Genomics 114 (2022) 110375

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Transcriptomic analysis reveals the significant effects of fertilization on the biosynthesis of sesquiterpenes in *Phoebe bournei*

Li Liu [a,1], Xu Wang [b,1], Yong Lai [c,1], Gongxiu He [a,*], Shizhi Wen [a], Hanjie He [d], Zhenshan Li [a], Baohong Zhang [e,*], Dangquan Zhang [c,*]

[a] School of Forestry, Central South University of Forestry and Technology, Changsha 410004, China
[b] Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou 510520, China
[c] College of Forestry, Henan Agricultural University, Zhengzhou 450002, China
[d] School of Life Sciences and Technology, Central South University of Forestry and Technology, Changsha 410004, China
[e] Department of Biology, East Carolina University, Greenville, NC 27858, USA

ARTICLE INFO

Keywords:
Sesquiterpenes
Compost
Compound fertilizer
Biosynthesis pathway
DEG expression

ABSTRACT

Phoebe bournei is a potential medicinal plant. Its essential oils (Eos) are mainly composed of sesquiterpenes that has potential activities of anti-bacteria and anti-tumors. In this study, we evaluated the effects of compost and compound fertilizer on the total amount and main components of Eos in *P. bournei*, we also studied the molecular mechanism undergoing this process by deep sequencing the genes involved in the biosynthesis of sesquiterpenes. Fertilization enhanced the total amount of main components in Eos from both leaves and twigs. Bicyclogermacrene, the primary sesquiterpene in the leaf EO, was significantly increased under compost treatment, while bicyclogermacrene and δ-cadinene (the second most abundant sesquiterpene) were decreased under compound fertilizer treatment. The two fertilizers had no significant effect on the abundance of the primary (+) -δ-cadinene in the twig EO, but had a positive effect on the second most abundant sesquiterpene copaene. Significant differences were observed in the number of differentially expressed genes (DEGs) with the leaves showing greater number of DEGs as compared to the twigs after compost treatment. Terpenoid backbone biosynthesis (TBB) is a key pathway of sesquiterpenes synthesis. The expression of genes regulating several important enzymes in TBB was altered after fertilization. After the compost treatment, the expression of the leaf DXS gene (*ACQ66107.1*), being closely related to the sesquiterpene biosynthesis in *P. bournei* leaves, was decreased. Compost and compound fertilizer altered the expression of the two important branch-point enzymes (FPPS and GGPPS) genes (*ART33314.1* and *ATT59265.1*), which contributed to the changes of the total amount and components of *P. bournei* sesquiterpenes. This study provides a new insight into the future use of *P. bournei* for Eos.

1. Introduction

Phoebe bournei (Hemsl). Yang (Lauraceae) is a unique, valuable timber, and ornamental tree species in China, and are mainly distributed in the subtropical regions of China. The wood of *P. bournei* has been considered a good timber for architecture, furniture, carving and shipbuilding. However, it takes a long growing time for the wood of *P. bournei* to meet the processing requirements, resulting in slow economic benefits. In recent years, it has been found that *P. bournei* is also a potential medicinal plant. The essential oils (EOs) of *P. bournei* had potential inhibitory activity against the growth of leukemia, breast and colon cancer cells as well as against *Epidermophyton floccosum* and *Microsporum gypseum* [1,2]. Therefore, the processing and utilization of *P. bournei* Eos may become a potential development direction in the future.

EOs are secondary metabolites of plants that are different from primary metabolites or intermediate metabolites and play an important role in plant adaptation to biological and abiotic environments, such as helping plants resist insect attacks or pathogenic microorganism invasion [3–5] and protecting plants from damage caused by ozone,

* Corresponding authors.
E-mail addresses: hegongxiu@163.com (G. He), zhangb@ecu.edu (B. Zhang), zhangdangquan@163.com (D. Zhang).
[1] These are co-first authors.

https://doi.org/10.1016/j.ygeno.2022.110375
Received 17 January 2022; Received in revised form 2 March 2022; Accepted 24 April 2022
Available online 29 April 2022

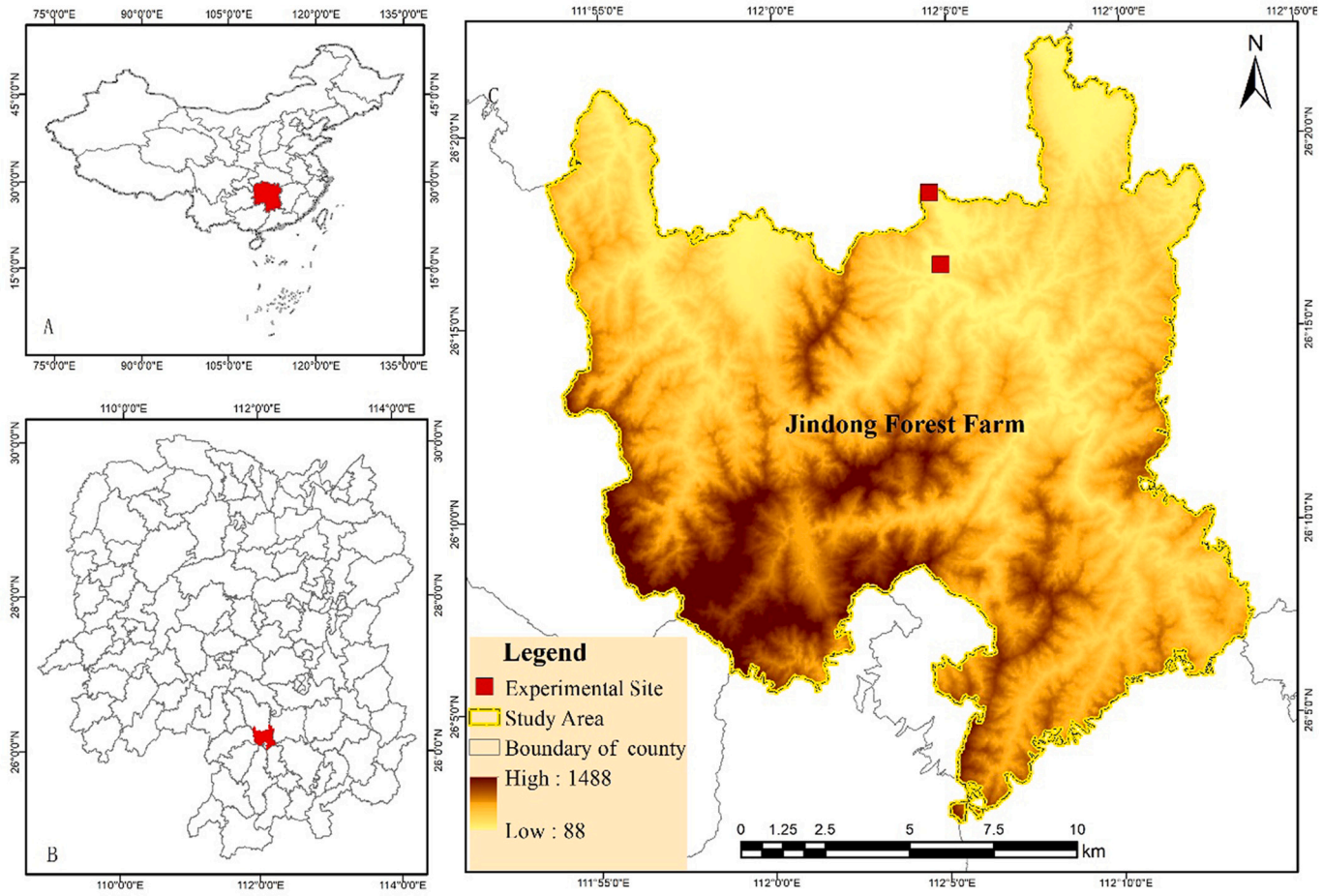

Fig. 1. Geographical location of test plot.

ultraviolet radiation or drought [6]. Eos also have many biological functions, such as antioxidant [7], antibacterial [8], and tumor growth inhibition [9] as well as defending against insects [10]. Nowadays, Eos are widely used in the food and cosmetics industries and even have great potential application value in the pharmaceuticals [11].

Eos are composed mainly of a variety of volatile organic compounds, such as terpenoids (mainly monoterpenes and sesquiterpenes), alcohols, ketones, and aldehydes [5,12–19]. Sesquiterpenes and monoterpenes are synthesized by the terpenoid backbone biosynthesis pathway [20], which consists of two metabolic pathways: the mevalonate (MVA) pathway and the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway [21].

Nutrient enrichment is a common and effective method of increasing plant biomass [22–24] and has a certain impact on the composition and biological activity of plant Eos [25]. Fertilization can not only promote the growth of plants but also modulate the composition of Eos, thus changing the EO biological activity at some extent [26]. A variety of methods and types of fertilization can be implemented, such as adding a single element (nitrogen, sulfur, zinc) [27–29] or adding a mixed fertilizer, such as compost [30].

In order to better understand the effects of nutrient enrichment on *P. bournei* Eos, two common fertilizers (compost and compound fertilizer) were applied in the present study to investigate how the total amount and composition of *P. bournei* Eos are regulated by the fertilization, and differential expressed genes involved in the biosynthesis of sesquiterpenes were identified to obtain a deep insight into the molecular mechanism in response to fertilization.

Table 1
Test result of elements in soil (The letters a, b and c represent the significant difference of elements between different fertilization treatments).

Element	Soil depth (cm)	Fertilization treatment		
		CK	Compost	Compound fertilizer
Organic matter (%)	0–10	1.61 ± 0.03b	2.77 ± 0.02c	1.39 ± 0.04a
	10–20	1.19 ± 0.02b	2.33 ± 0.09c	1.02 ± 0.01a
P (mg/kg)	0–10	21.83 ± 0.76a	254.47 ± 4.62c	38.23 ± 0.42b
	10–20	7.58 ± 0.29a	449.07 ± 9.60c	6.42 ± 0.47a
K (mg/kg)	0–10	100.67 ± 1.53a	174.67 ± 0.58c	99.11 ± 0.54a
	10–20	70.53 ± 1.05a	90.83 ± 1.11b	100.53 ± 1.80c
N (mg/kg)	0–10	75.93 ± 0.88a	121.72 ± 2.17b	77.77 ± 1.53a
	10–20	63.55 ± 0.88a	137.06 ± 1.79b	65.19 ± 0.80a

2. Materials and methods

2.1. Plant samples

The twigs and leaves of *P. bournei* trees grown for 6 years were collected from the domesticated clones of *P. bournei* plantation in the Jindong Forest Farm of Yongzhou city, Hunan Province, China.

2.2. Fertilization experiment

The *P. bournei* plantation was in Jindong Forest Farm (112°4′E, 26°18′N), Yongzhou city, Hunan Province, China, at an altitude of 195 m (Fig. 1). This area is part of the humid monsoon climate zone in the central subtropical zone, with an average temperature of 18.0 °C and an average annual precipitation of 1745 mm. The experimental trees of *P. bournei* were planted in the spring of 2013, with a planting density of 2.5 m × 2.5 m. On September 3, 2018, nine 20 m × 20 m sample plots in each experimental site were established for the fertilization treatments, including three groups treated with compost (organic matter 46%, 2.1% of N, 1.7% of P_2O_5, 1.6% of K_2O), the three ones treated with NPK compound fertilizer (20%–20%–20%) and the rest with no fertilizer (the control group). After 240 days post fertilization, on May 18, 2019, *P. bournei* trees with similar height and diameter at breast height were selected from each plot, and two-year-old twigs and one-year-old leaves were collected. Soils under different fertilization treatment were collected for the analysis of organic matter, P, N and K elements (Table 1). The EO components of the twigs and leaves from each plot were detected, and the samples randomly collected from each experimental site for RNA extraction, which were immediately treated with liquid nitrogen and then sent to Shanghai Majorbio Bio-pharm Technology for sequencing.

2.3. Distillation of essential oil

The extraction of Eos was performed according to a previous report [31]. Briefly, fresh leaves/twigs (1000 g) were mixed with 3000 g water, beat with beater for 2 min, and then distilled for 5 h. The total amount of EO (%) was calculated as follows: mass of EO/sample fresh weight × 100%. The EO (10 μL) was diluted with 1 mL pure ethanol, dehydrated with anhydrous sodium sulfate at room temperature (approximately 25 °C) for 2 h, and centrifuged, and the supernatant was used for the detection of the components of the EO via gas chromatography mass spectrometry by Agilent 7890B/5977 GC–MS.

2.4. Component analysis by GC/MS

The analysis method for the chemical components of the Eos in *P. bournei* was performed according to previously reported methods [1,32]. The GC detection conditions were as follows: the initial temperature was 50 °C, the temperature was increased to 260 °C by 5 °C/min, and the sample was injected into a HP-5 MS capillary column (30 m × 250 μm × 0.25 μm) at a split ratio of 10:1. The MS program information was input as follows: the scanning range was 30–550 AMU (m/z), with an ionizing voltage of 70 eV and an ionization current of 150 μA of electron ionization (EI). The flow velocity of helium was 1.2 mL/min. The ion source temperature was 230 °C, and the quadrupole temperature was 200 °C. The GC/MS ion chromatograms of *P. Bournei* leaf and twig have been shown in Fig. S1.

2.5. Determination of essential oil components content

The volatile components of *P. bournei* Eos detected by GC / MS were searched by NIST2.0 standard spectrogram library. Advanced qualitative detection and identification technology of components was employed to fully scan the essential oil samples. Each peak was corresponded to a full scan mass spectrum. After matching with the standard NIST08.L library, components with a matching degree of more than 95% were obtained, such as some main components in the EOs of twigs and leaves without fertilization were listed in Table S1. The relative contents of volatile components in *P. bournei* Eos were calculated by peak area normalization method. *t*-test was used to analyze the significance of the difference between groups, $P < 0.05$ was significant; The results were expressed as mean values ± S.D.

Table 2
The total amount of Eos from leaves and twigs of *P. bournei* after fertilization.

Tissues	Total amount (%)		
	Compost	Compound fertilizer	Control
Leaf	0.096 ± 0.003[a]	0.109 ± 0.012[a]	0.112 ± 0.006[a]
Twig	0.128 ± 0.006[a]	0.147 ± 0.031[a]	0.135 ± 0.013[a]

Note: The superscripted letters indicate the significance by least significance difference (LSD) at 0.05 level.

2.6. RNA sequencing, data processing, and gene annotation

Total RNA from each sample was isolated from frozen tissues (leaves and twigs) of *P. bournei* using TRIzol® reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA), and genomic DNA was removed by using DNase I (TaKara). The integrity and purity of the total RNA was determined by a 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara CA, USA), and the quantity was measured using an ND-2000 spectrophotometer (NanoDrop Thermo Scientific, Wilmington, DE, USA) [33,34]. Library preparation and sequencing were performed by Shanghai Majorbio Biopharm Technology Co. (Shanghai, China) with sequence runs of 2 × 150-bp paired-ends (200 cycles). The raw reads have been deposited in the Short Read Archive (SRA) of NCBI with the accession number PRJNA771755.

Transcriptome assembly was performed using the Trinity method [33]. The functions of *P. bournei* genes were annotated on the basis of information from the following databases: National Center for Biotechnology Information (NCBI) nonredundant protein sequences (Nr) database, the NCBI nonredundant nucleotide sequences (Nt) database, the Gene Ontology (GO) database, the protein family (Pfam) database, the Clusters of Orthologous Groups of proteins (KOG/COG) database, the manually annotated and reviewed SwissProt protein sequence database, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, and the KEGG Orthology (KO) database.

Gene expression levels were calculated using the fragments per kilobase per million reads (FPKM) method in RSEM software [35]. Differentially expressed genes (DEGs) were analyzed using the DESeq R package (1.10.1), and the genes with an adjusted q-value <0.05 identified by DESeq were considered differentially expressed.

2.7. Quantitative real-time PCR (qRT-PCR) verification

Based on the RNA-seq data, the expression of 13 genes were significantly altered associated with TBB and STB after different fertilizer treatments. Of which, five genes (TRINITY_DN60255_c0_g7, TRINITY_DN61719_c0_g1. TRINITY_DN79706_c2_g1, TRINITY_DN76855_c1_g3 and TRINITY_DN67962_c1_g3) were found to be important in the regulation of key enzymes and branching enzymes during terpenes synthesis. To confirm the expression profiles obtained from the transcriptome data, the expression levels of these five relatively important genes were selected for real-time qPCR (RT-qPCR) analysis. 18S rRNA were used as reference gene and its forward and revere primers were listed in Table S2.

The following experimental reaction conditions were used: pre denaturation at 95 °C for 5 min, and then run 35 cycles with each cycle denaturation at 95 °C for 30s, annealing at 50 °C for 30s, and extension at 72 °C for 1 min. The experiments were performed with three biological replicates and three technical replicates. The relative expression levels of the selected unigenes were calculated using the $2^{-\Delta\Delta C_T}$ method. The primer sequences used in this study are listed in Table S2.

2.8. Statistical analysis

All experiments were repeated at least three times. A probability value of $p < 0.05$ was considered a significant difference.

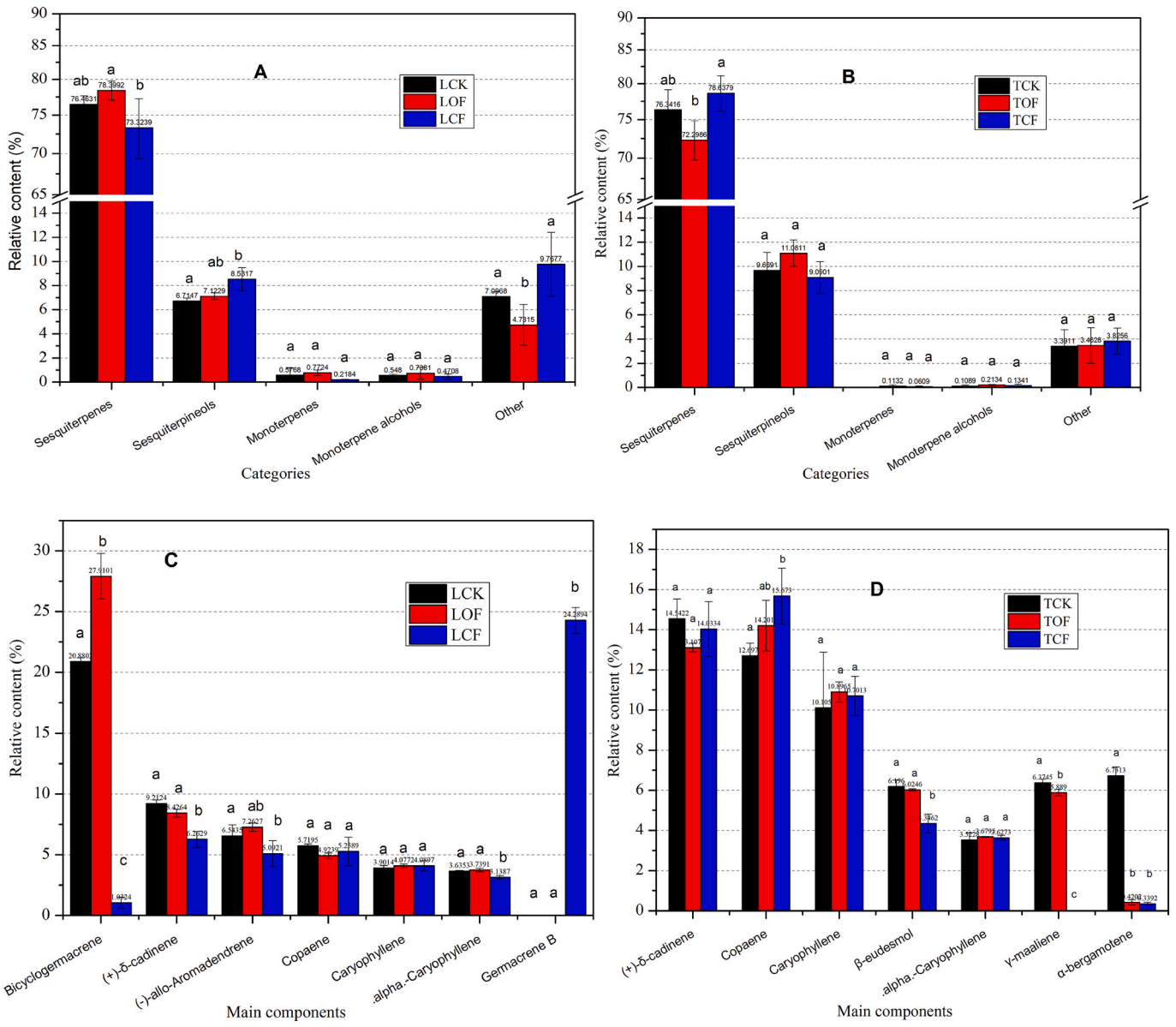

Fig. 2. Categories and main compounds of Eos from *P. bournei* leaves and twigs before and after fertilization. A and B show the categories of volatile components in the Eos of leaves and twigs, respectively; C and D show the main components in the Eos of leaves and twigs, respectively. The letters a, b and c indicate the significance by least significance difference (LSD) at 0.05 level. LCK, the leaf control samples; LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TCK, the twig control group; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer.

3. Results

3.1. Characteristics of the main components of P. bournei Eos after fertilization

The total amount of Eos were 0.112% and 0.135% in the leaves and twigs of *P. bournei*, respectively. These amount were not changed significantly by the compost and compound fertilizer treatment (Table 2). The GC/MS results showed that sesquiterpenes are the main EO components in *P. bournei* leaves and twigs (Fig. 2-A and B), which are counted for >95% of the total identified compounds. As shown in Fig. 2-C and D, the most abundant component in the leaves was bicyclogermacrene, and its relative content was more than 20%; the most abundant component in the twigs was (+)-δ-cadinene, with a relative content of more than 14%. The other main components (relative content >3%) in the Eos of *P. bournei* were as follows: δ-cadinene (leaf 9.21%, twigs 14.54%), copaene (leaf 5.72%, twigs 12.70%), caryophyllene (leaf 3.09%, twigs 10.11%) and α-caryophyllene (leaf 3.46%, twigs 3.52%). (−)–Allo-aromadendrene (6.54%) was detected only in leaves, while β-eudesmol (6.20%), γ-maaliene (6.37%) and α-bergamotene (6.73%) were detected only in twigs. Under fertilization, the categories of components in *P. bournei* Eos did not change significantly, but the contents of some of the main components changed. After the application of the compost, the content of bicyclogermacrene in the leaf EO was increased significantly, while the content of other main components did not change significantly (Fig. 2-C). Except α-bergamotene and γ-maaliene decreased significantly in the twig EO, other main components did not change significantly (Fig. 2-D). After compound fertilizer treatment, the contents of bicyclogermacrene, (+)-δ-cadinene, (−)-allo-aromadendrene and α-caryophyllene in the leaf EO were significantly decreased, and only germacrene B was significantly increased (Fig. 2-C); the content of copaene in the twig EO was significantly increased, while the contents of β-eudesmol, γ-maaliene and α-bergamotene were significantly decreased (Fig. 2-D). In general, compost increased the content of

Table 3
Summary of RNA-Seq data generated for control and treatment samples.

Parameters	Leaf			Twig		
	LCK	LCF	LOF	TCK	TCF	TOF
Raw reads	54,961,676	54,893,979	50,462,023	55,167,518	52,387,199	55,604,949
Clean reads	54,372,442	54,296,221	49,863,531	54,385,905	51,757,063	54,799,294
Total mapped	98.93%	98.91%	98.81%	98.58%	98.80%	98.55%

Note: LOF, leaf samples treated with compost; LCF, leaf samples treated with compound fertilizer; TOF, twig samples treated with compost; TCF, twig samples treated with compound fertilizer; LCK and TCK samples untreated with fertilization.

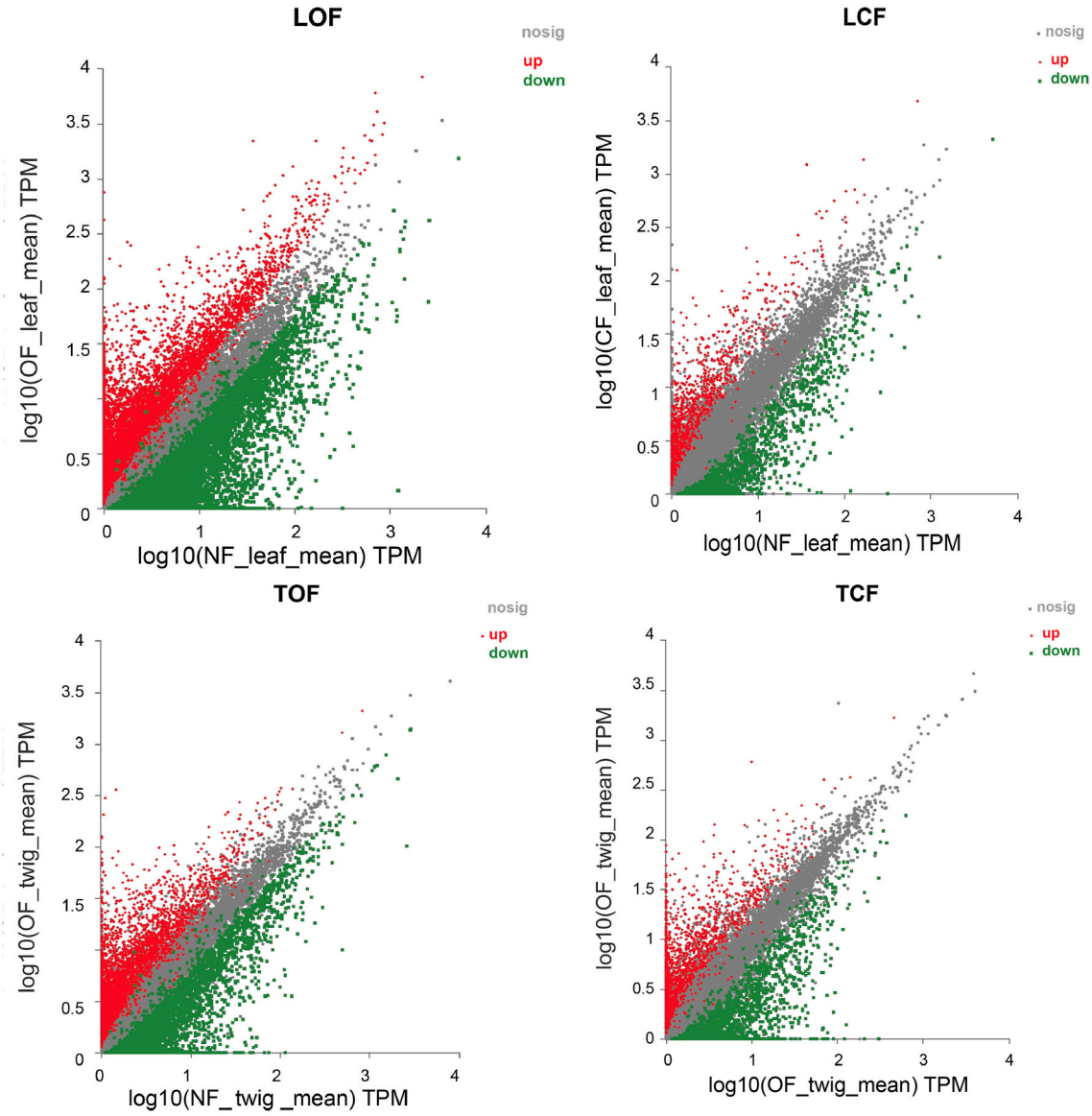

Fig. 3. Scatter plot of gene expression in samples, each dot represents a specific gene, the red is upregulated and the green is downregulated after fertilization. LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the most abundant component in the leaf EO but had no significant effects on the other components, while compound fertilizer decreased the content of the most abundant components in leaf and twig Eos.

3.2. Establishment and annotation of *P. bournei* transcriptomes

Raw data, generated by RNA-seq sequencing, were first cleaned up and low-quality reads and reads with less than 20 bp were removed. Finally, a total of more than 5.0×10^7 clean reads were obtained nearly in each sample, accounting for more than 98% of the total sequencing results (Table 3). The sequence data has been deposited in the NCBI database (Access number: PRJNA771755). After blasting the public databases (NR, Swiss-Prot, Pfam, COG, GO and KEGG), 87,595, 64,362, 62,401, 10,938, 54,370 and 42,768 unigenes were annotated respectively. After removing the duplicate genes in each database, a total of 95,682 unigenes were annotated. For all the results of the three samples

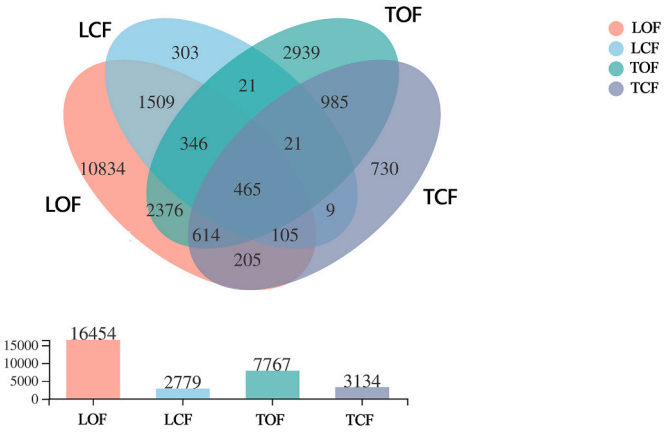

Fig. 4. The number of DEGs after fertilization. LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer.

tested repeatedly in each group, the correlation coefficients of the unigene expression in all samples were between 0.7–1, indicating that the biological repeatability of the experiment was good and that the sequencing data were accurate and reasonable (Fig. S2). Significant differences were found in the number of DEGs ($p < 0.05$) with the leaves and twigs after compost treatment showing greater number of DEGs as compared to compound fertilizer treatment (Fig. 3). There were 2779–16,454 differentially expressed genes (DEGs) annotated in the twig and leaf samples after the compost and compound fertilizer treatments. There were 465 common DEGs in LOF, LCF, TOF and TCF, and LOF had the greatest special DEGs, followed by TOF (Fig. 4).

3.3. Gene ontology annotation and KEGG pathway analysis of *P. bournei*

3.3.1. *P. bournei* GO functional annotation of DEGs

To study the functions of DEGs in the biosynthesis of Eos in *P. bournei*, GO annotation was carried out on the DEGs in the twigs and leaves of *P. bournei* under the fertilization treatments. The significant differences were observed in the number of annotated DEGs in the GO level 1 classification: cellular component (CC), molecular function (MF) and biological process (BP) (Table S3). The total proportion of upregulated and downregulated genes involved in CC was the highest in LOF, LCF, TOF and TCF. For LOF, more upregulated genes were present in BP and MF process, and almost the upregulated genes were the same as much as the downregulated ones in CC process. As for LCF, the number of up-regulated genes was nearly more twice that of down regulated genes in BP, CC and MF. For TOF, the number of upregulated genes in CC process was slightly less than that of downregulated genes, while there was no significant difference between upregulated and down regulated genes involved in BP and MF. It was different about TCF, the number of upregulated genes was less than that of downregulated genes in BP, CC and MF.

According to the GO classification of the DEGs (Fig. 5), more upregulated and downregulated genes ($p < 0.05$) were involved in the processes of binding, catalytic activity, cellular process, metallic process, cell, cellular part and membrane in the twigs and leaves after the compost treatment, compared with the compound fertilizer treatment. The number of downregulated genes involved in the processes of binding, catalytic activity, metallic process, membrane part and membrane were greater than that of upregulated genes in the leaves of *P. bournei* by the compost treatment, but the opposite happened in the cellular process, cell part and organelle (Fig. 5 - A). More downregulated genes existed in the majority of processes in the leaves under compound fertilization treatment (Fig. 5 - B), but a reverse result was observed in the twigs under compound fertilization treatment (Fig. 5 - D). The twigs treated with compost was different from the leaves treated with compost: the number of downregulated genes involved in binding, catalytic activity, membrane part and membrane were less than that of upregulated genes, but greater than that of upregulated genes in cellular process, cell part, cell, and organelle. The common DEGs in LOF, LCF, TOF and TCF were mainly existed in binding, catalytic activity, cellular process, metabolic process, membrane and membrane part (A in Fig. S3); the special DEGs of LOF and TOF were present in the same processes except "cellular component or biogenesis" instead of "nucleic acid binding factor activity", and the special DEGs of LCF and TCF were absent in some processes such as signaling (B in Fig. S3).

3.3.2. Identification of DEGs in KEGG pathways

Sesquiterpenes were the major components in Eos extracted from *P. bournei* (Fig. 3). The metabolic pathways directly related to their synthesis are the terpenoid backbone biosynthesis (TBB, map00900), sesquiterpenoid and triterpenoid biosynthesis (STB, map00909), and glycolysis/gluconeogenesis (GG) and pentose phosphate (PPP). Because the first four substrates (acetyl-CoA, acetoacetyl-CoA, pyruvate and D-glyceraldehyde-3P) in the TBB process are produced by the metabolism processes of the GG and PPP pathways (Fig. 6 and Fig. S4). Therefore, TBB can be regarded as the core metabolic pathway (Fig. 6). Fertilization had different effects on these metabolic pathways (Table 4). The percentage of DEGs involved in the four pathways was relatively high in the twigs and leaves under the compost treatment (Table 4).

TBB contains two metabolic pathways: the mevalonate (MVA) pathway and the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway [21], and involves several important enzymes (Fig. 6). According to the results of transcriptome analysis (Table 5), the expression of three genes related to 1-deoxy-D-xylulose-5-phosphate synthase (DXS) was altered in the leaves: one gene was upregulated (*ART66978.1*), and two genes were downregulated (*ART66976.1*, *ACQ66107.1*). One gene (*LOC111436948*) was probable a DXS gene. However, one downregulated DXS gene (*ART66976.1*) was only observed in the leaves under the compound fertilizer treatment. If an enzyme is regulated by two or more genes, these genes may belong to a multigene family or be part of a larger gene [36]. The expression of DXS genes is usually divided into three categories (DXS1, DXS2, DXS3), and most DXS genes are in the DXS2 category, which regulates plant secondary metabolite biosynthesis [37,38]. According to the SwissProt descriptions (Table 5), only the gene (*ACQ66107.1*) was one of genes regulating DXS2, and it could be closely related to the metabolism of sesquiterpenes in *P. bournei* (Fig. 6). The expression of this gene was downregulated only in the leaves of *P. bournei* treated with compost.

HMGCS is the first committed enzyme in the MVA pathway. The change of HMGCS expression has an important impact on the production of secondary metabolites [39]. The expression of two HMGCS genes (*LOC109728825* and *LOC109838068*) was upregulated in twigs under both compost treatment and compound fertilization, and that of the HMGCS gene (*LOC109728825*) was also upregulated in the leaves under compost (Table 5).

Another important step in the MVA pathway is mevalonate synthesis by 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR) catalyzing 3-hydroxy-3-methyl-glutaryl- CoA (HMG-CoA). The overexpression of HMGR can promote the synthesis of sesquiterpenes in plants [40]. HMGR is usually classified into HMGR1, HMGR2 and HMGR3 [41]. The overexpression of HMGR1 and HMGR3 can have a positive effect on the synthesis of secondary metabolites [41,42]. The expression level of the HMGR1 gene (*AAY87014.1*) was not altered significantly among the twigs under different treatments but clearly decreased in the leaves under the compost treatment. This indicated that the compost treatment would reduce the total amount of sesquiterpenes in *P. bournei* leaves. Similarly, the expression of the HMGR3 gene (*LOC107416066*) was decreased only in the leaves of *P. bournei* treated with compost and compound fertilizers (Table 5).

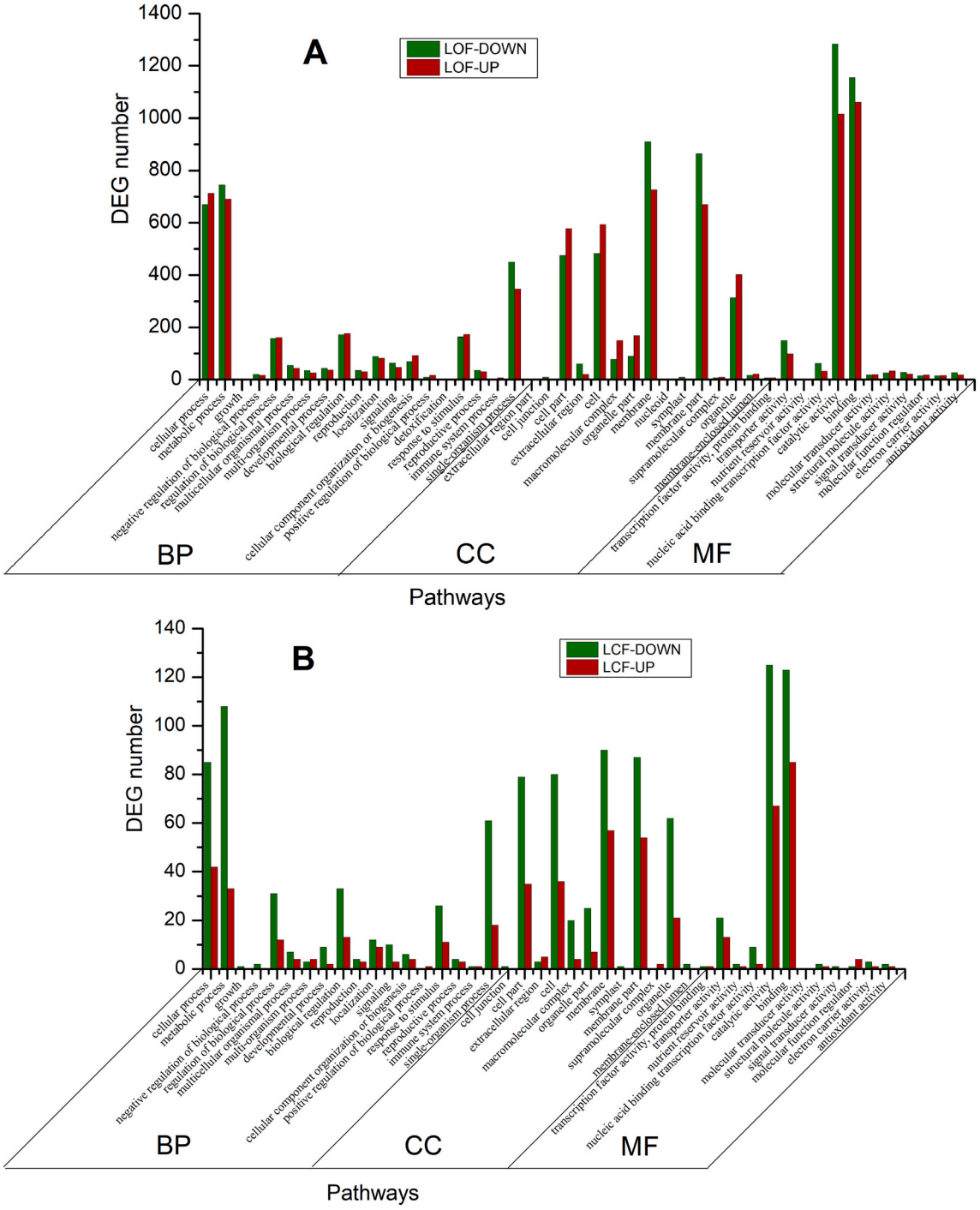

Fig. 5. Functional annotation and classification of DEGs from *P. bournei* after fertilization (p-adjust <0.05). LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer. The red columns represent the number of DEGs whose expression was upregulated after fertilization, and the green ones represent the number of DEGs whose expression was downregulated after fertilization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Farnesyl pyrophosphate synthase (FPPS) is an important branch-point enzyme whose activity can change the direction of isoprene metabolism in the TBB pathway and plays an important role in isoprene metabolism [43–45]. The expression of the FPPS gene (*ART33314.1*) was downregulated in *P. bournei* leaves and twigs under the fertilization treatment, but the most significant downregulation was present in the leaves under the compost treatment (Fig. S5). The change in the expression of the FPPS gene could be one main reason for the variation in the main components of the Eos in *P. bournei* twigs and leaves. Geranylgeranyl diphosphate synthase (GGPPS) is also an important branch-point enzyme in terpenoid biosynthesis [39]. In contrast to the expression of the FPPS gene, the expression of the GGPPS gene (*ATT59265.1*) was upregulated in both twigs and leaves under the compost treatment (Fig. S5). Therefore, changes in the expression of FPPS and GGPPS genes may be an important factor influencing the component species or contents of Eos in *P. bournei*.

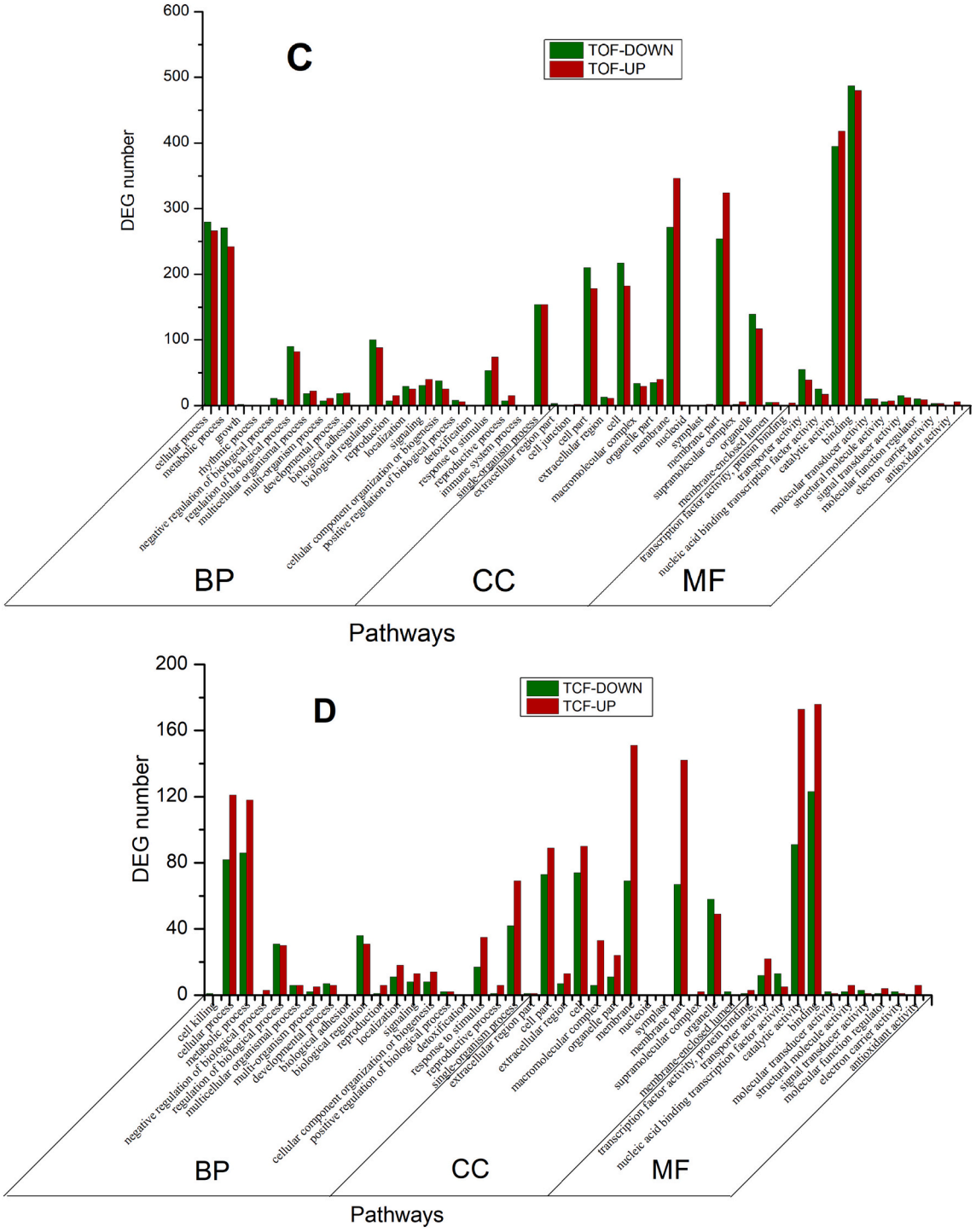

Fig. 5. (*continued*).

In addition to the TBB pathway, the synthesis of volatile components and their total amount in *P. bournei* Eos are also related to the GG and PPP pathways (Fig. S4). After fertilization, the expression of many genes regulating the two pathways were also affected. The above KEGG enrichment results revealed that the number of downregulated genes was higher than that of upregulated genes in all samples except the twigs of *P. bournei* trees treated with the compound fertilizer. Pyruvate was one of the important initial substrates in TBB pathway and was also involved in the metabolism of GG and PPP pathways. There was a significant difference in the number of DEGs regulating pyruvate with more DEGs in leaves compared to twigs after compost treatment (Fig. S6). Additionally, some genes existed in both twigs and leaves, but had different changes, such as the genes '*LOC105034612*' and '*LOC109826803*' (Fig. S4). These genes should indirectly affect the total amount and composition of *P. bournei* Eos. The expression of the two genes (*LOC110913581*, *AKQ19357.1*) were significantly altered in STB, and they are involved in the synthesis of (E,*E*-Farnesal and (−)-Germacrene D) whose content is relatively small in the essential oils and are not listed here (Fig. 6).

3.3.3. *Verification of genes expression*

The experimental results showed that there were significant changes

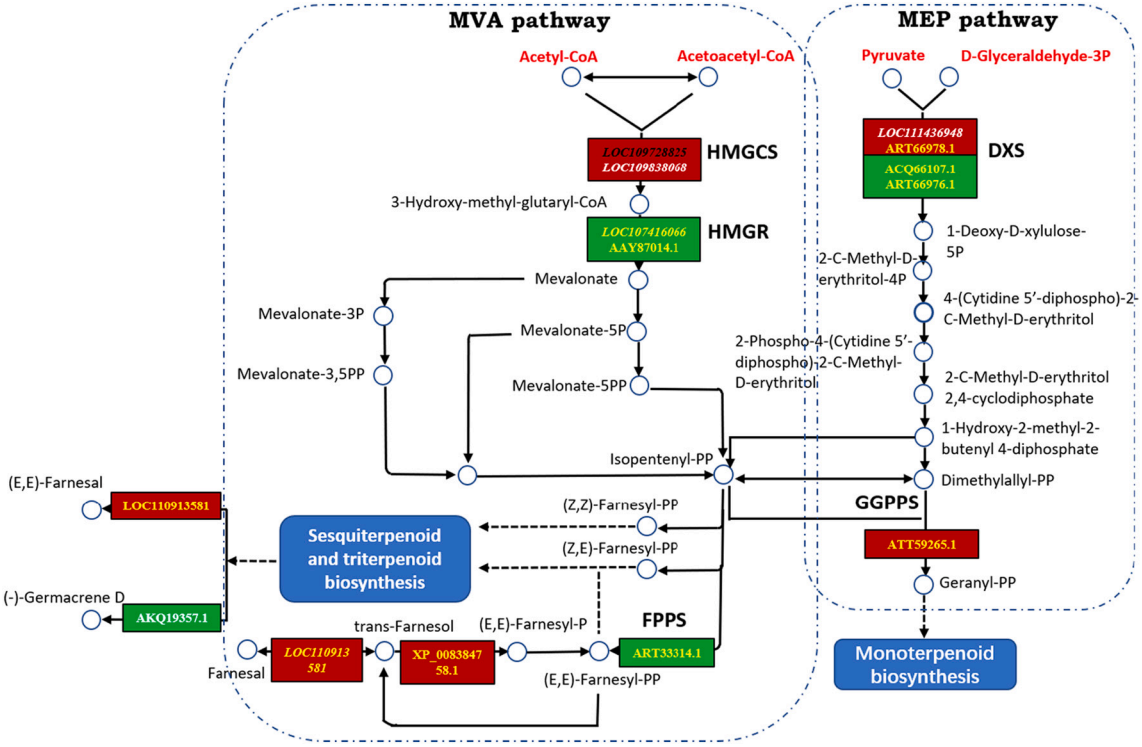

Fig. 6. Terpenoid backbone biosynthesis pathway [21]. This map was drawn with reference to the KEGG database. (https://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map00900&keyword=Terpenoid%20backbone%20biosynthesis) HMGCS: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; MVA: the mevalonate; MEP: 2C-methyl-D-erythritol 4-phosphate; HMGR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; DXS: 1-deoxy-D-xylulose-5-phosphate synthase; GGPPS: geranyl diphosphate synthase. The "yellow" in the red and green lattices represents the leaf gene, the "white" represents the twig gene, and the "black" represents a common gene of twig and leaf; the gene without gene symbol was marked with NCBI number. The expression of the genes in the red lattices are upregulated, and that of those in the green lattices was downregulated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Number of DEGs involved in sesquiterpene biosynthesis pathways after fertilization.

Groups	Pathways							
	TBB		STB		GG		PPP	
	DEGs	percentage/%	DEGs	percentage/%	DEGs	percentage/%	DEGs	percentage/%
LOF	18	9.73	1	2.78	96	6.34	55	9.89
LCF	4	2.16	0	0	17	0.11	12	2.16
TOF	4	2.16	1	2.78	26	0.09	7	1.26
TCF	4	2.16	0	0	23	0.05	5	0.9

Note: LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer; terpenoid backbone biosynthesis (TBB), glycolysis/gluconeogenesis (GG), pentose phosphate pathway (PPP), sesquiterpenoid and triterpenoid biosynthesis (STB).

in the expression of thirteen genes related to TBB and STB, and of which the five genes (TRINITY_DN60255_c0_g7, TRINITY_DN61719_c0_g1. TRINITY_DN79706_c2_g1, TRINITY_DN76855_c1_g3 and TRINITY_DN67962_c1_g3) were found to be important in the regulation of key enzymes and branching enzymes during terpenes synthesis, and therefore they were selected for real-time qPCR (RT-qPCR) analysis. The results (Fig. 7) of the qRT-PCR analysis of the selected genes were nearly consistent with the transcriptome data.

4. Discussion

4.1. The effect of nutrient enrichment on main sesquiterpenes of Eos in P. bournei

Phoebe bournei is a potential medicinal plant. Its essential oils (Eos) are mainly composed of sesquiterpenes with inhibitory activities against certain bacterium and tumor [1,2]. Fertilization can affect the composition and content of plant essential oil [46]. A certain amount of nitrogen and sulfur fertilization can significantly increase the content of *Z*-β-ocimene in *Tagetes minuta* L. essential oil [47]. Mineral fertilizer had a strong effect on the volume of essential oil in *Eryngium foetidum* L. leaves and roots [48]. The content of terpenoids in *Tanacetum vulgare* leaves changed with mineral fertilizers variation, but the terpenoid concentrations of the roots were unaffected by fertilization [49]. Here no significant changes were found in the component categories of the essential oils in *P. bournei* leaves and twigs, that is, sesquiterpenes were still the main components after the application of compost or compound fertilizer. However, compost with high organic matter content can increase the primary sesquiterpene bicyclogermacrene of the essential oil in *P. bournei* leaves. Compound fertilizer had a negative effect on bicyclogermacrene and the second most abundant sesquiterpene, (+)-δ-cadinene. Compost and compound fertilizer treatments had a

Table 5
Information on the DEGs in 'terpenoid backbone biosynthesis' and 'sesquiterpenoid and triterpenoid biosynthesis'.

Gene ID	Gene symbol/ NCBI	Swissprot Description	Log2FC	Pvalue	Pajust	Up/ Down	Group
TRINITY_DN79706_c2_g1	*LOC109728825*	Hydroxymethylglutaryl-CoA synthase	1.39516492	7.82E-03	4.90E-02	up	LOF
			1.707138136	3.68E-04	7.63E-03	up	TOF
TRINITY_DN84575_c6_g2	*LOC109838068*	Hydroxymethylglutaryl-CoA synthase	2.150896458	1.68E-03	2.41E-02	up	TOF
			2.493661561	2.26E-04	1.19E-02	up	TCF
TRINITY_DN56563_c0_g1	*ART66978.1*	1-deoxy-D-xylulose-5-phosphate synthase 1 (DXS1)	1.06898745	4.73E-04	5.48E-03	up	LOF
TRINITY_DN73974_c0_g2	*ART66976.1*	1-deoxy-D-xylulose-5-phosphate synthase 1 (DXS1)	−5.5884809	7.98E-05	1.26E-03	down	LOF
			−4.21307726	5.45E-06	1.31E-03	down	LCF
TRINITY_DN60255_c0_g7	*ACQ66107.1*	1-deoxy-D-xylulose 5-phosphate synthase 2 (DXS2)	−1.557267	2.84E-03	2.25E-02	down	LOF
TRINITY_DN70707_c0_g6	*LOC111436948*	Probable 1-deoxy-D-xylulose-5-phosphate synthase, chloroplastic	1.923531945	8.85E-07	5.23E-05	up	TOF
TRINITY_DN83281_c2_g2	*AAY87014.1*	3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 (HMGR1)	−1.92527724	9.50E-07	2.87E-05	down	LOF
TRINITY_DN76855_c1_g3	*LOC107416066*	3-hydroxy-3-methylglutaryl-coenzyme A reductase 3 (HMGR3)	−1.57011729	9.19E-06	2.04E-04	down	LOF
			−1.34758953	1.21E-03	4.35E-02	down	LCF
TRINITY_DN61719_c0_g1	*ART33314.1*	Farnesyl pyrophosphate synthase 2 (FPPS2)	−1.57943298	6.17E-05	1.02E-03	down	LOF
TRINITY_DN64139_c1_g1	*XP_008384758.1*	Probable phytol kinase 3	1.818575961	8.01E-08	3.31E-06	up	LOF
TRINITY_DN75454_c1_g3	*LOC110913581*	3 beta-hydroxysteroid dehydrogenase/Delta 5– > 4-isomerase	1.019357385	2.79E-03	2.22E-02	up	LOF
TRINITY_DN67962_c1_g3	*ATT59265.1*	Geranylgeranyl pyrophosphate synthase, chloroplastic (GGPPS)	1.132667099	1.63E-03	1.47E-02	up	LOF
TRINITY_DN64565_c0_g4	*AKQ19357.1*	TPSGD_VITVI/Terpene synthase family (Pfam)	−2.39226398	3.13E-03	3.79E-02	down	TOF

Note: LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer.

positive effect on the abundance of the second most abundant sesquiterpene, copaene, in the twig and had no effect on the most abundant sesquiterpene, (+)-δ-cadinene. The soil fertility was improved after the compost treatment with the contents of organic matter, N, P and K significantly greater than those after compound fertilizer treatment, and compared with the unfertilized group, slight changes occurred in these elements after applying compound fertilizer [50]. The changes of these elements seem to be greatly related to the synthesis of sesquiterpenes in *P. bournei* after compost treatment [28,51], because nitrogen-containing compounds are fundamental components of plant metabolism, including plant secondary metabolites [52,53], but phosphonate is an inhibitor of FPPS and HMGR [54–56], two important enzymes in the synthesis of sesquiterpenes [57]. Thus, the increase of N and P elements in the soil could probably have certain positive effects on the essential oils in *P. bournei* leaves and twigs after compost treatment, and therefore, it is necessary to implement an experiment of single nitrogen, phosphorus and potassium for clarifying the role of a single element on the yield and the components of *P. bournei* essential oils.

4.2. The effect of nutrient enrichment on the metabolism pathway of P. bournei sesquiterpenes

TBB is the basic pathway during sesquiterpenes biosynthesis [21,58,59]. 3-Hydroxy-3-methyl-glutaryl-CoA (HMG CoA) and 1-deoxy-d-xylulose-5phosphate (DXP) are two key substrates for the synthesis of sesquiterpenes (Fig. 6). DXP is produced by DXS catalyzing glyceraldehyde 3-phosphate (G3p) and pyruvate. DXS has been proven to be an important rate-limiting enzyme in the MEP pathway [21,38]. DXS is likely an important regulatory site for sesquiterpene biosynthesis in *P. bournei* twigs and leaves. The expression of genes regulating DXS is varied with the change of tissue type and environmental conditions [38]. The compost decreased the expression of DXS2 gene (*ACQ66107.1*) in the leaves [37,38]. The compost also decreased the expression of the leaf HMGCS gene catalyzing acetyl-CoA and acetoacetyl-CoA for the synthesis of HMG-CoA [60]. The expression of DXS2 and HMGCS genes decreased should be one of important factors in the decrease of the total amount of EOs in *P. bournei* leaf. In addition, compost and compound fertilizer can also make the expression of the two important branch-point enzymes (FPPS and GGPPS) genes changed [39,43–45], which should be the important factor that the components of *P. bournei* Eos were altered after fertilization treatments. The fertilization treatments also affected the GG and PPP biosynthesis pathways that provide the initial substrates for TBB biosynthesis. The number of downregulated genes in the GG and PPP pathways was greater than that of upregulated genes in all samples except the twigs under the compound fertilizer treatment, which might have indirectly affected the total amount and composition of Eos in *P. bournei*. Further experimental verification may be made by knocking out the above key genes by using some techniques such as CRISPR/cas [61,62,63].

5. Conclusions

In this study, the total amount and component changes of EOs and responding molecular mechanism to fertilization treatments were firstly identified in *P. bournei*. The total amount of EO was slightly decreased in *P. bournei* leaves and twigs under the compost and compound fertilizer treatments than that in the controls, but fertilization enhanced the total amount of main components in Eos. The content of the primary sesquiterpene, bicyclogermacrene, was increased in the leaves. The fertilization treatments had no significant effects on the total amount of

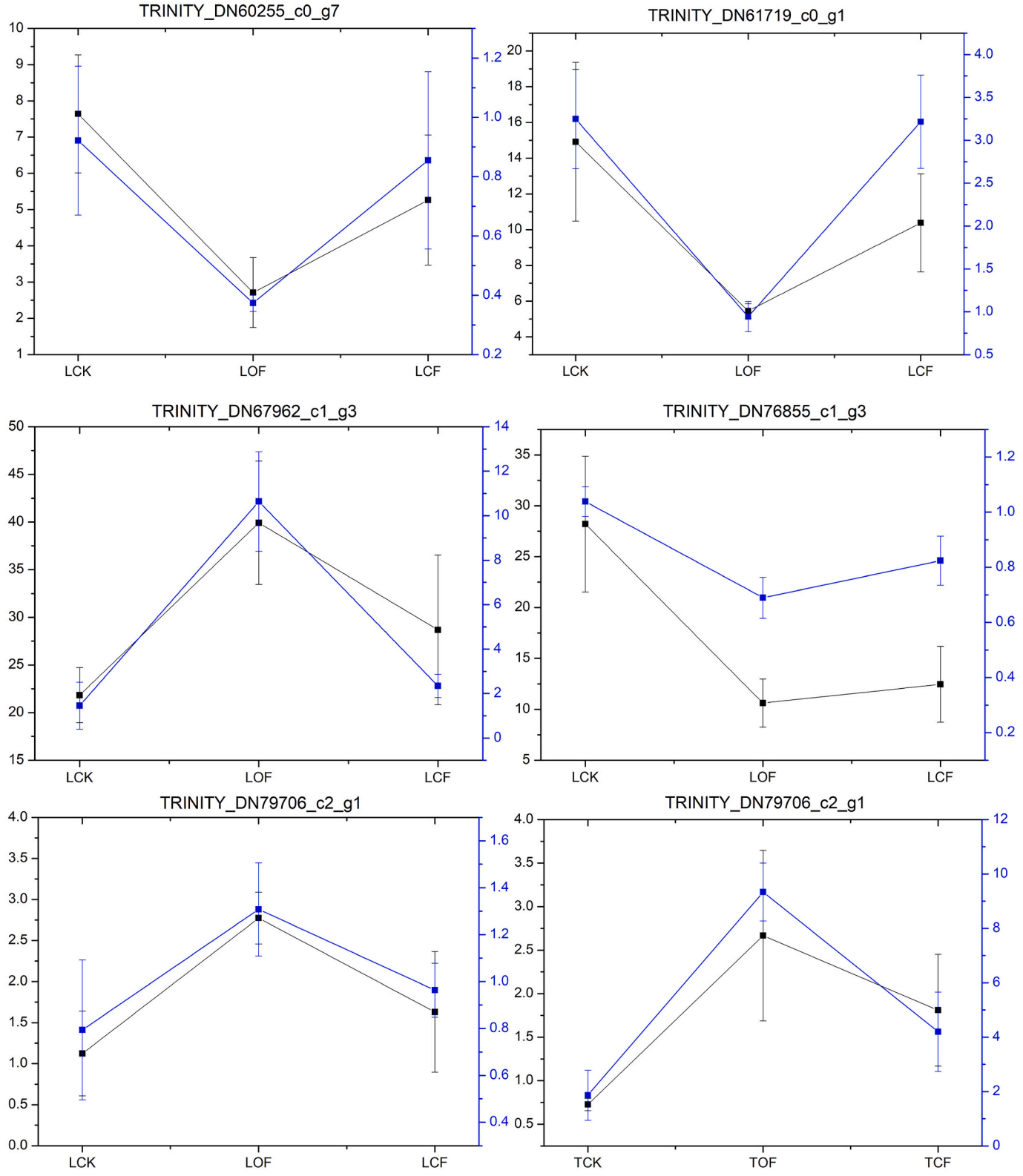

Fig. 7. qRT-PCR analysis of 5 DEGs in LCK, LOF, LCF, TCK, TOF and TCF. X-axis represents the different fertilization treatment: LCK, the leaf control samples; LOF, the leaf samples treated with compost; LCF, the leaf samples treated with compound fertilizer; TCK, the twig control group; TOF, the twig samples treated with compost; TCF, the twig samples treated with compound fertilizer. The left Y-axis represents the expression level of genes according to the FPKM value. The right Y-axis represents the expression level ($2^{-\Delta\Delta C_T}$ value) of genes. The black solid line represents the expression level of genes according to the FPKM value, and the blue solid line represents the expression level ($2^{-\Delta\Delta C_T}$ value) of genes using qRT-PCR. The qRT-PCR data are presented as the mean +/− SD of three biological replicates (each of the values is derived from the mean of three technical replicates). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

EO in *P. bournei* twigs and had a positive effect on the abundance of the second most abundant sesquiterpene, copaene, in the twigs. After the compost treatment, the high content of organic matter, nitrogen and phosphorus likely enhanced the biosynthesis of bicyclogermacrene in the leaves. The compost had different effects on plant different tissues. The experiment on the effects of single nitrogen, phosphorus and potassium on the composition and yield of Eos will be further implemented.

Abbreviations

EO	essential oil
DEGs	differentially expressed genes
TPS	terpene syntheses
HMGR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
GPP	geranyl diphosphate
FPP	farnesyl diphosphate
DXP	1-deoxy-d-xylulose-5phosphate
DXS	1-deoxy-D-xylulose-5-phosphate synthase
FPPS	farnesyl pyrophosphate synthase
GGPPS	geranyl diphosphate synthase
FPKM	fragments per kilobase of transcript per million mapped reads;
CRISPR / cas	clustered regularly interspaced short palindromic repeats associated nucleases.

Credit authorship contribution statement

Li Liu was responsible for data analysis and article writing; **Xu Wang** and **Yong Lai** participated in the experiment and data analysis; **Gongxiu He** was the manager of the project and provided financial support; **Shizhi Wen** was one of the important consultants for this project; and **Hanjie He** provided important help with the experiment and article writing; **Zhenshan Li** performed some of the fertilization, sample collection and data processing work; **Baohong Zhang** involved in the data analysis and revised the manuscript; **Dangquan Zhang** directed the data analysis and writing. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

We will provide the file in an editable file format (docx), and we are willing to share our manuscripts and related data. The experimental materials have been approved for use by the Forestry Bureau of Jindong Administration District, Yongzhou City, Hunan Province in China. Follow institutional, national or international guidelines.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

We thank Professor Zhiming Liu and Dr. Dan Cao for their revisions to the manuscript and Nature Research Editing Service for help with English usage. This research was supported by the Natural Science Foundation of China (32071752 and 32171835) and the Hunan Provincial Education Department, China (17A226). This project was also supported by the Science and Technology Program of Changsha City (kq2005002). The funders had no role in study design, data collection, analysis, decision to publish, or manuscript preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2022.110375.

References

[1] W. Ding, N. Liping, H. Xing, Z. Wei, Q. Zhoua, R. Nong, J. Chen, Essential oil extracted from leaf of Phoebe bournei (Hemsl.) yang: chemical constituents, antitumor, antibacterial, hypoglycemic activities, Nat. Prod. Res. (2018) 1–4, https://doi.org/10.1080/14786419.2018.1542393.
[2] W. Ding, L. Ning, S. Wei, H. Xing, Z.W. Li, Essential oil extracted from wood of Phoebe bournei (Hemsl.) Yang: chemical constituents, antitumor, antibacterial, and hypoglycemic activities, BioResources 14 (1) (2019) 858–869.
[3] Y. Chen, L. Liu, H. Wang, J. Ma, W. Peng, X. Li, Y. Lai, B. Zhang, D. Zhang, Environmentally friendly plant essential oil: Liquid gold for human health, Adv. Agron. 170 (2021) 289–337, https://doi.org/10.1016/bs.agron.2021.06.005.
[4] C.M. De Moraes, M.C. Mescher, J.H. Tumlinson, Caterpillar-induced nocturnal plant volatiles repel conspecific females, Nature 410 (6828) (2001) 577–580.
[5] J. Gershenzon, N. Dudareva, The function of terpene natural products in the natural world, Nat. Chem. Biol. 3 (7) (2007) 408–414, https://doi.org/10.1038/nchembio.2007.5.
[6] E.C. Palmer-Young, D. Veit, J. Gershenzon, M.C. Schuman, The Sesquiterpenes(E)-beta-Farnesene and (E)-alpha-Bergamotene quench ozone but fail to protect the wild tobacco *Nicotiana attenuata* from ozone, UVB, and drought stresses, PLoS One 10 (6) (2015).
[7] C. El-Kalamouni, P.R. Venskutonis, B. Zebib, O. Merah, C. Raynaud, T. Talou, Antioxidant and antimicrobial activities of the essential oil of *Achillea millefolium* L. grown in France, Medicines 4 (2) (2017), https://doi.org/10.3390/medicines4020030.
[8] Y.G. Kim, J.H. Lee, G. Gwon, S.I. Kim, J.G. Park, J. Lee, Essential oils and eugenols inhibit biofilm formation and the virulence of *Escherichia coli* O157:H7, Sci. Rep. 6 (2016) 36377, https://doi.org/10.1038/srep36377.
[9] F. Salehi, H. Behboudi, G. Kavoosi, S.K. Ardestani, Monitoring ZEO apoptotic potential in 2D and 3D cell cultures and associated spectroscopic evidence on mode of interaction with DNA, Sci. Rep. 7 (1) (2017) 2553, https://doi.org/10.1038/s41598-017-02633-z.
[10] T. Li, T. Holst, A. Michelsen, R. Rinnan, Amplification of plant volatile defence against insect herbivory in a warming Arctic tundra, Nature Plants 5 (6) (2019) 568–574, https://doi.org/10.1038/s41477-019-0439-3.
[11] Y. Zhou, W. Zhao, Y. Lai, B. Zhang, D. Zhang, Edible plant oil: global status, health issues, and perspectives, Front. Plant Sci. 11 (2020) 1315, https://doi.org/10.3389/fpls.2020.01315.
[12] M. Dicke, J.J. van Loon, R. Soler, Chemical complexity of volatiles from plants induced by multiple attack, Nat. Chem. Biol. 5 (5) (2009) 317–324, https://doi.org/10.1038/nchembio.169.
[13] R.A. Dixon, Natural products and plant disease resistance, Nature 411 (2001) 843–847.
[14] R.P. Ferraz, G.M. Cardoso, T.B. da Silva, J.E. Fontes, A.P. Prata, A.A. Carvalho, M. O. Moraes, C. Pessoa, E.V. Costa, D.P. Bezerra, Antitumour properties of the leaf essential oil of *Xylopia frutescens* Aubl. (Annonaceae), Food Chem. 141 (1) (2013) 196–200, https://doi.org/10.1016/j.foodchem.2013.02.114.
[15] K. Fidyt, A. Fiedorowicz, L. Strzadala, A. Szumny, beta-caryophyllene and beta-caryophyllene oxide-natural compounds of anticancer and analgesic properties, Cancer Med-Us 5 (10) (2016) 3007–3017.
[16] B.C. Gibb, 101 libations, Nat. Chem. 9 (8) (2017) 725–726, https://doi.org/10.1038/nchem.2832.
[17] J.K. da Silva, R. da Trindade, N.S. Alves, P.L. Figueiredo, J.G.S. Maia, W.N. Setzer, Essential oils from Neotropical Piper species and their biological activities, Int. J. Mol. Sci. 18 (12) (2017), https://doi.org/10.3390/ijms18122571.
[18] M.K. Swamy, M.S. Akhtar, U.R. Sinniah, Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review, Evidence-Based Complementary Alternative Med 2016 (2016) 3012462, https://doi.org/10.1155/2016/3012462.
[19] M. Sylvestre, A. Pichette, A. Longtin, F. Nagau, J. Legault, Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe, J. Ethnopharmacol. 103 (1) (2006) 99–102, https://doi.org/10.1016/j.jep.2005.07.011.
[20] Z.J. Sun, Z.X. Li, The terpenoid backbone biosynthesis pathway directly affects the biosynthesis of alarm pheromone in the aphid, Insect Mol. Biol. 27 (6) (2018) 824–834, https://doi.org/10.1111/imb.12521.
[21] J.M. Estevez, A. Cantero, A. Reindl, S. Reichler, P. Leon, 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants, J. Biol. Chem. 276 (25) (2001) 22901–22909, https://doi.org/10.1074/jbc.M100854200.

[22] J. Guo, Y. Wu, B. Wang, Y. Lu, F. Cao, G. Wang, The effects of fertilization on the growth and physiological characteristics of *Ginkgo biloba* L, Forests 7 (12) (2016) 293, https://doi.org/10.3390/f7120293.
[23] C. Silva Ade, A.F. Blank, W.M. dos Santos, P.S. Prata, P.B. Alves, F. Arrigoni-Blank Mde, Fertilization and colors of plastic mulch affect biomass and essential oil of sweet-scented geranium, Sci. World J. 2014 (2014), 828259, https://doi.org/10.1155/2014/828259.
[24] T. Van Do, N.T. Thang, V.T. Lam, D. Van Thuyet, P.D. Trung, T.H. Quy, N.T. T. Phuong, L.T.T. Huyen, N.H. Thinh, N. Van Tuan, D.T. Duc, D.T.H. Ha, D. Q. Trung, H.T. Luong, N.T.H. Anh, P. Nykiel, Monitoring fine root growth to identify optimal fertilization timing in a forest plantation: a case study in Northeast Vietnam, PLoS One 14 (11) (2019), e0225567, https://doi.org/10.1371/journal.pone.0225567.
[25] M.P. Geneva, I.V. Stancheva, M.M. Boychinova, N.H. Mincheva, P.A. Yonova, Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity, and essential oil composition, J. Sci. Food Agric. 90 (4) (2010) 696–702, https://doi.org/10.1002/jsfa.3871.
[26] A. Kasrati, C. Alaoui Jamali, R. Spooner-Hart, L. Legendre, D. Leach, A. Abbad, Chemical characterization and biological activities of essential oil obtained from mint Timija cultivated under mineral and biological fertilizers, J. Analytical Mmethods Chem 2017 (2017) 1–7, https://doi.org/10.1155/2017/6354532.
[27] S. Najafian, M. Zahedifar, Antioxidant activity and essential oil composition of *Satureja hortensis* L. as influenced by sulfur fertilizer, J. Sci. Food Agric. 95 (12) (2015) 2404–2408, https://doi.org/10.1002/jsfa.6959.
[28] D. Sugier, K. Olesinska, P. Sugier, M. Wojcik, Chemical composition of essential oil from flower heads of *Arnica Chamissonis* less. Under a nitrogen impact, Molecules 24 (24) (2019), https://doi.org/10.3390/molecules24244454.
[29] V. Tavallali, S. Rahmati, A. Bahmanzadegan, Antioxidant activity, polyphenolic contents and essential oil composition of *Pimpinella anisum* L. as affected by zinc fertilizer, J. Sci. Food Agric. 97 (14) (2017) 4883–4889, https://doi.org/10.1002/jsfa.8360.
[30] S. Seyedalikhani, J. Esperschuetz, N.M. Dickinson, R. Hofmann, J. Breitmeyer, J. Horswell, B.H. Robinson, Biowastes to augment the essential oil production of *Leptospermum scoparium* and Kunzea robusta in low-fertility soil, Plant Physiol. Biochem. 137 (2019) 213–221, https://doi.org/10.1016/j.plaphy.2019.02.008.
[31] Suchismita Raha, Seong Min Kim, Ho Jeong Lee, Sang Joon Lee, Jeong Doo Heo, Venu Venkatarame Gowda Saralamma, Sang Eun Ha, Eun Hee Kim, Mun SP, Kim GS, Essential oil from Korean *Chamaecyparis obtusa* leaf ameliorates respiratory activity in Sprague-Dawley rats and exhibits protection from NF-κB-induced inflammation in WI38 fibroblast cells, Int. J. Mol. Med. 43 (2019) 393–403.
[32] L.D. Linh, P.H. Ban, D.N. Dai, N.V. Hung, D.B. Thin, V.T. Dung, I.A. Ogunwande, Analysis of essential oils from the leaf of Phoebe paniculata (wall. Ex Nees) Nees, leaf and stem of Phoebe tavoyana (Meissn.) Hook. f. from Vietnam, J. Essential Oil Bearing Plants 22 (1) (2019) 231–238, https://doi.org/10.1080/0972060x.2019.1599735.
[33] M.G. Grabherr, 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, Full-length transcriptome assembly from RNA-Seq data without a reference genome, Nat. Biotechnol. 29 (7) (2011) 644–652, https://doi.org/10.1038/nbt.1883.
[34] L. Qiu, H. Chen, Z. Zhou, H. Zhang, R. Liu, Q. Yi, C. Yang, L. Gao, L. Wang, Transcriptomic profile of oyster *Crassostrea gigas* hemocyte after short-term cadmium exposure and bacteria stimulation, Fish Shellfish Immunol. 98 (2020) 138–146, https://doi.org/10.1016/j.fsi.2019.12.083.
[35] B. Li, CN D, RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, BMC Bioinform. 12 (323) (2011) (RSEM).
[36] M. Soltani Howyzeh, S.A. Sadat Noori, J.V. Shariati, M. Amiripour, Comparative transcriptome analysis to identify putative genes involved in thymol biosynthesis pathway in medicinal plant *Trachyspermum ammi* L, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-31618-9.
[37] H. Paetzold, S. Garms, S. Bartram, J. Wieczorek, E.-M. Urós-Gracia, M. Rodríguez-Concepción, W. Boland, D. Strack, B. Hause, M.H. Walter, The Isogene 1-deoxy-D-Xylulose 5-phosphate synthase 2 controls isoprenoid profiles, precursor pathway allocation, and density of tomato Trichomes, Mol. Plant 3 (5) (2010) 904–916, https://doi.org/10.1093/mp/ssq032.
[38] F. Zhang, W. Liu, J. Xia, J. Zeng, L. Xiang, S. Zhu, Q. Zheng, H. Xie, C. Yang, M. Chen, Z. Liao, Molecular characterization of the 1-deoxy-D-Xylulose 5-phosphate synthase gene family in *Artemisia annua*, Front. Plant Sci. 9 (2018) 952, https://doi.org/10.3389/fpls.2018.00952.
[39] Y.-R. Tong, Y.-F. Zhang, Y.-J. Zhao, T.-Y. Hu, J.-D. Wang, L.-Q. Huang, W. Gao, Differential expression of the TwHMGS gene and its effect on triptolide biosynthesis in Tripterygium wilfordii, Chin. J. Nat. Med. 17 (8) (2019) 575–584, https://doi.org/10.1016/s1875-5364(19)30059-7.
[40] A.A. Song, J.O. Abdullah, M.P. Abdullah, N. Shafee, R. Othman, E.F. Tan, N. M. Noor, A.R. Raha, Overexpressing 3-hydroxy-3-methylglutaryl coenzyme a reductase (HMGR) in the lactococcal mevalonate pathway for heterologous plant sesquiterpene production, PLoS One 7 (12) (2012), e52444, https://doi.org/10.1371/journal.pone.0052444.
[41] Gábor Nagy, Anita Farkas, Árpád Csernetics, Ottó Bencsik, András Szekeres, Ildikó Nyilasi, Csaba Vágvölgyi, T. Papp, Transcription of the three HMG-CoA reductase genes of Mucor circinelloides, BMC Microbiol. 14 (2014) 93.
[42] R. Jayashree, P.A. Nazeem, K. Rekha, S. Sreelatha, A. Thulaseedharan, R. Krishnakumar, R.G. Kala, M. Vineetha, P. Leda, U. Jinu, P. Venkatachalam, Over-expression of 3-hydroxy-3- methylglutaryl-coenzyme A reductase 1 (hmgr1) gene under super-promoter for enhanced latex biosynthesis in rubber tree (*Hevea brasiliensis* Muell. Arg.), Plant Physiol. Biochem. 127 (2018) 414–424, https://doi.org/10.1016/j.plaphy.2018.04.011.
[43] S. Anna, Po Danuta, Farnesyl diphosphate synthase; regulation of product specificity, Acta Biochim. Pol. 52 (2005) 45–55.
[44] M.K. Dhar, A. Koul, S. Kaul, Farnesyl pyrophosphate synthase: a key enzyme in isoprenoid biosynthetic pathway and potential molecular target for drug development, New Biotechnol. 30 (2) (2013) 114–123, https://doi.org/10.1016/j.nbt.2012.07.001.
[45] L. Guo, J.Q. Kong, cDNA cloning and expression analysis of farnesyl pyrophosphate synthase from Ornithogalum saundersiae. Zeitschrift fur Naturforschung C, J. Biosci. 69 (5–6) (2014) 259–270, https://doi.org/10.5560/znc.2013-0130.
[46] F. Senatore, F. Oliviero, E. Scandolera, O. Taglialatela-Scafati, G. Rosciano, M. Zaccardelli, E. De Falco, Chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp. vulgare var. azoricum (Mill.) Thell], Fitoterapia 90 (2013) 214–219, https://doi.org/10.1016/j.fitote.2013.07.021.
[47] S. Walia, R. Kumar, Nitrogen and sulfur fertilization modulates the yield, essential oil and quality traits of wild Marigold (*Tagetes minuta* L.) in the Western Himalaya, Front. Plant Sci. 11 (2020), 631154, https://doi.org/10.3389/fpls.2020.631154.
[48] T.L.M. Rodrigues, G.L.S. Castro, R.G. Viana, E.S.C. Gurgel, S.G. Silva, M.S. de Oliveira, E.H.A. Andrade, Physiological performance and chemical compositions of the *Eryngium foetidum* L. (Apiaceae) essential oil cultivated with different fertilizer sources, Nat. Prod. Res. (2020) 1–5, https://doi.org/10.1080/14786419.2020.1795653.
[49] S. Kleine, C. Müller, Differences in shoot and root terpenoid profiles and plant responses to fertilisation in *Tanacetum vulgare*, Phytochemistry 96 (2013) 123–131, https://doi.org/10.1016/j.phytochem.2013.09.018.
[50] S.O. Dahunsi, S. Oranusi, V.E. Efeovbokhan, A.T. Adesulu-Dahunsi, J.O. Ogunwole, Crop performance and soil fertility improvement using organic fertilizer produced from valorization of *Carica papaya* fruit peel, Sci. Rep. 11 (1) (2021) 4696, https://doi.org/10.1038/s41598-021-84206-9.
[51] M. Ram, D. Ram, S.K. Roy, Influence of an organic mulching on fertilizer nitrogen use efficiency and herb and essential oil yields in geranium (*Pelargonium graveolens*), Bioresour. Technol. 87 (2003) 273–278.
[52] D. Ristova, C. Carré, M. Pervent, A. Medici, G.J. Kim, D. Scalia, S. Ruffel, K. D. Birnbaum, B. Lacombe, W. Busch, G.M. Coruzzi, G. Krouk, Combinatorial interaction network of transcriptomic and phenotypic responses to nitrogen and hormones in the *Arabidopsis thaliana* root, Sci. Signal. 9 (451) (2016) rs13.
[53] B. Wen, W. Xiao, Q. Mu, D. Li, X. Chen, H. Wu, L. Li, F. Peng, How does nitrate regulate plant senescence? Plant Physiol. Biochem. 157 (2020) 60–69, https://doi.org/10.1016/j.plaphy.2020.08.041.
[54] S. Hani, L. Cuyas, P. David, D. Secco, J. Whelan, M.C. Thibaud, R. Merret, F. Mueller, N. Pochon, H. Javot, O. Faklaris, E. Marechal, E. Bertrand, L. Nussaume, Live single-cell transcriptional dynamics via RNA labelling during the phosphate response in plants, Nature Plants 7 (8) (2021) 1050–1064, https://doi.org/10.1038/s41477-021-00981-3.
[55] J. Park, V.R. Pandya, S.J. Ezekiel, A.M. Berghuis, Phosphonate and bisphosphonate inhibitors of farnesyl pyrophosphate synthases: a structure-guided perspective, Front. Chem. 8 (2020), 612728, https://doi.org/10.3389/fchem.2020.612728.
[56] B.M. Wasko, J.P. Smits, L.W. Shull, D.F. Wiemer, R.J. Hohl, A novel bisphosphonate inhibitor of squalene synthase combined with a statin or a nitrogenous bisphosphonate in vitro, J. Lipid Res. 52 (11) (2011) 1957–1964, https://doi.org/10.1194/jlr.M016089.
[57] M.J. Wassen, J. Schrader, J. van Dijk, M.B. Eppinga, Phosphorus fertilization is eradicating the niche of northern Eurasia's threatened plant species, Nature Ecol. Evolution 5 (1) (2021) 67–73, https://doi.org/10.1038/s41559-020-01323-w.
[58] J. Degenhardt, T.G. Kollner, J. Gershenzon, Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants, Phytochemistry 70 (15–16) (2009) 1621–1637, https://doi.org/10.1016/j.phytochem.2009.07.030.
[59] L.K. Henry, S.T. Thomas, J.R. Widhalm, J.H. Lynch, T.C. Davis, S.A. Kessler, J. Bohlmann, J.P. Noel, N. Dudareva, Contribution of isopentenyl phosphate to plant terpenoid metabolism, Nature Plants 4 (9) (2018) 721–729, https://doi.org/10.1038/s41477-018-0220-z.
[60] T. Bock, J. Kasten, R. Muller, W. Blankenfeldt, Crystal structure of the HMG-CoA synthase MvaS from the gram-negative bacterium *Myxococcus xanthus*, Chembiochem 17 (13) (2016) 1257–1262, https://doi.org/10.1002/cbic.201600070.
[61] D. Zhang, B. Zhang, SpRY: engineered CRISPR/Cas9 harnesses new genome-editing power, Trends Genetics 36 (8) (2020) 546–548, https://doi.org/10.1016/j.tig.2020.05.001.
[62] D. Zhang, Z. Zhang, T. Unver, B. Zhang, CRISPR/Cas: a powerful tool for gene function study and crop improvement, J. Adv. Res. 29 (2021) 207–221, https://doi.org/10.1016/j.jare.2020.10.003.
[63] H. Basim, E. Basim, H. Tombuloglu, T. Unver, Comparative transcriptome analysis of resistant and cultivated tomato lines in response to Clavibacter michiganensis subsp. michiganensis, Genomics 113 (4) (2021) 2455–2467, https://doi.org/10.1016/j.ygeno.2021.05.033.