

Transcriptome Characterization of *Panax Quinquefolius* L. seedlings in Response to Arbuscular Mycorrhizal Fungi using RNA-seq

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Abstract

Panax quinquefolius L. has been considered as an important traditional Chinese medicine with a history of more than 300 years in China. Ginsenoside is the main bioactive component. Our research group has found that the accumulation of ginsenoside could be affected by arbuscular mycorrhizal fungi (AMF). However the underlying mechanism how AMF affected the biosynthesis of ginsenoside in *P. quinquefolius* is still unclear. In this study, the RNA-seq analysis was used to evaluate the effects of AMF (*Rhizophagus intraradices*, *R. intraradices*) on the expression of ginsenoside synthesis related genes in *P. quinquefolius* root. The results indicated that a symbiotic relationship between *R. intraradices* and *P. quinquefolius* was established. RNA-seq achieved approximately 48.62 G reads of all samples. Assembly of all the reads involved in all samples produced 63420 transcripts and 24137 unigenes. Differential expression analysis was performed between the control and AMF group. A total of 111 differentially expressed genes (DEGs) in response to AMF vs control were identified, 78 and 33 transcripts were upregulated and downregulated, respectively. Based on the functional analysis, Gene ontology (GO) analysis revealed that most DEGs were related to stress responses and cellular metabolic processes. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis identified transduction, plant hormone signal transduction and terpenoids and polyketides biosynthesis pathways. Furthermore, the expression of glycolysis-related genes and ginsenoside synthesis related genes was largely induced by AMF. In conclusion, our results comprehensively elucidated the molecular mechanism how AMF affected the biosynthesis of ginsenoside in *P. quinquefolius* by transcriptome profiling.

Introduction

Panax quinquefolius L., which belongs to the Araliaceae family, has been commonly known as American ginseng and considered as one of the most valuable traditional Chinese medicines with a history of more than 300 years in China (China Pharmacopoeia Committee 2015; Schlag and McIntosh 2013). American ginseng has been recorded as "cool" and listed as superior with a tonic effect in "Chinese Materia Medica" (Schlag and McIntosh 2013; Dharmananda 2002). Ginsenoside (Rg₁, Rb₁, Re and Rb₂) has been regarded as the main bioactive ingredient of American ginseng and important index for evaluating the quality of American ginseng (Wang et al. 2014; Shi et al. 2013). Modern pharmacology researches have proved its extensive effects of pharmacological, including antitumor, anti-stress, anti-ageing, anti-fatigue, cardioprotective and hepatoprotective properties (Shi et al. 2013; Lu et al. 2016; Wang et al. 2016; Chen et al. 2007). In the past few years, American ginseng has been widely used in medicine, food and cosmetic products (Sharma and Pandit 2009; Dong et al. 2016; Zhou et al. 2019). With the increasing demand for American ginseng, it is important to take effective measures to improve the accumulation of ginsenoside in *P. quinquefolius*.

Arbuscular mycorrhizal fungi (AMF) is one of the symbiotic microorganisms of soil that can form mutualistic symbiotic associations with more than 80% of terrestrial plant species (Bouwmeester et al. 2007). It has been well investigated that inoculation with AMF could improve plant growth and crop productivity (Liu et al. 2013; Chang et al. 2018; Zhang et al. 2019). The biosynthesis of secondary

metabolites such as phenolic acids, flavonoids, alkaloids, essential oil and etc in plants was also affected by AMF, which has attracted extensive attention in recent years (Zhu et al. 2015; Raghuwanshi and Sinha 2014; Rydlová et al. 2016). The expression of related genes involved in secondary metabolic pathway was regulated by AMF, resulting in a significant increase in the content of active components in medicinal plants (Rydlová et al. 2016; Andrade et al. 2013). For instance, the expression of genes encoding tryptophan decarboxylase (TDC), strictosidine synthase (STR), deacetylindoline (DAT), putrescine n-methyltransferase (PMT) in the biosynthesis of alkaloid in *Catharanthus roseus* was significantly improved by AMF (Andrade et al. 2013). Zhang et al. (2020) found that AMF could promote the accumulation of flavonol-glycosides by regulating the expression of phenylalanine ammonia-lyase (PAL), 4-coumarate CoA ligase (4CL), chalcone synthase (CHS), glucosyltransferase (GT) and rhamnosyltransferase (RT) genes in flavonoid biosynthesis in *Anoectochilus roxburghii*. In genus of *Panax*, it has been observed that the content of total ginsenoside in *Panax ginseng* was increased by *Glomus intraradices* (Tian et al. 2019; Cho et al. 2009). While the effects of AMF on the accumulation of ginsenosides in *P. quinquefolius* have been poorly understood. Our research group has showed that a positive correlation between the mycorrhizal colonization (*Rhizophagus intraradices*) and the content of ginsenoside in *P. quinquefolius* has been successfully obtained based on the field investigation (Ran et al. 2020). Here, we hypothesized that AMF could improve the biosynthesis of ginsenoside by regulating the expression of ginsenoside synthesis related genes, which might be a new effective strategy to promote the quality of American ginseng.

Transcriptome sequencing technology (RNA-Seq) is a new technology used to explore new gene pathways and mechanisms, which possesses the advantages of higher sensitivity and the ability to detect splicing isomers and somatic mutations (Lou et al. 2020; Zhang et al. 2013). It has been extensively employed in the study of genome-wide gene expression in living organisms (Jung et al. 2020; Li et al. 2020; Wang et al. 2019). Recently, transcriptome analysis has been widely reported on the detection of whole transcripts of AMF and non-AMF colonized plants, and identified the differential expression of plant genes induced by AMF (Frenzel et al. 2005; Zouari et al. 2014; Handa et al. 2015). Based on the RNA-Seq data, Mandal et al. (2015) signified that compared with non-AMF, AM symbiosis upregulated the transcription of eleven steviol glycosides (SGs) biosynthesis genes. Buisson et al. (2016) showed that the expression of phenylalanine ammonia-lyase (PAL), stilbene synthase (STS) and ROMT (encoding a resveratrol O-methyltransferase) genes involved in the stilbenoid biosynthesis pathway was up-regulated in Grapevine by inoculation with AMF using the RNA-Seq. However the underlying molecular mechanism that how AMF influences the secondary metabolites of *P. quinquefolius* has been rarely reported. Therefore, in this study, the AMF (*R.intraradices*) and *P. quinquefolius* were used to establish the AM symbiosis, and the molecular mechanism of AMF improving the accumulation of ginsenoside in *P. quinquefolius* will be studied using the transcriptome technique, which will provide basic data for the elaboration of the mechanism of AMF regulated secondary metabolites.

Materials And Methods

Experimental materials and design

The mycorrhizal fungus *Rhizophagus intraradices* (*R.intraradices* (No. BGC BJ09)) used in this study was purchased from the Institute of Plant Nutrition and Resources, Beijing Academy of Agricultural and Forestry Sciences, China. The inoculum was composed of spore (approximately 500 spores per gram inoculum), hypha, host root segment and culture medium.

Seeds of *P. quinquefolius* were sterilized with 10% H₂O₂ for 30 min, washed with sterilized water for three times, and then germinated at 25°C in an incubator after soaking for 24 h. There were two treatments in the present study, including control and AMF treatment (*R. intraradices*). For treatments, a mixture of soil and sand (2:1, V:V) was used as growth medium, then autoclaved at 121°C, 0.10 MPa for 2 h to kill indigenous AMF propagules and other microorganisms. The selected soil belongs to the Meadow soils (Semiaqueous soil Order) according to the China soil classification system. Approximately 3.50 kg of the medium and 30 g of inoculum were mixed, put in a plastic pot (180×145×155 mm) for the growth of *P. quinquefolius*. There were 3 replicates of each treatment. The non-AMF pot was added with the same amount of inactivated inoculum. We also added 30 ml of filtered inoculant (0.25mm filter membrane), which was free from mycorrhizal propagules to the non-AM treatment to provide the same microorganism biota. The plants were grown in a day/night temperature of 25/20°C, a relative day/night humidity of 70/65%, a day/night of 16/8 h and a photosynthetic photon flux density (PPFD) at the height of the plant of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light was provided by a fluorescent lamp. Pots were repositioned weekly to reduce environmental error. All pots were irrigated with 100 ml Hoagland solution with 1/10 P (0.1 mM) strength. The fresh roots of *P. quinquefolius* seedlings were harvested at 6 months old. Each plant of *P. quinquefolius* was divided into branch root and taproot. The branch root of each *P. quinquefolius* was used to detect the colonization of AMF and the corresponding taproot was further divided equally into two parts, an aliquot of the taproot was used to determine the content of ginsenoside, and another aliquot was frozen in liquid nitrogen and stored at -80°C for further analysis.

The analysis of AMF colonization in roots of *P. quinquefolius*

The AMF colonization in roots of *P. quinquefolius* was determined according to the method reported by Shu et al. (2016). The branch roots were removed from the FAA (Formalin 5 ml + Acetic Acid 5 ml + Alcohol 90 ml) solution, washed several times with distilled water, and then placed in a test tube. Subsequently, root samples were added with 10% (w/v) KOH at 99°C for 1.5 h, stained with 0.05% (w/v) trypan blue, and estimated the mycorrhizal colonization using a modified line intersect method (Rufykir et al. 2000; McGonigle et al. 1990). 30 root segments per root sample were observed for the presence of AMF structures with repeated three times. The degree of root infection by AMF was assessed using a light microscope (Olympus-BH-2, Japan). The AMF infection was calculated according to the following formula: frequency of AM fungal colonization = (Infected root segments / All root segments)×100%. The AMF colonization intensity according to the following formula: AMF colonization intensity =

$(95 \times n_5 + 70 \times n_4 + 30 \times n_3 + 5 \times n_2 + n_1) / \text{All root segments} \times 100\%$, n_5 represented the number of root segments infected at level 5; n_4 represented the number of root segments infected by level 4; n_3 represented the number of root segments infected at level 3, and so on.

Analysis of the content of ginsenoside

The content of ginsenoside was conducted using the methods reported by Liu et al. (2014) with slightly changes. A total of 0.50 g air-dried *P. quinquefolius* roots were ground into a powder, with 50 ml methanol for extraction. The extracts were obtained with ultrasound for 40 min, and then left to stand for 10 min. The supernatant was filtered with a 0.45 mm microporous membrane and injected with 10 ul for HPLC analysis. The content of ginsenoside was determined using a Kromasil C₁₈ (4.60×250 mm, 5 mm) column. The mobile phase solution was (A) acetonitrile and (B) 0.10% phosphoric acid water with a speed of 1.00 ml min⁻¹ and the following gradient program was used: 0-25 min, 19-20% A; 25-60 min, 20-40% A; 60-90 min, 40-55% A; 90-100 min, 55-60% A. The wavelength was detected at 203 nm, and the temperature of column was set at 40°C. The content of ginsenoside was calculated according to the regression equation of standard curve.

Total RNA Extraction, cDNA library construction and RNA Sequencing

Total RNA was extracted from control and AMF treated root samples using the RNA prep Pure Plant Kit (Tiangen Biotech Co., Ltd., Beijing, China) based on the manufacturer's manual. DNA was then removed using the RNAClean Kit (Tiangen, Beijing, China). The quality and quantity of total RNA was assessed with the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). The library construction and RNA-Seq assay were performed by the Novogene Biotechnology Corporation (Beijing, China). The cDNA library was constructed using A TruSeq™ RNA Sample Preparation Kit (Illumina, Inc.). First, Poly-(A)-containing mRNA was purified from the total RNA using oligo (dT) magnetic beads and Oligotex mRNA kits (Qiagen, Germany), following the manufacturer's instructions. Fragmentation was carried out using divalent cations. Fragmentary RNAs were used as template for first strand cDNA synthesis by an cDNA preparation kit (Illumina, San Diego, CA, USA).

Second-stranded cDNA was synthesized using RNase H and DNA polymerase I. Then cDNAs were subjected to end-repair, phosphorylation, and ligation to sequencing adapters. Afterward, the products enriched by PCR amplification were purified through 2% agarose gelelectrophoresis and quantified by TBS380 (Picogreen). Finally, cDNA libraries were subsequently sequenced using an Illumina HiSeq™ 2000 platform.

Transcriptome assembly, annotation and function enrichment

Before assembly, raw data were filtered to remove reads containing adapter, reads containing ploy-N, and low quality reads to generate highquality clean data. At the same time, Q20, Q30, GC-content, and sequence duplication level of the clean data were calculated. After removing the low quality reads, a denovo strategy was used to assemble the clean reads into distinct contigs using Trinity assembly software (Grabherr et al. 2011). All of the contigs were clustered, and the contigs with the longest sequences were defined as unigenes. For functional annotation, the unigenes, through a BLASTall algorithmbased program with a threshold of E-value $< 10^{-10}$, were searched against different databases, including NCBI non redundant protein sequences (Nr), NCBI non-redundant nucleotide sequences (Nt), Protein family (Pfam), Protein primogenomic cluster (KOG/COG), Artificial annotated and reviewed protein sequence database (Swiss-Prot), KEGG Ortholog database (KO), Gene Ontology (GO).

Analysis of differential genes

The expression level of gene was determined using the the reads per kilobase per million mapped reads (RPKM) method (Trapnell et al. 2010) by RSEM software (Dewey and Li 2011). The transcript RPKM values were estimated using RNA-Seq by Expectation Maximization (RSEM) with Bowtie read mapping. Differentially expressed genes (DEGs) analysis in pair-wise comparisons was conducted using the DESeq software (Anders and Huber 2010), and the input data of the differentially expressed genes (DEGs) was based on the read counts. The false discovery rate (FDR) method was applied to correct the threshold of the P values in multiple tests for identifying the differences between two groups. A FDR < 0.001 and an absolute value of $\log_2(\text{ratio}) > 2$ were applied as thresholds to identify significant differences between two groups (Mortazavi et al. 2008). Furthermore, the DEGs were then analyzed through GO and KEGG pathway enrichment analyses.

Simple sequence repeats (SSRs) detection and primer design

In order to check SSRs in *P. quinquefolius*, the microsatellite identification tool (MISA) was used to identify the SSR. The parameters were used to distinguish di-, tri-, tetra-, penta- and hexa nucleotide motifs with the lowest limit of repeats of 6, 5, 4, 4 and 4 respectively, and Primer 3 software was used to devise primers for each SSR. The design parameters of major primer were set as follows: 100 to 300 nt of PCR products, 18 to 24 nt of primer lengths, 60°C optimal annealing temperature, and 40% to 65% of GC content from (Zeng et al. 2019).

Quantitative real-time RT-PCR

A total of 5.0 ug RNA of each treatment root sample was used for first-stand cDNA synthesis using the RNA prep Pure Plant Kit (Tiangen Biotech Co., Ltd., Beijing, China) based on the manufacture's instructions. The synthesised cDNA was used as a template for quantitative real-time polymerase chain reaction (qRT-PCR). The qRT-PCR reaction system with 20 ml volume of reaction mixture made up of 2 ml of dilute template cDNA, 2 ml of primer pairs, 10 ml of GoTaq® qPCR Master Mix (Promega, USA), and 6 ml of deionized water. The setting program of qPCR reaction was carried out as follows: denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing at 57°C for 1 min, 72°C for 50 s and then extension at 72°C for 1 min (Wang et al. 2016). After the reactions, the specificity was assessed by melt curve and size estimation of the amplified product. Each gene was quantified using three biological replicates. The relative expression of the selected genes was normalized by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). β -Actin was used as internal standard and was amplified with the forward primer 5'-AGGAACCACCGATCCAGACA-3' and reverse primer 5'-GGTGCCCTGAGGTCCTGTT-3'. The gene primers used for RT-PCR are shown in Table S1.

Statistical analysis

The data were subjected to analysis of one-way ANOVA using Statistical Product and Service Solutions 17.0 software (SPSS Institute Inc. Chicago, IL, USA) and differences were compared by Duncan's test with a significance level of $P < 0.05$.

Results

AM colonization of *P. quinquefolius* roots

The colonization of AMF in the *P. quinquefolius* roots is shown in Fig. 1. Roots of inoculated *P. quinquefolius* were extensively colonized with treatment of *R. intraradices*. Excellent symbiotic relationship between *R. intraradices* and *P. quinquefolius* was established with the formation of vesicles and hypha. The frequency of AM fungal colonization and AMF colonization intensity in *P. quinquefolius* root reached 87.78% and 57.50% under the *R. intraradices* inoculation respectively (Fig S1). No mycorrhizal colonization was observed in control.

Changes in ginsenoside content under AMF inoculation

The content of ginsenosides, including Rb₁, Rg₁, Re and Rb₂, was determined during the AMF inoculation (Fig. 2). The content of Rb₁ was significantly increased by *R. intraradices*, which was estimated as 26.15% higher ($P < 0.05$) than that of control respectively. It was observed that the content of Re was also markedly enhanced by 22.38% ($P < 0.05$) by *R. intraradices*. No significant differences in content of Rg₁ and Rb₂ was detected between non-AMF and AMF colonized plant ($P > 0.05$).

Sequencing, assembly and splicing

A total of 172600168 raw reads in AMF inoculation and 156278548 raw reads in the control were obtained. After filtering out the low-quality reads, there were 170350412 clean reads in the AMF inoculation and 153757750 clean reads in the control. A total clean base number of 25.55 G (AMF) and 23.07 G (the control), was obtained respectively. The quality of clean reads included an error of less than 0.03%, the percentage of Q30 base was 92.21% or higher, and the G/C content was approximately 43%. 267266 transcripts were obtained by Trinity software with average lengths of 1291 bp and N50 lengths of 1854 bp (Table 1 and Fig S2a). Furthermore, 75031 unigenes with a mean length of 1137 bp and N50 length of 1729 bp were assembled. Among these unigenes, 24137 (32.17%) were within the range of 300–500 bp, 22444 (29.91%) were within 500 bp-1 kbp, 16394 (21.85%) were within 1–2 kbp, and 12056 (16.07%) were longer than 2 kbp (Table 2 and Fig S2b).

Table 1
Summary of sample sequencing data quality

sample	raw_reads	clean_reads	clean_bases	error_rate	Q20	Q30	GC_pct
Control-1	58667970	57649802	8.65 G	0.03	97.33	92.35	42.72
Control-2	47966440	47178500	7.08 G	0.03	97.25	92.21	42.60
Control-3	49644138	48929448	7.34 G	0.03	97.44	92.55	42.69
<i>R. intraradices</i> -1	60821978	60025026	9.00 G	0.03	97.58	92.93	42.54
<i>R. intraradices</i> -2	47867660	47239488	7.09 G	0.03	97.48	92.76	42.79
<i>R. intraradices</i> -3	63910530	63085898	9.46 G	0.03	97.45	92.55	42.53

Table 2
Summary of splicing transcripts

Length_interval	300 bp-500 bp	500 bp-1 kbp	1 kb-2 kbp	> 2 kbp	Total
Number of transcripts	63420	75415	75718	52713	267266
Number of Unigenes	2i4137	22444	16394	12056	75031

Functional annotation and classification

In order to obtain comprehensive gene function information, the unigenes were successfully annotated using seven public databases (Nr, Nt, Pfam, KOG/COG, Swiss-prot, KEGG, GO). As shown in Table 3, out of 75031 unigenes, 45199 genes (60.24%) were annotated in at least one database, and 5112 genes (6.81%) were annotated in all seven databases. The annotation success rate of unigenes was 38341 in

NR (51.10%), 30185 in NT (40.23%), 14586 in KO (19.44%), 27435 in SwissProt (36.56%), 26802 in PFAM (35.72%), 26802 in GO (35.72%), and 9165 in KOG (12.21%). The functions of the predicted unigenes were classified in KOG, GO and KEGG.

KOG annotation showed that a total of 10315 unigenes were classified into 26 categories (Fig. 3). Within these genes, the cluster of post translational modification represented the largest group (O) (1353, 13.12%), followed by translation, ribosomal structure and biogenesis (J) (1245, 12.07%), general function prediction only (R) (938, 9.26%), RNA processing and modification (688, 6.67%) and intracellular trafficking, secretion, and vesicular transport (660, 6.40%). In addition, 121 unigenes (1.17%) were related to secondary metabolites biosynthesis, transport and catabolism and 79 as defense mechanism unigenes (V).

All unigenes were classified into three GO categories, including biological process (BP), cellular component (CC), and molecular function (MF) (Fig. 4). For the biological process category, the majority of the GO terms were assigned to cellular processes (2487) and metabolic processes (1098). With the cellular components category, 953 and 2803 were classified to cell and cell parts respectively. For molecular function, the assignments were mostly binding (675) and catalytic activity (1013). Transcripts related to GO term binding were abundant in the molecular function category.

KEGG database was used to systematically analyze the gene function associated with genomic information. For AMF inoculation, a total of 13806 transcripts were classified into 19 KEGG pathways (Fig. 6). As shown in Fig. 5, the majority of KEGG assigned transcripts were involved in transduction (1569), a large pool of transcripts were attributed to the area of carbohydrate metabolism biosynthesis (1201), additional transcripts were mapped to the area of lipid metabolism biosynthesis (651) and metabolism of terpenoids and polyketides biosynthesis (318).

Table 3
Function annotation of Unigene

Database	Number.of.Unigenes	Percentage
Annotated in NR	38341	51.10
Annotated in NT	30185	40.23
Annotated in KO	14586	19.43
Annotated in SwissProt	27435	36.56
Annotated in PFAM	26802	35.72
Annotated in GO	26802	35.72
Annotated in KOG	9165	12.21
Annotated in all Databases	5112	6.81
Annotated in at least one Database	45199	60.24
Total Unigenes	75031	100

Analysis of SSRs

In order to further evaluate the quality of assembly, MISA software was used to perform simple sequence repeat (SSR) analysis based on the selected unigene (length > 1 kb). We successfully screened 4958 SSRs, including mono-, di-, tri-, tetra-, penta- and hexa-nucleotide SSRs (Fig. 6). The di-nucleotide SSR has been regarded as the most abundant, followed by mono- and tri-nucleotide SSRs. Table S2 has showed a series of primer pairs for each SSR that match the primer design parameters to further investigate the potential of these SSRs as genetic markers.

Identification and analysis of DEGs

A total of 111 DEGs (78 up-regulated, 33 down-regulated) were identified in the AMF vs control comparison (Table 4). To further understand the specific processes at the transcriptional level as well as the gene functions and interactions, GO and KEGG enrichment analyses were performed in this study. Based on the GO enrichment, DEGs were significantly (KS value ≤ 0.05) enriched in 10 terms of biological processes (Table 5). These enriched terms were highly linked to biological processes in the adaptive response to AMF inoculation, such as homoserine metabolic process (GO:0009092), L-methionine biosynthetic process (GO:0071265) and L-methionine biosynthetic process from L-homoserine via cystathionine (GO:0019279). Further genes were identified from the DEGs data by KEGG annotations, which might be assigned to nine pathways in the KEGG database. The KEGG pathway analyses revealed that the most significant metabolic pathways enriched and the most enriched differential genes were Plant hormone signal transduction and Ribosome (Fig. 7). Additionally, Venn diagrams were constructed for displaying the number of shared and exclusively expressed genes between the AMF and control groups. There were 68541 and 66759 expressed genes were detected from the AMF group and control

group, respectively. Among them, 61315 were shared, 7227 were exclusive to the AMF treatment, and 5445 were presented only in the control (Fig. 8).

Table 4
Differences of DEGs in *P. quinquefolius* roots system

Compare	all	up	down	threshold
<i>Ri</i> vs Control	111	78	33	DESeq2 padj<0.05 log2FoldChange >1

Table 5
The GO classification of enriched DEGs using the topGO tool

GO_accession	Description	Term type	DEG item	P-Value
GO:0071266	de novo' L-methionine biosynthetic process	biological_process	2	0.00004124
GO:0009067	aspartate family amino acid biosynthetic process	biological_process	2	0.0089157
GO:0006541	glutamine metabolic process	biological_process	2	0.0036623
GO:0009092	homoserine metabolic process	biological_process	2	0.00004124
GO:0071265	L-methionine biosynthetic process	biological_process	2	0.00004124
GO:0019281	L-methionine biosynthetic process from homoserine via O-succinyl-L-homoserine and cystathionine	biological_process	2	0.00004124
GO:0019279	L-methionine biosynthetic process from L-homoserine via cystathionine	biological_process	2	0.00004124
GO:0009086	methionine biosynthetic process	biological_process	2	0.00033687
GO:0006555	methionine metabolic process	biological_process	2	0.0055536
GO:0000097	sulfur amino acid biosynthetic process	biological_process	2	0.00057345
GO:0048046	apoplast	cellular_component	2	0.002188
GO:0005507	copper ion binding	molecular_function	3	0.0030746
GO:0004819	glutamine-tRNA ligase activity	molecular_function	1	0.010066
GO:0008899	homoserine O-succinyltransferase activity	molecular_function	2	0.00004124
GO:0008374	O-acyltransferase activity	molecular_function	2	0.009336
GO:0016750	O-succinyltransferase activity	molecular_function	2	0.00004124
GO:0016715	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced ascorbate as one donor, and incorporation of one atom of oxygen	molecular_function	1	0.0080787
GO:0016748	succinyltransferase activity	molecular_function	2	0.00055321
GO:0016747	transferase activity, transferring acyl groups other than amino-acyl groups	molecular_function	4	0.0089139

GO_accession	Description	Term type	DEG item	P-Value
GO:0016762	xyloglucan:xyloglucosyl transferase activity	molecular_function	2	0.002188

Identification of glycolysis-related DEGs

Glycometabolism has been regarded as an important primary metabolic process. A large number of DEGs in *P. quinquefolius* were homologous with well-known related genes of glycosylsis in model plants. In detail, seven genes encoding for hexokinase (HK), two genes encoding for phosphoglycerate isomerase (PGI), four genes encoding for 6-phosphofructokinase (PFK), eight genes encoding for fructose-bisphosphate aldolase (FBA), four genes of triosephosphate isomerase (TPI), 11 genes of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), two genes of phosphoglycerate kinase (PGK), three genes encoding for probable phosphoglycerate mutase (GPM), six enolase (ENO) genes and ten pyruvate kinase (PK) genes were identified in *P. quinquefolius* (Fig. 9). Moreover, the changes of glycolysis-related genes expression with AMF treatment were analyzed. The result showed that most of the genes involved in glycolysis were largely up-regulated during the AMF inoculation, suggesting that AMF could induce the rising of glycolysis in *P. quinquefolius*.

Identification and analysis of DEGs

Genes involved in biosynthesis of ginsenoside with AMF treatment were retrieved from the DEG group according to the KEGG annotation. As shown in Fig. 10, the analysis of the transcriptome data showed that 29 unigenes coded for six enzymes in the mevalonate (MVA) pathway were located in the cytoplasm and that 25 unigenes coded for seven enzymes in the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway were located in plastid, which synthesized the common precursor isopentenyl diphosphate (IPP) for downstream terpenoid pathways. In MVA pathway, four genes of Acetyl-CoA acetyltransferase (AACT), four hydroxymethylglutaryl-CoA synthase (HMGS) genes, eight genes coded for hydroxymethylglutaryl-CoA reductase (HMGR), one mevalonate kinase (MVK) gene, six genes coded for dioxiphosphate methylhydroxyvalerate kinase (PMK), one diphosphomevalonate decarboxylase (MVD) gene, were identified in *P. quinquefolius*. In MEP pathway, eight encoding genes of 1-deoxy-D-xylulose-5-phosphate synthase (DXS), three 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) encoding genes, one 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase (CMS) gene, two genes coded for 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MCS), four genes of HDS and five IDS genes were identified in *P. quinquefolius*. One unigene was predicted to code for isopentenyl-diphosphate delta-isomerase (IPPI), which catalyzes the isomerization of IPP to dimethylallyl diphosphate. Seven geranylgeranyl diphosphate synthase GPS genes, three squalene e-poxidase (SS) genes and seven squalene synthetase (SE) genes were identified in downstream terpenoid pathways (Fig. 10a). For the key enzyme genes involved in MVA pathway (Fig. 10b), the result revealed that a number of genes were up-regulated during AMF inoculation,

especially, the expressions of HMGR, GPS and SS genes were induced nearly two-fold with AMF inoculation. The key enzymes of the MEP pathway did not change significantly between the AMF and control (Fig. 10c). These data indicated that AMF might promote the ginsenoside biosynthesis and the formation of secondary metabolites of *P. quinquefolius* mainly activate MVA pathway.

qRT-PCR validation analysis

In order to verify the changes of expression level of several key enzymes in ginsenoside biosynthesis, a qRT-PCR assay was conducted. A total of 9 key enzymes genes (HMGR, PMK, GPS, FPS, DXS, DXR, SS, SE and DS) were chosen to test the RNA-seq data in AMF treatment (Fig S3). As shown in Fig S3, the results indicated that the expression of HMGR, GPS, SS and SE was significantly increased ($P < 0.05$) with AMF inoculation, which was approximately 1.33, 1.56, 1.47 and 1.35-fold higher than that in control respectively. Compared with control, the expression of DXS and DXR showed an increasing trend by treatment of AMF, while there was no obvious difference ($P > 0.05$). The results obtained from the qRT-PCR analysis revealed that unigenes acquired from the assembled transcriptomes and the expression of gene profiles from RNA-Seq data were reliable.

Discussion

Previous studies have shown that AMF colonization could lead to the changes of morphological and functional in host roots, and then affected plant growth (Geneva et al. 2010; Subramanian and Charest 1997; Armstrong and Peterson 2002). Our research group has found that *Rhizophagus intraradices* was widely distributed in the root of *P. quinquefolius* during the field investigation (Ran et al. 2020). In present work, we found that the a symbiotic relationship between *R. intraradices* and *P. quinquefolius* was established, and *R. intraradices* significantly promoted the content of ginsenoside, which laid a foundation to further reveal the effect of AMF on the secondary metabolism in *P. quinquefolius*. To investigate the mechanism of AMF influencing the secondary metabolism in *P. quinquefolius*, transcriptome analyses were performed in this study. Based on the evaluation of all transcripts, a number of key enzyme genes related to the ginsenoside biosynthesis of *P. quinquefolius* were obtained, which would provide a basis for further research the molecular mechanisms of AMF to promote the ginsenosides content in *P. quinquefolius*. Zhao et al. (2014) also found that mycorrhizal fungi could induce the biosynthesis of secondary metabolites and hormone balance of *Cymbidium hybridum* based on the transcriptome analysis. In addition, several DEGs involved in plant hormone signal transduction have been found. We speculate that there may be some signal molecule communication between AMF and *P. quinquefolius*, which will promote the colonization of AMF. Tsuzuki et al. (2016) indicated that AMF also secreted signaling molecules to the host plants including lipochitooligosaccharides and chitin oligomers. The possible mechanisms of signal recognition, exchange and transmission between *P. quinquefolius* and AMF need to be further studied. To our knowledge, it was the first study to investigate the transcriptomic responses of *P. quinquefolius* to AMF inoculation. Next generation sequencing techniques could be conveniently used for analysis of transcriptome profiling during plant/fungus

interactions (Wang et al. 2009). In summary, RNA-Seq technology not only provides a comprehensive gene expression change for *P. quinquefolius* in response to AMF inoculation, but also provides a new technical means for further analysis the regulation of secondary metabolism by AMF.

Glycolysis has been considered to be the key way to convert glucose into pyruvate, which provides metabolic intermediates for other pathways and energy for organisms (Yang et al. 2018). In general, the transformation of fructose into pyruvate through glycolysis requires the catalytic reaction of many enzymes, which not only act as catalyst and energy regulator, but also act as a signal transmission device to respond to environmental changes (Khanna et al. 2014). In order to study whether the mycorrhizal fungi could affect the glycolysis of *P. quinquefolius*, all related enzymes unigenes involved in the glycolysis pathway were identified by transcriptomes. The results showed that the genes involved in glycolysis of *P. quinquefolius* were induced by AMF inoculation and most genes were largely up-regulated. Thus, under the treatment of AMF, the expression of genes in glycolysis was accelerated, and the metabolism of *P. quinquefolius* might be changed.

Ginsenosides are supposed to be the main secondary metabolites in *P. quinquefolius* and play significant impacts on the quality of *P. quinquefolius* (Liu et al. 2008). In order to investigate the mechanism of AMF regulating ginsenoside synthesis, we have analysed the gene changes in the biosynthesis of triterpenoid saponins pathway in *P. quinquefolius* at the transcription level. From our investigation, the expression of ginsenoside biosynthesis-related genes was changed and the most abundant up-regulated unigenes were assigned to HMGR and GPS in response to AMF inoculation. HMGR has been considered as an important rate-limiting enzyme in MVA pathway, which affects the biosynthesis of ginsenoside by affecting the production of its precursor IPP and DMAPP (Tholl 2015; Haralampidis et al. 2002). Ohyama et al. (2007) found that *Arabidopsis thaliana* lacking the gene *hmgr-1* exhibited growth dwarfism and other characteristics. In response to the inoculation of AMF, the expression of SS and SE genes also was up-regulated with different levels. Han et al. (2010) silenced the *PgSE1* gene by RNAi technology and found that the content of ginsenoside was significantly decreased, indicating that the *PgSE1* gene regulates the biosynthesis of ginsenoside. In addition, seven related enzymes involved in the biosynthesis of ginsenoside were detected in MEP pathway, and there was no significant difference in gene expression. Among these enzymes, DXS was constricted for the formation of 1-deoxy-D-xylulose 5-phosphate through the condensation of pyruvate and glyceraldehydes-3-phosphate (Niu et al. 2014; Wu et al. 2007). It suggested that the mechanism of AMF affecting the ginsenosides synthesis in *P. quinquefolius* might be mainly through the activation of the MVA pathway. Studies had showed that the changes of genes involved in saponin biosynthesis were closely associated with the saponin content and composition (Zu et al. 2018; Li et al. 2016). Under the regulation of AMF, the key enzyme genes were efficiently transcribed and expressed in order to increase the accumulation of ginsenoside with high value, and ultimately improved the quality and economic value of *P. quinquefolius*.

Conclusion

In this work, the results showed that a symbiotic relationship between AMF and *P. quinquefolius* was established. Compared with non-AMF inoculation, a total of 111 differential DEGs in the roots of *P. quinquefolius* were identified and 78 DEGs were up-regulated with the AMF treatment. The expression level of genes involved in glycolysis and ginsenoside biosynthesis showed significantly changes during the AMF inoculation. Our data elucidated the mechanism of AMF induced secondary metabolite synthesis of *P. quinquefolius* and provided new insights for improving the quality of ginsenosides of *P. quinquefolius*.

Declarations

Acknowledgements

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Compliance with ethical standards

Conflict of interest

Authors declare that there are no conflicts of interest regarding the publication of this work.

References

1. Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome Biol* 11:R106
2. Andrade SAL, Malik S, Sawaya ACHF, Bottcher A, Mazzafera P (2013) Association with arbuscular mycorrhizal fungi influences alkaloid synthesis and accumulation in *Catharanthus roseus* and *Nicotiana tabacum* plants. *Acta Physiol Plant* 35:867-880
3. Armstrong L, Peterson RL (2002) The interface between the arbuscular mycorrhizal fungus *Glomus intraradices* and root cells of *Panax quinquefolius*: a Paristype mycorrhizal association. *Mycologia* 94:587-595
4. Bouwmeester HJ, Roux C, Lopez-Raez JA, Becard G (2007) Rhizosphere communication of plants, parasitic plants and AM fungi. *Trends Plant Sci* 12:224-230
5. Buisson S, Maillot P, Schellenbaum P, Walter B, Gindro K, Deglène-Benbrahim L (2016) Arbuscular mycorrhizal symbiosis stimulates key genes of the phenylpropanoid biosynthesis and stilbenoid production in grapevine leaves in response to downy mildew and grey mould infection. *Phytochemistry* 131:92-99

6. Cervantes-Gómez RG, Bueno-Ibarra MA, Cruz-Mendivil A, Calderón-Vázquez CL, Ramírez-Dourietet CM, Maldonado-Mendoza IE, Villalobos-López MÁ, Ángel Valdez-Ortíz Á, López-Meyer M (2016) Arbuscular mycorrhizal symbiosis-Induced expression changes in *solanum lycopersicum* leaves revealed by RNA-seq analysis. *Plant Mol Biol Rep* 34:89-102
7. Chang Q, Diao FW, Wang QF, Pan L, Dang ZH, Guo W (2018) Effects of arbuscular mycorrhizal symbiosis on growth, nutrient and metal uptake by maize seedlings (*Zea mays* L.) grown in soils spiked with Lanthanum and Cadmium. *Environ pollut* 241:607-615
8. Chen J, Xie M, Fu Z, Lee SC, Wang X (2007) Development of a quality evaluation system for *Panax quinquefolius*. L based on HPLC chromatographic fingerprinting of seven major ginsenosides. *Microche J* 85:201-208
9. China Pharmacopoeia Committee (2015) Pharmacopoeia of Peoples Republic of China. Ch.P, Beijing. P:131
10. Cho EJ, Lee DJ, Wee CD, Kim HL, Cheong YH, Cho JS, Sohn BK (2009) Effects of AMF inoculation on growth of *Panax ginseng* C.A. Meyer seedlings and on soil structures in mycorrhizosphere. *Sci horticulture-amsterdam* 122:0-637
11. Dharmananda S (2002) The nature of ginseng: Traditional use, modern research, and the question of dosage. *HerbalGram* 54:34-51
12. Dong LL, Xu J, Feng GQ, Li XW, Chen SL (2016) Soil bacterial and fungal community dynamics in relation to *Panax notoginseng* death rate in a continuous cropping system. *Sci Rep* 6:31802
13. Frenzel A, Manthey K, Perlick AM, Meyer F, Pühler A, Küster H (2005) Combined transcriptome profiling reveals a novel family of arbuscular mycorrhizal-specific *Medicago truncatula* Lectin genes. *Mol Plant-Microbe Interact* 8:771-782
14. Geneva M, Hristozkova M, Yonova P, Boychinova M, Stancheva I (2010) Effect of endomycorrhizal colonization with *Glomus intraradices* on growth and antioxidant capacity of *Sideritis scardica* Griseb. *Gen Appl Plant Physiol* 36:47-54
15. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* 29:644–652
16. Han JY, In JG, Kwon YS, Choi YE (2010) Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squalene epoxidase gene in *Panax ginseng*. *Phytochemistry* 71:36-46
17. Handa Y, Nishide H, Takeda N, Suzuki Y, Kawaguchi M, Saito K (2015) RNA-seq transcriptional profiling of an arbuscular mycorrhiza provides insights into regulated and coordinated gene expression in *Lotus japonicus* and *Rhizophagus irregularis*. *Plant Cell Physiol* 56:1490-1511
18. Haralampidis K, Trojanowska M, Osbourn AE (2002) Biosynthesis of triterpenoid saponins in plants. *Adv Biochem Eng/Biotechnol* 75:31-49
19. Jung JH, Kim HY, Kim HS, Jung SH (2020) Transcriptome analysis of *Panax ginseng* response to high light stress. *J Ginseng Res* 44:312-320

20. Khanna SM, Taxak PC, Jain PK, Saini R, Srinivasan R (2014) Glycolytic enzyme activities and gene expression in cucumber roots exposed to water-deficit stress. *Appl Biochem Biotechnol* 173:2241-2253
21. Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 12:323
22. Li JX, Liu SJ, Wang J, Li J, Liu DH, Li JL, Gao WY (2016) Fungal elicitors enhance ginsenosides biosynthesis, expression of functional genes as well as signal molecules accumulation in adventitious roots of *Panax ginseng* C. A. Mey. *J Biotechnol* 239:106-114
23. Li HH, Wu L, Tang N, Liu R, Jin Z, Liu YQ, Li ZG (2020) Analysis of transcriptome and phytohormone profiles reveal novel insight into ginger (*Zingiber officinale* Rose) in response to postharvest dehydration stress. *Postharvest Biol Tec* 161:111087
24. Liu J, Blaylock LA, Endre G, Cho J, Town CD, VandenBosch KA, Harrison MJ (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. *Plant Cell* 15:2106-2123
25. Liu WC, Zheng YN, Han LK, Wang HM, Saito M, Ling MS, Kimura Y, Feng YX (2008) Saponins (Ginsenosides) from stems and leaves of *Panax quinquefolius* prevented high-fat diet-induced obesity in mice. *Phytomedicine* 15:1140-1145
26. Liu ZL, Li YJ, Hou HY, Zhu XC, Rai V, He XY, Tian CJ (2013) Differences in the arbuscular mycorrhizal fungi-improved rice resistance to low temperature at two N levels: aspects of N and C metabolism on the plant side. *Plant Physiol Biochem* 71:87-95
27. Liu H, Wang J, Gao WY, Wang Q, Zhang LM, Man SL (2014) Optimization and quality assessment of adventitious roots culture in *Panax quinquefolius* L. *Acta Physiol Plant* 36:713–719
28. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402-408
29. Lou LN, Su XJ, Liu XH, Liu Z (2020) Transcriptome analysis of *Luffa cylindrica* (L.) Roem response to infection with Cucumber mosaic virus (CMV). *Gene* 737:144451
30. Lu C, Zhao S, Wei G, Zhao H, Qu Q (2016) Functional regulation of ginsenoside biosynthesis by RNA interferences of a UDP-glycosyltransferase gene in *Panax ginseng* and *Panax quinquefolius*. *Plant Physiol Biochem* 111:67
31. Mandal S, Upadhyay S, Singh VP, Kapoor R (2015) Enhanced production of steviol glycosides in mycorrhizal plants: A concerted effect of arbuscular mycorrhizal symbiosis on transcription of biosynthetic genes. *Plant Physiol Biochem* 89:100-106
32. McGonigle TP, Miller MH, Evans DG, Fairchild DL, Swan GA (1990) A new method which gives an objective measure of colonisation of roots by vesicular arbuscular mycorrhizal fungi. *New Phytol* 115:495-501
33. Mo GY, Huang F, Fang Y, Han LT, Kayla KP, Bu LJ, Du XW, Joan WB, Yin GH (2019) Transcriptomic analysis in *Anemone flaccida* rhizomes reveals ancillary pathway for triterpene saponins biosynthesis and differential responsiveness to phytohormones. *Chin J Nat Medicines* 17:01310-0144

34. Mortazavi A, Williams BA, Mccue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods* 5:621
35. Ohyama K, Suzuki M, Masuda K, Yoshida S, Muranaka T (2007) Chemical phenotypes of the hmg1 and hmg2 mutants of Arabidopsis demonstrate the in-planta role of HMG-CoA reductase in triterpene biosynthesis. *Chem Pharm Bull* 55:1518-1521
36. Pallara G, Todeschini V, Lingua G, Camussi A, Racchi ML (2013) Transcript analysis of stress defence genes in a white poplar clone inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* and grown on a polluted soil. *Plant Physiol Biochem* 63:131-139
37. Raghuwanshi R, Sinha S (2014) Linking mycorrhizal technology with medicinal plant secondary metabolites. *Microbial Diversity and Biotechnology in Food Security*. Springer India.
38. Ran ZF, Yang XT, Ding WN, Zhou J, Guo LP, Zhang YQ (2020) A preliminary investigation on the arbuscular mycorrhiza of the roots of cultivated *Panax quinquefolius* L. *Chin J Chin Mater Med* 9:2020-2056 (in Chinese)
39. Rufyikiri G, Declerck S, Dufey JE, Delvaux B (2000) Arbuscular mycorrhizal fungi might alleviate aluminium toxicity in banana plants. *New Phytol* 148:343-352
40. Rydlová J, Jelínková M, Dusek K, Dusková E, Vosátka M, Püschel D (2016) Arbuscular mycorrhiza differentially affects synthesis of essential oils in coriander and dill. *Mycorrhiza* 26:123-131
41. Schlag EM, McIntosh MS (2013) The relationship between genetic and chemotypic diversity in American ginseng (*Panax quinquefolius* L.). *Phytochemistry* 93:96-104
42. Sharma SK, Pandit MK (2009) A new species of *Panax* L. (Araliaceae) from Sikkim Himalaya, India. *Syst Bot* 34:434e8
43. Shi Y, Sun C, Zheng B, Gao B, Sun A (2013) Simultaneous determination of ten ginsenosides in American ginseng functional foods and ginseng raw plant materials by liquid chromatography tandem mass spectrometry. *Food Anal Method* 6:112-122
44. Shu B, Li WC, Liu LQ, Wei YZ, Shi SY (2016) Transcriptomes of Arbuscular Mycorrhizal Fungi and Litchi Host Interaction after Tree Girdling. *Front Microbiol* 7:408
45. Subramanian KS, Charest C (1997) Nutritional, growth, and reproductive responses of maize (*Zea mays* L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasselling. *Mycorrhiza* 7:25-32
46. Tholl D (2015) Biosynthesis and biological functions of terpenoids in plants. *Adv Biochem Eng Biotechnol* 148:63-106
47. Tian L, Shi SH, Ma LN, Zhou X, Luo SS, Zhang JF, Lu BH, Tian CJ (2019) The effect of *Glomus intraradices* on the physiological properties of *Panax ginseng* and on rhizospheric microbial diversity. *J Gins Res* 41:1-9
48. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol* 28:511–515

49. Tsuzuki S, Handa Y, Takeda N, Kawaguchi M (2016) Strigolactone-induced putative secreted protein 1 is required for the establishment of symbiosis by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Mol Plant Microbe In* 29:277-286
50. Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10:57-63
51. Wang L, Zhao SJ, Liang YL, Sun Y, Cao HJ, Han Y (2014) Identification of the protopanaxatriol synthase gene *CYP6H* for ginsenoside biosynthesis in *Panax quinquefolius*. *Funct Integr Genomics* 14:559-570
52. Wang J, Li J, Li J, Liu S, Wu X, Li J, Gao WY (2016) Transcriptome profiling shows gene regulation patterns in ginsenoside pathway in response to methyl jasmonate in *Panax quinquefolius* adventitious root. *Sci Rep-Uk* 6:37263
53. Wang WJ, Wu Y, Xu HH, Shang Y, Chen YC, Yana M, Li ZH, Walt DR (2019) Accumulation mechanism of indigo and indirubin in *Polygonum tinctorium* revealed by metabolite and transcriptome analysis. *Ind Crop Prod* 141:111783
54. Yang YJ, Jinag ZF, Guo JN, Yang XL, Xu N, Chen ZH, Hao J, Li JX, Pang JL, Shen CJ, Xu MJ (2018) Transcriptomic analyses of *Chrysanthemum morifolium* Ramat under UV-B radiation treatment reveal variations in the metabolisms associated with bioactive components. *Ind Crop Prod* 124:475-486
55. Zeng S, Xiao G, Guo J, Fei Z, Xu Y, Roe BA, Wang Y (2010) Development of a EST dataset and characterization of EST-SSRs in a traditional Chinese medicinal plant, *Epimedium sagittatum* (Sieb. Et Zucc.) Maxim. *BMC Genomics* 11:94
56. Zhang Z, Zhang P, Li W, Zhang J, Huang F, Yang J, Bei Y, Lu Y (2013) De novo transcriptome sequencing in *Frankliniella occidentalis* to identify genes involved in plant virus transmission and insecticide resistance. *Genomics* 101:296-305
57. Zhang XH, Han CZ, Gao HM, Cao YP (2019) Comparative transcriptome analysis of the garden asparagus (*Asparagus officinalis* L.) reveals the molecular mechanism for growth with arbuscular mycorrhizal fungi under salinity stress. *Plant Physiol Biochem* 141:20-29
58. Zhang Y, Li YY, Chen XM, Meng ZX, Guo SX (2020) Combined metabolome and transcriptome analyses reveal the effects of mycorrhizal fungus *Ceratobasidium* sp. AR2 on the flavonoid accumulation in *Anoectochilus roxburghii* during different growth stages. *Int J Mol Sci* 21:564
59. Zhao H, Feng SS, Zhou W, Kai GY (2019) Transcriptomic analysis of postharvest toon buds and key enzymes involved in terpenoid biosynthesis during cold storage. *Sci Hortic-Amsterdam* 257:108747
60. Zhao XL, Zhang JX, Chen CL, Yang JZ, Zhu HY, Liu M, Lv FB (2014) Deep sequencing-based comparative transcriptional profiles of *Cymbidium hybridum* roots in response to mycorrhizal and non-mycorrhizal beneficial fungi. *BMC Genom* 15:747
61. Zhou J, Ran ZF, Yang XT, Li J (2019) Postharvest UV-B irradiation stimulated ginsenoside Rg1 biosynthesis through nitric oxide (NO) and jasmonic acid (JA) in *Panax quinquefolius* roots. *Molecules* 24:1462

62. Zouari I, Salvioli A, Chialva M, Novero M, Miozzi L, Tenore G, Bagnaresi P, Bonfante P (2014) From root to fruit: RNA-seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. *BMC Genomics* 15:1-19
63. Zhu HH, Zhang RQ, Chen WL, Gu ZH, Xie XL, Zhao HQ, Yao Q (2015) The possible involvement of salicylic acid and hydrogen peroxide in the systemic promotion of phenolic biosynthesis in clover roots colonized by arbuscular mycorrhizal fungus. *J Plant Physiol* 178:27-34
64. Zu YQ, Li ZR, Mei XY, Wu J, Cheng SC, Jiang YQ, Li Y (2018) Transcriptome analysis of main roots of *Panax notoginseng* identifies genes involved in saponin biosynthesis under arsenic stress. *Plant Gene* 16:1-7

Figures

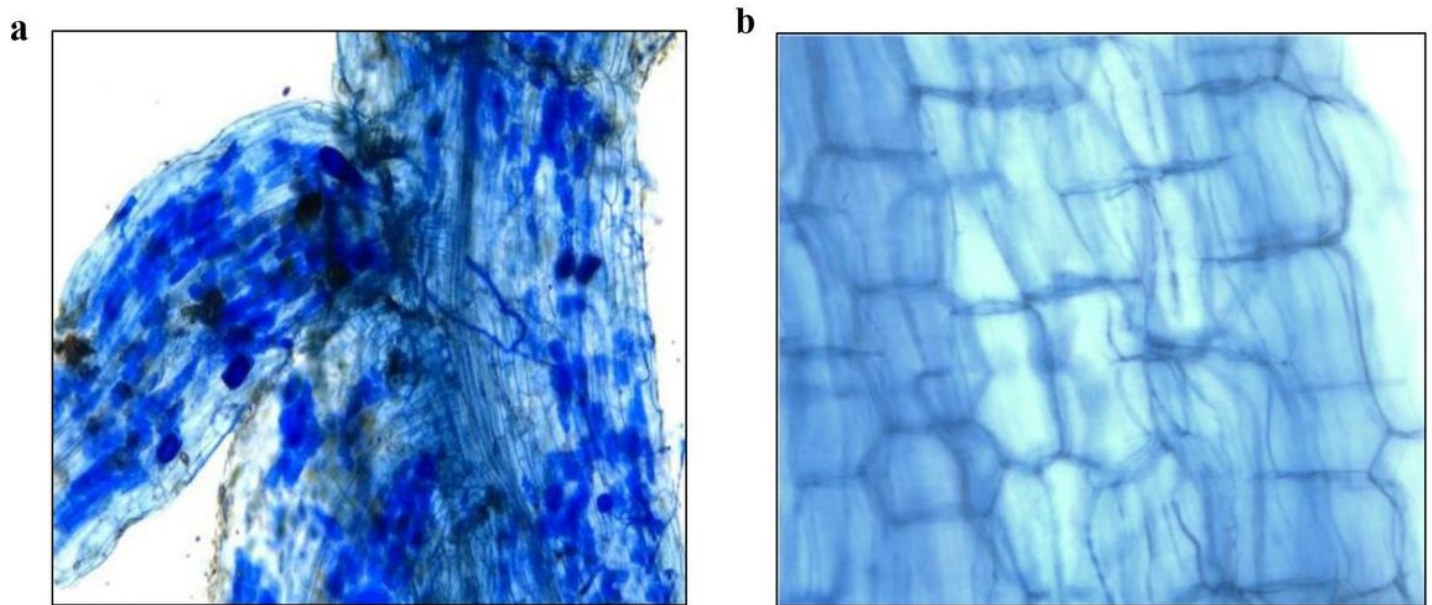


Figure 1

The colonization characteristics of AMF in roots of *P. quinquefolius* under the different treatment. a. The colonization of *R. intraradices* in *P. quinquefolius* roots; b. No-AMF in *P. quinquefolius* roots.

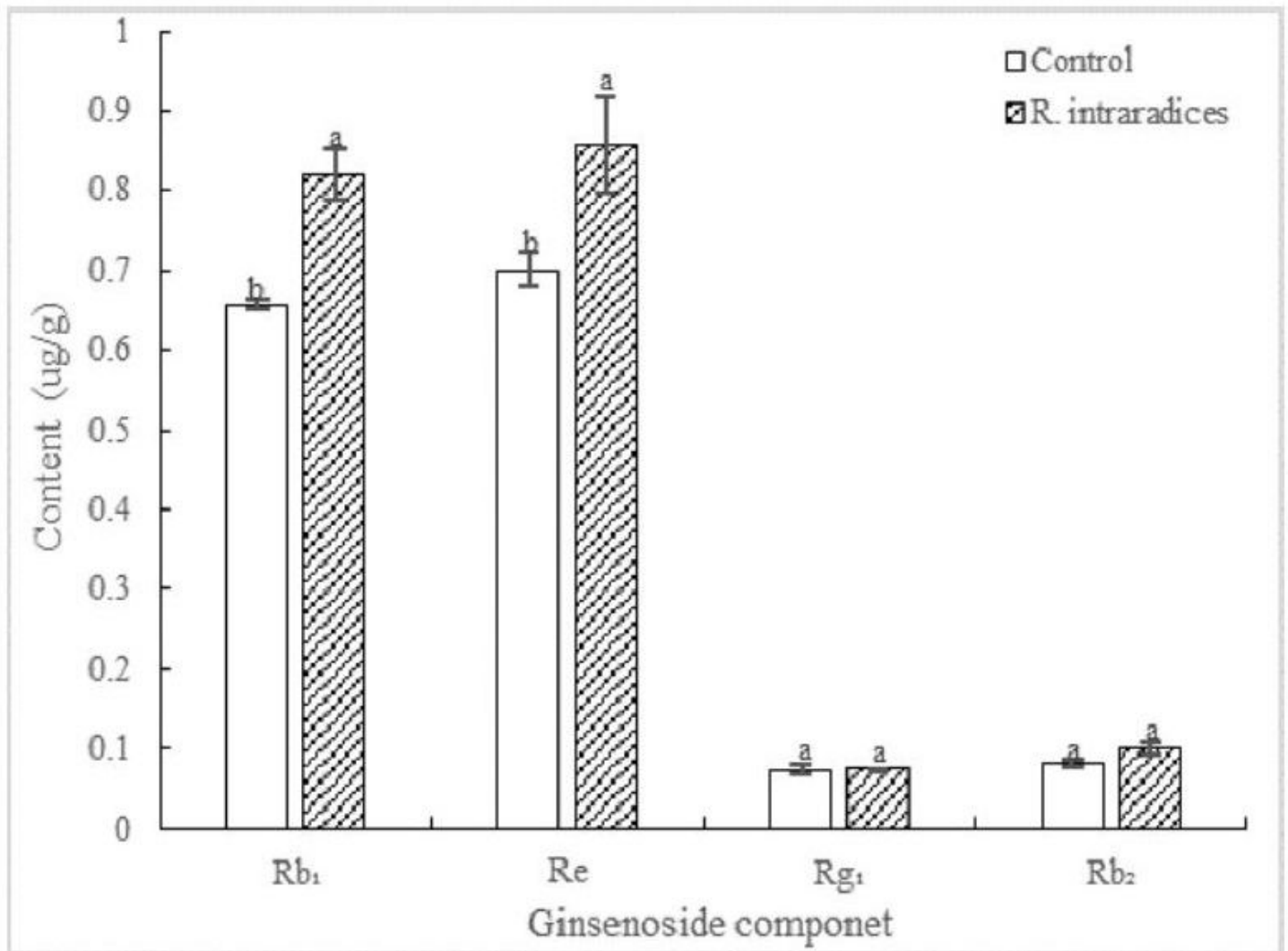


Figure 2

Effects of AMF (*R. intraradices*) on ginsenosides (Rb₁, Re, Rg₁ and Rb₂) content of *P. quinquefolius*. Data was expressed as mean $\pm$ sd of three replicates. Different letters show significant differences ($P < 0.05$, Duncan's tests) between the AMF inoculation and the control group.

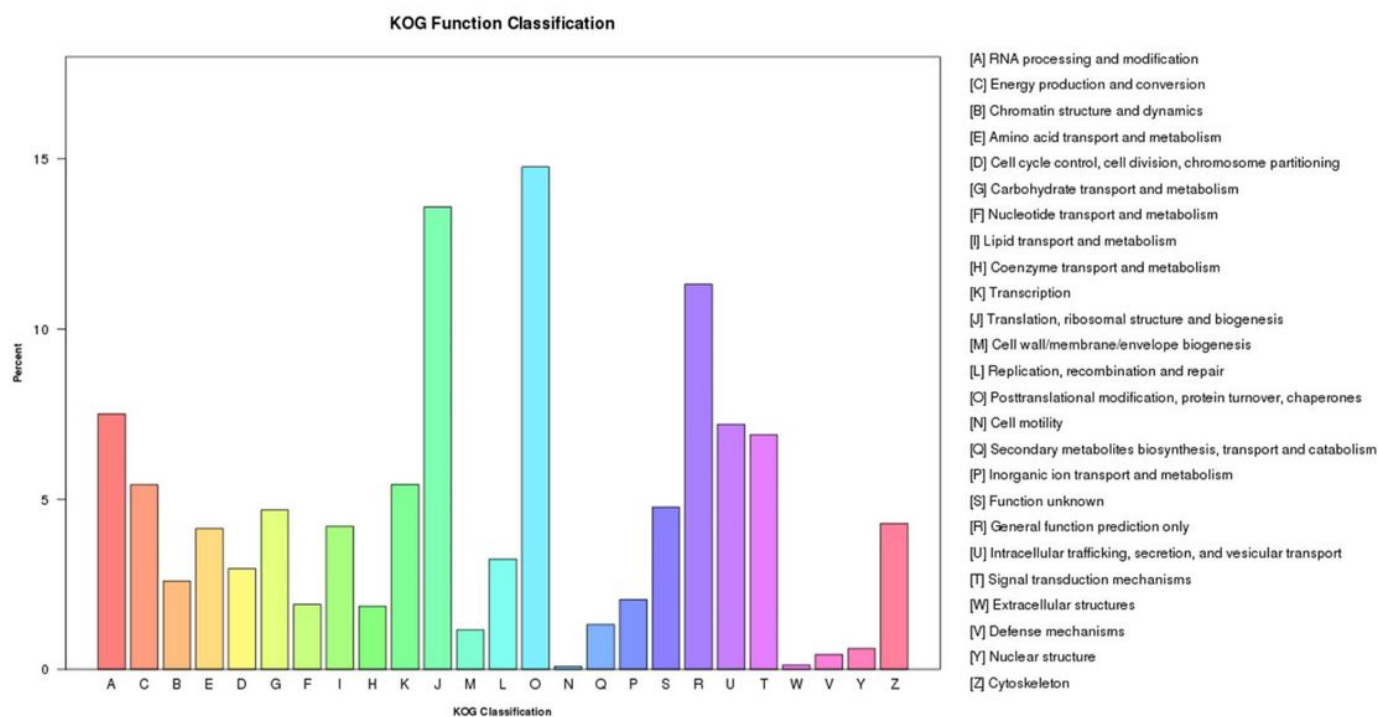


Figure 3

Classification of the unigenes annotated in KOG. The X-axis represents the names of the 25 groups, and the Y-axis corresponds to the percentage of the number of genes in the group accounting for the total number of annotated genes.

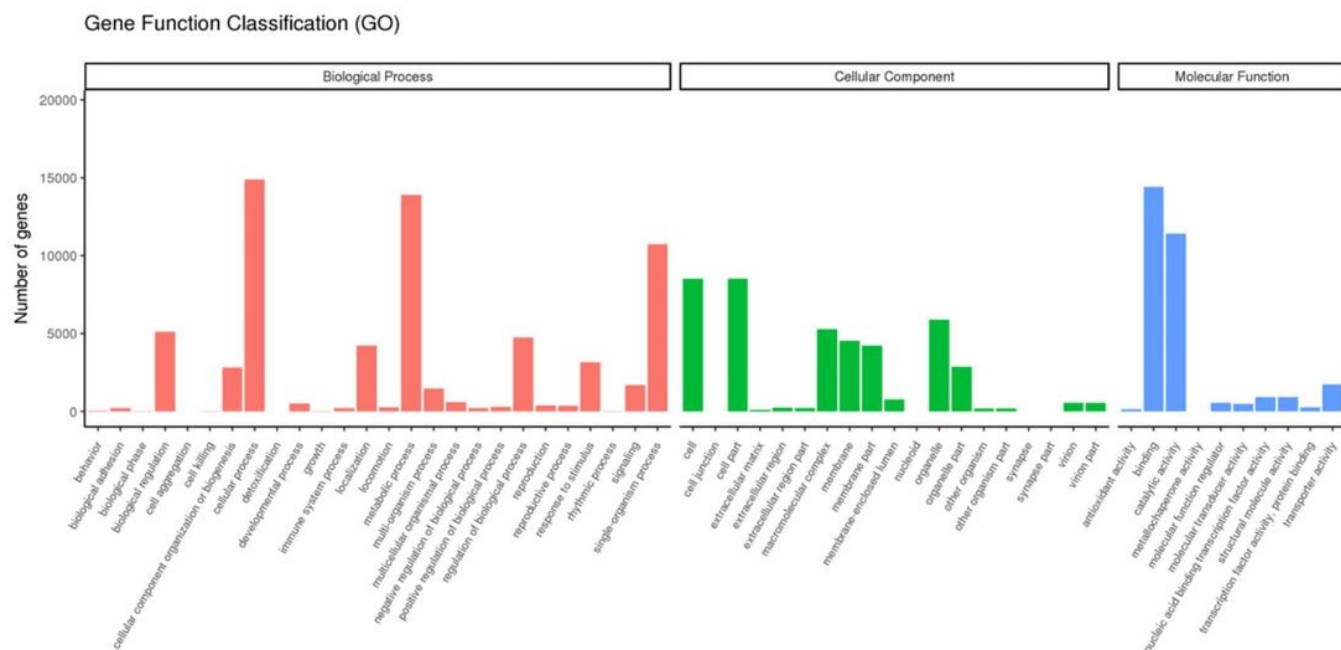


Figure 4

Gene ontology classifications of the assembled unisequences. The results are summarized in three main categories: biological process, cellular component and molecular function.

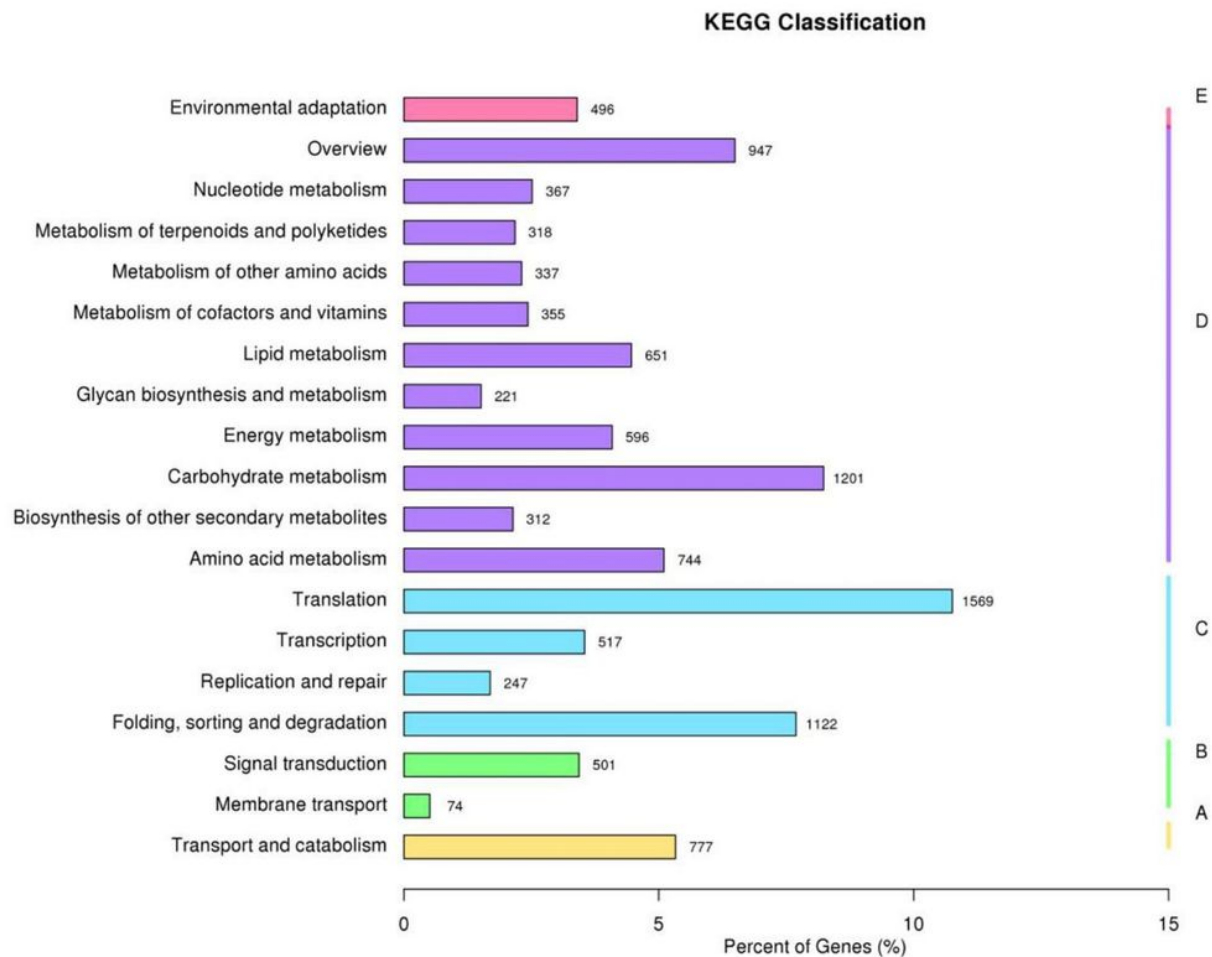


Figure 5

KEGG classification diagram of Unigene. The ordinate is the name of KEGG metabolic pathway, and the abscissa is the number of genes annotated to the pathway and the proportion of the number of genes annotated to the total number of genes annotated.

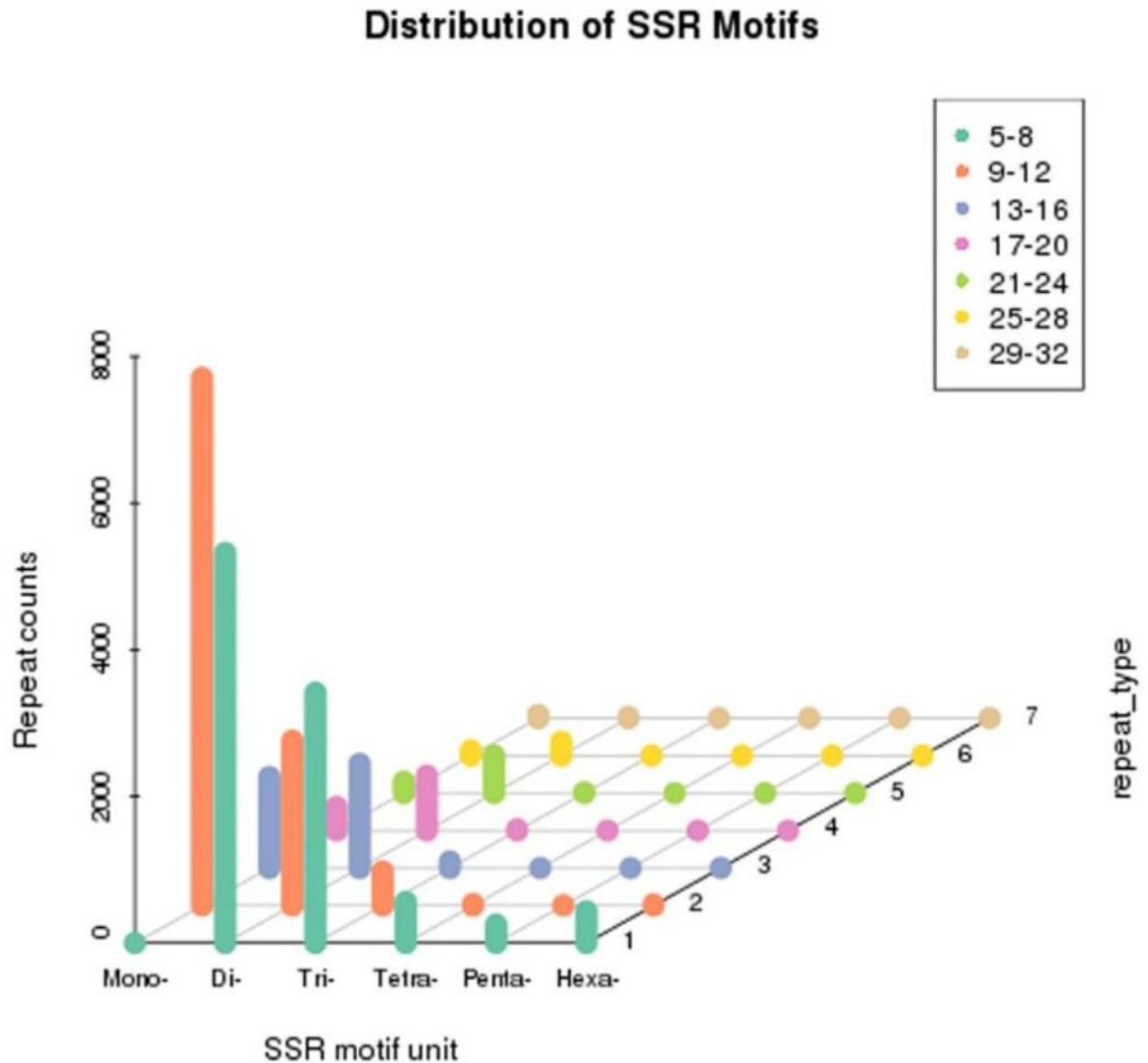


Figure 6

Distribution diagram of simple sequence repeats. X-axis represents the type of simple sequence repeats. Z-axis represents the number of simple sequence repeats. The numerical value of Y-axis is coordinate. The number of specific repeat should be in accordance with illustrations according to the color.

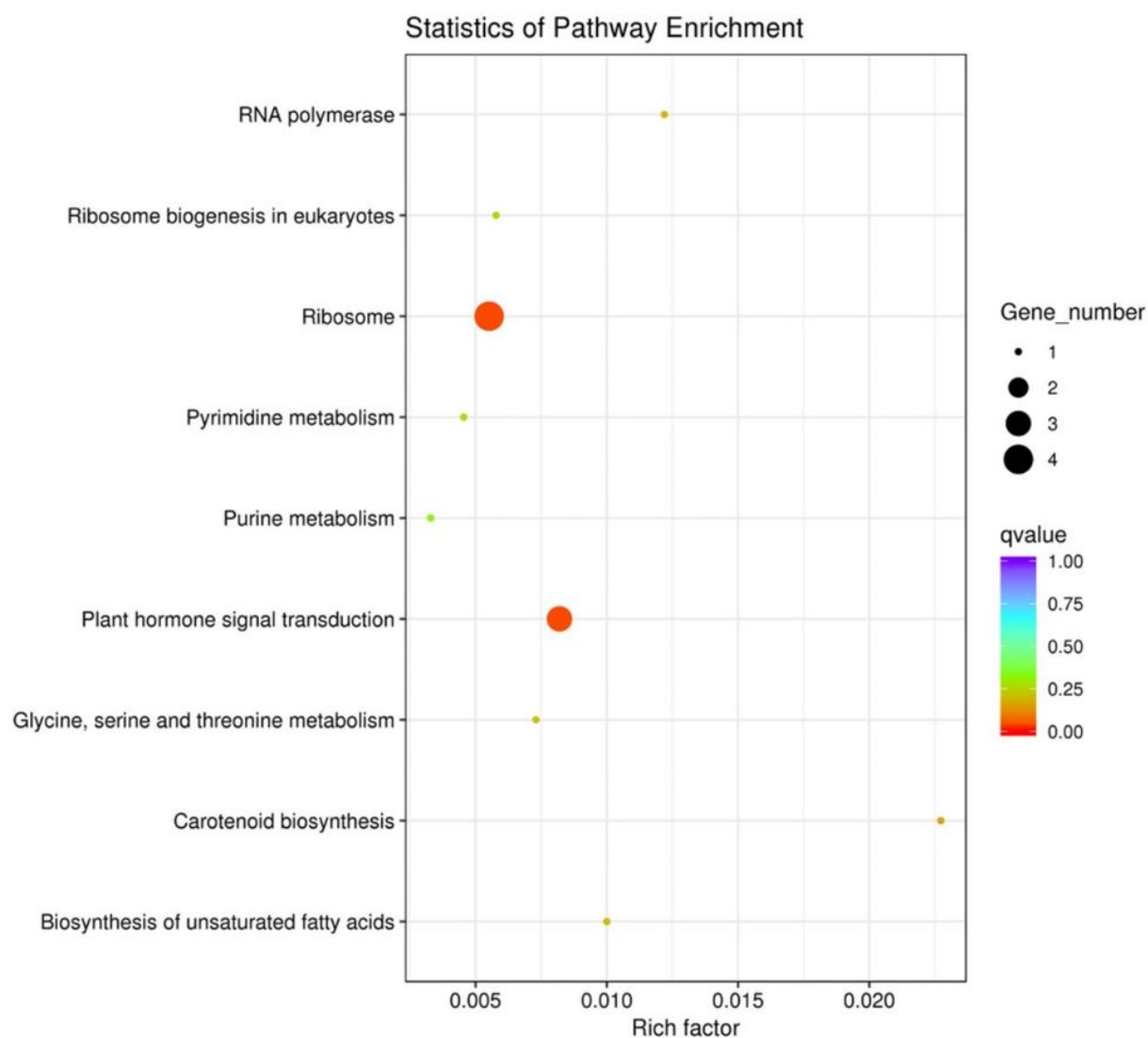


Figure 7

KEGG pathway enrichment scatter map of differentially expressed transcripts in the roots of *P. quinquefolius* with AMF inoculation. The X-axis corresponds to the rich factor of pathway, and the Y-axis represents a different pathway. The magnitude of the dots displays gene number ranged from 1 to 4, and the q-value is described by the color classification.

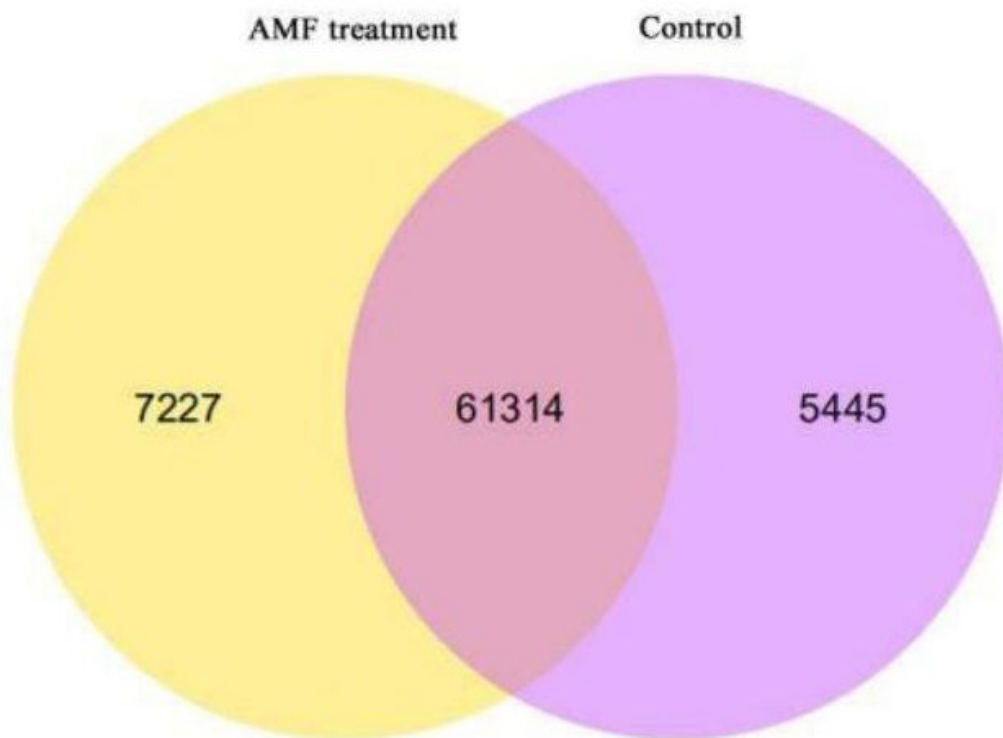


Figure 8

Venn diagram of the quantitative gene classification into those specifically expressed in one library or those expressed in both libraries.

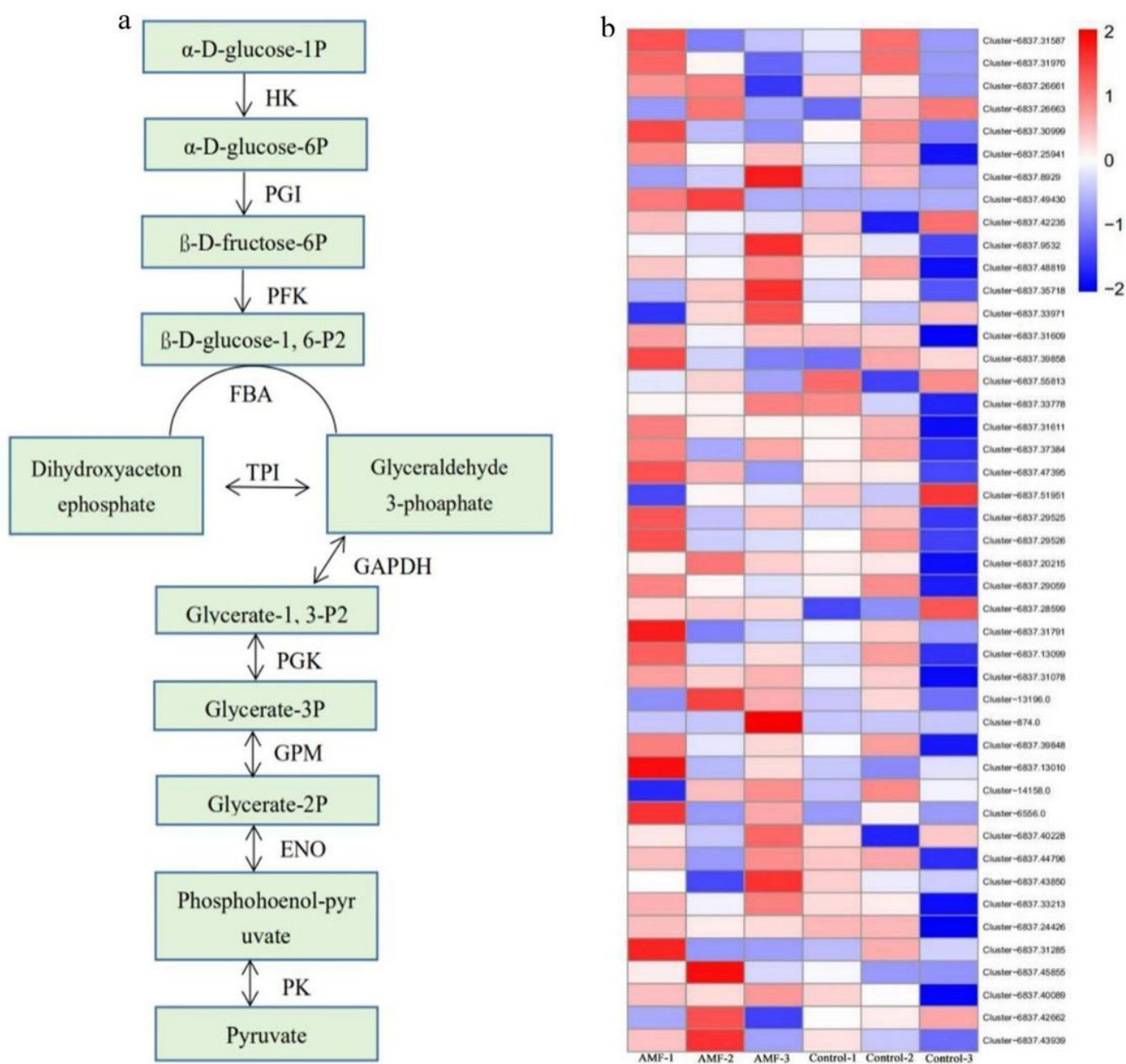


Figure 9

Transcript abundance changes of glycolysis-related genes. (a) Overview of glycolysis in *P. quinquefolius*. HK: hexokinase; PGI: phosphoglycerate isomerase; PFK: 6-phosphofructokinase 1; FBA: fructose-bisphosphate aldolase; TPI: triosephosphate isomerase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PGK: phosphoglycerate kinase; GPM: probable phosphoglycerate mutase; ENO: enolase; PK: pyruvate kinase. (b) Expression changes of genes associated with glycolysis under AMF inoculated in *P. quinquefolius*. Red indicates up-regulated genes and blue indicates down-regulated gene

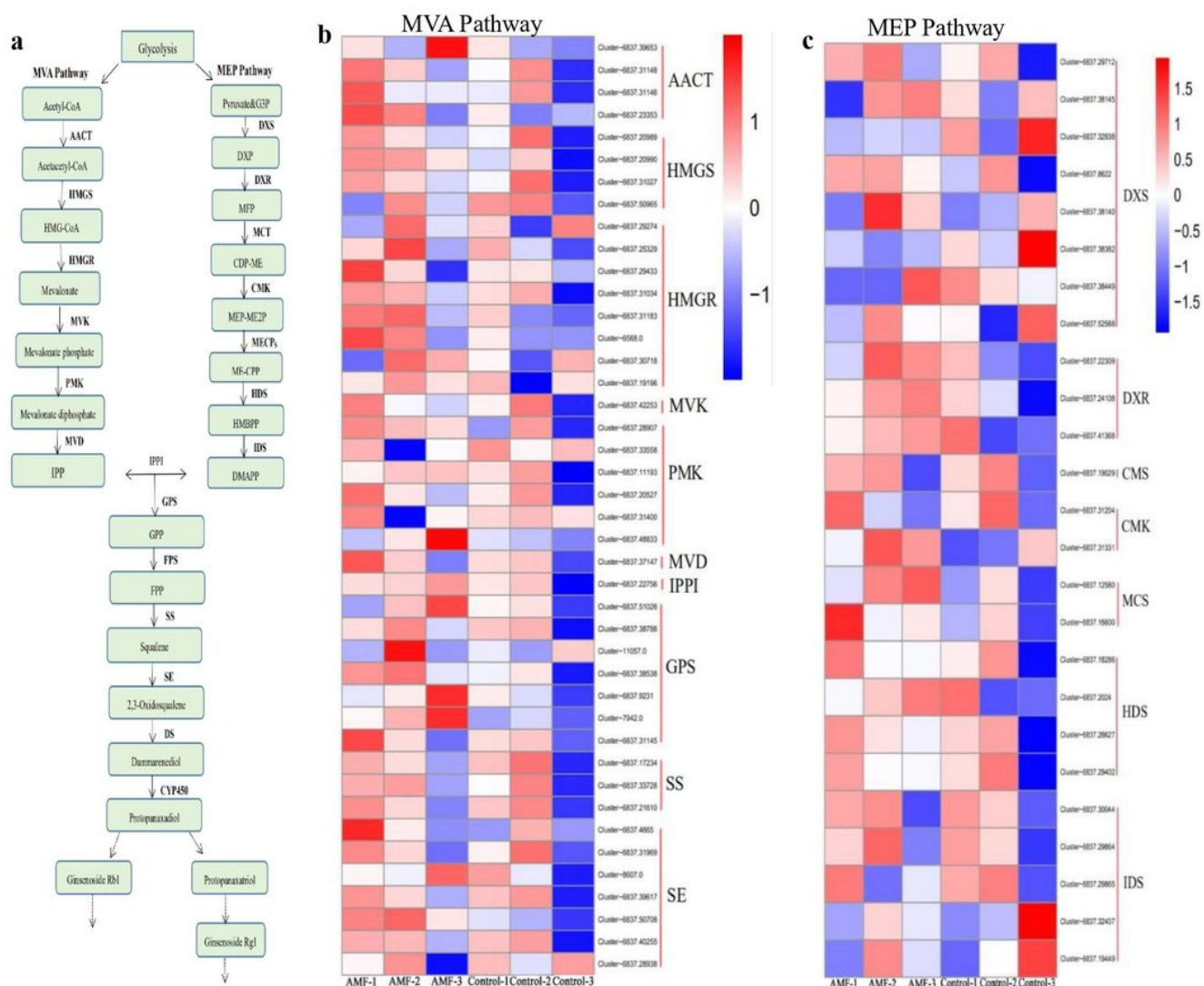


Figure 10

Changes in the expression of ginsenoside synthesis pathway. (a) Candidate genes identified in this study are shown in bold. Acetyl-CoA acetyltransferase (AACT), HMG-CoA synthase (HMGS), HMG-CoA reductase (HMGR), mevalonate kinase (MVK), phosphomevalonate kinase (PMK), mevalonate diphosphate decarboxylase (MVD), isopentenyl diphosphate isomerase (IDI), 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (CMS), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MCS), (E)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase (HDS), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase (IDS), geranylgeranyl pyrophosphate synthase (GPS), farnesyl diphosphate synthase (FPS), squalene synthase (SS), squalene epoxidase (SE) and dammarendiol synthase (DS). (b) Expression changes of genes related to MVA pathway of ginsenoside metabolism under AMF treatment in *P. quinquefolius*. (c) Expression changes of genes related to MEP pathway of ginsenoside metabolism under AMF treatment in *P. quinquefolius*. Red indicates up-regulated genes and blue indicates down-regulated genes.

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