for metabolite and RNA extraction.

## RNA Extraction, cDNA Library Preparation, and Transcriptome Sequencing

RNA was extracted from the root tissues of three biological individuals with MeJA (0.5 mM) treatment for 24 hours and of three individuals with ethanol and water solution treatment as controls using the RNeasy pure Tissue Kit (Qiagen, Beijing, China), in accordance with the manufacturer's protocol. The RNA quality and purity was verified using 2100 Agilent Bioanalyzer and Qubit 2.0. Equal quantities of high-quality RNA were pooled from three biological replicates for cDNA synthesis. The pooled mRNA was enriched with Oligo (dT) beads (Thermo Fisher Scientific, USA), and fragmented into short sequences from 200 to 400 bp. The cleaved RNA fragments were reverse-transcribed into double-stranded cDNA using random hexamer primers and then purified and ligated to sequencing adapters. The products were purified and enriched by PCR to generate the final cDNA library. The Illumina HiSeq<sup>TM</sup> 2500 was used to sequence the libraries following the manufacturer's instructions.

## Illumina Sequencing Data Analysis

To improve the sequence quality, reads with poly-N and low-quality fragments were removed to obtain high-quality reads. The clean reads were mapped onto the reference genome of the species using HISAT2 [68]. The fragments per kilobase per million map reads (FPKM) was calculated by Cufflinks to estimate the level of gene expression. DEGSeq was used to detect differently expressed genes (DEGs) between CK and MeJA treated samples. Genes were identified significantly differently expressed with a False Discovery Rate (FDR) of  $<0.05$  and  $|\log_2(\text{fold change})| > 2$ . Functional GO and KEGG enrichment

analyses of DEGs were respectively performed. P-value and FDR correction were used to determine the significance of enriched pathways. Multi-Experiment Viewer (MeV) (version 4.9.0) was used to generate heatmaps of DEGs.

## Analysis of Differentially Expressed Genes (DEGs)

Differential expression analysis was designed to identify genes with differential expressions between different samples, according to the negative binomial distribution test in DESeq [69] software (<http://bioconductor.org/packages/release/bioc/html/DESeq.html>). The amount of differential expression of the genes was calculated while the NB (negative binomial distribution test) was used to test the difference in the number of reads. The gene expression was estimated by using the baseMean value. To compare and analyze whether the same gene in two samples is differentially expressed, two criteria were selected: one is FoldChange, which is the fold change of the same gene expression level in the two samples; the other is the p-value or FDR (adjusted p-value). The default screening difference condition was p-value<0.05 and the differential multiple was greater than 2. To identify genes related to bibenzyl biosynthesis, unigenes were searched against the flavonoid biosynthetic pathway.

## Validation of genes related to bibenzyl biosynthesis by qRT-PCR

Four differentially expressed genes in the bibenzyl biosynthesis pathway were selected to determine their expression patterns in response to MeJA treatment. These four genes are bibenzyl synthase-like (BBS), tryptamine hydroxycinnamoyltransferase 1-like (THT1), trans-cinnamate 4-monooxygenase (C4H) and putative caffeoyl-CoA O-methyltransferase (CAMT). All gene IDs and their primer sequences are listed in the Additional file 3. Both RNA and cDNA were extracted and synthesized using TransScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR kit (TransGen Biotech, Beijing, China). qRT-PCR was conducted using TransStart Tip Green qPCR SuperMix (TransGen Biotech, Beijing, China) on the Bio-Rad CFX96 system. All primers were designed using the Primer Premier v5.0 software and shown in the Additional file 3. The relative expression levels were calculated using the  $2^{-\Delta\Delta CT}$  model and normalized with actin as the internal control in *D. officinale*. The expression level of the control sample was normalized to 1. Analyses of qRT-PCR were carried out on three biological and technical replicates per sample to validate our transcriptomic data.

## Abbreviations

**4CL:** 4-coumarate: CoA ligase

**4CL:** 4-coumarate-CoA ligase

**AOS:** allene oxide synthase

**AP2/ERF:** APELATA2/ethylene response factor

**BBS:** bibenzyl synthase

**C4H:** cinnamate 4-hydroxylase

**CCoAOMT:** Caffeoyl-CoA O-methyltransferase

**CK:** control group

**CMK:** 4-diphosphocytidyl-2-C-methyl-d-erythritol kinase

**CYP450:** cytochrome P450

**DAD1:** phospholipase A1

**DEGs:** differentially expressed genes

**DXR:** 1-deoxy-d-xylulose-5-phosphate reductoisomerase

**DXS:** 1-deoxy-d-xylulose-5-phosphate synthase

**GO:** Gene Ontology

**HCT:** Hydroxycinnamoyl-CoA shikimate/quinic acid hydroxycinnamoyltransferase

**HDR:** 4-hydroxy-3-methylbut-2-enyl diphosphate reductase

**HMGS:** hydroxymethylglutaryl-CoA synthase

**JA:** jasmonate

**JAZ:** jasmonate ZIM domain-containing protein

**LOX:** lipoxygenase

**MDS:** 2-C-methyl-d-erythritol 2,4-cyclodiphosphate synthase

**MeJA:** methyl-jasmonate

**MEP:** methylerythritol 4-phosphate

**MJ:** treatment group

**MK:** mevalonate kinase

**MVA:** mevalonate

**PAL:** phenylalanine ammonia-lyase

**PLD:** phospholipase D

**PMK:** phosphomevalonate kinase

**TFs:** transcription factors

## **Declarations**

### **Ethics approval and consent to participate**

No specific permits/license were required because the Dendrobium plants used in this study were collected from the Experimental Base of Dendrobium Breeding and Planting, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China. The authors declared that experimental research works on the Dendrobium plants described in this study comply with institutional, national and international guidelines.

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The datasets generated and/or analysed during the current study are available in the Sequence Read Archive (SRA) under SRR9866323 and SRR9866324.

### **Competing interests**

The authors declare that they have no competing interests.

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### **Authors' Contributions**

AL designed the experiments and analyzed the data, OIA, JG, LY and JMH performed the experiment. OIA, AY, SMM and AL analyzed the data. YW participated in the experimental design and provided fanatical

support. AL and OIA wrote this manuscript. All authors discussed and commented on the manuscript.

## References

1. Dixon RA. Natural products and plant disease resistance. *Nat.* 2001; 411: 843–847.
2. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Adv. Nutr.* 2011; 2: 32–50.
3. Kliebenstein DJ, Osbourn A. Making new molecules - evolution of pathways for novel metabolites in plants. *Curr. Opin. Plant Biol.* 2012; 15: 415–23.
4. Goossens AH, Laakso ST, Seppanen-Laakso I, Biondi T, De Sutter S, Lammertyn V, et al. A functional genomics approach toward the understanding of secondary metabolism in plant cells. *Proc Natl Acad Sci U S A.* 2003; 100: 8595–600.
5. Hussain MSF, Ansari S, Rahman S, Ahmad MA, Saeed IZ. Current approaches toward production of secondary plant metabolites. *J Pharm Bioallied Sci.* 2012; 4: 10–20.
6. Yang DL, Yao J, Mei CS, Tong XH, Zeng LJ, Li Q. et al. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc Natl Acad Sci U S A.* 2012; 109: E1192–200.
7. Wu ZY, Raven PH, Hong DY. *Flora of China.* Harvard Papers in Botany. 2010; 25: 347–347
8. Yang S. *Comprehensive Utilization of Citrus By-Products Methods for Determining the Functional Components of Citrus Peel.* Academic Press. 2016; 15–30.
9. Zhang J, He C, Wu K, Teixeira da Silva JA, Zeng S, Zhang X, Yu Z, et al. Transcriptome Analysis of *Dendrobium officinale* and its Application to the Identification of Genes Associated with Polysaccharide Synthesis. *Front Plant Sci.* 2016; 7: 5.
10. Tang H, Zhao T, Sheng Y, Zheng T, Fu L, Zhang Y. *Dendrobium officinale* Kimura et Migo: A Review on Its Ethnopharmacology, Phytochemistry, Pharmacology and Industrialization. *Evid. Based Complement Alternat Med.* 2017; 2017: 7436259.
11. Kreis LM, Carreira EM. Total synthesis of (-)-dendrobine. *Angew. Chem. Int. Ed.* 2012; 51: 3436- 3439.
12. Li Q, Ding G, Li B, Guo S. Transcriptome Analysis of Genes Involved in Dendrobine Biosynthesis in *Dendrobium nobile* Lindl. Infected with Mycorrhizal Fungus MF23 (*Mycena* sp.). *Sci Rep.* 2017; 7:316.
13. Li J, Li S, Huang D, Zhao X, Cai G. Advances in the of resources, constituents and pharmacological effects of *Dendrobium officinale*. *Sci. Technol. Rev.* 2011; 29: 74–79.
14. Xu J, Zhao WM, Qian ZM. Guan J, Li SP. Fast determination of five components of coumarin, alkaloids and bibenzyls in *Dendrobium* spp. using pressurized liquid extraction and ultra-performance liquid chromatography. *J. Sep. Sci.* 2010; 33: 1580–1586.
15. Gong YQ. *Mechanisms of Erianin Anti-Tumor Angiogenesis.* China Pharm Uni, Nanjing, China. 2003.
16. Barbosa EG, Bega LA, Beatriz A, Sarkar T, Hamel E, do Amaral MS, et al. A diaryl sulfide, sulfoxide, and sulfone bearing structural similarities to combretastatin A–4. *Eur. J. Med. Chem.* 2009; 44:

2685–2688.

17. Li Y, Wang C, Wang F, Dong H, Guo S, Yang J, et al. Chemical constituents of *Dendrobium candidum*. *China Journal of Chinese Materia Medica*. 2010; 35: 1715–1719.
18. Su P. Research on the Molecular Mechanism of Erianin Anti-Hepatoma Effect. University of Chinese Academy of Sciences, Beijing, China. 2011.
19. Cai HL, Huang XJ, Nie SP, Xie MY, Phillips GO, Cui SW. Study on *Dendrobium officinale* O-acetylglucomannan (Dendronan®): Part III-Immunomodulatory activity in vitro. *Bioactive Carbohydrates and Dietary Fibre*. 2015; 5: 99–105.
20. Zhang X, Xu JK, Wang J, Wang NL, Kurihara H, Kitanaka S. et al. Bioactive bibenzyl derivatives and fluorenones from *Dendrobium nobile*. *J. Nat. Prod.* 2007; 70: 24–28.
21. Hossain MM. Therapeutic orchids: traditional uses and recent advances-an overview. *Fitoterapia*. 82,102–40. 2011
22. Majumder PL, Sen S, Majumder S. Phenanthrene derivatives from the orchid *Coelogyne cristata*. *Phytochemistry*. 2001; 58: 581–6.
23. Yahyaa M, Ali S, Davidovich-Rikanati R, Ibdah M, Shachtier A, Eyal Y, et al. Characterization of three chalcone synthase-like genes from apple (*Malus x domestica* Borkh.). *Phytochemistry* 2017; 140: 125–133.
24. Ibdah M, Martens S, Gang DR. Biosynthetic Pathway and Metabolic Engineering of Plant Dihydrochalcones. *J. Agric. Food. Chem.* 2018; 66: 2273–2280.
25. Jeong YJ, An CH, Woo SG, Park JH, Lee KW, Lee SH, et al. Enhanced production of resveratrol derivatives in tobacco plants by improving the metabolic flux of intermediates in the phenylpropanoid pathway. *Plant Mol. Biol.* 2016; 92: 117–129.
26. Peled-Zehavi H, Oliva M, Xie Q, Tzin V, Oren-Shamir M, Aharoni A, et al. Metabolic Engineering of the Phenylpropanoid and Its Primary, Precursor Pathway to Enhance the Flavor of Fruits and the Aroma of Flowers. *Bioengineering (Basel)*. 2015; 2: 204–212.
27. Jimenez-Garcia Sandra N, Vazquez-Cruz Moises A, Guevara-Gonzalez Ramon G, Torres-Pacheco Irineo, Cruz-Hernandez Andres, Feregrino-Perez Ana A. Current Approaches for Enhanced Expression of Secondary Metabolites as Bioactive Compounds in Plants for Agronomic and Human Health Purposes—a Review. *Pol. J. Food Nutr. Sci.* 2013; 63: 67–78.
28. Devi K, Mishra SK, Sahu J, Panda D, Modi MK, Sen P. Genome wide transcriptome profiling reveals differential gene expression in secondary metabolite pathway of *Cymbopogon winterianus*. *Sci. Rep.* 2016; 6: 21026.
29. Guzman F, Kulcheski FR, Turchetto-Zolet AC, Margis R. De novo assembly of *Eugenia uniflora* L. transcriptome and identification of genes from the terpenoid biosynthesis pathway. *Plant Sci.* 2014; 229: 238–246.
30. Jia XL, Wang GL, Xiong F, Yu XR, Xu ZS, Wang F, et al. De novo assembly, transcriptome characterization, lignin accumulation, and anatomic characteristics: novel insights into lignin biosynthesis during celery leaf development. *Sci. Rep.* 2015; 5: 8259.

31. Zhu JH, Cao TJ, Dai HF, Li HL, Guo D, Mei WL, et al. De Novo transcriptome characterization of *Dracaena cambodiana* and analysis of genes involved in flavonoid accumulation during formation of dragon's blood. *Sci. Rep.* 2016; 6: 38315.
32. Kumar A, Kumar S, Bains S, Vaidya V, Singh B, Kaur., et al. De novo Transcriptome Analysis Revealed Genes Involved in Flavonoid and Vitamin C Biosynthesis in *Phyllanthus emblica* (L.). *Front Plant Sci.* 2016; 7: 1610.
33. Pandey S, Goel R, Bhardwaj A, Asif MH, Sawant SV, Misra P. Transcriptome analysis provides insight into prickle development and its link to defense and secondary metabolism in *Solanum viarum* Dunal. *Sci. Rep.* 2018; 8: 17092.
34. Lei Z, Zhou C, Ji X, Wei G, Huang Y, Yu W, et al. Transcriptome Analysis Reveals genes involved in flavonoid biosynthesis and accumulation in *Dendrobium catenatum* From Different Locations. *Sci. Rep.* 2018; 8: 6373.
35. Meng Y, Yu D, Xue J, Lu J, Feng S, Shen C, et al. A transcriptome-wide, organ-specific regulatory map of *Dendrobium officinale*, an important traditional Chinese orchid herb. *Sci. Rep.* 2016; 6: 18864.
36. Yuan Y, Yu M, Jia Z, Song X, Liang Y, Zhang J. Analysis of *Dendrobium huoshanense* transcriptome unveils putative genes associated with active ingredients synthesis. *BMC Genomics* 2018; 19: 978.
37. Mu HM, Wang R, Li XD, Jiang YM, Wang CY, Quan JP, et al. Effect of abiotic and biotic elicitors on growth and alkaloid accumulation of *Lycoris chinensis* seedlings. *Z. Naturforsch. C.* 2009; 64: 541–550.
38. Wang H, Yu M, Paek KY, Piao XC, Lian ML. An efficient strategy for enhancement of bioactive compounds by protocorm-like body culture of *Dendrobium candidum*. *Ind Crops Prod.* 2016; 84: 212–130.
39. Zhang XN, Liu J, Liu Y, Wang Y, Abozeid A, Yu ZG, et al. Metabolomics Analysis Reveals that Ethylene and Methyl Jasmonate Regulate Different Branch Pathways to Promote the Accumulation of Terpenoid Indole Alkaloids in *Catharanthus roseus*. *J. Nat. Prod.* 2018; 81: 335–342.
40. Charoenrungruang S, Chanvorachote P, Sritularak B, Pongrakhananon V. Gigantol, a bibenzyl from *Dendrobium draconis*, inhibits the migratory behavior of non-small cell lung cancer cells. *J Nat Prod.* 2014; 77: 1359–1366.
41. Shen C, Guo H, Chen H, Shi Y, Meng Y, Lu J, et al. Identification and analysis of genes associated with the synthesis of bioactive constituents in *Dendrobium officinale* using RNA-Seq. *Sci. Rep.* 2017; 7: 187.
42. Schwab W, Wust M. Understanding the Constitutive and Induced Biosynthesis of Mono- and Sesquiterpenes in Grapes (*Vitis vinifera*): A Key to Unlocking the Biochemical Secrets of Unique Grape Aroma Profiles. *J. Agric. Food Chem.* 2015; 63: 10591–10603.
43. Ng TB, Liu J, Wong JH, Ye X, Wing Sze, SC, Tong Y, et al. Review of research on *Dendrobium*, a prized folk medicine. *Appl. Microbiol Biotechnol.* 2012; 93: 1795–803.
44. Li Y, Li F, Gong Q, Wu Q, Shi J. Inhibitory effects of *Dendrobium* alkaloids on memory impairment induced by lipopolysaccharide in rats. *Planta Med.* 2011; 77: 117–121.

45. Ge YH, Wang J, Yang F, Dai GH, Tong YL. Effects of fresh *Dendrobium officinale* polysaccharides on immune function in mice with Lewis lung cancer. *Zhejiang J Tradic Chin Med*. 2014; 49: 277–279.
46. Li M, He Y, Peng C, Xie X, Hu G. Erianin inhibits human cervical cancer cell through regulation of tumor protein p53 via the extracellular signal-regulated kinase signaling pathway. *Oncol. Lett*. 2018; 16: 5006–5012.
47. Gong YQ, Fan Y, Wu DZ, Yang H, Hu ZB, Wang ZT. In vivo and in vitro evaluation of erianin, a novel anti-angiogenic agent. *Eur. J. Cancer*. 2004; 40: 1554–65.
48. He C, Zhang J, Liu X, Zeng S, Wu K, Yu Z, et al. Identification of genes involved in biosynthesis of mannan polysaccharides in *Dendrobium officinale* by RNA-seq analysis. *Plant Mol. Biol*. 2015; 88: 219–31.
49. Unahabhokha T, Chanvorachote P, Sritularak B, Kitsongsermthon J, Pongrakhananon V. Gigantol Inhibits Epithelial to Mesenchymal Process in Human Lung Cancer Cells. *Evid. Based Complement Alternat. Med*. 2016; 2016: 1–10.
50. Chen Y, Wang Y, Lyu P, Chen L, Shen C, Sun C. Comparative transcriptomic analysis reveal the regulation mechanism underlying MeJA-induced accumulation of alkaloids in *Dendrobium officinale*. *J. Plant Res*. 2019; 132: 419–429.
51. Ge Q, Zhang Y, Hua WP, Wu YC, Jin XX, Song SH, et al. Combination of transcriptomic and metabolomic analyses reveals a JAZ repressor in the jasmonate signaling pathway of *Salvia miltiorrhiza*. *Sci. Rep*. 2015; 5: 14048.
52. Cheong JJ, Choi YD. Methyl jasmonate as a vital substance in plants. *Trends Genet*. 2003; 19: 409–13.
53. Guo X, Li Y, Li C, Luo H, Wang L, Qian J, et al. Analysis of the *Dendrobium officinale* transcriptome reveals putative alkaloid biosynthetic genes and genetic markers. *Gene*. 2013; 527: 131–138.
54. Yanfang Y, Kaikai Z, Liying Y, Xing L, Ying W, Hongwei L, et al. Identification and characterization of MYC transcription factors in *Taxus sp*. *Gene*. 2018; 675: 1–8.
55. De Boer K, Tilleman S, Pauwels L, Vanden Bossche R, De Sutter V, Vanderhaeghen R, et al. APETALA2/ETHYLENE RESPONSE FACTOR and basic helix-loop-helix tobacco transcription factors cooperatively mediate jasmonate-elicited nicotine biosynthesis. *Plant J*. 2011; 66: 1053–1065.
56. Toledo-Ortiz G, Huq E, Quail PH. The Arabidopsis basic/helix-loop-helix transcription factor family. *Plant Cell*. 2003; 15: 1749–1770.
57. Sun H, Fan HJ, Ling HQ. Genome-wide identification and characterization of the bHLH gene family in tomato. *BMC Genomics*. 2015; 16: 9.
58. Guo XJ, Wang JR. Global identification, structural analysis and expression characterization of bHLH transcription factors in wheat. *BMC. Plant Biol*. 2017; 17: 90.
59. Niu X, Guan Y, Chen S, Li H. Genome-wide analysis of basic helix-loop-helix (bHLH) transcription factors in *Brachypodium distachyon*. *BMC Genomics*. 2017; 18: 619.

60. Chen Y, Shen Q, Lin R, Zhao Z, Shen C, ad Sun C. De novo transcriptome analysis in *Dendrobium* and identification of critical genes associated with flowering. *Plant Physiol. Biochem.* 2017; 119: 319–327.
61. Xu M, Liu X, Wang JW, Teng SY, Shi,JQ, Li YY, et al. Transcriptome sequencing and development of novel genic SSR markers for *Dendrobium officinale*. *Molec. Breed.* 2017; 37: 18.
62. Hassimotto NM, Genovese MI, Lajolo FM. Identification and Characterisation of Anthocyanins from Wild Mulberry (*Morus Nigra* L.) Growing in Brazil. *Int J Food Sci Tech.* 2007; 13: 17–25.
63. Rasool S, Mohamed R. Plant cytochrome P450s: nomenclature and involvement in natural product biosynthesis. *Protoplasma*, 2016; 253: 1197–209.
64. Chen Y, Li F, Tian L, Huang M, Deng R, Li X, et al. The Phenylalanine Ammonia Lyase Gene LjPAL1 Is Involved in Plant Defense Responses to Pathogens and Plays Diverse Roles in *Lotus japonicus*-*Rhizobium* Symbioses. *Mol. Plant Microbe Interact.* 2017; 30: 739–753.
65. Jiang Y, Liao Q, Zou Y, Liu Y, Lan J. Transcriptome analysis reveals the genetic basis underlying the biosynthesis of volatile oil, gingerols, and diarylheptanoids in ginger (*Zingiber officinale* Rosc.). *Bot. Stud.* 2017; 58: 41.
66. Cao YP, Fang Z, Li SM, Yan CC, Ding QQ, Cheng X, et al. Genome-wide identification and analyses of 4CL gene families in *Pyrus bretschneideri* Rehd. *Yi Chuan.* 2015; 37: 711–719.
67. Yamada Y, Shimada T, Motomura Y, Sato F. Modulation of benzyloquinoline alkaloid biosynthesis by heterologous expression of CjWRKY1 in *Eschscholzia californica* cells. *PLoS One.* 2017; 12.
68. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol.* 2019; 37: 907–915.
69. Anders S, Huber W. Differential expression of RNA-Seq data at the gene level–the DESeq package[J]. European Molecular Biology Laboratory (EMBL). Heidelberg, Germany, 2012; 23.

## Table 1

Table 1

Comparison of bibenzyls contents (erianin and gigantol) in different tissues of *D. officinale* (Mean  $\pm$  Standard error). ND denotes “not detectable”.

Compounds	Erianin $\mu\text{g/g}$	Gigantol $\mu\text{g/g}$	Total content $\mu\text{g/g}$
Roots	2.63 $\pm$ 0.69	37.01 $\pm$ 2.16	39.64 $\pm$ 4.14
basal stems	0.61 $\pm$ 0.01	22.67 $\pm$ 0.15	23.28 $\pm$ 0.25
upper stems	ND	12.15 $\pm$ 1.87	12.15 $\pm$ 1.87
Leaves	ND	ND	ND

## Figures

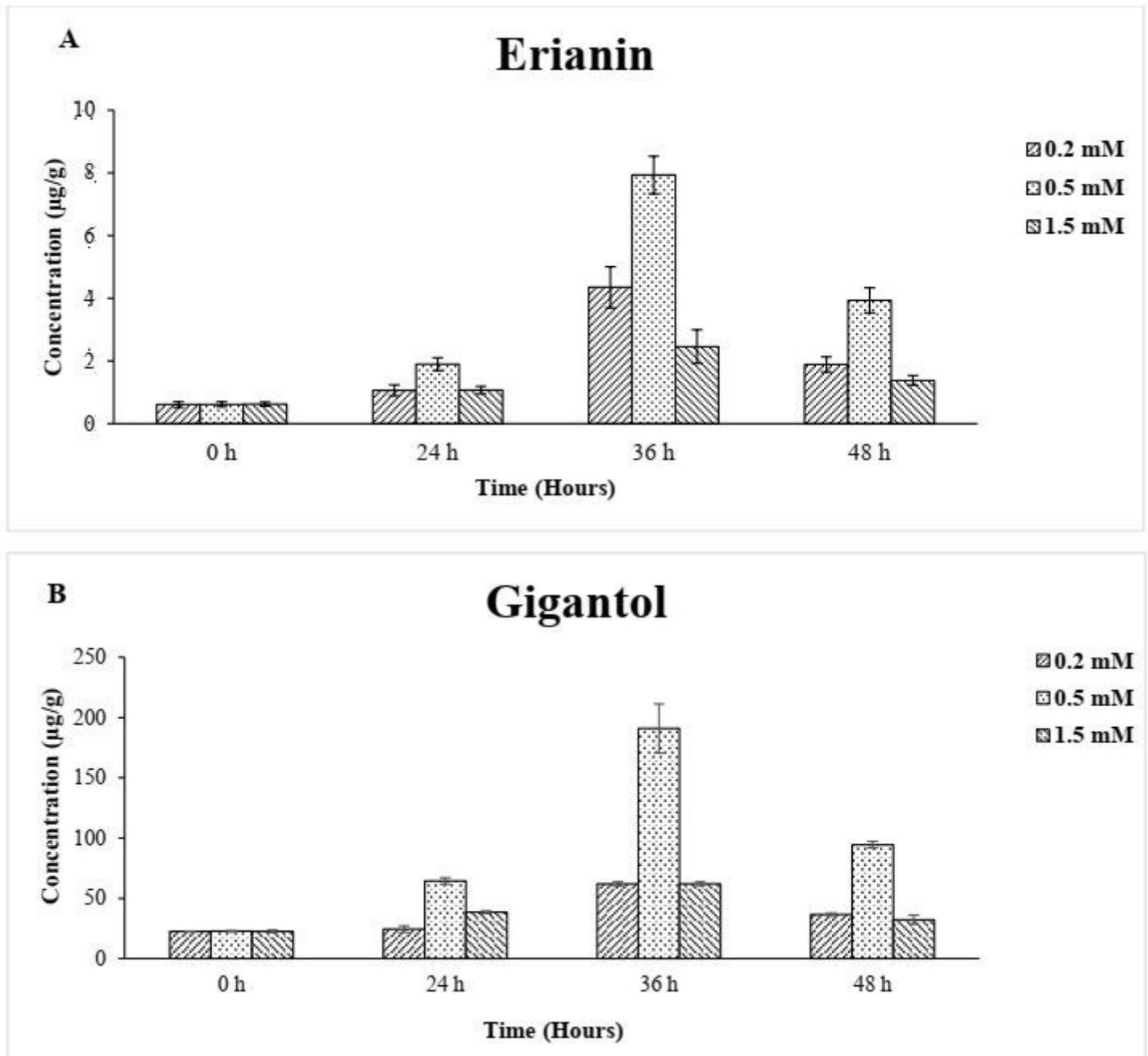
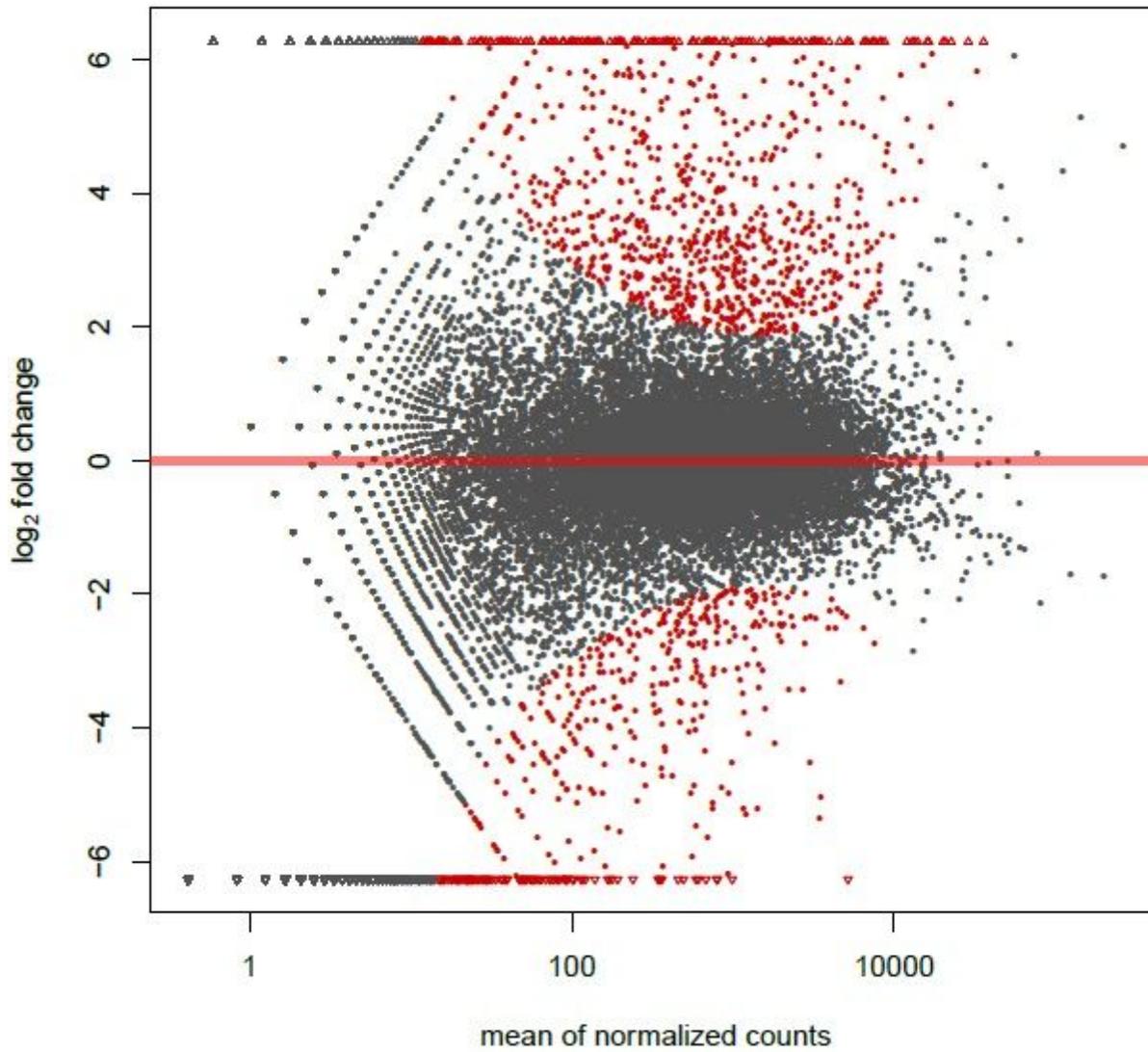


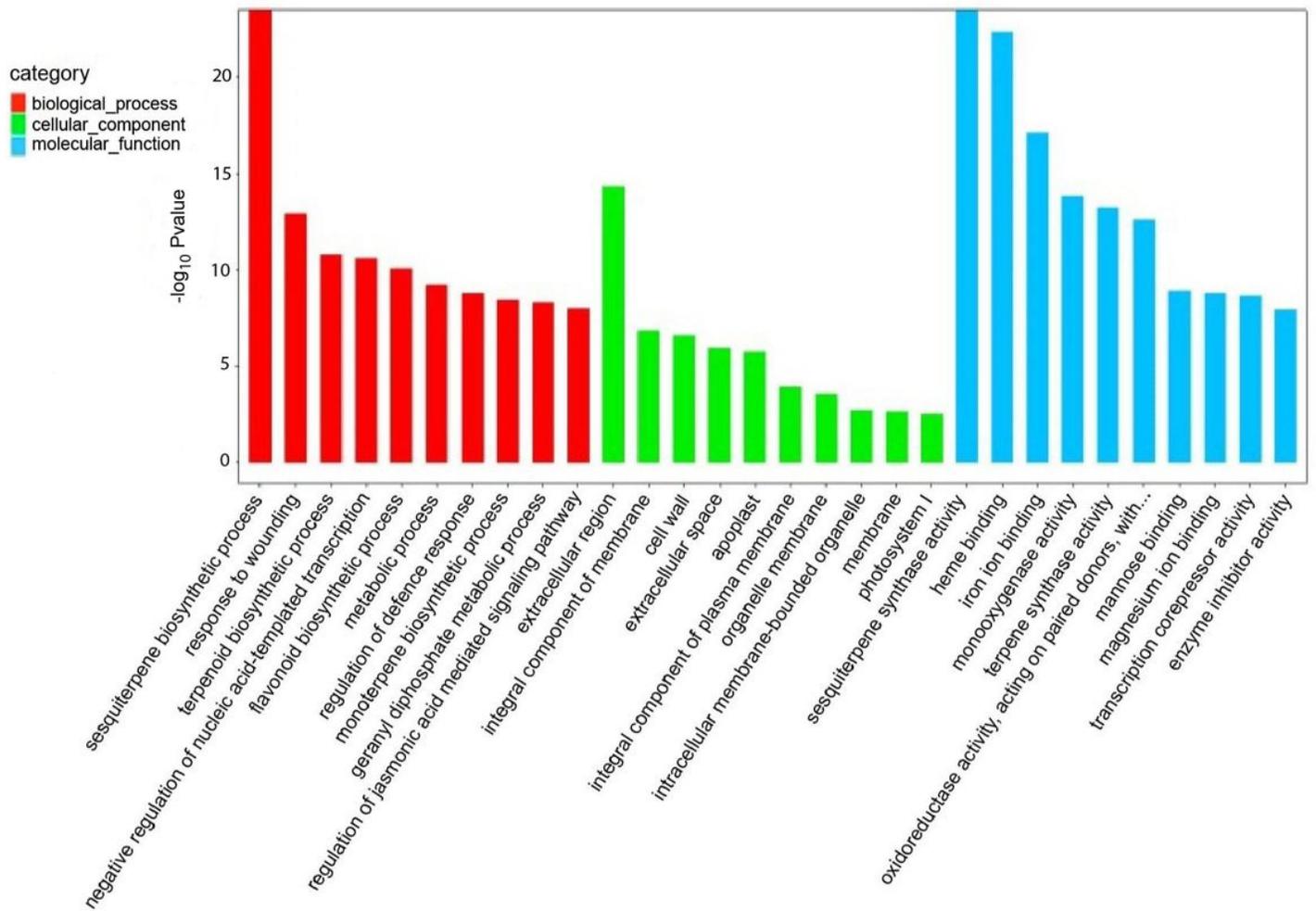
Figure 1

Changes of erianin (a) and gigantol (b) contents under different treatment of exogenous MeJA concentration using 0.2 mM, 0.5 mM & 1.5 mM from 0 h to 48 h.



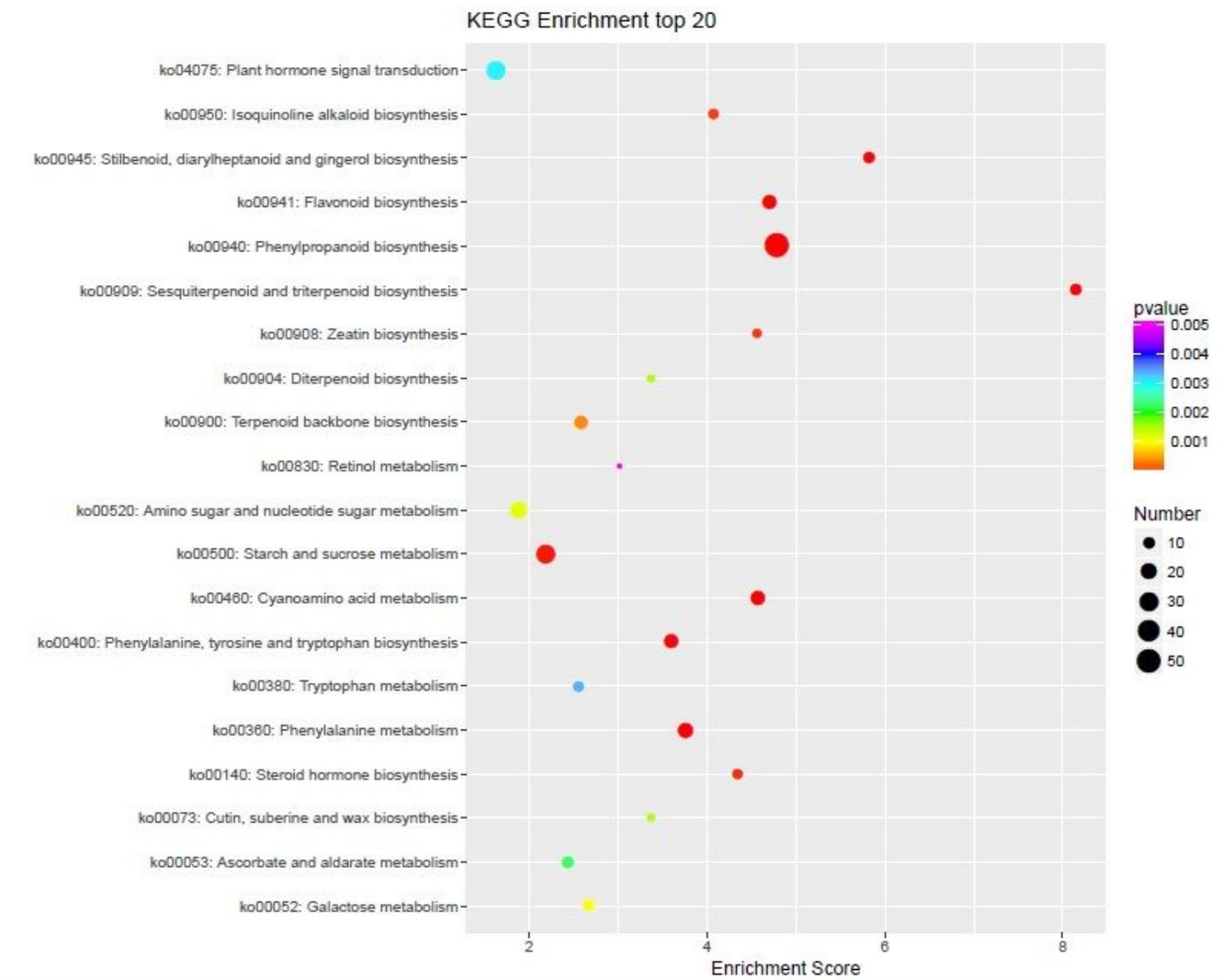
**Figure 2**

Identification of differentially expressed genes (DEGs). The dots in gray indicates genes that are not differentially expressed and dots in red denote the Up-regulated and down-regulated DEGs, respectively.



**Figure 3**

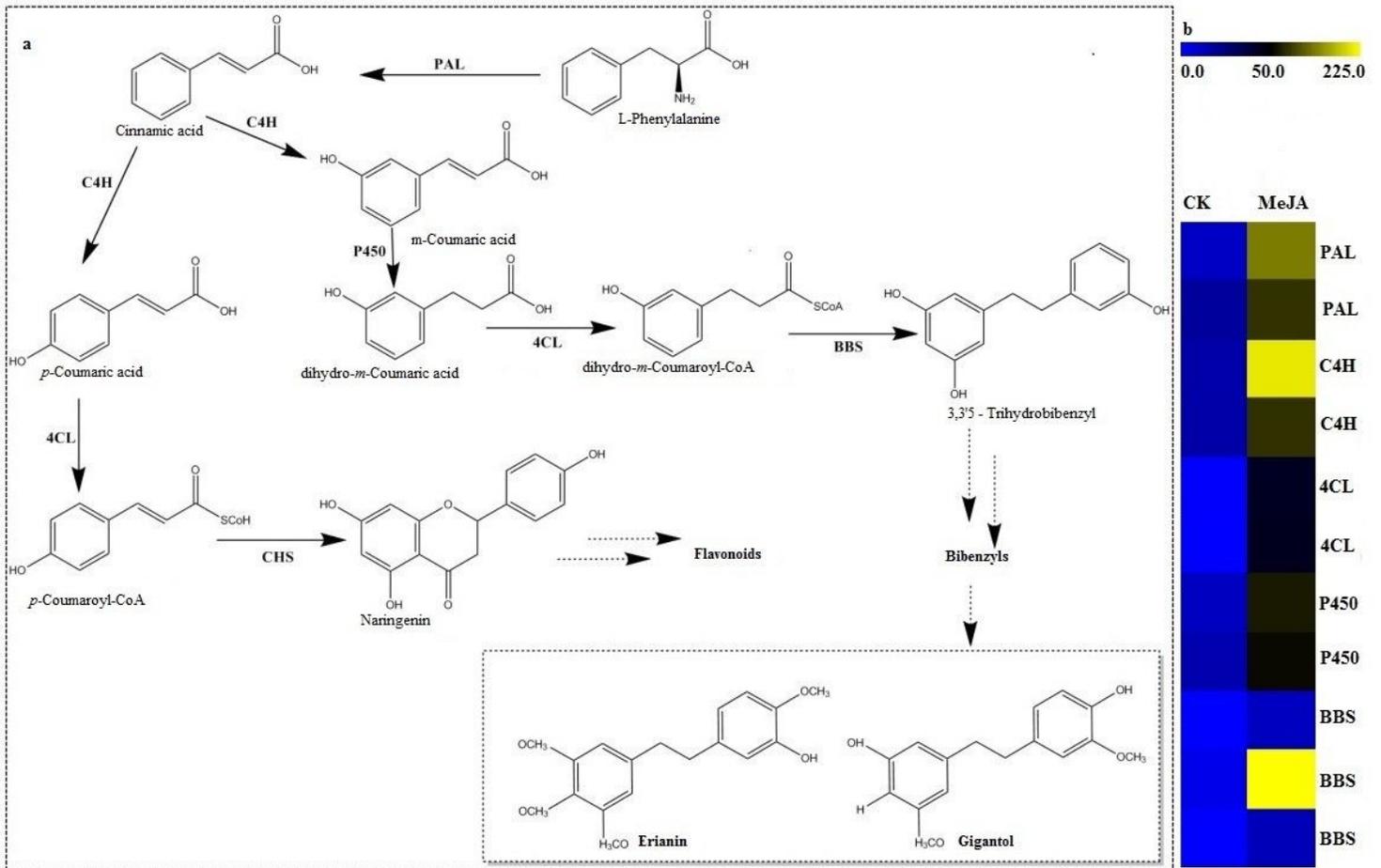
Functional GO enrichment analysis for the identified DEGs. GO enrichment analysis of DEGs identified from RNA-Seq analysis. The red, green and blue bars represent biological process, cellular component, and molecular function, respectively.



**Figure 4**

The top 20 pathways within KEGG analysis. KEGG pathway enrichment analysis of DEGs identified from RNA-Seq analysis. The different colors represent p-value, ranging from 0.001 to 0.005 while the black circle represents gene number.





**Figure 6**

(a): Putative bibenzyl biosynthesis pathways and identification of differentially expressed enzyme genes involved in bibenzyl biosynthesis. PALs denote phenylalanine ammonia-lyase, C4H denotes trans-cinnamate 4-monooxygenase, P450 denotes cytochrome P450s, 4CL denotes 4-coumarate-CoA ligase 1,2-like and BBS denotes bibenzyl synthase (or bibenzyl synthase-like). (b): Expressional changes of the identified DEGs involved in bibenzyl biosynthesis.

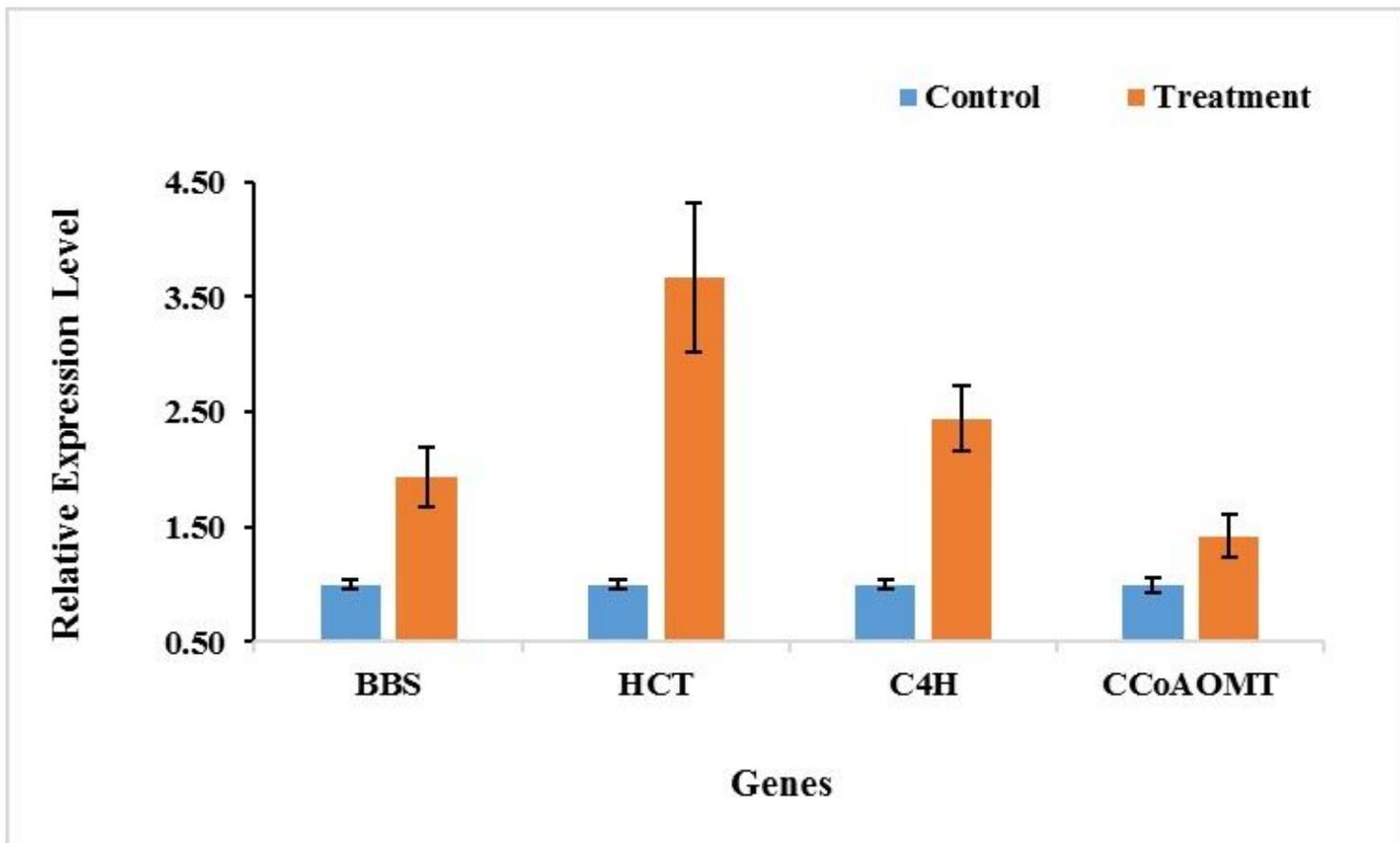


Figure 7

Quantitative real-time PCR analysis of four unigenes associated with the bibenzyl biosynthesis. Error bars were calculated based on three biological replicates. The expression level of the control sample was normalized to 1.

## Supplementary Files

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