



## Transcriptome changes in *Polygonum multiflorum* Thunb. roots induced by methyl jasmonate<sup>\*#</sup>

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**Abstract:** Transcriptome profiling has been widely used to analyze transcriptomic variation in plants subjected to abiotic or biotic stresses. Although gene expression changes induced by methyl jasmonate (MeJA) have been profiled in several plant species, no information is available on the MeJA-triggered transcriptome response of *Polygonum multiflorum* Thunb., a species with highly valuable medicinal properties. In this study, we used transcriptome profiling to investigate transcriptome changes in roots of *P. multiflorum* seedlings subjected to a 0.25 mmol/L-MeJA root-irrigation treatment. A total of 18 677 differentially expressed genes (DEGs) were induced by MeJA treatment, of which 4535 were up-regulated and 14 142 were down-regulated compared with controls. These DEGs were associated with 125 metabolic pathways. In addition to various common primary and secondary metabolic pathways, several secondary metabolic pathways related to components with significant pharmacological effects were enriched by MeJA, including arachidonic acid metabolism, linoleic acid metabolism, and stilbenoid biosynthesis. The MeJA-induced transcriptome changes uncovered in this study provide a solid foundation for future study of functional genes controlling effective components in secondary metabolic pathways of *P. multiflorum*.

**Key words:** *Polygonum multiflorum* Thunb., Methyl jasmonate, Transcriptome change, Differentially expressed genes  
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### 1 Introduction

Methyl jasmonate (MeJA), the methyl ester derivative of jasmonic acid, is a signaling molecule in plant stress responses that regulates many aspects of plant development and growth including flowering, fruit ripening, and senescence (Mukkun and Singh, 2009; Rivera-Dominguez *et al.*, 2012). MeJA is present in low concentrations in various parts of plants, including buds, shoots, leaves, flowers, fruits, and seeds, with the largest amounts present in fruits

(Sayyari *et al.*, 2011; Repčák and Suvak, 2013; Nopo-Olazabal *et al.*, 2014). The effects of MeJA have been analyzed in different plant species and vary depending on the MeJA concentration, treated plant organ, and treatment duration (Takahashi and Hara, 2014). Exogenous application of MeJA involving quantities much higher than those found in nature induces synthesis of jasmonate-induced proteins (Li *et al.*, 2014). These proteins activate the plant defense system in response to pathogen attack, mechanical wounding, insect herbivory, and general stress (Zhang *et al.*, 2013; Sabater-Jara *et al.*, 2014).

MeJA participates in the signal transduction pathway that induces particular enzymes to catalyze the formation of low molecular weight defensive compounds in plants (Sivanandhan *et al.*, 2012; Ku and Juvik, 2013; Misra *et al.*, 2014). Production of these compounds, such as alkaloids, terpenoids, and

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phenylpropanoids, is affected through an extensive transcriptional reprogramming of plant metabolism (Sun G.L. *et al.*, 2013; Zheljaskov *et al.*, 2013). The induction of secondary metabolite accumulation is a stress response, which depends on MeJA to coordinate the activation of the expression of multiple biosynthetic genes and the activity of diversifying enzymes (Gai *et al.*, 2010). MeJA-induced transcriptome changes have been analyzed in many different plant species including wheat and sweet basil (Diallo *et al.*, 2014; Misra *et al.*, 2014).

Next generation sequencing (NGS) is a rapid and cost-effective means for analyzing the genome and transcriptome of non-model species. It is also powerful for quantifying gene expression because of its improvement in the *de novo* assembly of high-throughput sequencing data and relatively accurate estimation of gene expression levels (Sun G.L. *et al.*, 2013). *Polygonum multiflorum*, a perennial plant in the Polygonaceae family, is used commercially to make a hair-darkening agent and a potent kidney tonic. The species has been widely used in traditional medicine for treating various ailments, and is known for its diverse pharmacological properties (Lv *et al.*, 2010). Great efforts have been made to increase the production of stilbene glucoside, one of the major active ingredients of *P. multiflorum*, by using bio-transformation (Yan *et al.*, 2007) and micropropagation (Lin *et al.*, 2003) but current production processes suffer from low yields. Some compounds are known to stimulate the production of stilbenoids when added to the medium. Among them, MeJA has been found to elicit significantly the production of stilbenoids in cultured cells of grapevine (Jeandet *et al.*, 2002; Vannozzi *et al.*, 2012). Our experimental data showed that a 0.20 mmol/L-MeJA solution could significantly increase stilbene glucoside production in *P. multiflorum* (Fig. S1). However, the detailed biological mechanism of MeJA stimulation of stilbenoid production and concomitant transcriptome changes associated with the response to MeJA remains poorly understood. A detailed knowledge of the biosynthesis of stilbene glucoside and its regulation by MeJA is required before directed bioengineering can be implemented.

Diverse secondary metabolites with potent biological activities have been identified in roots of *P. multiflorum*, but the biosynthetic pathways pro-

ducing these compounds are poorly understood. In addition, genomic and transcriptomic information for *P. multiflorum* is scarce in public databases. To identify genes involved in the biosynthesis of the biologically active materials, we used NGS to analyze transcriptomic changes in *P. multiflorum* roots following MeJA treatment.

## 2 Materials and methods

### 2.1 Plant materials and root-irrigation treatment of plantlets with MeJA solution

Plants were propagated from robust branches of cultivated *P. multiflorum* originally acquired from the He-Shou-Wu GAP cultivation base (Shibing County, Guizhou, China). These plants were cultivated in controlled conditions with a 16-h photoperiod, 25 °C/20 °C (day/night) temperature, and 75% relative humidity. Plants were watered once every three days with Hoagland nutrient solution (Hoagland and Arnon, 1950). The 45-d old plants with five to seven leaves were used for MeJA solution root-irrigation experiments. Our pre-experiment results showed that production of the main active component (stilbene glucoside) of *P. multiflorum* roots could be induced significantly 26 h after treatment with 0.25 mmol/L MeJA. Therefore, in this study, the final concentration of MeJA solution with wetting agent Triton X-100 at 0.1% was 0.25 mmol/L. The solution (500 ml) was added to each 10-L flowerpot containing 4 kg of vermiculite and expanded perlite (1:1) and two *P. multiflorum* plantlets. Control plantlets were irrigated with the Triton solution at 0.1%. Roots were collected 26 h after treatment, immediately frozen in liquid nitrogen, and stored at -80 °C until RNA extraction. Treatment and control experiments were performed with 135 plants in triplicate, respectively, and all chemicals were purchased from Sigma Co., Ltd. (Guangzhou, China) unless otherwise stated.

### 2.2 Total RNA isolation, complementary DNA (cDNA) library construction and sequencing

Total RNA was extracted from 135 roots of *P. multiflorum* plantlets from control and treatment plants, respectively, following the protocol of the E.Z.N.A.<sup>TM</sup> Plant RNA Kit (OMEGA, GA, USA). A Thermo Scientific Multiskan GO spectrophotometer

(Thermo Fisher Scientific, USA) and gel electrophoresis were used to analyze the quality and quantity of the total RNA, respectively. For cDNA synthesis, equal quantities of high-quality RNA from control and treatment samples were pooled.

An mRNA-seq Sample Preparation Kit (Illumina Inc., San Diego, CA, USA) was used for constructing an mRNA-seq library following the manufacturer's instructions, and then poly-(A) mRNA was isolated from total RNA samples using poly-T oligo-attached magnetic beads. The mRNA was cleaved into short fragments using an RNA Fragmentation Kit (Ambion, Austin, TX, USA), which were then used as templates to reverse-transcribe first-strand cDNA using random hexamer primers and reverse transcriptase (TaKaRa, Japan). Second-strand cDNA was synthesized in a reaction mixture containing buffer, DNA polymerase I, RNase H, and dNTPs (TaKaRa, Japan). A Genomic Sample Preparation Kit (Illumina, USA) was used for synthesizing a paired-end library according to the manufacturer's instructions. A MinElute polymerase chain reaction (PCR) Purification Kit (QIAGEN, Dusseldorf, Germany) was used for purifying short fragments, which were eluted in 10  $\mu$ l of ethidium bromide (EB) buffer. Short fragments were connected using sequencing adaptors, and agarose gel electrophoresis and a Gel Extraction Kit (Axygen Biosciences, CA, USA) were used for separating and purifying desired fragments ((200 $\pm$ 25) bp). Finally, the paired-end sequencing library was constructed by PCR amplification with 15 cycles and sequenced using an Illumina Solexa Genome Analyzer Iix sequencing platform (BGI-Huada Genomics Institute, Shenzhen, China). Illumina instrument software was used for performing data analysis and base calling.

### 2.3 Sequence analysis, assembly, and annotation

All raw reads were cleaned by removing reads with adapters or with unknown nucleotides larger than 5%, or reads in which the percentage of low quality bases (base quality  $\leq$ 10) was more than 20%. Qualifying reads were extended into contigs using Trinity software (unpublished software of BGI-Huada Genomics Institute, Shenzhen, China) through the overlap between the sequences, and then contigs were connected to form transcript sequences according to paired-end information of sequences (Li et al., 2010; Grabherr et al., 2011). The overlap settings used for

this assembly were 31 bp long and have 80% similarity, and the group pairs distance (the maximum length expected between a fragment pair) was set to 500 bp, with all the other parameters set to their default values. The longest transcripts were selected as unigene sequences of our samples. Transcript levels were quantified as reads per kilobase of exon model per million mapped reads (RPKM) (Mortazavi et al., 2008). Genes with high expression levels were screened and listed. Optimal assembly results were chosen according to the assembly evaluation, and then clustering analysis was performed to achieve a unigene database that comprised potential alternative splicing transcripts.

The assembled sequences were compared against the National Center for Biotechnology Information (NCBI) Nr, Nt, and Swiss-Prot databases using BLASTn (Version 2.2.14) with an *E*-value of  $10^{-5}$ , and then each assembled sequence was assigned to gene names based on the highest scoring BLAST hit. For each query, to increase computational speed, searches were limited to the first 10 significant hits. The "getorf" program of EMBOSS software package (Rice et al., 2000) was used to predict open reading frames (ORFs), with the longest ORF extracted for each unigene. The Swiss-Prot BLAST results were imported into Blast2GO to obtain the gene ontology (GO) annotations of the unigenes (Conesa et al., 2005; Conesa and Gotz, 2008) for annotating the assembled sequences with GO terms. ANNEX (Myhre et al., 2006; Ye et al., 2006) was used for enriching and refining the obtained annotation. The unigene sequences were also aligned to the Clusters of Orthologous Group (COG) database for predicting and classifying functions (Tatusov et al., 2000). The online Kyoto encyclopedia of genes and genome (KEGG) automatic annotation server (<http://www.genome.jp/kegg/kaas>) was used to assign genes from the KEGG pathways to the assembled sequences. KEGG orthology assignment (Moriya et al., 2007) was obtained using the bi-directional best hit method. If results from different databases conflicted with each other, a priority order of Nr, Nt, Swiss-Prot, KEGG, and COG was followed when deciding the sequence direction of unigenes. When a unigene happened to be aligned to none of the above databases, ESTScan software (Iseli et al., 1999) was introduced to decide its sequence direction. For unigenes with

sequence directions, their sequences from the 5' end to 3' end were provided; for those without direction, their sequences were provided from the assembly software.

## 2.4 Unigene differential expression analysis

We performed this analysis to predict genes with different expression levels, and then carry out GO functional analysis and KEGG pathway analysis on them. The unigene expression was calculated using the fragments per kilobase of transcript per million fragments mapped (FPKM) method (Mortazavi *et al.*, 2008). We have developed a rigorous algorithm to identify genes expressed differentially between two samples, which is based on “the significance of digital gene expression profiles” which has been cited hundreds of times (Audic and Claverie, 1997; Benjamini *et al.*, 2001).

## 2.5 Real-time PCR and PCR product sequence validation

To validate RNA-seq gene expression results, quantitative real-time PCR (qRT-PCR) was run using SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II (Perfect Real Time; TaKaRa, Japan) and a real-time PCR thermal cycler (qTower 2.2, Analytikjena, Germany) using the same cDNA samples as used in the RNA-seq experiment. A first-strand cDNA fragment was synthesized from total RNAs using the TaKaRa PrimerScript<sup>™</sup> RT Reagent Kit with gDNA eraser (Perfect Real Time; TaKaRa, Japan). Gene-specific primers were designed for target transcript sequences and *UBQ14*, *UBQ4-1*, and *SAMS* were selected as reference genes (RGs) in our experiments (Tables S1–S3). The comparative threshold cycle method was used to calculate the relative gene expression (Livak and Schmittgen, 2001). Each qRT-PCR was carried out three times. qRT-PCR products of 13 target transcripts were sequenced for comparing sequence similarity with RNA-seq data.

Primers of RGs and genes of interest (GOIs), which putatively coded for key enzymes in the stilbenoid biosynthesis pathway, were designed using Primer Premier 6.0 software (Premier Biosoft, CA, USA) and assessed with Oligo 7.0 software (Molecular Biology Insights, CO, USA), based on the following criteria: 55–65 °C annealing temperature, 40%–60% GC content, 18–24-bp primer length, and

75–300-bp amplicon length. The products of PCR amplification were sequenced to confirm the specificity of RG primer annealing. Reaction mixtures contained 10.0 μl of 2× SYBR Premix Ex Taq II, 0.4 μmol/L of each primer, and 2.0 μl cDNA as a template in a total volume of 20 μl. The following amplification program was used in all qRT-PCR reactions: 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 2 min. The specificity of each amplification reaction was verified by dissociation curve (melting curve) analysis by heating the amplicon from 55 to 95 °C at the end of the 40-cycle reaction. Non-template controls were included for each primer pair. Three biological samples were used for each RG and GOI, with expression levels in each sample based on three technical replicates. Root samples subjected to the non-MeJA root-irrigation treatment were used to normalize the values obtained from MeJA root-irrigation-treated seedlings.

A standard curve based on a five-fold dilution series was calculated according to the equation  $(1+E)=10^{\text{slope}}$ , which was used to determine PCR efficiencies (*E*) of the RGs (Vandesompele *et al.*, 2002). Mean *E* values were obtained for each biological replicate. RG and GOI transcript abundances in all samples were determined according to the cycle quantity (*C<sub>q</sub>*) value in the exponential phase of the PCR. *C<sub>q</sub>* values were converted using the formula  $Q=E^{\Delta C_q}$  (Huggett *et al.*, 2005), where  $\Delta C_q$  = the lowest *C<sub>q</sub>* value of the calibrator (corresponding to the sample with the highest expression)–the *C<sub>q</sub>* value of the sample tested. Data obtained from each biological replicate were analyzed independently.

## 3 Results

### 3.1 Effect of MeJA on gene expression

We identified 79 565 consensus sequences from non-elicited and elicited root transcriptome data of *P. multiflorum* seedlings, with 89 440 and 78 409 sequences detected from non-elicited and elicited root transcriptome data, respectively. The total number and length (nt) of consensus sequences and the number of distinct clusters and singletons were smaller under MeJA treatment conditions, suggesting that MeJA may inhibit the expression of certain genes (Table S4). The entire set of consensus sequences

was subjected to differential expression analysis (false discovery rate  $\leq 0.001$  and  $|\log_2 \text{Ratio}| \geq 1$ ) and then processed to eliminate putative redundancies (Fig. S2). A total of 18 677 differentially expressed genes (DEGs) were identified, with 4535 genes up-regulated and 14 142 down-regulated compared with the controls.

### 3.2 GO functional classification of DEGs

DEGs were classified into three main GO categories, namely biological process, cellular component, and molecular function. Genes differentially regulated upon exogenous MeJA treatment were most heavily represented in the biological process category, followed by cellular component and molecular function. With respect to biological processes, most DEGs were related to cellular and metabolic processes, indicating the greater sensitivity of these genes to MeJA, followed by response to stimulus, biological regulation, and single-organism process. Gene expression related to cells and cell parts dominated activity in the cellular component category; genes associated with cells and cell parts were also responsible for the highest expression observed in any of the three main GO categories. Other important categories were organelles as well as membranes and organelle parts. In regard to molecular function, the most predominant category was binding and catalytic activity, followed

by transporter activity, structural molecule activity, and nucleic acid-binding transcription factor activity as shown in Fig. 1 (Liu *et al.*, 2015).

### 3.3 Pathway enrichment analysis of DEGs

MapMan software using a specifically designed mapping file was used for the functional analysis of DEGs (Thimm *et al.*, 2004). We functionally classified all genes from the transcriptome sequences into 125 major metabolic pathways to build the mapping file (Table 1). Genes involved in the 125 major pathways of the KEGG database were found to be significantly up- or down-regulated by MeJA treatment. Only one pathway, “thiamine metabolism”, was found to be fully up-regulated, while 12 of 125 major pathways, including “synthesis and degradation of ketone bodies”, “other glycan degradation”, and “glycosaminoglycan degradation”, were completely down-regulated (Table 1). These results indicate that 0.25 mmol/L MeJA can completely inhibit gene activity in these pathways, thereby likely inhibiting the activity of their corresponding enzymes.

#### 3.3.1 Effect of MeJA on the “plant-pathogen interaction” pathway

Different functional categories were specifically adjusted after MeJA treatment in *P. multiflorum* roots,

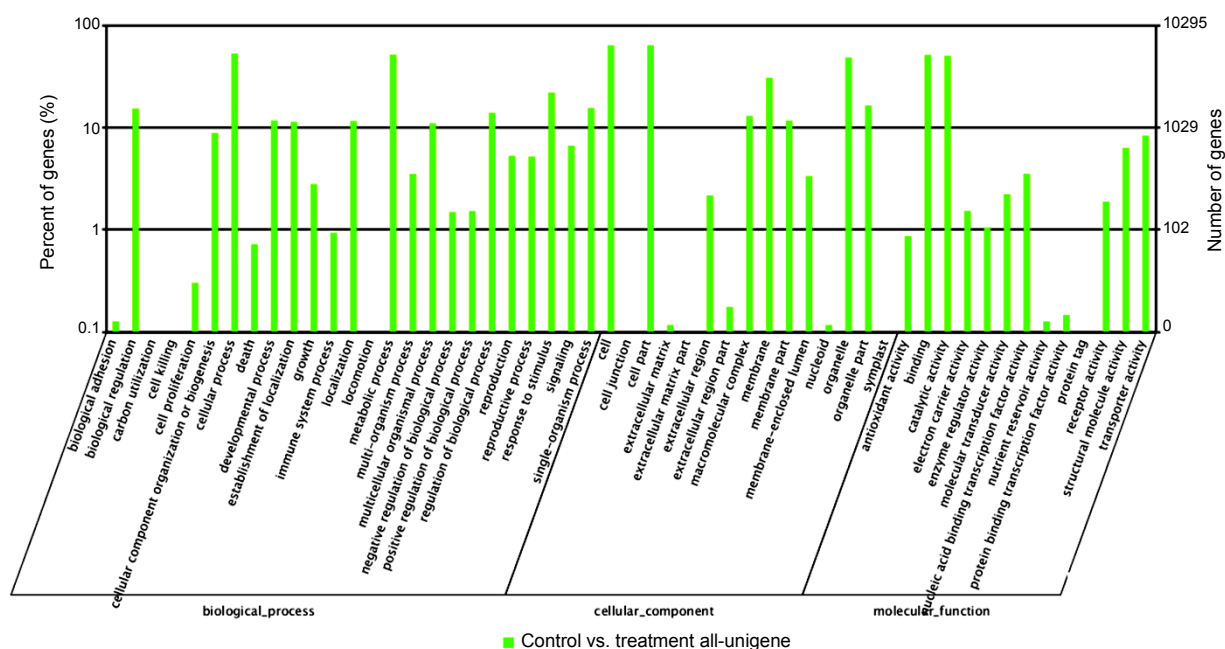


Fig. 1 GO function classification of DEGs (Liu *et al.*, 2015)

**Table 1 Significantly modulated pathways in *P. multiflorum* root transcriptome subjected to MeJA treatment**

| No. | Pathway   | Up-regulated genes | Down-regulated genes | Genes with pathway annotation |              |
|-----|---|--------------------|----------------------|-------------------------------|--------------|
|     |   |                    |                      | DEGs (8936)                   | All (32 790) |
| 1   | Glycolysis/gluconeogenesis                          | 25                 | 74                   | 99 (1.11%)                    | 460 (1.40%)  |
| 2   | Citrate cycle (TCA cycle)                           | 7                  | 20                   | 27 (0.30%)                    | 151 (0.46%)  |
| 3   | Pentose phosphate pathway                           | 19                 | 34                   | 53 (0.59%)                    | 171 (0.52%)  |
| 4   | Pentose and glucuronate interconversions            | 58                 | 145                  | 203 (2.27%)                   | 457 (1.39%)  |
| 5   | Fructose and mannose metabolism                     | 7                  | 45                   | 52 (0.58%)                    | 217 (0.66%)  |
| 6   | Galactose metabolism                                | 9                  | 62                   | 71 (0.79%)                    | 284 (0.87%)  |
| 7   | Ascorbate and aldarate metabolism                   | 25                 | 59                   | 84 (0.94%)                    | 189 (0.58%)  |
| 8   | Fatty acid biosynthesis                             | 7                  | 13                   | 20 (0.22%)                    | 77 (0.23%)   |
| 9   | Fatty acid elongation                               | 8                  | 9                    | 17 (0.19%)                    | 59 (0.18%)   |
| 10  | Fatty acid metabolism                               | 8                  | 40                   | 48 (0.54%)                    | 181 (0.55%)  |
| 11  | Synthesis and degradation of ketone bodies          |                    | 4                    | 4 (0.04%)                     | 29 (0.09%)   |
| 12  | Cutin, suberine and wax biosynthesis                | 19                 | 18                   | 37 (0.41%)                    | 115 (0.35%)  |
| 13  | Steroid biosynthesis                                | 1                  | 30                   | 31 (0.35%)                    | 111 (0.34%)  |
| 14  | Ubiquinone and other terpenoid-quinone biosynthesis | 6                  | 18                   | 24 (0.27%)                    | 93 (0.28%)   |
| 15  | Oxidative phosphorylation                           | 84                 | 34                   | 118 (1.32%)                   | 609 (1.86%)  |
| 16  | Photosynthesis                                      | 4                  | 12                   | 16 (0.18%)                    | 114 (0.35%)  |
| 17  | Photosynthesis-antenna proteins                     | 4                  | 1                    | 5 (0.06%)                     | 24 (0.07%)   |
| 18  | Purine metabolism                                   | 59                 | 202                  | 261 (2.92%)                   | 919 (2.80%)  |
| 19  | Pyrimidine metabolism                               | 52                 | 205                  | 257 (2.88%)                   | 895 (2.73%)  |
| 20  | Alanine, aspartate and glutamate metabolism         | 3                  | 34                   | 37 (0.41%)                    | 208 (0.63%)  |
| 21  | Glycine, serine and threonine metabolism            | 10                 | 44                   | 54 (0.60%)                    | 167 (0.51%)  |
| 22  | Cysteine and methionine metabolism                  | 28                 | 64                   | 92 (1.03%)                    | 290 (0.88%)  |
| 23  | Valine, leucine and isoleucine degradation          | 5                  | 50                   | 55 (0.62%)                    | 174 (0.53%)  |
| 24  | Valine, leucine and isoleucine biosynthesis         | 3                  | 21                   | 24 (0.27%)                    | 76 (0.23%)   |
| 25  | Lysine biosynthesis                                 | 4                  | 7                    | 11 (0.12%)                    | 40 (0.12%)   |
| 26  | Lysine degradation                                  | 4                  | 22                   | 26 (0.29%)                    | 86 (0.26%)   |
| 27  | Arginine and proline metabolism                     | 9                  | 51                   | 60 (0.67%)                    | 268 (0.82%)  |
| 28  | Histidine metabolism                                | 1                  | 21                   | 22 (0.25%)                    | 71 (0.22%)   |
| 29  | Tyrosine metabolism                                 | 19                 | 26                   | 45 (0.50%)                    | 167 (0.51%)  |
| 30  | Phenylalanine metabolism                            | 27                 | 87                   | 114 (1.28%)                   | 251 (0.77%)  |
| 31  | Tryptophan metabolism                               | 17                 | 35                   | 52 (0.58%)                    | 117 (0.36%)  |
| 32  | Phenylalanine, tyrosine and tryptophan biosynthesis | 6                  | 21                   | 27 (0.30%)                    | 134 (0.41%)  |
| 33  | Benzoxazinoid biosynthesis                          | 4                  | 10                   | 14 (0.16%)                    | 46 (0.14%)   |
| 34  | $\beta$ -Alanine metabolism                         | 7                  | 30                   | 37 (0.41%)                    | 205 (0.63%)  |
| 35  | Taurine and hypotaurine metabolism                  | 2                  | 8                    | 10 (0.11%)                    | 29 (0.09%)   |
| 36  | Selenocompound metabolism                           | 6                  | 19                   | 25 (0.28%)                    | 72 (0.22%)   |
| 37  | Cyanoamino acid metabolism                          | 12                 | 35                   | 47 (0.53%)                    | 137 (0.42%)  |
| 38  | Glutathione metabolism                              | 34                 | 33                   | 65 (0.73%)                    | 268 (0.82%)  |
| 39  | Starch and sucrose metabolism                       | 75                 | 243                  | 318 (3.56%)                   | 897 (2.74%)  |
| 40  | <i>N</i> -glycan biosynthesis                       | 3                  | 19                   | 22 (0.25%)                    | 137 (0.42%)  |
| 41  | Other glycan degradation                            |                    | 67                   | 67 (0.75%)                    | 214 (0.65%)  |
| 42  | Other types of <i>o</i> -glycan biosynthesis        | 3                  | 3                    | 6 (0.07%)                     | 37 (0.11%)   |
| 43  | Amino sugar and nucleotide sugar metabolism         | 31                 | 89                   | 120 (1.34%)                   | 450 (1.37%)  |
| 44  | Glycosaminoglycan degradation                       |                    | 28                   | 28 (0.31%)                    | 132 (0.40%)  |

To be continued

Table 1

| No. | Pathway   | Up-regulated genes | Down-regulated genes | Genes with pathway annotation |              |
|-----|---|--------------------|----------------------|-------------------------------|--------------|
|     |   |                    |                      | DEGs (8936)                   | All (32 790) |
| 45  | Glycerolipid metabolism                               | 21                 | 24                   | 45 (0.50%)                    | 146 (0.45%)  |
| 46  | Inositol phosphate metabolism                         | 20                 | 80                   | 92 (1.03%)                    | 299 (0.91%)  |
| 47  | Glycosylphosphatidylinositol-anchor biosynthesis      | 10                 | 45                   | 55 (0.62%)                    | 141 (0.43%)  |
| 48  | Glycerophospholipid metabolism                        | 169                | 334                  | 503 (5.63%)                   | 1345 (4.10%) |
| 49  | Ether lipid metabolism                                | 138                | 311                  | 449 (5.02%)                   | 1165 (3.55%) |
| 50  | Arachidonic acid metabolism                           | 2                  | 3                    | 5 (0.06%)                     | 30 (0.09%)   |
| 51  | Linoleic acid metabolism                              | 12                 | 11                   | 23 (0.26%)                    | 55 (0.17%)   |
| 52  | $\alpha$ -Linolenic acid metabolism                   | 36                 | 36                   | 72 (0.81%)                    | 168 (0.51%)  |
| 53  | Sphingolipid metabolism                               | 1                  | 47                   | 48 (0.54%)                    | 141 (0.43%)  |
| 54  | Glycosphingolipid biosynthesis-globo series           |                    | 4                    | 4 (0.04%)                     | 24 (0.07%)   |
| 55  | Glycosphingolipid biosynthesis-ganglio series         |                    | 26                   | 26 (0.29%)                    | 79 (0.24%)   |
| 56  | Pyruvate metabolism                                   | 16                 | 59                   | 75 (0.84%)                    | 321 (0.98%)  |
| 57  | Glyoxylate and dicarboxylate metabolism               | 4                  | 46                   | 50 (0.56%)                    | 206 (0.63%)  |
| 58  | Propanoate metabolism                                 | 11                 | 24                   | 35 (0.39%)                    | 146 (0.45%)  |
| 59  | Butanoate metabolism                                  | 2                  | 18                   | 20 (0.22%)                    | 92 (0.28%)   |
| 60  | C <sub>5</sub> -branched dibasic acid metabolism      | 3                  | 4                    | 7 (0.08%)                     | 14 (0.04%)   |
| 61  | One carbon pool by folate                             | 2                  | 10                   | 12 (0.13%)                    | 53 (0.16%)   |
| 62  | Carbon fixation in photosynthetic organisms           | 13                 | 38                   | 51 (0.57%)                    | 284 (0.87%)  |
| 63  | Thiamine metabolism                                   | 7                  |                      | 7 (0.08%)                     | 27 (0.08%)   |
| 64  | Riboflavin metabolism                                 | 3                  | 13                   | 16 (0.18%)                    | 55 (0.17%)   |
| 65  | Vitamine B <sub>6</sub> metabolism                    | 11                 | 4                    | 15 (0.17%)                    | 41 (0.13%)   |
| 66  | Nicotinate and nicotinamide metabolism                | 3                  | 3                    | 6 (0.07%)                     | 63 (0.19%)   |
| 67  | Pantothenate and CoA biosynthesis                     | 2                  | 23                   | 25 (0.28%)                    | 99 (0.30%)   |
| 68  | Lipoic acid metabolism                                |                    | 2                    | 2 (0.02%)                     | 5 (0.02%)    |
| 69  | Folate biosynthesis                                   |                    | 7                    | 7 (0.08%)                     | 26 (0.08%)   |
| 70  | Porphyrin and chlorophyll metabolism                  | 15                 | 60                   | 75 (0.84%)                    | 179 (0.55%)  |
| 71  | Terpenoid backbone biosynthesis                       | 16                 | 57                   | 73 (0.82%)                    | 245 (0.75%)  |
| 72  | Indole alkaloid biosynthesis                          | 4                  | 4                    | 8 (0.09%)                     | 24 (0.07%)   |
| 73  | Monoterpenoid biosynthesis                            | 4                  | 1                    | 5 (0.06%)                     | 25 (0.08%)   |
| 74  | Limonene and pinene degradation                       | 35                 | 54                   | 89 (1.00%)                    | 215 (0.66%)  |
| 75  | Diterpenoid biosynthesis                              | 38                 | 22                   | 60 (0.67%)                    | 146 (0.45%)  |
| 76  | Brassinosteroid biosynthesis                          | 4                  | 19                   | 23 (0.26%)                    | 65 (0.20%)   |
| 77  | Carotenoid biosynthesis                               | 14                 | 55                   | 69 (0.77%)                    | 183 (0.56%)  |
| 78  | Zeatin biosynthesis                                   | 13                 | 63                   | 76 (0.85%)                    | 215 (0.66%)  |
| 79  | Sesquiterpenoid and triterpenoid biosynthesis         | 38                 | 4                    | 42 (0.47%)                    | 78 (0.24%)   |
| 80  | Nitrogen metabolism                                   | 6                  | 31                   | 37 (0.41%)                    | 136 (0.41%)  |
| 81  | Sulfur metabolism: reduction and fixation             | 13                 | 21                   | 34 (0.38%)                    | 79 (0.24%)   |
| 82  | Phenylpropanoid biosynthesis                          | 68                 | 148                  | 216 (2.42%)                   | 622 (1.90%)  |
| 83  | Flavonoid biosynthesis                                | 75                 | 83                   | 158 (1.77%)                   | 340 (1.04%)  |
| 84  | Anthocyanin biosynthesis                              | 6                  | 1                    | 7 (0.08%)                     | 17 (0.05%)   |
| 85  | Isoflavonoid biosynthesis                             | 4                  | 11                   | 13 (0.15%)                    | 29 (0.09%)   |
| 86  | Flavone and flavonol biosynthesis                     | 20                 | 41                   | 61 (0.68%)                    | 144 (0.44%)  |
| 87  | Stilbenoid, diarylheptanoid and gingerol biosynthesis | 44                 | 72                   | 116 (1.30%)                   | 282 (0.86%)  |
| 88  | Isoquinoline alkaloid biosynthesis                    | 2                  | 8                    | 10 (0.11%)                    | 42 (0.13%)   |

To be continued

Table 1

| No. | Pathway  | Up-regulated genes | Down-regulated genes | Genes with pathway annotation |               |
|-----|--|--------------------|----------------------|-------------------------------|---------------|
|     |  |                    |                      | DEGs (8936)                   | All (32 790)  |
| 89  | Tropane, piperidine and pyridine alkaloid biosynthesis | 2                  | 9                    | 11 (0.12%)                    | 45 (0.14%)    |
| 90  | Glucosinolate biosynthesis                             | 3                  | 23                   | 26 (0.29%)                    | 43 (0.13%)    |
| 91  | Aminoacyl-t-RAN biosynthesis                           | 9                  | 17                   | 26 (0.29%)                    | 243 (0.74%)   |
| 92  | Biosynthesis of unsaturated fatty acids                | 5                  | 12                   | 17 (0.19%)                    | 85 (0.26%)    |
| 93  | ABC transporters                                       | 52                 | 45                   | 97 (1.09%)                    | 387 (1.18%)   |
| 94  | Ribosome biogenesis in eukaryotes                      | 24                 | 152                  | 176 (1.97%)                   | 750 (2.29%)   |
| 95  | Ribosome   | 74                 | 570                  | 644 (7.21%)                   | 1402 (4.28%)  |
| 96  | RNA transport  | 87                 | 257                  | 354 (3.96%)                   | 1240 (3.78%)  |
| 97  | mRNA surveillance pathway                              | 66                 | 136                  | 202 (2.26%)                   | 800 (2.44%)   |
| 98  | RNA degradation  | 24                 | 133                  | 157 (1.76%)                   | 744 (2.27%)   |
| 99  | RNA polymerase   | 51                 | 150                  | 201 (2.25%)                   | 572 (1.74%)   |
| 100 | Basal transcription factors (eukaryotes)               | 3                  | 21                   | 24 (0.27%)                    | 186 (0.57%)   |
| 101 | DNA replication  | 1                  | 48                   | 49 (0.55%)                    | 191 (0.58%)   |
| 102 | Spliceosome  | 57                 | 156                  | 213 (2.38%)                   | 1085 (3.31%)  |
| 103 | Proteasome   | 3                  | 15                   | 18 (0.20%)                    | 143 (0.44%)   |
| 104 | Protein export   |                    | 19                   | 19 (0.21%)                    | 179 (0.55%)   |
| 105 | Base excision repair                                   | 1                  | 27                   | 28 (0.31%)                    | 127 (0.39%)   |
| 106 | Nucleotide excision repair                             | 2                  | 59                   | 61 (0.68%)                    | 261 (0.80%)   |
| 107 | Mismatch repair  |                    | 29                   | 29 (0.32%)                    | 122 (0.37%)   |
| 108 | Homologous recombination                               | 4                  | 45                   | 49 (0.55%)                    | 265 (0.81%)   |
| 109 | Non-homologous end-joining                             |                    | 6                    | 6 (0.07%)                     | 45 (0.14%)    |
| 110 | Phosphatidylinositol signaling system                  | 35                 | 55                   | 90 (1.01%)                    | 303 (0.92%)   |
| 111 | Plant hormone signal transduction                      | 156                | 465                  | 621 (6.95%)                   | 1746 (5.32%)  |
| 112 | Ubiquitin mediated proteolysis                         | 18                 | 97                   | 115 (1.29%)                   | 589 (1.80%)   |
| 113 | Sulfur relay system                                    |                    | 4                    | 4 (0.04%)                     | 19 (0.06%)    |
| 114 | Snare interactions in vesicular transport              | 11                 | 21                   | 32 (0.36%)                    | 131 (0.40%)   |
| 115 | Regulation of autophagy                                | 11                 | 21                   | 32 (0.36%)                    | 198 (0.60%)   |
| 116 | Protein processing in endoplasmic reticulum            | 30                 | 168                  | 198 (2.22%)                   | 895 (2.73%)   |
| 117 | Endocytosis  | 154                | 378                  | 532 (5.95%)                   | 1517 (4.63%)  |
| 118 | Phagosome  | 18                 | 92                   | 110 (1.23%)                   | 438 (1.34%)   |
| 119 | Peroxisome   | 14                 | 27                   | 41 (0.46%)                    | 289 (0.88%)   |
| 120 | Plant-pathogen interaction                             | 232                | 395                  | 627 (7.02%)                   | 1990 (6.07%)  |
| 121 | Natural killer cell mediated cytotoxicity              | 14                 | 24                   | 38 (0.43%)                    | 121 (0.37%)   |
| 122 | Circadian rhythm                                       |                    | 10                   | 10 (0.11%)                    | 105 (0.32%)   |
| 123 | Circadian rhythm-plant                                 | 28                 | 80                   | 108 (1.21%)                   | 293 (0.89%)   |
| 124 | Metabolic pathways                                     |                    |                      | 2441 (27.32%)                 | 8071 (24.61%) |
| 125 | Biosynthesis of secondary metabolites                  |                    |                      | 1079 (12.07%)                 | 3557 (10.85%) |

being either induced or repressed. MeJA treatment had the strongest inductive effect on the “plant-pathogen interaction” pathway, which had the greatest number of expressed genes identified in the *P. multiflorum* root transcriptome (Table 1). Of the 1990 genes, 627 were differentially expressed by MeJA treatment, with 232 up-regulated and 395 down-

regulated. Genes coding for disease-resistance proteins such as RPS2, RPS3, RPS5, and (pathogenesis-related protein 1) PR1 were heavily represented in this pathway (Fig. S3). The effect of MeJA on the induction of these biotic/abiotic stress associated-genes suggests that they could be exploited to improve plant resistance to pathogens and pests.



### 3.3.2 Effect of MeJA on the “phenylpropanoid biosynthesis” pathway

One of the most effective protective mechanisms against potential damage due to induction of senescence by MeJA treatment is the reduction of MeJA penetration into plant tissues (Malladi *et al.*, 2012). Studies of the biosynthesis of diverse secondary metabolites, especially the biosynthesis of flavonoids and other phenolic compounds, may provide insights into the molecular events underlying MeJA induction in plants (Nopo-Olazabal *et al.*, 2014). Treatment of *P. multiflorum* roots with MeJA had a significant inductive effect on genes in the “secondary metabolism” functional category, particularly those involved in the “phenylpropanoid biosynthesis” pathway (Fig. S4) and subcategories of “phenylpropanoid general pathway-flavonoid biosynthesis”, “anthocyanin biosynthesis”, “isoflavonoid biosynthesis”, “flavone and flavonol biosynthesis”, and “phytoalexins-stilbenoid, diarylheptanoid and gingerol biosynthesis”. Genes coding for key enzymes in the “phenylpropanoid biosynthesis” pathway, including phenylalanine ammonialyase (EC 4.3.1.24), *trans*-cinnamate 4-monooxygenase (EC 1.14.13.11), 4-coumarate-CoA

ligase (EC 6.2.1.12), cinnamate 4-hydroxylase (EC 1.14.13.11), and stilbene synthase (EC 2.3.1.95), were up- or down-regulated in response to MeJA (Fig. 2). Of 17 genes expressed in the “anthocyanin biosynthesis” pathway, mainly those encoding anthocyanidin 3-*O*-glucosyltransferase (EC 2.4.1.115) and anthocyanidin 3-*O*-glucoside 5-*O*-glucosyltransferase (EC 2.4.1.298), seven were differentially expressed under MeJA treatment: six were up-regulated and one down-regulated (Fig. S5).

Stilbene synthase also can use chalcone synthase substrates for the production of stilbenes which are classified as phytoalexins because of their roles in plant defense mechanisms against fungal pathogens (Lin *et al.*, 2015). Many genes assigned to the functional category “stilbenoid, diarylheptanoid and gingerol biosynthesis” were affected by MeJA (Table 1). Among them, genes encoding mainly stilbene synthase and resveratrol synthesis were significant in this functional category (Fig. 2). The enhanced representation of the “phenylpropanoid general pathway” and “stilbenoid, diarylheptanoid and gingerol biosynthesis” categories demonstrates that MeJA promotes the expression of defense system genes.

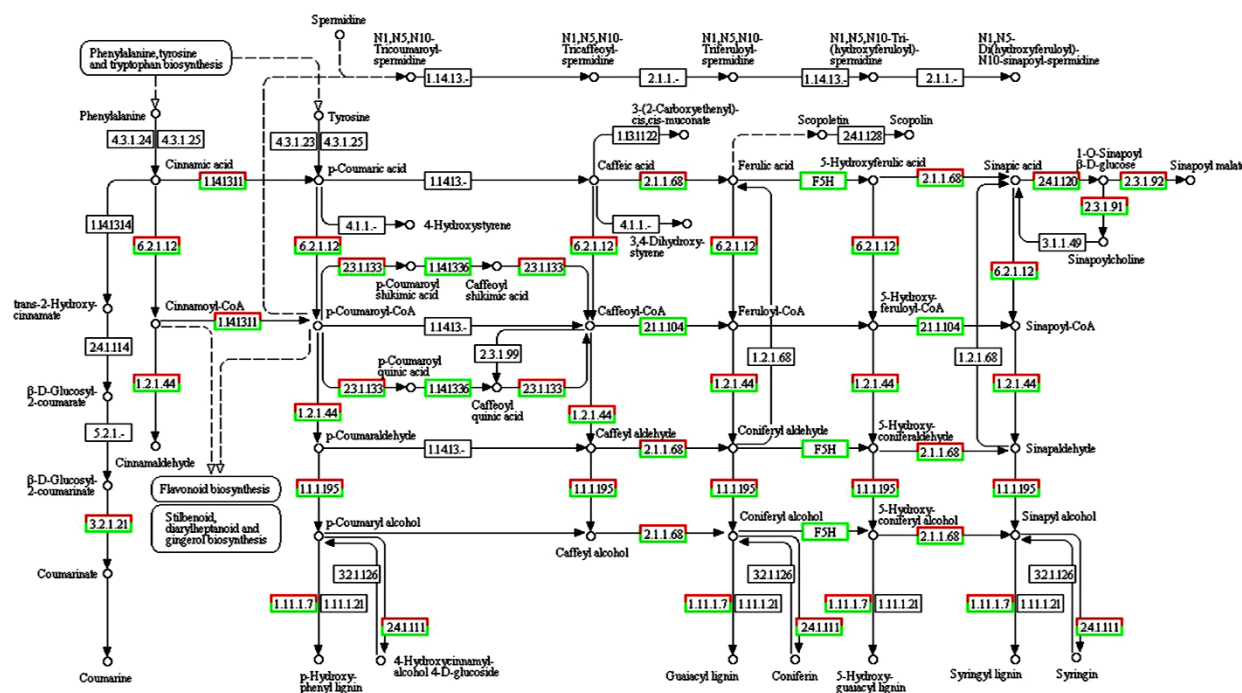


Fig. 2 “Stilbenoid, diarylheptanoid and gingerol biosynthesis” pathway induced by MeJA in root transcriptome of *P. multiflorum*

### 3.3.3 Effect of MeJA on the “plant hormone signal transduction” pathway

Plant hormones play a vital role in various growth and developmental processes in response to abiotic/biotic stresses. Some plant hormones, such as auxins, abscisic acid, and ethylene, have been associated with MeJA-induced phenotypes (Mukkun and Singh, 2009). The number of genes expressed in the “plant hormone signal transduction” pathway was second only to that of genes in the “plant-pathogen interaction” pathway, which comprised 1746 genes, including 621 DEGs (Table 1). Among the DEGs, 156 genes were up-regulated and 465 were down-regulated. Many genes related to plant hormones, including genes encoding gibberellin receptor, ethylene receptor (EC 2.7.13.-), and mitogen-activated protein kinase 6 (EC 2.7.11.24), were up- or down-regulated to variable degrees (Fig. S6).

The functional categories of “brassinosteroid biosynthesis” (Fig. S7) and “zeatin biosynthesis” (Fig. S8) were significantly over-represented under MeJA treatment. We found that a large number of genes, such as those encoding brassinosteroid insensitive 1-associated receptor kinase 1 (EC 2.7.10.1 and 2.7.11.1), protein brassinosteroid insensitive 1 (EC 2.7.10.1 and 2.7.11.1), protein brassinosteroid insensitive 2 (EC 2.7.11.1), brassinosteroid-6-oxidase 1 (EC 1.14.-.-), and brassinosteroid-6-oxidase 2 (EC 1.14.-.-), were induced under MeJA treatment. Genes encoding cytokinin dehydrogenase (EC 1.5.99.12) and cytokinin-*N*-glucosyltransferase (EC 2.4.1.-) were also differentially expressed.

### 3.3.4 Effect of MeJA on the vitamin pathway

Vitamins play key roles in many plant physiological processes, including promotion of plant growth and development, adjustment of physiological processes, and enhancement of production and quality (Miret *et al.*, 2014). Our study found that the biosynthesis of eight types of vitamins was affected by MeJA, with many genes up- or down-regulated to different degrees. Among these biosynthetic pathways, 189 genes involved in the “ascorbate and aldarate” pathway (Fig. S9) were expressed, including 84 DEGs (25 up-regulated and 59 down-regulated) mainly encoding GDP-D-mannose-3',5'-epimerase (EC 5.1.3.18 and 5.1.3.-), L-ascorbate oxidase (EC 1.10.3.3), and L-ascorbate peroxidase (EC 1.11.1.11).

A total of 99, 63, 55, and 41 genes were induced in pathways associated with “thiamine metabolism” (Fig. S10), “riboflavin metabolism” (Fig. S11), “nicotinate and nicotinamide metabolism” (Fig. S12), and “vitamin B<sub>6</sub> metabolism” (Fig. S13), respectively. Genes encoding cysteine desulfurase (EC 2.8.1.7), thiamine pyrophosphokinase (EC 2.7.6.2), riboflavin synthase (EC 2.5.1.9), riboflavin kinase (EC 2.7.1.26), nicotinamide-nucleotide adenylyltransferase (EC 2.7.7.1), nicotinate-nucleotide adenylyltransferase (EC 2.7.7.18), pyridoxal phosphatase (EC 3.1.3.74), and pyridoxine 4-dehydrogenase (EC 1.1.1.65) were up- or down-regulated to different degrees following MeJA treatment.

### 3.3.5 Effect of MeJA on the “starch and sucrose metabolism” pathway

Starch and sucrose metabolism provides energy for plant growth and development and other metabolic activities. In our experiment, 897 genes related to starch and sucrose metabolism were expressed in roots subjected to exogenous MeJA application. Among them, 318 genes were DEGs, with 75 genes up-regulated and 243 genes down-regulated (Fig. S14) including genes encoding pectinesterase (EC 3.1.1.11), sucrose synthase (EC 2.4.1.13), starch phosphorylase (EC 2.4.1.1),  $\beta$ -amylase (EC 3.2.1.2),  $\alpha$ -amylase (EC 3.2.1.1), glycogen debranching enzyme (EC 2.4.1.25), and  $\beta$ -glucosidase (EC 3.2.1.21).

### 3.3.6 Effect of MeJA on the terpenoid biosynthesis pathway

Plant terpenoids, which play an important role in the basic life activities of plants, are increased by foliar applications of MeJA (Han *et al.*, 2007; Chang *et al.*, 2013; Misra *et al.*, 2014). We found that genes involved in eight types of terpenoid biosynthesis pathways were induced. Among them, 245 genes related to the “terpenoid backbone biosynthesis” pathway (Fig. S15) were expressed, including 73 DEGs (16 up-regulated and 57 down-regulated). Among genes encoding key enzymes in this metabolic pathway, hydroxymethylglutaryl-CoA synthase (EC 2.3.3.10), hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34), mevalonate kinase (EC 2.7.1.36), phosphomevalonate kinase (EC 2.7.4.2), and farnesyl diphosphate synthase (EC 2.5.1.1 and 2.5.1.10) were differentially expressed. The next most-enriched

pathway was the “limonene and pinene degradation” pathway (Fig. S16), with 215 expressed genes including 89 DEGs, of which 35 genes were up-regulated and 54 were down-regulated. A total of 183 genes associated with the “carotenoid biosynthesis” pathway (Fig. S17) were expressed, of which 69 were DEGs, with 14 and 55 genes up- and down-regulated, respectively, under MeJA treatment. In the “diterpenoid biosynthesis” pathway (Fig. S18), 146 genes were expressed, including 60 DEGs, with 38 genes up-regulated and 22 genes down-regulated (Table 1).

### 3.3.7 Effect of MeJA on alkaloid biosynthesis pathways

Genes involved in three alkaloid pathways were expressed under exogenous MeJA application. Eleven of 45 expressed genes participating in the “tropane, piperidine and pyridine alkaloid biosynthesis” pathway (Fig. S19) were differentially expressed under MeJA treatment, with 2 genes up-regulated and 9 genes down-regulated. These genes mainly encoded the key enzymes such as primary-amine oxidase (EC 1.4.3.21), tropinone reductase 1 (EC 1.1.1.206), aspartate aminotransferase (EC 2.6.1.1), and tyrosine aminotransferase (EC 2.6.1.5). Of the 42 genes identified in the “isoquinoline alkaloid biosynthesis” pathway (Fig. S20), 10 were differentially expressed (2 up-regulated and 8 down-regulated), including genes encoding polyphenol oxidase (EC 1.10.3.1) and tyrosine decarboxylase (EC 4.1.1.25). Many key enzymes, such as EC 2.6.1.1, EC 2.6.1.5, and EC 4.1.1.25, are common to these two metabolic pathways. Eight of 24 expressed genes related to the “indole alkaloid biosynthesis” pathway (Fig. S21) were differentially expressed under exogenous MeJA treatment; 4 genes were up-regulated and 4 genes were down-regulated. They encoded mainly strictosidine synthase (EC 4.3.3.2) and polyneuridine-aldehyde esterase (EC 3.1.1.78).

### 3.3.8 Effect of MeJA on the basic physiological metabolic pathways

“Endocytosis”, a basic physiological metabolic process, was the third most significantly expressed pathway. Among the 1517 genes associated with this pathway, 532 were DEGs (154 up-regulated and 378 down-regulated) (Table 1, Fig. S22). Other basic

physiological metabolic pathways, such as “ribosome”, “glycerophospholipid metabolism”, “RNA transport”, “ether lipid metabolism”, “RNA polymerase”, “ubiquitin mediated proteolysis”, and “RNA degradation”, were differentially up- or down-regulated, with the number of affected genes varying from 572 to 1402. Genes in common plant pathways such as “glycolysis/gluconeogenesis”, “pentose phosphate pathway”, and “pentose and glucuronate interconversions”, were also significantly induced by MeJA treatment (Table 1).

## 3.4 Validation results

To validate the results of the transcriptome sequencing analysis, we carried out qRT-PCR assays on 70 genes, 50 up-regulated and 20 down-regulated, using gene-specific primers and RGs (Tables S1 and S2) based on the corresponding consensus sequences. Linear regression analysis revealed highly significant correlations between RG transcriptome sequencing and qRT-PCR results. The validation results showed that the expression profile of the 70 genes was consistent with corresponding RNA-seq data (Table S3). In addition, PCR products of 13 genes were sequenced to validate the accuracy of raw sequences. The similarity of sequences varied from 91.05% to 100% between the two methods (Table S5). These results further support the reliability of the RNA-seq data.

## 4 Discussion

Down-regulation of gene expression associated with active plant growth is an important effect of MeJA treatment. The concentration of MeJA partially determines whether genes are up- or down-regulated (Sun J.W. *et al.*, 2013; Misra *et al.*, 2014). In our study, some metabolic pathways were characterized by strong down-regulation due to 0.25 mmol/L MeJA. It is well known that MeJA can accelerate chlorophyll degradation, stimulate ethylene synthesis, improve hydrolase activity, and ultimately promote plant senescence when the exogenous MeJA concentration is higher than the plant’s physiological concentration (Matsuoka *et al.*, 2015). Consistent with this reported activity, annual cutting seedlings of *P. multiflorum* treated with 0.25 mmol/L MeJA in our study started

wilting after 4 d and then slowly began to defoliate, with tender plant parts affected first. Similar symptoms have been observed in rabbiteye and southern highbush blueberry (Malladi *et al.*, 2012). However, plants did not die, and new leaf growth recommenced after 20 d. This result indicates that MeJA concentrations above the plant physiological concentration have adverse effects on plant growth and development. Under these circumstances, it is likely advantageous for *P. multiflorum* plants to temporarily halt physiological activities, allowing DNA damage to be repaired and preventing the introduction of MeJA molecules into daughter cell DNA (Matsuoka *et al.*, 2015), but the mechanism needs further research.

## 5 Conclusions

The root transcriptome of *P. multiflorum* was differentially affected by root-irrigation treatment with MeJA. Three times as many genes were down-regulated than up-regulated. Analysis of the functional categories differentially represented under MeJA treatment revealed the existence of common stress responses. General protective responses, such as the induction of pathways regulating the synthesis of alkaloids, terpenoids, and stilbene compounds, the induction of the plant-pathogen interaction system, and the activation of the plant hormone signal transduction pathway associated with biotic or abiotic stress responses, played critical roles. Some common physiological metabolic pathways, such as “amino acid metabolism”, “glucose metabolism”, “fatty acid metabolism”, “citrate cycle”, “photosynthesis”, and “oxidative phosphorylation”, were also greatly induced. Furthermore, exogenous application of 0.25 mmol/L MeJA caused down-regulation of a large number of genes involved in numerous primary and secondary metabolic processes, especially “ribosome”, “plant hormone signal transduction”, “plant-pathogen interaction”, and “endocytosis” metabolic pathways. Down-regulation of a large number of genes under high MeJA concentrations may be an advantageous protective mechanism in plants. In addition, a large number of DEGs associated with various metabolic pathways were identified in the *P. multiflorum* root transcriptome, which will serve as a useful reference for future studies of func-

tional genes controlling effective components of metabolic pathways in *P. multiflorum*. More importantly, these findings provide a preliminary functional genomics framework to understand the complexity of the response of *P. multiflorum* to MeJA treatment.

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## Compliance with ethics guidelines

Hong-chang LIU, Wei WU, Kai HOU, Jun-wen CHEN, and Zhi ZHAO declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

- Audic, S., Claverie, J.M., 1997. The significance of digital gene expression profiles. *Genome Res.*, **10**(7):986-995.
- Benjamini, Y., Drai, D., Elmer, G., *et al.*, 2001. Controlling the false discovery rate in behavior genetics research. *Behav. Brain Res.*, **125**(1-2):279-284. [doi:10.1016/S0166-4328(01)00297-2]
- Chang, L.L., Zhang, Y.T., Wang, G.X., *et al.*, 2013. The effects of exogenous methyl jasmonate on *FaNES1* gene expression and the biosynthesis of volatile terpenes in strawberry (*Fragaria X ananassa* Duch.) fruit. *J. Hortic. Sci. Biotech.*, **88**(4):393-398.
- Conesa, A., Gotz, S., 2008. Blast2GO: a comprehensive suite for functional analysis in plant genomics. *Int. J. Plant Genomics*, **2008**:619832. [doi:10.1155/2008/619832]
- Conesa, A., Gotz, S., Garcia-Gomez, J.M., *et al.*, 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, **21**(18):3674-3676. [doi:10.1093/bioinformatics/bti610]
- Diallo, A.O., Agharbaoui, Z., Badawi, M.A., *et al.*, 2014. Transcriptome analysis of an *mvp* mutant reveals important changes in global gene expression and a role for methyl jasmonate in vernalization and flowering in wheat. *J. Exp. Bot.*, **65**(9):2271-2286. [doi:10.1093/jxb/eru102]
- Gaige, A.R., Ayella, A., Shuai, B., 2010. Methyl jasmonate and ethylene induce partial resistance in *Medicago truncatula* against the charcoal rot pathogen *Macrophomina phaseolina*. *Physiol. Mol. Plant Pathol.*, **74**(5-6):412-418. [doi:10.1016/j.pmpp.2010.07.001]
- Grabherr, M.G., Haas, B.J., Yassour, M., *et al.*, 2011. Full-length transcriptome assembly from RNA-seq data

- without a reference genome. *Nat. Biotechnol.*, **29**(7): 644-652. [doi:10.1038/nbt.1883]
- Han, J.L., Li, Z.Q., Liu, B.Y., et al., 2007. Metabolic engineering of terpenoids in plants. *Chin. J. Biotech.*, **23**(4): 561-569.
- Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Sta. Circular*, **347**:32.
- Huggett, J., Dheda, K., Bustin, S., et al., 2005. Real-time RT-PCR normalization; strategies and considerations. *Genes Immun.*, **6**(4):279-284. [doi:10.1038/sj.gene.6364190]
- Iseli, C., Jonqeneel, C.V., Bucher, P., 1999. ESTScan: a program for detecting, evaluating, and reconstructing potential coding regions in EST sequences. International Conference on Intelligent Systems for Molecular Biology, p.138-148.
- Jeandet, P., Douillet-Breuil, A.C., Bessis, R., et al., 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.*, **50**(10):2731-2741. [doi:10.1021/jf011429s]
- Ku, K.M., Juvik, J.A., 2013. Environmental stress and methyl jasmonate-mediated changes in flavonoid concentrations and antioxidant activity in Broccoli Florets and Kale leaf tissues. *Hortscience*, **48**(8):996-1002.
- Li, R.Q., Zhu, H.M., Ruan, J., et al., 2010. De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.*, **20**(2):265-272. [doi:10.1101/gr.097261.109]
- Li, Y.F., Nie, Y.F., Zhang, Z.H., et al., 2014. Comparative proteomic analysis of methyl jasmonate induced defense responses in different rice cultivars. *Proteomics*, **14**(9): 1088-1101. [doi:10.1002/pmic.201300104]
- Lin, L.C., Nalawade, S.M., Mulabagal, V., et al., 2003. Micropropagation of *Polygonum multiflorum* Thunb. and quantitative analysis of the anthraquinones emodin and physcion formed *in vitro* propagated shoots and plants. *Biol. Pharm. Bull.*, **26**(10):1467-1471. [doi:10.1248/bpb.26.1467]
- Lin, L.F., Ni, B.R., Lin, H.M., et al., 2015. Traditional usages, botany, phytochemistry, pharmacology and toxicology of *Polygonum multiflorum* Thunb.: a review. *J. Ethnopharmacol.*, **159**:158-183. [doi:10.1016/j.jep.2014.11.009]
- Liu, H.C., Wu, W., Hou, K., et al., 2015. Deep sequencing reveals transcriptome re-programming of *Polygonum multiflorum* Thunb. roots to the elicitation with methyl jasmonate. *Mol. Genet. Genomics*, in press. [doi:10.1007/s00438-015-1112-9]
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta C_t}$  method. *Methods*, **25**(4):402-408. [doi:10.1006/meth.2001.1262]
- Lv, L.S., Shao, X., Wang, L.Y., et al., 2010. Stilbene glucoside from *Polygonum multiflorum* Thunb.: a novel natural inhibitor of advanced glycation end product formation by trapping of methylglyoxal. *J. Agric. Food Chem.*, **58**(4): 2239-2245. [doi:10.1021/jf904122q]
- Malladi, A., Vashisth, T., Johnson, L.K., 2012. Ethephon and methyl jasmonate affect fruit detachment in rabbiteye and southern highbush blueberry. *Hortscience*, **47**(12):1745-1749.
- Matsuoka, D., Yasufuku, T., Furuya, T., et al., 2015. An abscisic acid inducible *Arabidopsis* MAPKKK, MAPKKK18 regulates leaf senescence via its kinase activity. *Plant Mol. Biol.*, **87**(6):565-575. [doi:10.1007/s11103-015-0295-0]
- Miret, J.A., Cela, J., Bezerra, L.A., et al., 2014. Application of a rapid and sensitive method for hormonal and vitamin E profiling reveals crucial regulatory mechanisms in flower senescence and fruit ripening. *J. Plant Growth Regul.*, **33**(1):34-43. [doi:10.1007/s00344-013-9375-z]
- Misra, R.C., Maiti, P., Chanotiya, C.S., et al., 2014. Methyl jasmonate-elicited transcriptional responses and pentacyclic triterpene biosynthesis in sweet basil. *Plant Physiol.*, **164**(2):1028-1044. [doi:10.1104/pp.113.232884]
- Moriya, Y., Itoh, M., Okuda, S., et al., 2007. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.*, **35** (Suppl. 2):W182-W185. [doi:10.1093/nar/gkm321]
- Mortazavi, A., Williams, B.A., Mccue, K., et al., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods*, **5**(7):621-628. [doi:10.1038/nmeth.1226]
- Mukkun, L., Singh, Z., 2009. Methyl jasmonate plays a role in fruit ripening of 'Pajaro' strawberry through stimulation of ethylene biosynthesis. *Sci. Hortic.*, **123**(1):5-10. [doi:10.1016/j.scienta.2009.07.006]
- Myhre, S., Tveit, H., Mollestad, T., et al., 2006. Additional gene ontology structure for improved biological reasoning. *Bioinformatics*, **22**(16):2020-2027. [doi:10.1093/bioinformatics/btl334]
- Nopo-Olazabal, C., Condori, J., Nopo-Olazabal, L., et al., 2014. Differential induction of antioxidant stilbenoids in hairy roots of *Vitis rotundifolia* treated with methyl jasmonate and hydrogen peroxide. *Plant Physiol. Biochem.*, **74**:50-69. [doi:10.1016/j.plaphy.2013.10.035]
- Vandesompele, J., de Preter, K., Pattyn, F., et al., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.*, **3**(7):research0034.1-0034.11. [doi:10.1186/gb-2002-3-7-research0034]
- Vannozzi, A., Dry, I.B., Fasoli, M., et al., 2012. Genome-wide analysis of the grapevine stilbene synthase multigenic family: genomic organization and expression profiles upon biotic and abiotic stresses. *BMC Plant Biol.*, **12**(1):130. [doi:10.1186/1471-2229-12-130]
- Repčák, M., Suvak, M., 2013. Methyl jasmonate and *Echinothrips americanus* regulate coumarin accumulation in leaves of *Matricaria chamomilla*. *Biochem. Syst. Ecol.*, **47**:38-41. [doi:10.1016/j.bse.2012.10.009]
- Rice, P., Longden, I., Bleasby, A., 2000. EMBOSS: the European

- molecular biology open software suite. *Trends Genet.*, **16**(6):276-277. [doi:10.1016/S0168-9525(00)02024-2]
- Rivera-Dominguez, M., Astorga-Cienfuegos, K.R., Tiznado-Hernandez, M.E., et al., 2012. Induction of the expression of defence genes in *Carica papaya* fruit by methyl jasmonate and low temperature treatments. *Electron J. Biotech.*, **15**(5):6. [doi:10.2225/vol15-issue5-fulltext-7]
- Sabater-Jara, A.B., Almagro, L., Pedreno, M.A., 2014. Induction of extracellular defense-related proteins in suspension cultured-cells of *Daucus carota* elicited with cyclodextrins and methyl jasmonate. *Plant Physiol. Biochem.*, **77**:133-139. [doi:10.1016/j.plaphy.2014.02.006]
- Sayyari, M., Babalar, M., Kalantari, S., et al., 2011. Vapour treatments with methyl salicylate or methyl jasmonate alleviated chilling injury and enhanced antioxidant potential during postharvest storage of pomegranates. *Food Chem.*, **124**(3):964-970. [doi:10.1016/j.foodchem.2010.07.036]
- Sivanandhan, G., Arun, M., Mayavan, S., et al., 2012. Optimization of elicitation conditions with methyl jasmonate and salicylic acid to improve the productivity of withanolides in the adventitious root culture of *Withania somnifera* (L.) Dunal. *Appl. Biochem. Biotech.*, **168**(3):681-696. [doi:10.1007/s12010-012-9809-2]
- Sun, G.L., Yang, Y.F., Xie, F.L., et al., 2013. Deep sequencing reveals transcriptome re-programming of taxus X media cells to the elicitation with methyl jasmonate. *PLoS ONE*, **8**(4):e62865. [doi:10.1371/journal.pone.0062865]
- Sun, J.W., Zhang, H., Wang, F.Y., et al., 2013. Effects of methyl jasmonate on accumulation and release of main tropane alkaloids in liquid cultures of *Datura stramonium* hairy root. *China J. Chin. Mater. Med.*, **38**(11):1712-1718 (in Chinese).
- Takahashi, I., Hara, M., 2014. Enhancement of starch accumulation in plants by exogenously applied methyl jasmonate. *Plant Biotech. Rep.*, **8**(2):143-149. [doi:10.1007/s11816-013-0304-1]
- Tatusov, R.L., Galperin, M.Y., Natale, D.A., et al., 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.*, **28**(1):33-36. [doi:10.1093/nar/28.1.33]
- Thimm, O., Blasing, O., Gibon, Y., et al., 2004. Mapman: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.*, **37**(6):914-939. [doi:10.1111/j.1365-313X.2004.02016.x]
- Yan, C.Y., Zhang, Z., Yu, R.M., et al., 2007. Studies on biotransformation of arbutin by 4-hydroxy phenol in hairy root of *Polygonum multiflorum*. *China J. Chin. Mater. Med.*, **32**(3):192-195 (in Chinese).
- Ye, J., Fang, L., Zheng, H.K., et al., 2006. WEGO: a web tool for plotting GO annotations. *Nucleic Acids Res.*, **34**(Suppl. 2):W293-W297. [doi:10.1093/nar/gkl031]
- Zhang, L.J., Wang, G., Zhang, F.M., et al., 2013. Soaking seeds in methyl jasmonate or benzothiadiazole induces resistance to an insect pest and stem decay in *Brassica campestris* L. ssp. *chinensis*. *J. Hortic. Sci. Biotech.*, **88**(6):715-720. [doi:10.1080/14620316.2013.11513029]
- Zheljazkov, V.D., Astatkie, T., Jeliaskova, E., 2013. Effect of foliar application of methyl jasmonate and extracts of juniper and sagebrush on essential oil yield and composition of 'Native' spearmint. *HortScience*, **48**(4):462-465.

## List of electronic supplementary materials

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## 中文概要

**题目:** 茉莉酸甲酯处理对何首乌根转录组的影响

**目的:** 研究外源茉莉酸甲酯 (MeJA) 处理对中药何首乌 (*Polygonum multiflorum*) 根转录组的影响, 分析在外源 MeJA 刺激下何首乌根各代谢途径中差异表达基因的变化, 为寻找影响何首乌根主要药效成分合成的关键酶基因奠定基础。

**创新点:** 首次在中药何首乌上利用外源 MeJA 处理研究何首乌根转录组的变化, 且获得了根中各代谢途径的大量差异表达基因。

**方法:** 将何首乌植株分为对照组 (Hoagland 营养液灌根) 和实验组 (含 0.25 mmol/L MeJA 的 Hoagland 营养液灌根)。灌根处理 26 h 后, 分别提取对照组和实验组根中的总 RNA, 利用新一代测序技术分析对照组和实验组根的转录组变化并进行测序。同时, 利用美国国家生物技术信息中心 (NCBI) 的蛋白质数据库和基因数据库信息, 对所测 cDNA 序列进行基因功能注释和代谢途径归类, 寻找 MeJA 刺激下何首乌根各条代谢途径中的差异表达基因。

**结论:** 外源 MeJA 刺激处理可影响中药何首乌根转录组的表达变化 (表 S1)。与对照相比, 外源 MeJA 处理可导致何首乌根中 4535 个基因上调, 14 142 个基因下调变化, 并分别在 125 个代谢途径中富集 (表 1)。代谢途径差异表达基因分析的结果表明, 可在相应代谢途径中找到影响何首乌某些主要药效成分合成的关键酶基因 (图 2)。差异表达基因的代谢途径归类分析结果提示今后可对何首乌根各代谢途径中的关键酶基因分别进行研究 (表 1)。综上所述, 利用外源 MeJA 处理后进行转录组测序对寻找何首乌中主要有效成份合成的关键酶基因有重要价值。

**关键词:** 何首乌; 茉莉酸甲酯; 转录组变化; 差异表达基因