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Full Length Article

Analysis of Transcriptome differences in Two Leaf-Type Cultivars of FuZi (*Aconitum armichaelii*)

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Abstract

FuZi (*Aconitum armichaelii*) is an important medicinal plant in China, as well as agricultural and economic crops. There are many cultivars with different agronomic characters. According to the major differences of agricultural characters among various FuZi cultivars, the lateral roots of Ai-leaf and Dahua-leaf FuZi plants were selected as the research objects. Illumina Hiseq high-throughput platform was used for transcriptome sequencing, assembly and annotation. We mostly focused the activity differential transcripts, metabolism pathways and enrichment functions. The results showed that a total of 52.23 Gb nucleotide bases were obtained from six FuZi transcriptome databases, with 52,471 unigenes and 28,765 matched annotation. There were 1,052 transcripts of the two kinds of FuZi with a difference of more than two times, 808 of which were annotated. Through Gene Ontology (GO) and Clusters of Orthologous Groups (COG) analysis, they were found to mainly concentrate in metabolic processes, cell processes, catalytic processes and transport processes, connections and other functions. Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis showed that 262 differentially expressed unigenes (DEGs) were enriched in 78 metabolic pathways, such as starch and sucrose metabolism, plant hormone signaling, carbon compounded transport etc. It was implied that many genes in Dahua-leaf FuZi regulated the conversion of starch to small molecules such as sucrose, glucose and maltose, while some other genes regulated the accumulation of amino acids, which may be the important biological principles for the formation of the differences between the quality and disease resistance of two leaf types of FuZi. This study will provide reference data for FuZi breeding research.

Keywords: FuZi; Cultivation type; Transcriptome; Quality formation; Disease resistance

Introduction

FuZi is a typical genuine medicinal material in China, and also an important agricultural economic crop in Southwest China. It has a cultivation history of more than 1300 years, mainly produced in Sichuan, Shaanxi, Yunnan, Guizhou, Chongqing and other regions (Huang *et al.* 2011; Zhang *et al.* 2017a). Both the Chinese government and Chinese companies have invested a lot in FuZi. It is a biennial herb, native to the northwest and east of the Indian Himalayas. It is diverse in the East Asian mountains, widely distributed from New York to the south of George Asia, and also distributed in the divided areas of Ohio, Wisconsin and Iowa. It usually grows in woodland, and its tuberous root is usually a therapeutic component of traditional Indian medicine, which is used to treat dyspepsia, abdominal pain, diabetes, etc. Some East Asian countries, such as Japan, South Korea, Mongolia and India, import the drug from China in large quantities.

Aconitum carmichaelii, the basic plant of FuZi, is the most widely distributed species with the most different characters, which leads to a variety of leaf types of FuZi (Gao *et al.* 2018). The leaf type is usually selected according to 11 leaf characteristics, such as leaf size, leaf shape, leaf division degree, leaf surface, leaf back and hair state etc. In the planting base of Sichuan province, FuZi has many common names such as pumpkin leaf, Dahua leaf, Xiaohua leaf, goose palm leaf, berry leaf, oil leaf and Maoshi Miao according to leaf shape (Xiao *et al.* 1991), and there is a big regional difference in the definition of plant and leaf characters. At present, the main leaf types of FuZi cultivation are flower leaves (including Dahua leaves and pumpkin leaves), mixed with a small amount of Ai leaves (due to the small yield, it belongs to the type gradually selected and eliminated by the base). The Hua-leaf FuZi is favored by the farmers due to its high single yield. The saying "Jiangyou FuZi is big and of high quality " refers to the type of Hua-leaf, but the Hua-leaf FuZi is more sensitive to external pathogens, and is very vulnerable to root rot (Gao *et al.* 2017). Compared with Hua-leaf FuZi, Ai-leaf FuZi has higher resistance to root rot and white silk disease, though its plant is weak and its yield is small.

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At present, there are some basic researches on the biology development and intraspecific variation of FuZi in China (Huang *et al.* 1980; Xiao *et al.* 1990; Xiao *et al.* 1991; Hu *et al.* 2008), and some explorations on the transcriptome molecular markers of leaf type differences and stress resistance (Wen *et al.* 2016; Zhang *et al.* 2017b). However, there are few special academic reports on the formation of type quality and agricultural disease resistance, and the molecular biological mechanism is even less in this paper, we selected Dahua-leaf and Ai-leaf FuZi, and compared their transcriptome and expression activity of related genes, to explore the molecular biological mechanism of leaf quality differences between the two FuZi types from the perspective of growth metabolism network and key gene differences in them.

Materials and Methods

Plant collection

In this study, the main cultivated leaf types of FuZi are Dahua-leaf and Ai-leaf FuZi plants, which were collected in GAP base of Taiping Town, Jiang you City, Sichuan Province (N31 ° 44 ", E104 ° 42 ') in the middle of June 2018, and the plants of FuZi were photographed respectively (Fig. 1). The roots with soil were extracted and brought back to the greenhouse of National Resources Bank for planting traditional Chinese medicine of Chengdu University of Traditional Chinese Medicine for cultivation.

According to the flora of China, the leaves of FuZi are thin leathery or papery, pentagonal, 6–11 cm long and 9–15 cm wide, with shallow heart-shaped trifid at or near the base, wide rhombus in the center, sometimes obovate rhombus or rhombus, acute, sometimes short acuminate and nearly pinnate, with about two pairs of secondary lobes, obliquely triangular, with 1–3 teeth, occasionally or completely, and lateral complete lobes Unequal dichroism, surface sparsely pubescent, abaxial surface usually sparsely pubescent only along veins (Editorial Committee of flora of China 1993). On this basis, the stems of Dahua-leaf are thick and glossy (slightly yellow in June), the teeth of the leaves are gentle, and the leaves split shallowly; while the stems of Ai-leaf FuZi are thin and weak, and there are more tillers in the axils of the leaves, with small leaf surface, sharp leaf tip, narrow and long leaf bases, and the leaves split deep to the petiole, whose shape is like that of *A. argyi*.

Subroot processing

After the two types of FuZi were adapted to grow in the greenhouse for two weeks, three plants were selected with similar growth potential for root extraction and numbered it (D1, D2, D3 for Dahua-leaf, x1, x2, x3 for Ai-leaf), cleaned the surface soil with distilled water and washed with 75% alcohol. After drying, the seeds were frozen at -80℃ immediately.

DNA extraction and cDNA library construction

The total RNA of each sample tissue was extracted with Trizol Kit (Burlington Co. Canada) by using the cryopreserved roots of Dahua-leaf and Ai-leaf FuZi plants, using oligo (DT) magnetic beads to separate and purify mRNA, decomposing it into short gene segments, and then reverse transcribing it into cDNA chains, and establishing six transcriptome libraries of FuZi.

High throughput sequencing, assembly and functional annotation of transcriptome

High-throughput sequencing platform Illumina Hillseq2500 of Illumina Company was used to sequence the transcriptome library of FuZi. The primers of sequencing splices were intercepted and low-quality data of the original base sequence was filtered to obtain clean reads. The long fragment contig was assembled with Trinity software to obtain the long fragment set component (Unigene). BLAST software was used to compare the Unigene sequence with the NCBI non-redundant protein sequence (NR), Swiss-Prot protein (Swiss-pro)t, Gene Ontology (GO), Clusters of Orthologous Groups (COG), euKaryotic Orthologous Groups (KOG), eggNOG4.5, Kyoto Encyclopaedia of Genes and Genomes (KEGG) and other databases. The kOBAS2.0 was used to get the Unigene of KEGG ontology result in KEGG, and got the annotation information of Unigene.

Gene structure and expression analysis

TransDecoder software was used to predict the coding region sequence of Unigene and its corresponding amino acid sequence. SSR Analysis of Unigene with more than 1 kB was screened by MISA software. Reads of each sample were compared with Unigene sequences using STAR software, and single nucleotide polymorphism (SNP) sites were identified through GATK's SNP calling process for RNA Seq.

The bowtie software was used to compare reads obtained from sequencing with the Unigene library, and the expression level was estimated based on the comparison results and RESM. FPKM value is used to express the expression abundance of corresponding Unigene. On the basis of FPKM expression analysis, set the error rate (FDR $\leq$ 0.5) and absolute value log2FC $\geq$ 2. Used DEGseq R software for the differential expression analysis of transcriptome samples of FuZi. Goseq R and KOBAS software were used for enrichment analysis of differential expression genes (DEGs), such as GO, COG, KEGG, KOG, Protein family (Pfam) and eggNOG etc.

Fluorescence quantitative PCR detection

Real-time quantitative polymerase chain reaction (QRT-PCR) was used to detect the expression of the representatively differential genes in the growth,

development and metabolism of FuZi transcriptome data (D1, D2, D3, X1, X2, X3). Actin was used as the internal reference gene, and fluorescent quantitative PCR primers were designed according to each nucleotide sequence fragment (Table 1). SsoFast^{TM} Eva Green®Superemix (Bio-Rad, USA) was used to carry out fluorescence quantitative PCR experiment on Bio-Rad iCycler MyiQ Real-Time PCR System platform according to the instructions. The PCR reaction mixture was 20 μL: moderate cDNA samples, 10 μL of Ssofast TM Eva Green ® Superemix, 1.0 μL of positive and 1.0 μL of negative primers and 7 μL of DD H2O. PCR program was: 95°C, 20 s, 1 cycle; 95°C, 5 s, Tm, 20 s, 45 cycles, dissolution curve from 65–95°C, temperature program was 0.5°C/s. Results calculation was done using 2 − ΔΔCt method. Three repeated experiments were designed in total.

Results

Library sequencing and assembly

A total of 52.23Gb clean data was obtained by sequencing quality control of the transcriptome libraries of 6 samples of Dahua-leaf and Ai-leaf FuZi plants, including 90,064,627 reads of Dahua-leaf, 85,252,760 reads of Ai-leaf and the percentage of Q30 base of each sample was not less than 92.75%. After assembly, 52,471 unigenes were obtained, with a total length of 49,038,560. The N50 of Unigene was 1,364 bp, among which 15,898 Unigene were longer than 1 kB.

Annotation analysis of gene structure and function

The coding region of the gene is indispensable for the growth and development. Through the structural analysis of the Dahua-leaf and Ai-leaf FuZi plants transcriptional group, we obtained 15,568 CDs, of which 15,219 (97.75%) were within 1000 bp, 317 (2.02%) were within 1000–2000 bp, 26 (0.17%) were within 2000–3000 bp, and 6 (0.04%) were above 3000 bp. Simple sequence repeat (SSR) and single nucleotide polymorphisms (SNP) are the important markers types for the selection of transcriptome sequence differences between Dahua leaf and AI leaf. In this study, 3,347 SSR markers were obtained from the single gene sequence structure analysis of six transcriptome libraries, with single base repeat (1,700 base factors), followed by three base repeats (1,012 genes) and two base repeats (450 genes). In terms of single nucleotide polymorphism, there are 697,214 SNP loci in the transcriptome library of three large flower leaf samples and 693,486 SNP loci in three AI leaf samples. The number of the two leaf type loci is similar, and the repetitive sites are removed, 271,958 SNP loci are included in the transcriptome library.

By setting the BLAST parameter E-value not greater than 1e-5 and HMMER parameter E-value not greater than 1e-10, 28,765 Unigenes with annotation information were finally obtained. Among them, were 13,963 GO notes (the most "biological process" was 7,121 "metabolic process",

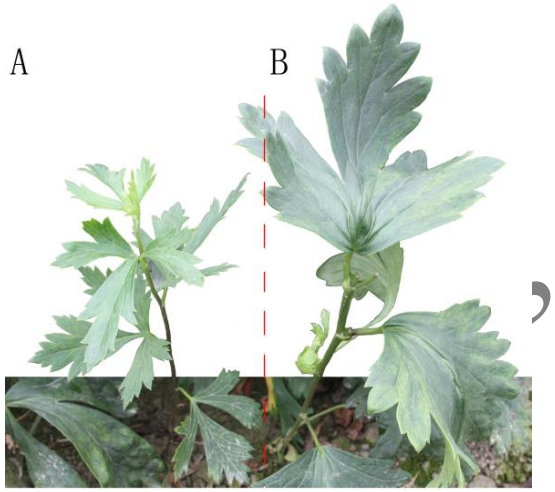

Fig. 1: The FuZi plants with two type of leaves (**A)** Ai-Type FuZi plants and (B) Dahua-Type FuZi plants

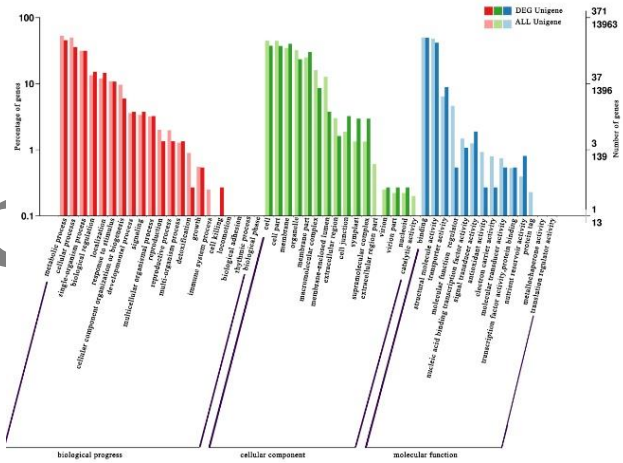

Fig. 2: Histogram of GO classification of all assembled unigens and DEGs

the most "molecular function" was 7,009 "catalytic activity", the most "cell component" is 6,219 "(Fig. 2), 8,460 COG notes (the most abundant are 1,026" transcription, ribosome structure and biosynthesis", 931 "general function prediction", and carbon hydration 810 compounds were transcribed and metabolized, 10,443 were annotated by KEGG (13.1% of the first three pathways were starch and sucrose metabolism, 11.7% were plant hormone signaling, and 7.6% were carbon metabolism).

Screening of differentially expressed genes (DEGs)

Using false discovery rate (FDR) less than 0.05 and difference multiple FC (fold change) greater than or equal to 2 as the standard to screen the differentially expressed transcript sequences of Dahua-leaf and Ai-leaf FuZi plants, a total of 1,052 differential Unigene were obtained, of which 411 were up-regulated and 641 were down-regulated (Fig.

Table 1: Details of genes and primers used in real-time PCR

Gene ID	Primer sequence（5'–3'）	*T*m (°C)	Amplification length per bp
Glucan endo-1, 3-beta-glucosidase (c75304.graph_c0)	Upstream: CCCACTTCGGAGAGAAACTATGGGC Downstream: TGCGTCCTCGTTCTCGTTCTCATTC	52	165
Alpha-1, 4 glucan phosphorylase L isozyme (c81496.graph_c0)	Upstream: CTGTTCCCAGTTCAGTGTCATGGCT Downstream: GCCAAGAGGAAGTTGACAAGGCAT	65	317
beta-fructofuranosidase (c86619.graph_c1)	Upstream: CCGTAATCATACCTCAACCCCGTGG Downstream: TCCCGGTTTCATTGAAGGGAAGCAA	64	196
Heat shock protein 90 (c69909.graph_c0)	Upstream: TCGGATGATGAGGACGAGGAAGAGA Downstream: TGGCTTCCTCATCCAGATTGGCTTC	65	165
beta-amylase (c72189.graph_c0)	Upstream: ATCCTCCACCTTGGGCACATCTCTA Downstream: CGGGTTGTCGTTTGATTCTGCATCG	64	155
S-phase kinase-associated protein (c73748.graph_c0)	Upstream： TGTTCGAGACGGAGGAGATCGAGAA Downstream： CACTTTTTCACCTCCTCCTCGTCCC	64	292
abscisic acid receptor PYR/PYL (c70275.graph_c0)	Upstream: AACCATGGTTTCACACCCCCAGTAC Downstream: CAGTTGGGGGTTATCGAAACGACGA	65	199
ABA responsive element binding factor (c73411.graph_c0)	Upstream: CGGACTTGGCCCTTGGTAGAGTAAA Downstream: GCCAAACCAGTGATCTCCCCTTCTT	63	129
Chalcone synthase (c71781.graph_c0)	Upstream: GTCGGCTCCAACTATAAGTGCAGCT Downstream: ACAAAGATGCACGTGTACTCGTCGT	64	140

3). *In toto* 808 of these differentially expressed sequences were annotated, with the highest up-regulation being 6.9 times (c63821. graph_ c0) and the lowest being about 1 / 8 (c81071. graph _c0).

Among the DEGs, 371 were annotated into the go database, and the functions related to "biological process" were mainly enriched in "metabolic process" (GO: 0008152, 169), "cellular process" (GO: 0009987, 133), "single tissue process" (117) etc., including glucan-1, 3-beta-glucosidase (c75304. graph_c0), glutamate acyltransferase (c89091. graph_c0), methyltransferase (c81444. graph_c0), etc. The functions related to "cell components" are mainly enriched in "cells" (go: 0005623, 139), "cell parts" (go: 0044464, 138), "membranes" (GO: 0016020, 149), such as leucine repeat (c82305. graph_c0), post-translational modification/protein conversion chaperone (c69283. graph_c0), glycosylhydrolase (c76304. graph_c0), etc. The enrichment function related to molecular function mainly had catalytic activity (GO: 0003824, 184)."Connection" (GO: 0005488,155), "transport activity" (GO: 0005215,33) Among them, beet glucosides are typical: gossypol/seed inhibitor protein (c70620. graph_c0), sucrose –galactosyl transferase (c70620. graph_c0), LEA protein (c77003. graph_c0) and so on. Similar to the results of GO functional enrichment, the difference analysis of COG in the transcriptome of Dahua-leaf and Ai-leaf showed that 263 genes were mainly enriched in "carbon transport and metabolism" (42), "general functional prediction" (31), "signal transduction mechanism" (30).

Enrichment analysis of metabolic pathway of DEGs

Through KEGG enrichment and retrieval, 262 transcriptome differential genes were found in 78 metabolic pathways. These metabolic pathways can be classified into six categories, most of which are metabolism (covering 90 differential genes and 53 metabolic pathways), followed by genetic information processing (28 differential genes and 13 pathways) and environmental signal processing (20 differential genes and 3 pathways).

In the metabolic pathway, the most abundant differential gene was "starch and sucrose metabolism" (ko00500), which was consistent with the results of GO differential analysis. There are 19 different genes in this pathway. Compared with Ai-leaf FuZi, the alpha-1, 4-glucan phosphatase (EC: 2.4.1.1, c80590.graph_c1, log2FC = 1.736955; c81496.graph_c0, log2FC = 1.807435) was up-regulated in Dahua-leaf, the down-regulated were beta-fructofuranosidase (EC: 3.2.1.26, c86619.graph_c1, log2FC = -1.5398), beta-amylase (EC: 3.2.1.2., c66708.graph_c0, log2FC = -2.22859; c72189.graph_c0, log2FC = -2.70389; c75146.graph_c0, log2FC= -2.61579), sucrose-phospho synthase (EC: 2.4.1.14, c90035.graph_c0, log2FC = -1.20994), trehalose-6-phosphatase (EC: 3.1.3.12., c84278.graph_c0, log2FC = -1.71061), UTP-glucose-1-phosphouridinase (EC:2.7.7.9, c84554.graph.c0, log2FC = -1.26431), 4-alpha-glucan transferase (EC: 2.4.1.13., c88414.graph_c0 , log2FC = -1.09065) etc.

In the genetic information processing pathway, “endoplasmic reticulum protein processing” (ko04141, including 10 differential genes) was the most abundant differentially expressed gene, and the up-regulated gene in this pathway contain heat shock proteins 90 (Hsp90, c69909.graph_c0, log2FC = 1.08). The down-regulated genes included kinase-related protein 1 (Skp1, c73748.graph_c0, log2FC = -1.61), endoplasmic reticulum oxidoreductase (Ero1, c88371.graph_c0, log2FC = -1.07), Luminal- junction protein (Bip, c85414.graph_c0, log2FC = -1.13), other heat sensitive proteins (c72477.graph_c0, log2FC = -1.22; c73978.graph_c0, log2FC = -3.01; c74099.graph_c0, log2FC = -2.67; c78831.graph_c0, log2FC = -2.31; c79477.graph_c0, log2FC = -2.22; c80311.graph_c0, log2FC = -2.24) etc.

In the environmental signal processing pathway, the most abundant differential gene was "plant hormone signal transduction" (KO04075, including 17 differential genes).

The up-regulated genes in this pathway included ABA receptor PYR/PYL (c66471.graph_c0, log2FC = 2.20; c70275.graph_c0, log2FC = 1.93; c79132.graph_c0, log2FC = 2.91) two-component response regulator ARR-A, (c72468.graph_c0, log2FC = 1.35; c87201.graph_c1, log2FC = 1.96), ethylene response transcription factor ½ (ERF1/2, c80070.graph_c0, log2FC = 2.43), The down-regulated genes in this pathway include ABA response element binding factor (ABF, c72751.graph_c0, log2FC = -1.27; c72819.graph_c0, log2FC = -1.19; c73411.graph_c0, log2FC = -1.96; c75230.graph_c0, log2FC = -1.80; c89735.graph_c0, log2FC = -2.05), Protein phosphatase 2C (PP2C, c77439.graph_c0, log2FC = -2.42; c81169.graph_c0, log2FC = -4.32; c88118.graph_c1, log2FC = -4.91), plant hormone response protein IAA (AUX-IAA, c79601.graph_c0, log2FC = -1.97), Serine/threonine protein kinase 2 (SnRK2，c83136.graph_c0, log2FC = -1.05), transducer inhibitor protein 1 (c86202.graph_c0, log2FC = -1.76) etc.

In many pathways, there were also genes that interact with the habitat. For example, were three genes of plant pathogen interaction class (ko04626). Heat shock protein 90 (HSP90, c69909.graph_c0, log2FC = 1.08), calcium-binding protein CML (CaMCML, c81386.graph_c0, log2FC = -1.06), RPM1 interacting protein 4 (RIN4, c88439.graph_c0, log2FC = 1.38). In addition, there were four genes related to the plant rhythm of FuZi (ko04712): chalcone synthase (CHS, EC: 2.3.1.74, c71781.graph_c0, log2FC = 2.29; c81482.graph_c0, log2FC = -3.63), phytase inhibitor protein 1 (SPA1, c87667.graph_c0, log2FC = -1.53), two-component responder regulatory protein 1 (TOC1, c88696.graph_c0, log2FC = -1.07).

Activity detection of DEGs

The expression of the representative differential genes in the growth, development and metabolism of the Dahua-leaf and Ai-leaf FuZi plants were detected by fluorescence quantitative PCR. The results showed that the relative expression and transformation of nine gene sequences including glucan-1, 3-glucosidase (GG), glucan phosphatase l isoenzyme (GP), fructofuranosidase (FFS), amylase (Amy), heat shock protein 90 (Hsp90), S-phase kinase helper protein 1 (Skp1), abscisic acid receptor PYR/PYL (ARPP), ABA responsive element binding factor (AREB), chalcone synthetase (CHS) were basically consistent with the corresponding FPKM change trend in the transcriptome database (Fig. 4). Among them, beta-amylase gene had the highest transcription expression, and it is also the sequence with the largest difference in transcriptional activity between the two species.

Discussion

The clear record of cultivating FuZi in China can be traced back to the Northern Song Dynasty, and it was only in modern times that the original plant was FuZi. In view of the wide adaptability and complex evolution of FuZi plants,

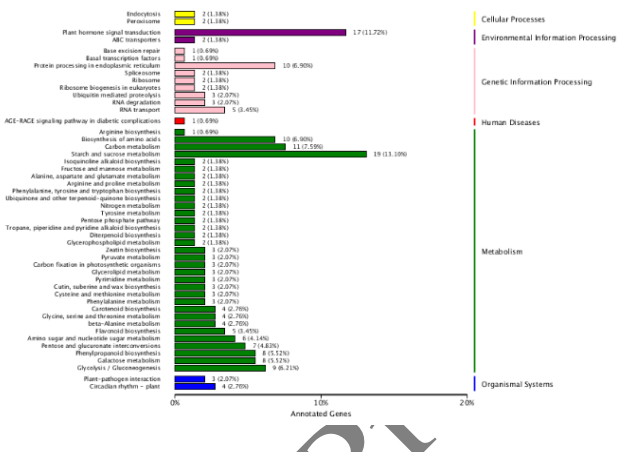

Fig. 3: The KEGG classification of enriched DEGs in FuZi transcriptomes

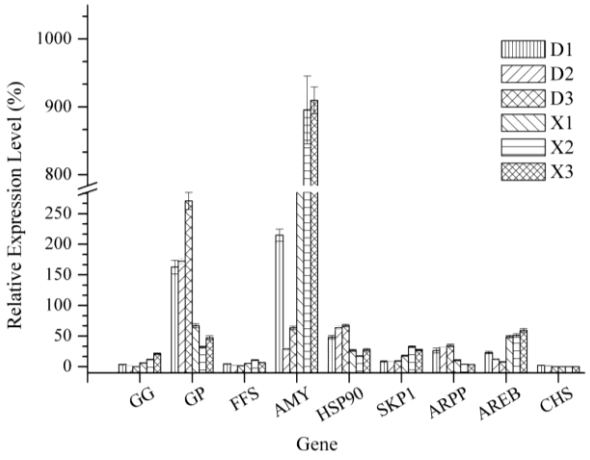

Fig. 4: Real-time PCR verification for some important DEGs in FuZi transcriptomes. GG: glucan-1, 3-glucosidase, GP: glucan phosphatase l isoenzyme, FFS: fructofuranosidase, AMY: amylase, Hsp90: heat shock protein 90, Skp1:S-phase kinase helper protein 1, ARPP: abscisic acid receptor PYR / PYL, AREB: ABA responsive element binding factor, CHS: chalcone synthetase.

Chinese scholars who study the authenticity of traditional Chinese medicine have paid attention to the influence of these complex biological characteristics of FuZi on the quality of traditional Chinese medical materials for a long time (Xiao *et al.* 1990; Xiao *et al.* 1991; Xiao *et al.* 2009). In recent years, these plants which can visually distinguish leaf types have been the important material basis for the cultivation of aconite varieties, and there are many certified varieties (Hu *et al.* 2008; Xia *et al.* 2009; Xia *et al.* 2014). However, there is little research on the quality differences among several natural leaf type varieties, and the concept of "variety" and the extension of planting are still weak, so that the farmers in the large-scale cultivation areas such as Sichuan, Shaanxi and Yunnan still rely on the traditional experience to select their own production every year. In this paper, the comparative transcriptome data analysis of

Dahua-leaf and Ai-leaf FuZi plants is helpful to accumulate the relevant basis of quality formation from the perspective of genetic genes and habitat co-evolution, and to promote the systematic research of FuZi.

In this study, the comparative transcriptome analysis of aconite between Dahua-leaf and Ai-leaf FuZi showed that the mainly enriched GO functions of 808 DEGs of two FuZi including "metabolic process" (GO:0008152), "Cell process" (GO:0009987), "Single organization process", "Cell" (GO:0005623), "Cell location", "membrane" (GO:0016020), "catalytic activity" (GO:0003824), "Connect" (GO:0005488), "Transport activity" (GO:0005215) and so on. The functional classification of COG mainly includes "carbon transport and metabolism ", "general function prediction ", "signal transduction mechanism", etc. The above functional categories are basically attributed to basic metabolism (sugar and amino acid metabolism), which contains many basic catalytic protein enzymes (such as dextran-1, 3-beta-glucosidase, glutamate-acyltransferase, glycosyl hydrolase, sucrose-galactose transferase, etc.), which can show significant differences in comparing the gene pool of the transcriptome, suggesting the types and the difference of the content may be an important aspect of the quality difference between the leaves of Dahua-leaf and Ai-leaf FuZi.

In line with the results of GO and other differential analysis, KEGG differential analysis (especially in the pathway ko00500) further deepened the above speculation. The decrease in the activity of beta-fructofuranosidase (EC: 3.2.1.26, log2fc > 0) accelerated the sucrose synthesis in FuZi, and the decrease of the activity of sucrose-phosphosynthetase (EC: 2.4.1.14, log2fc < 0), 4-alpha-glucan transferase (EC: 2.4.1.13, log2fc < 0) also promoted the transformation of sucrose to UDP glucose, the latter through utp-glucose-1-phosphouridine-transferase (EC: 2.7.7.9, log2FC < 0) At the same time, the increase in alpha-1, 4-glucan phosphatase (EC: 2.4.1.1, log2fc > 0) transcriptional activity also promoted the transformation of the starch to glucose-1-phosphate. In addition, the decreased expression of trehalose-6-phosphatase (EC: 3.1.3.12, log2fc < 0), beta-amylase (EC: 3.2.1.2, log2fc < 0) and other genes also promoted the accumulation of other small molecular sugar (maltose, trehalose etc.).

The disease resistance is one of the most important indexes in agricultural breeding. There have been a few reports on the difference of disease resistance and pathogenic bacteria of FuZi. In traditional areas, the Hua-leaf FuZi is more sensitive to external pathogens and is very vulnerable to root rot. Although the plants of Ai-leaf are thin and the yield lesser; their resistance to root rot and white silk disease is high (Gao *et al.* 2017). Compared with the soil fungi of the two types, the results showed that the diversity and abundance of dominant fungi, pathogenic bacteria and antagonistic bacteria on the root surface of two kinds were significantly different, which were the external factors of the diseases (Wang *et al.* 2018).

Conclusion

As for the internal disease factors of aconite plants, this study showed that compared with Ai-leaf, Dahua-leaf preferred to accumulate more small molecular active sugars and amino acids. It has been proved that sucrose, glucose, maltose and so on can obviously promote the growth of hyphae of white silk and root rot, increase the growth of mycelia, cause colony thickening, and increase the production of sclerotia or conidia which is consistent with the objective situation that Dahua-leaf FuZi is more susceptible to root rot and it is also suggested that we should further study about the sugar types and contents in different leaves of aconite, as well as the correlation with the quality formation and disease resistance of FuZi (Bolton *et al.* 2010; Fang *et al.* 2011; Liebe and Varrelmann 2016; Strausbaugh 2018).

Acknowledgements

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Authors Contribution

Sha zhong, Yanpeng Yin and Dingkun Zhang planned the experiments, Yanan He and Manjia Li interpreted the results, Sha Zhong, Cheng Peng and Jihai Gao made the write up and Min Zhang statistically analyzed the data and made illustrations.

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