



**Full Length Article**

## Effects of Arsenic on Flavonoids Accumulation and Metabolic Pathway in Main Roots of *Panax notoginseng*

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### Abstract

*Panax notoginseng* (Burk.) F.H. Chen is one of herbaceous, perennial medicinal plants. Flavonoids are the key medicinal compositions of *P. notoginseng*, but its regulating molecular mechanism under arsenic stress was not elucidated. The contents of flavonoids and arsenic, its relative key enzymes PAL (phenylalanine ammonia lyase) and CHS (chalcone synthase) in roots of *P. notoginseng* under different arsenic treatment levels ( $As^{5+}$ : 0, 20, 140 mg kg<sup>-1</sup>) were determined at early flowering stage. Meanwhile, the transcriptomes of *P. notoginseng* roots were compared using RNA sequencing (RNA-seq) to identify the molecular mechanism and candidate genes involved in flavonoids. The qRT-PCR assays were implemented to verify the expression patterns of nine representatives of regulated DEGs. The results showed that: (1) Flavonoid contents increased with 20 mg kg<sup>-1</sup> arsenic treatment, while decreased with 140 mg kg<sup>-1</sup> arsenic treatment. PAL activities increased and CHS activities decreased with increase in arsenic treatment concentrations. (2) Genes relating to arsenic stress showed distinct patterns with biosynthesis of secondary metabolism. We identified metabolism processing associated with flavonoid. (3) Genes of key enzymes relating to the pathway of flavonoid (relative genes: *DcCHS2*, *LOC100221899*) biosynthesis were identified. The results would be important in revealing the genetic data that affects flavonoid biosynthesis and provides novel gene resources for *P. notoginseng* breeding under arsenic stress. © 2018 Friends Science Publishers

**Keywords:** Medicinal plant; Heavy metal stress; Differential expressed genes; RNA-sequencing; Secondary metabolism

### Introduction

Arsenic (As) widely distributed in soils. Soil arsenic pollution has been paid a more and more attention (Dudka and Adriano, 1997; Zhou *et al.*, 2016). Some  $9.4 \times 10^7$  kg arsenic per year was input into soil in the world (Xiao *et al.*, 2008). According to The Report on the National General Survey of Soil Contamination of China from 2005 to 2013, some 16.1% of soils were contaminated in China and soil arsenic content in 2.7% of investigated sites was higher than the national standard values (The Report on the National General Survey of Soil Contamination, China, 2014). There are some arsenic mines with an average content 17.80 mg kg<sup>-1</sup> in red soil in Yunnan Province, China (Li *et al.*, 2009; Cai *et al.*, 2010; Yan *et al.*, 2011). Herbal medicines contaminated by arsenic were reported and limited to market (Liu *et al.*, 2014; Yan *et al.*, 2013). Due to the arsenic mining and the application of arsenic containing pesticides, soils in *Panax notoginseng* production area are contaminated by arsenic in Wenshan prefecture, Yunnan Province, China (Liu *et al.*, 2014; Liu *et al.*, 2016), which could result in poisoning reactions (Nnorom, 2014; Zhu *et al.*, 2017).

*Panax notoginseng* (Burk.) F. H. Chen, which has long cultivation history in Wenshan prefecture in Yunnan province, China, is belonging to the genus of *Panax*, family of Araliaceae (Guo *et al.*, 2010). There are thousands of years for *P. notoginseng* using as traditional Chinese medicine with many pharmacological roles (Xia *et al.*, 2014; Wang *et al.*, 2016). *P. notoginseng* could enhance blood circulation and pain relieving (Cui and Liao, 2001; Lee *et al.*, 2017), which are relative to flavonoids compounds. The flavonoids compounds were related to plants secondary metabolism, energy metabolism and environmental information processing. Flavonoids compounds as a part of plant integrated defense system could decrease cell damage and eliminate oxygen radicals (Hou *et al.*, 2016). Flavonoids contents increased under environmental stress. Flavonoids are related to quercetin biosynthesis pathway. The metabolic pathway of flavonoids are controlled by some key enzymes, including PAL (phenylalanineammoniallyase), C4H (cinnamate 4-hydroxylase), 4CL (4-coumaroyl-CoA ligase), CHS (chalcone synthase), CHI (chalcone isomerase), F3H (flavonone 3-hydroxylase), F3'H (flavonoid 3'-hydroxylase) and FLS (flavonol synthase) (Tu *et al.*, 2014). PAL and

CHS are the key regulatory enzymes. RNA-seq is widely used in plant response mechanism to environmental stresses (Zhang et al., 2013). However, the impact of soil arsenic on flavonoids metabolism of *P. notoginseng* is not known well.

This aims are to understand the enzymatic and transcriptome mechanisms of flavonoids contents from *P. notoginseng* with different arsenic treatment levels in soil under field condition based on RNA-seq.

## Materials and Methods

### Plant Materials and Experiment Procedure

Field experiments were carried out in Dalongtan village (22°39' N, 103°06' E, elevation 2251m), Xundian County, Kunming prefecture, Yunnan province, China. The area of plot was 3 m<sup>2</sup>. Soil from the 0–15 cm was mixed with the arsenic stock solutions with As<sup>5+</sup> 0, 20, 140 mg kg<sup>-1</sup> (prepared using H<sub>3</sub>AsO<sub>4</sub>). The arsenic treated soil was incubated for 15 days. The background of soil characteristics before arsenic treatment had a pH 5.32, organic matter content 16.77 g kg<sup>-1</sup>, total N content 0.60 g kg<sup>-1</sup>, total P content 1.20 g kg<sup>-1</sup> and arsenic content 12.00 mg kg<sup>-1</sup> with clay loam texture.

One-year old *P. notoginseng*, which was named by Huaiman Chen (1900–1993), a taxonomist from South China Botanical Garden, Chinese Academy of Sciences), were transplanted with 132 plants per plot in Jan, 2015. The cold frame plastic greenhouse covered with two layers of shade cloth provided 17% of natural light intensity. Some 15 plants were harvested in each plot when plants were at the early flowering stage. Fresh root tissues were washed with tap water followed by rinsing in deionized water and frozen in liquid nitrogen and stored at -80°C for analysis of RNA-seq, Real Time PCR (qRT-PCR) and the activities of PAL and CHS. The other roots tissues were dried at 105°C for 30 min following by 65–70°C until constant weight for biomass and then were ground to a fine powder for testing arsenic and flavonoids contents.

### Determination and RNA-seq Analysis

**Arsenic contents determination:** After 0.1 g main root powder of *P. notoginseng* was added into aerenmeyer flask (150 mL), the following order of 7 mL sulphuric acid, 10 mL nitric acid, and 2 mL perchloric acid were added in the reagent solutions. The mixture was heated on an electric heating plate to evaporate the nitric acid. After cooling and dissolving in distilled water, the filtrates were adjusted to a 25 mL final volume. AsH<sub>3</sub> was generated after the addition of thiourea and potassium borohydride (KBH) solutions. Arsenic concentrations were tested with atomic fluorescence spectrometer (AFS-933, Beijing Jitian Instrument Ltd. Company). Arsenic content was converted to mg kg<sup>-1</sup> (dry weight).

**Flavonoids contents determination:** A root powder of 0.1 g was dissolved in 50 mL 70% methanol followed by

ultrasonic extraction 2 h and then centrifuged at 4000 r min<sup>-1</sup>. One mL of supernatant liquor was taken and diluted 12 times, which were analyzed using ultraviolet spectrophotometer (T6-1650E, Beijing Persee Instrument Limited Company, China) at 249 nm. Flavonoid content was converted into % of dry weight.

**Enzyme activities determination:** Samples of 1 g were added (1: 10, w:v) into a buffer consisting of 2 mL 100 mmol L<sup>-1</sup> phosphate buffered saline (pH 7.5), 2 mL 250 mmol L<sup>-1</sup> sucrose, 2 mL 4 mmol L<sup>-1</sup> magnesium chloride hexahydrate, and 2 mL 5 mmol L<sup>-1</sup> 2-Mercaptoethanol. Tissues were homogenized in an icy cold water bath. After centrifugation at 10,000 rpm for 10 min, the supernatant was collected as the crude enzyme extracts.

**Phenylalanine ammonia lyase (PAL):** Enzymatic assay was determined with a phenylalanine ammonia lyase assay kit (Tszelisa, USA) on a ELISA Reader (DNM-9602, Beijing Perlong New technology Limited Company). Enzymatic activity of PAL was indicated by units per gram of fresh weight (U mg<sup>-1</sup> prot).

**Chalcone synthase (CHS):** Enzymatic assay was determined with a chalcone synthase assay kit (Tszelisa, USA) on a ELISA Reader (DNM-9602, Beijing Perlong New technology Limited Company). Enzymatic activity of chalcone synthase was showed in units per gram of fresh weight (U mg<sup>-1</sup> protein).

### RNA-seq Analysis

The Trizol Kit (Promega, USA) was used to isolate RNA of root tissue with RNase-free DNase I (Takara Bio, Japan) for 30 min at 37°C to remove residual DNA. A 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) was used to ensure the RNA quality, which was checked by RNase free agarose gel electrophoresis. The concentration of the total RNA was measured by a 2100 Bioanalyzer at 260 nm and 280 nm, whose ratio of 260 nm to 280 nm between 1.8 and 2.0 were used for subsequent analyses.

The extracted RNA samples were used for the cDNA synthesis. Oligo-dT beads (Qiagen) was used to isolate Poly (A) mRNA. After mRNA was broken into small fragments by adding fragmentation buffer, following agarose gel electrophoresis, the final cDNA library was construct with the RNA fragments (~300 bp). Random hexamer-primed reverse transcription was used to obtain first-strand cDNA, which followed by the synthesis of the second-strand cDNA using RNase H and DNA polymerase I. AQIAquick PCR extraction kit was used to purify the cDNA fragments, which were washed with EB buffer for end reparation poly (A) addition and ligated to sequencing adapters. The Illumina sequencing platform (IlluminaHiSeq™ 2500) was used to sequence the cDNA library by GeneDenovo, Guangzhou, China.

After clean reads were selected with aperl program, Trinity 2.0 to construct unique consensus sequences was used to assemble the clean reads. The transcripts were

annotated with BLASTx, against databases of NCBI non-redundant protein database (Nr) (<http://www.ncbi.nlm.nih.gov/>), clusters of Orthologous Groups of proteins database (COG) (<http://www.ncbi.nlm.nih.gov/COG/>), KEGG and Swiss-Prot (<http://www.expasy.ch/prot>). Blast2GO was used to get the annotation results of Unigenes in Gene Ontology (GO) (<http://www.geneontology.org/>).

The Bowtie2 was used to map and reference sequence the clean reads. RPKM (the number of uniquely mapped reads per kilobase of exon region per million mappable reads) was used to measure the gene expression level. The significance of the gene expression differences was based on the false discovery rate (FDR)  $\leq 0.05$  and an absolute value of  $\log_2 \text{Ratio} \geq 1$ . Different expression genes (DEGs) were annotated by the GO database with Blast2GO.WEGO (<http://wego.genomics.org.cn/cgi-bin/wego/index.pl>) was used to obtain functional classifications for all the DEGs and cluster analysis of gene expression patterns with Java Treeview software.

### Quantitative Real Time PCR (qRT-PCR) Validation

For qRT-PCR, transcripts of target genes were amplified in a 20  $\mu$ l reaction containing 2  $\mu$ l cDNA (corresponding to 20 ng RNA), 1  $\mu$ l primers and 5  $\mu$ l SsoFast™ EvaGreen R Supermix (Bio-Rad, USA). Quantitation of each transcript was repeated using total RNA from two independent samples as starting materials and each qPCR was performed in triplicate.

### Data Statistical Analysis

Data were analyzed with Microsoft Excel 2000. The correlation between arsenic treatment levels, and arsenic contents, biomass of root were analyzed with SPSS (11.0).

Arsenic accumulation rate = mean of biomass  $\times$  arsenics content.

Flavonoids accumulation rate = mean of biomass  $\times$  flavonoids content.

## Results

### Effects of Arsenic on Biomass and Flavonoids Accumulation in Roots of *Panax notoginseng*

Biomass in roots tissues under 20 mg kg<sup>-1</sup> arsenic treatment was higher than that under non-arsenic treatment. Arsenic contents in roots increased with increases in arsenic treatment concentration. Compared with non-arsenic treatment, arsenic contents in roots increased by 4.02 and 18.93 times under 20 and 140 mg kg<sup>-1</sup> arsenic treatments, respectively (Table 1). Flavonoid contents and accumulation increased with 20 mg kg<sup>-1</sup> and decreased with 140 mg kg<sup>-1</sup> arsenic treatments (Table 2).

### Differentially Expressed Genes with Different Arsenic Treatment Concentrations

Based on flavonoid contents in main roots of *P. notoginseng* could not simply estimate through PAL and CHS activities, the differentially expressed genes (DEGs) from the collection of 48,325 Unigenes were screened (Table 3). Based on the criteria of fold-change  $\geq 2$  and FDR  $< 0.05$ , 72 and 228 genes were identified up- and down-regulated under 20 mg kg<sup>-1</sup> arsenic treatment, respectively, compared with non-arsenic treatment. Compared with 0 mg kg<sup>-1</sup> arsenic treatment, 90 and 186 genes were up- and down-regulated under 140 mg kg<sup>-1</sup> arsenic treatment, respectively; Compared with 20 mg kg<sup>-1</sup> arsenic treatment, 42 and 33 genes were up- and down-regulated under 140 mg kg<sup>-1</sup> arsenic treatment, respectively (Fig. 1). The DEGs under 20 mg kg<sup>-1</sup> arsenic treatment was higher than the response to 140 mg kg<sup>-1</sup> arsenic treatment compared with 0 mg kg<sup>-1</sup> arsenic treatment. With increase in arsenic treatment concentrations, the number of up-regulated DEGs increased, but the down-regulated DEGs decreased. In total, the DEGs were mainly down-regulated. A set of 474 DEGs for subsequent identification of genes involved in the regulation of *P. notoginseng* with arsenic stress were obtained.

Nine DEGs were selected for qRT-PCR, which were relative to biosynthesis of secondary metabolism. The qRT-PCR data were similar tendency to those from RNA-seq for these DEGs ( $P < 0.05$ ) with the different fold change values (Fig. 2).

### Main Categories of Differentially Expressed Genes Associated with Flavonoids under Different Arsenic Treatments

The contents of flavonoid of *P. notoginseng* should be comprehensive results response to arsenic stress. Based on FDR  $< 0.05$ , we identified 283 and 286 biological process (BP) related DEGs, 95 and 90 molecular function (MF) related DEGs with 20 and 140 mg kg<sup>-1</sup> arsenic treatments compared with 0 mg kg<sup>-1</sup> arsenic treatment, respectively. There were 74 biological process and 23 molecular function related DEGs under 140 mg kg<sup>-1</sup> arsenic treatment compared with 20 mg kg<sup>-1</sup> arsenic treatment. Genes clustered in GO terms of biosynthesis of secondary metabolism were significantly enriched under arsenic treatments compared with 0 mg kg<sup>-1</sup> arsenic treatment (Table 4).

Of 52106 Unigenes in *P. notoginseng*, 11042 could be annotated and mapped to different pathway based on KEGG analysis. Transcripts encoding proteins involved in biosynthesis of secondary metabolites were significantly enriched (Table 5), showing that 7 of the 52 annotated DEGs in the arsenic 20 mg kg<sup>-1</sup> treatment were relative to these pathways. The phenylpropanoid biosynthesis, phenylalanine metabolism, pyruvate metabolism and flavonoid biosynthesis were identified, which were contributed to flavonoid metabolism.

**Table 1:** Biomass and arsenic contents in main roots of *Panax notoginseng* under different arsenic treatments

Arsenic treatment concentration (mg kg <sup>-1</sup> )	Biomass (gplant <sup>-1</sup> )	Arsenic content (mg kg <sup>-1</sup> )	Arsenic accumulation (μgplant <sup>-1</sup> )
0	2.32±0.15b	2.48±0.08c	5.75
20	2.84±0.21a	12.45±1.42b	35.36
140	2.54±0.13b	49.43±11.80a	125.55

Data were mean ± standard deviation. The different letter in the same line meant significant differences between the treatments at  $P < 0.05$  level. The same was as below

**Table 2:** Activities of PAL, CHS and flavonoids contents in main roots of *Panax notoginseng* under different arsenic treatments

Arsenic treatment concentration (mg kg <sup>-1</sup> )	PAL (Umg <sup>-1</sup> prot)	CHS (Umg <sup>-1</sup> prot)	Flavonoids content (mg kg <sup>-1</sup> )	Flavonoids accumulation (μgplant <sup>-1</sup> )
0	3.91±0.02c	9.49±0.23a	2.98±0.35b	6.91
20	4.26±0.05b	6.40±0.20b	3.51±0.19a	9.97
140	6.10±0.10a	4.23±0.12c	2.60±0.51b	6.60

**Table 3:** Summary of the mapping reads and unigenes identified by RNA-seq

	Number of unigenes
Total unigenes	52106
Total mapped unigenes	48325(92.80%)
Mapped in sample	
0 mg kg <sup>-1</sup> arsenic treatment	46547 (89.33%)
20 mg kg <sup>-1</sup> arsenic treatment	47164 (90.52%)
140 mg kg <sup>-1</sup> arsenic treatment	46905 (90.02%)

**Table 4:** log<sub>2</sub><sup>Ratio(Fold)</sup> of DEGs with enriched GO terms of biosynthesis of secondary metabolism

Unigene ID	Description	log <sub>2</sub> <sup>Ratio(T1/CK)</sup>	log <sub>2</sub> <sup>Ratio(T2/CK)</sup>	log <sub>2</sub> <sup>Ratio(T2/T1)</sup>
Unigene0018486	Pyruvate decarboxylase isozyme 2	-2.8	-3.4	-0.6
Unigene0030569	alcohol dehydrogenases homologous	-2.6	-2.3	0.3
Unigene0036356	1-aminocyclopropane-1-carboxylate	-2.72	-3.27	-0.66
Unigene0040190	glyceraldehyde 3-phosphate dehydrogenase	-2.5	-2.8	-0.3
Unigene0051271	gibberellin 2-beta-dioxygenase	-5.2	-4.6	0.6
Unigene0051338	Cytochrome P450 CYP73A100	-3.29	-4.01	0.72

CK: arsenic 0 mg kg<sup>-1</sup>, T1: arsenic 20 mg kg<sup>-1</sup>, T2: arsenic 140 mg kg<sup>-1</sup>

**Table 5:** The number of DEGs in the flavonoids corresponding pathway differentially regulated by arsenic treatment

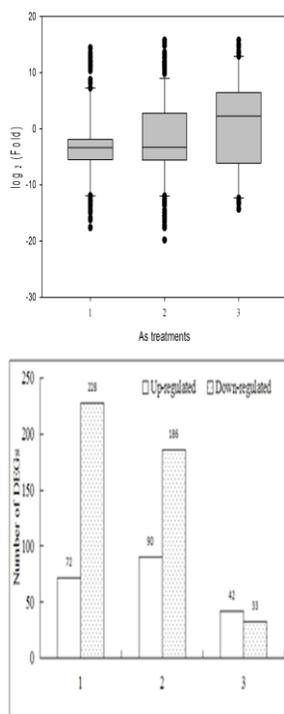
Pathway	Arsenic 0 vs 20 mg kg <sup>-1</sup>	Arsenic 0 vs 140 mg kg <sup>-1</sup>	Arsenic 20 vs 140 mg kg <sup>-1</sup>
Metabolic pathways	14 (26.92%)	13(31.71%)	3(23.08%)
Photosynthesis	1 (1.92%)	1(2.44%)	1(7.69%)
Biosynthesis of secondary metabolites	7 (13.46%)	9(21.95%)	-
Plant hormone signal transduction	7 (13.46%)	4(9.56%)	1(7.69%)
Phenylpropanoid biosynthesis	2 (3.85%)	2(4.88%)	-
Phenylalanine metabolism	1 (1.92%)	2(4.88%)	-
Pyruvate metabolism	-	2(4.88%)	-
Flavonoid biosynthesis	1 (1.92%)	1(2.44%)	-

Data in the parenthesis were percentage of DEGs number in the corresponding pathway to total different DEGs. “-” meant no DEGs

### Expression Levels of Differentially Expressed Genes Associated with Flavonoids Pathway Enriched under Different Arsenic Treatments

There were three sets DEGs annotated biosynthesis of secondary metabolism, phenylalanin metabolism and flavonoid biosynthesis according to the KEGG annotation loaded into MeV for hierarchical clustering analysis based on whether they showed a trend toward up- (red) or down-regulation (blue) (absolute ratios of log<sub>2</sub> value > 10 between 0–20, 0–140, 20–140 mg kg<sup>-1</sup> arsenic treatments

comparison groups) (Fig. 3). DEGs involved in biosynthesis of secondary metabolism were significantly inhibited with 20 mg kg<sup>-1</sup> arsenic treatment to 140 mg kg<sup>-1</sup> arsenic treatment, indicating constitutive inhibition by arsenic stress. 140 mg kg<sup>-1</sup> arsenic treatment led to a increase in transcript abundance of Unigene 0017947 (pyruvate metabolism) compared with 20 mg kg<sup>-1</sup> arsenic treatment (Fig. 3A and Table 4). Genes encoding phenylalanine relative to PAL were similar with flavonoid metabolic process gene family, with PAL gene Unigene 0030404 slight down-regulated by arsenic treatment (Fig. 3B).



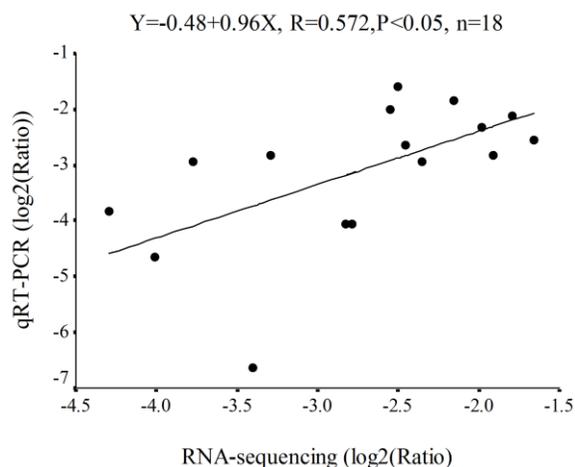
**Fig. 1:** Global comparisons of transcript profiles and DEGs in responses to different arsenic treatments. Note: 1: arsenic 0 vs 20 mg kg<sup>-1</sup>; 2: arsenic 0 vs 140 mg kg<sup>-1</sup>, 3: arsenic 20 vs 140 mg kg<sup>-1</sup>

Most of flavonoid metabolic process gene families were activated by arsenic treatments, while part of genes was inhibited (Fig. 3C).

RPKM of PAL gene (Unigene0030404) was low than 10, which resulted in the limiting influence on PAL activities (Fig. 4).

## Discussion

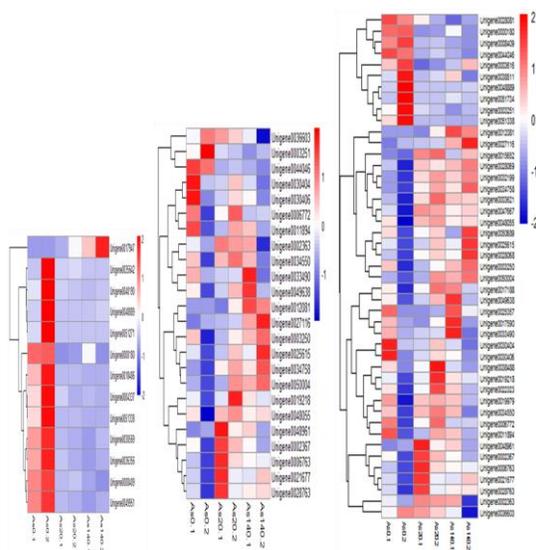
Considering the relative enzymes, PAL activities increased and CHS activities decreased with increase in arsenic contents in main roots and arsenic treatment concentrations, which showed the PAL activity induced and the CHS activity inhibited by arsenic stress (Zu *et al.*, 2016). Other researches results showed PAL and CHS activities in *Erigeron breviscapus* increased under environmental stress (Feng *et al.*, 2009). CHS could be induced by UV-B radiation, fungal and trace elements, which changed the flavonoid contents (Hou *et al.*, 2016). CHS activities were on the contrary to PAL activities under arsenic stress. It suggested that flavonoids contents should be depended on PAL activities under 20 mg kg<sup>-1</sup> arsenic treatment and on CHS activities under 140 mg kg<sup>-1</sup> g arsenic treatment. More information with RNA-sequencing analysis showed 300 and 276 DEGs under 20 and 140 mg kg<sup>-1</sup> arsenic treatments, respectively, of which 139 Unigenes were common in both datasets, including biological process and molecular



**Fig. 2:** Correlation between qRT-PCR and RNA-sequencing for the nine selected genes. Each point represents a value of fold change of expression level at T1 or T2 comparing with at T0. Fold-change values were log<sub>2</sub> transformed

function, especial environmental information processing, biosynthesis of secondary metabolism and energy metabolism. Transcript changes in metabolism of carbohydrate and energy, membrane transport of *S. algae* were response to salt stress and reactive oxygen species stress in *Litchi Chinese* (Fu *et al.*, 2014; Lu *et al.*, 2014).

Focused on genes involved in flavonoid metabolism processing, some DEGs were up- or down-regulated according to the KEGG annotation with arsenic treatments (Zhu, 2014; Zhou *et al.*, 2016). The metabolic pathway of flavonoids is controlled by some key enzymes (Tu *et al.*, 2014). The PAL gene transcript level is responsive to environmental stress including nutrient deficiency, UV irradiation and extreme temperatures. For instance, most of PAL family genes were up-regulated in allelopathic rice PI312777 under nitrogen deficiency, while only PAL8 and PAL11 genes up-regulated in non-allelopathic rice Lemont (Fang *et al.*, 2011). Gene expressions of C4H, CHS and CHI were inhibited under 140 mg kg<sup>-1</sup> arsenic stress, which CHS gene expression was similar with CHS enzyme activity and flavonoids contents (Liu *et al.*, 2014). The shading condition hindered the synthesis of anthocyanin in the petals of 'spectra', as well as inhibited the expression of genes CHS (Fang *et al.*, 2013). It was suggested that the flavonoid contents of *P. notoginseng* could be more depended on activities of CHS (Unigene0025357 gene, *DcCHS2*), other than PAL activity. Both of F3H and FLS were based on gene expressions of Unigene0032199 (*LOC108221899*), which increased under arsenic treatments. Flavonoids are one of medical components in *P. notoginseng* and are resistant substance to environmental stresses (Bi *et al.*, 2015). F3H and FLS genes expressions were activated under arsenic treatments resulted in increase in flavonoids contents and resistance of *P. notoginseng*.



**Fig. 3:** Clustering of flavonoid relative genes expression profiles with arsenic treatments. Note: Expression ratios are expressed as log 2 values. Red color represents increasing level of the gene expression and blue color indicates decreasing of the gene expression

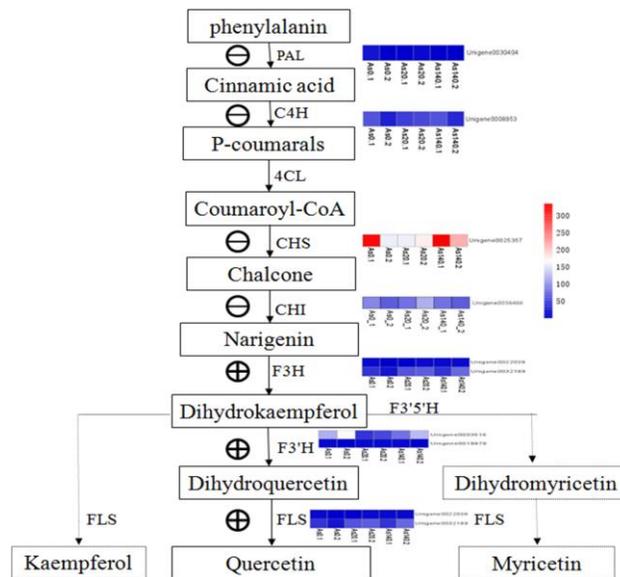
A: Biosynthesis of secondary metabolism B: Phenylalanin metabolism (PAL) C: Flavonoid biosynthesis

F3'5'H relative gene expression was not detected in *P. notoginseng*, which suggested that the dihydromyricetin and myricetin were not mainly flavonoids in *P. notoginseng* under arsenic stress. Kaempferol and quercetin should be the mainly flavonoids in *P. notoginseng*. Quercetin was extracted from *P. notoginseng* (Wei and Wang, 1987). Changes in gene expression of CHI, F3H and FLS were consistent with that of the total flavonoids, suggesting these genes might play an important role in the adaptation of *Fagopyrum tataricum* to environmental stresses (salt (NaCl), drought (PEG-6000), and UV-B radiation) during florescence (Hou et al., 2016).

In general, the results should be of important value to provide a new theoretical basis and it could be possible to obtain novel gene resources for Chinese herbal medicine plant breeding under environmental stress.

### Conclusion

Flavonoids accumulation and metabolism pathway of *Panax notoginseng* could be explained by enzymetic activities and comprehensive differentially expressed genes under arsenic stress. Flavonoids metabolism could be activated by arsenic treatment through F3H and FLS genes expressions increased, but limited by CHS and CHI genes expressions. F3H and FLS should be the most important key rate-limiting enzymes for flavonoids metabolism. The flavonoids in *P. notoginseng* should be dominant with kaempferol and quercetin. Up-regulated genes (*DcCHS2*, *LOC100221899*) were the candidate genes for flavonoids metabolism of *P. notoginseng* under arsenic stress. It is important for genomic studies of *P. notoginseng*.



**Fig. 4:** The metabolic pathway and DEGs of flavonoids. PAL: phenylalanine ammonia lyase, C4H: cinnamate 4-hydroxylase, 4CL: 4-coumaroyl-CoA ligase, CHS: chalcone synthase, CHI: chalconeisomerase, F3H: flavonone-3-hydroxylase, F3'5'H: flavonoid 3', 5'-hydroxylase, F3'H: flavonoid 3'-hydroxylase, FLS: flavonol synthase. up-regulated, ⊖ down-regulated

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