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Comparison of the transcriptomes and expression patterns of genes involved in key medicinal secondary metabolites from *Astragalus membranaceus* and *Astragalus membranaceus* var. *mongholicus*

Y. CUI, M. JIANG, K.-C. YU, D.-Z. MA, J.-H. LI, K.-Y. ZHANG*, J.-C. LIU*, and H. LI*

Department of Science and Technology, Qiqihar Medical University, Qiqihar 161006, Heilongjiang, China

*Corresponding authors: E-mails: zkyyxy@126.com; qyyliu@126.com; lihui314220@qmu.edu.cn

Abstract

Radix astragali, from the roots of *Astragalus* L. species, is regarded as an important traditional medicinal plant and has been widely used as a Qi-Invigorating medicine for more than 2 000 years. Considering the different metabolites or functional compounds of *Radix astragali* from distinct species, the underlying genetic information among the two species is important. Here, we compared the two different root transcriptomes and expression patterns of genes involved in key medicinal secondary metabolites of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao and *Astragalus membranaceus* (Fisch.) Bge. A total of 98 017 578 and 102 635 956 reads, including 98 294 560 and 92 494 416 high-quality reads, were obtained for *A. mongholicus* and *A. membranaceus*, respectively. In general, 73 785 (39.47%) and 60 739 (34.93%) unigenes for *A. mongholicus* and *A. membranaceus* were differentially expressed, indicating that the two species had many homologous genes. In comparison, the carbon metabolism category contained the most abundant unigenes in *A. mongholicus,* and it contained many more genes than those found in *A. membranaceus* based on the *KEGG* results. Genes that may participate in the biosynthesis of flavonoids, diterpenoids, triterpenoids, steroids, isoquinoline alkaloids, and carotenoids, such as *CHS*, *F3H*, *GA3*, and *CYPs*, were identified based on annotations. A total of 5 227 and 5 101 transcription factors (TFs) were identified in *A. mongholicus* and *A. membranaceus*, respectively. Three TF categories were found and showed twice as many numbers for *A. mongholicus* than *A. membranaceus*, *i.e.*, SOH1 (4:2), LIM (18:5), and GRF (19:5), while MED7 had more unigenes in *A. membranaceus* (6:12). These results indicated that the different pathways may be involved in the synthesis of active ingredients in the two germplasm resources, and the data obtained will provide valuable information for further utilization and investigation of these two species.

Keywords: *Radix astragali*, secondary metabolites, transcriptome.

Introduction

Radix astragali (named "Huangqi") contains the roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (*A. mongholicus*) and *Astragalus membranaceus* (Fisch.) Bge. (*A. membranaceus*). *Radix astragali* has been widely used for Qi-Invigoration as a traditional Chinese medicine for approximately 2 000 years to treat liver disease, cancer, cardiovascular disease, immune disorders, depression, as well as blood sugar and hepatic system diseases (Li *et al.* 2019a, 2021; Zhang *et al.* 2021). The pharmacology and phytochemistry of *Radix astragali*

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Abbreviations: BP - biological process; CC - cellular components; *COG* - the Cluster of Orthologous Groups database; DEG - differential expressed genes; *KEGG* - the Kyoto Encyclopedia of Genes and Genomes database; MF - molecular function; *Nr* - the *NCBI* nonredundant protein database; *Nt* - the *NCBI* nonredundant nucleotide sequence database; TFs - transcription factors.

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have been widely studied recently (Zhang *et al*. 2020, Yang *et al*. 2021). *Radix astragali* extracts are also utilized as protective foods, cosmetic additives, and veterinary drugs (Ji *et al*. 2011). The *Radix astragali* is mainly derived from different species containing *Astragalus membranaceus* var. *mongholicus*, *Astragalus membranaceus*, *Hedysarum polybotrys*, and *Hedysarum multijugum* (Liu *et al*. 2012, 2015; Huang *et al*. 2019). Considering the different metabolites or functional compounds of *Radix astragali* from distinct species, the underlying genetic information among the two species is important.

Astragalus saponins, flavonoids, aminophenols, and polysaccharides have been identified as bioactive constituents (Zhang *et al*. 2021). The saponins found in *Radix astragali* included astragaloside I - VIII, isoastragaloside I, II, and IV, agroastragaloside I - IV, and other compounds. Among these constituents, astragaloside IV is recognized as the core active component of *Radix astragali* and has very important anti-inflammatory, antioxidant, and antitumor activities (Zhang *et al*. 2017, Sui *et al*. 2020, Ying *et al*. 2021). Flavonoids have been widely studied as an important category of natural constituents distributed in several medicinal plants. To date, more than 40 flavonoids have been found in *Radix astragali* that contain four different skeletal structures and novel insights into triterpenoid and flavonoid biosynthesis were analyzed (Chen *et al*. 2023). The neuroprotective, antioxidant, bone protection, antiviral and melanin inhibition functions of flavonoids have been frequently investigated (Yu *et al*. 2005, Kim *et al*. 2009, Zhu *et al*. 2009). The polysaccharides isolated from *Radix astragali* possess immunostimulatory and anticancer effects (Zhao *et al*. 2019). The crude polysaccharide extract from *Radix astragali* had an effect on oxidative stress in mice under an exhaustive swimming exercise (Wu *et al*. 2014).

Previous studies have demonstrated several differences in the considerable number of bioactive constituents between *A. mongholicus* and *A. membranaceus*, and the taxonomy of the two species has been debated for half a century. A GC-TOF/MS-based metabolic analysis revealed that content of fatty acids, proline, and polyamines was obviously lower, but content of several soluble sugars, such as mannose, xylose, and pentanate, was higher in *A. membranaceus* compared with *A. mongholicus* (Duan *et al*. 2012). The content of isoflavane and several isoflavones in *A. mongholicus* was much higher than in *A. membranaceus*; however, content of saponins in roots and hesperidin in aboveground parts was evidently higher in *A. membranaceus* (Li *et al*. 2019b). Thirteen bioactive compounds of flavonoids and triterpenoids have been recognized as remarkable elements in the differentiation of the samples between these two species (Liu *et al*. 2018). Pharmacological studies revealed that the polysaccharide constituents were the only fraction that had an effect on type 1 diabetes mellitus. However, all of the major compounds of *Radix astragali* have been demonstrated to differentially lower blood glucose in type 2 diabetes mellitus (Agyemang *et al*. 2013). Although the literature regarding comparative research of these two species is limited, it should be noted that the two herbs need to be utilized separately, which will benefit the advancement of related drugs and/or health products.

Regarding the biosynthesis of active constituents in *Radix astragali*, saponins have received the most attention, especially triterpene saponins. The putative triterpene saponin biosynthetic pathway begins with the mevalonate (MVA) and 2-C-methyl-D-erythritol-4-phosphate (MEP) pathways, continues with the synthesis of 2,3-oxidosqualene, and then proceeds through modifications of oxidation, reduction, and glycosylation (Sawai and Saito 2011). A few cDNAs related to the triterpene saponin biosynthetic pathway have been studied in *Radix astragali*, such as cytochrome P_{450} and GBSS (Wu *et al*. 2012, Chen *et al*. 2015). The enzyme-encoding genes related to the biosynthesis of flavonoids in *Radix astragali* are limited. The purpose of the present study was to exploit the molecular differences between *A. mongholicus* and *A. membranaceus* and identify key genes responsible for the biosynthesis of active constituents in these two different species.

Materials and methods

Plant materials: Two different cultivars belonging to *A. mongholicus* and *A. membranaceus* with high content of bioactive ingredients were used in this study. The pharmaceutical compounds contained saponins, polysaccharides, flavonoids, terpenoids, and other ingredients. The roots of 2-year-old plants were collected in December and grown at Qiqihar Medical University, Qiqihar, Heilongjiang Province, China. Three fresh roots of these two medicinal species were collected, immediately frozen in liquid nitrogen and stored at -80°C after washing in distilled water.

RNA extraction and quality evaluation: For transcriptome sequencing, total RNA was isolated from the roots of *A. mongholicus* and *A. membranaceus* using *TRIzol* reagent (*Beyotime Biotech*, Shanghai, China) based on the manufacturer's instructions. The RNA samples were treated with RNase-free DNase I (*TransGen Biotech*, Beijing, China), and then the purified and enriched RNA concentrations were checked with a *Nanodrop* spectrophotometer (*Shimadzu*, Shanghai, China). The quality of the RNA was detected on a 1% denaturing gel with TBE (108 g Tris, 55 g boric acid, 40 ml of 0.5 M EDTA dissolved in 600 ml of deionized water) buffer. Then, 10 μg of extracted RNA was used to construct a cDNA library.

The cDNA library construction and *Illumina* sequencing: The cDNA library used for sequencing was produced with a *NEXTflex*™ prep kit based on the manufacturer's instructions. The poly(A) mRNA was separated using oligo(dT)-attached magnetic beads and then fragmented using an RNA fragmentation kit (*New England Biolabs*, Beijing, China). The short fragments were prepared for adenine addition in EB

buffer (100 mM Tris-HCl). The first-strand cDNA was reverse transcribed with random hexamer primers, and then the second strand was synthesized. The appropriate fragments (approximately 180 bp) were chosen and purified with agarose gel electrophoresis to connect adaptors after the addition of poly(A). The double-stranded cDNA was sequenced based on the *Illumina HiSeq™ 2000* platform (*Novogene Bioinformatics Technology Company*, Beijing, China). The raw data were obtained after sequencing and were stored in fastq format.

***HiSeq™ 2000* data processing and assembly:** A Perl script was used to remove the adaptor sequences, low-quality sequences, poly(A) tails and empty reads to obtain high-quality clean reads, which were then assembled based on the Trinity method (Grabherr *et al*. 2011). The clean sequences were assembled into contigs based on the program *Inchworm* and were then associated with transcripts based on paired-end information. The transcripts were clustered, and the longest unit was recognized as the unigene sequence.

Functional annotation and classification: The assembled unigenes were functionally searched against the *NCBI* nonredundant nucleotide sequence database (*Nt*) by *BLASTn* (*E* value < $1e^{-5}$), the *Swiss-Prot* protein database with *BLASTX* (*E* value < $1e^{-5}$), and the *NCBI* nonredundant protein database (*Nr*). The functional analysis of unigenes was carried out with the Kyoto Encyclopedia of Genes and Genomes database (*KEGG*), the Cluster if Orthologous Groups database (*COG*), and the *Pfam* database. Finally, the *Blast2GO* program was executed to obtain the *GO* annotation results, and *GO* classifications containing biological processes, cellular components, and molecular functions were obtained. The homologous unigenes between *A. mongholicus* and *A. membranaceus* were checked with *BLAST*. These provide an overview of gene functions and enhance our understanding of classification in a species-independent manner.

Mining of genes related to main metabolite biosynthesis: The genes encoding key enzymes related to the biosynthesis of important active ingredients were identified by searching related literature. The unigenes that were considered pivotal genes were screened from the two different datasets on the basis of enzyme codes, names or abbreviations from the *Nr*, *KEGG*, and *SwissProt* databases. Results were analyzed statistically using *Microsoft Office Excel 2016*.

Identification of TFs: The related protein sequences of all plant transcription factors and the assignment information were downloaded from the plant transcription factor database (http://plntfdb.bio.uni-potsdam.de/v3.0/downloads.php). Red transcripts were subjected to *BLASTx* analysis against the PlnTFDB peptide sequences with an *E*-value cutoff of $<10^{-5}$.

Quantitative real-time RT-PCR analysis: The quantitative real-time RT-PCR (qPCR) analysis was employed to evaluate the expressions of differentially expressed genes (DEGs) obtained from transcriptome data. RNA extracted was used to synthesize the first strand cDNAs based on instruction of *TransScript* cDNA synthesis super mix kit (*TransGen*, Beijing, China). Three house-keeping genes (*GAPDH*, *CYP*, *UBQ*) were employed and the *GAPDH* was identified as the most stable reference gene. The qPCR analysis was performed using *SYBR Green* qPCR master mix (*Takara*, Dalian, China). The relative gene expressions were calculated with the method of Ct ($2^{-\Delta\Delta Ct}$).

Results

The transcriptome profiles of two species of *Radix astragali* were sequenced by the *Illumina HiSeq-2000* platform, and 98 017 578 and 102 635 956 reads, including 98 294 560 and 92 494 416 high-quality reads, were obtained for *A. mongholicus* and *A. membranaceus*, respectively. After assembly, we obtained 419 484 transcripts with a mean length of 828 bp, and these transcripts were clustered into 296 618 unigenes with a mean length of 1 041 bp (Table 1).

The unigenes obtained for *A. mongholicus* and *A. membranaceus* were successfully annotated based on sequence similarity across different public databases, including the *Nt*, *Nr*, *KEGG*, *SwissProt*, *COG*, and *GO* databases. Based on the results, 58.84% (109 974) and 59.71% (103 815) of the unigenes from *A. mongholicus*

Table 1. Length distribution of assembled contigs and unigenes from *Radix astragali*.

Nucleotide length [bp]	Transcripts	Unigenes
200 - 500	205 307 (48.12%)	90 893 (18.74%)
500 - 1 000	99 676 (18.99%)	92 235 (28.01%)
1 000 - 2 000	78 860 (18.14%)	77 906 (29.20%)
> 2 000	35 641 (14.75%)	35 584 (24.05%)
Total number	419 484	296 618
Total length [bp]	347 394 147	308 816 896
N50 length [bp]	1 298	1 459
N90 length [bp]	338	495
Mean length [bp]	828	1 041

and *A. membranaceus* were aligned to the *Nt* database. For *A. mongholicus*, 16.87% to 64.57% of the unigenes were aligned the remaining protein databases, and 17.39% to 65.50% were matched for *A. membranaceus.* Comparable numbers of unigenes for the two species were detected from the same database (Table 2). In the aggregate, 73 785 (39.47%) and 60 739 (34.93%) unigenes for *A. mongholicus* and *A. membranaceus* showed differential expression, indicating that the two species had many homologous genes but still employed a high proportion of specifically expressed genes or genes with low similarity (Fig. 1 Suppl.).

The predicted unigenes were annotated and used for *GO* category annotation to understand their general expression. All the unigenes were classified into 56 categories based on annotation. There were 25, 21, and 10 different groups aligned to three categories biological process (BP), cellular components (CC), and molecular function (MF), respectively. Cellular process, metabolic process, and single-organism process were the most abundant terms in the biological processes category. For the cellular components, many genes were annotated to cell and cell part. A large number of unigenes were annotated to binding and catalytic activity for the molecular function category. A relatively high percentage of unigenes were differentially annotated across the three categories when *A. mongholicus* and *A. membranaceus* were compared. External encapsulating structure organization and oxidoreductase activity were the most highly represented groups in the BP and MF categories for *A. mongholicus*, while positive regulation of macromolecule metabolic processes and steroid dehydrogenase activity were abundant in the BP and MF categories for *A. membranaceus* (Fig. 1). Based on homology analysis, 457 DEGs were annotated to the BP category, which contained plant-type cell wall organization or biogenesis (126), halogenated hydrocarbon metabolic process (96), fatty acid derivative metabolic process (35), and external encapsulating structure organization (200) functional groups.

The annotations from the *KOG* database were used to predict the functions of all unigenes in *Radix astragali* based on sequence homology. A total of 53 088 matched unigenes were divided into 25 classification categories (Fig. 2). The posttranslational modification, protein turnover, and chaperones category was the largest group, with 6 375 unigenes (12.01%), followed by translation, ribosomal structure, and biogenesis (4 711, 8.87%), intracellular trafficking, secretion, and vesicular transport (3 440, 6.48%), and RNA processing and modification (3 358, 6.33%).

Based on the *KEGG* results, 71 779 unigenes were annotated. Carbohydrate metabolism was the most abundant category (6 822), followed by translation (6 430), folding, sorting, and degradation (4 800), and overview (4 615) (Fig. 3). In comparison, the carbon metabolism category employed the most abundant unigenes for *A. mongholicus*, with many more than those for *A. membranaceus* in the same group (1 945 to 1 790). The DEGs annotated in the *KEGG* database were analyzed and elucidated to determine the characteristics of the DEGs and explain the enriched metabolic pathways. Among the 3 919 DEGs related to *KEGG* annotation, 1 528 DEGs were identified between the two plants. DEGs were significantly enriched in plant hormone signal transduction, starch and sucrose metabolism, spliceosome and ubiquitin-mediated proteolysis (Fig. 3). A total of 204 and 184 DEGs were related to the biosynthesis of flavonoids, and 41 and 57 DEGs were related to the biosynthesis of diterpenoids in the two datasets. These DEGs seem to be linked to the different content of active ingredients in *A. mongholicus*, with many more DEGs being present in *A. mongholicus* than in *A. membranaceus*. Most DEGs related to categories linked to the metabolism of other important secondary metabolites were associated with phenylpropanoid biosynthesis (735 to 662) (Fig. 3).

To compare the metabolism of the main active ingredients in the roots of *A. mongholicus* and *A. membranaceus*, presumptive genes that may participate in the biosynthesis of flavonoids, diterpenoids, triterpenoids, steroids, isoquinoline alkaloids, and carotenoids were identified based on the annotations. Flavonoid biosynthesis-related genes were discovered in the two datasets, such as chalcone synthase (CHS), *trans*-cinnamate 4-monooxygenase (CYP73A), chalcone isomerase (CHI), naringenin 3-dioxygenase (F3H), flavonol synthase (FLS), flavonoid 3'-monooxygenase (CYP75B1), shikimate O-hydroxycinnamoyltransferase (HCT), 5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase (CYP98A), caffeoyl-CoA O-methyltransferase (cCoAOMT), isoflavone-7-O-methyltransferase (7-IOMT), 2-hydroxyisoflavanone dehydratase (HIDH), isoflavone 7-O-glucoside-6"-O-malonyltransferase

Table 2. Number of annotated unigenes in *Radix astragali*.

Database	*Astragalus membranaceus*	*Astragalus mongholicus*
Nt	109 974 (58.84%)	103 815 (59.71%)
Nr	120 690 (64.57%)	113 880 (65.50%)
KEGG	47 294 (25.30%)	45 143 (25.96%)
SwissProt	86 544 (46.30%)	82 050 (47.19%)
Pfam	80 155 (42.88%)	75 467 (43.41%)
GO	6 737 (32.50%)	64 976 (37.37%)
KOG	31 534 (16.87%)	30 178 (17.39%)
Total	186 911	173 865

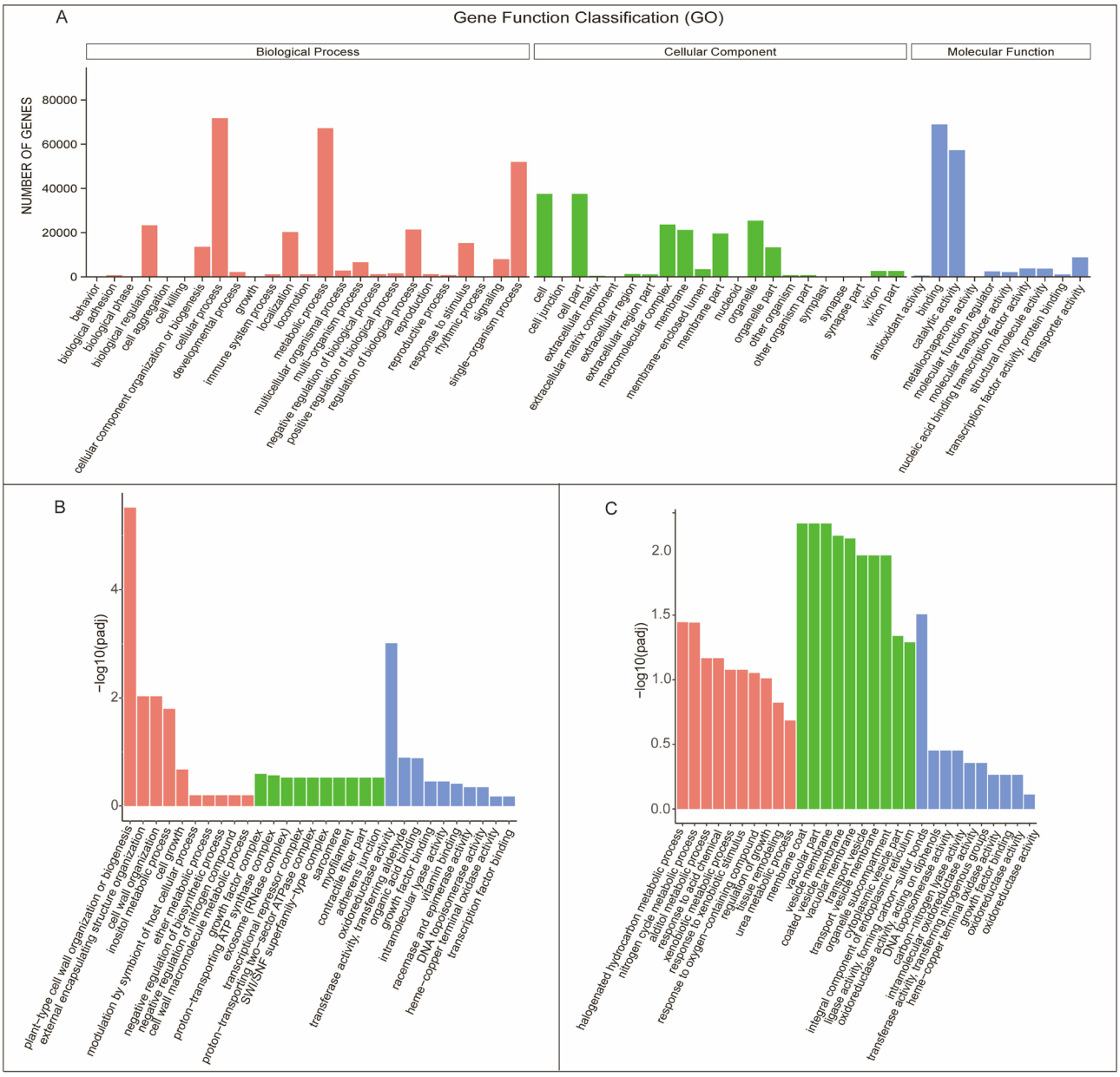

Fig. 1. Functional annotations of all unigenes (*A*), the unigenes of *Astragalus membranaceus* var. *mongholicus* (*B*) and *Astragalus membranaceus* (*C*).

(IF7MAT), vestitone reductase (VR), and isoflavone/4'-methoxyisoflavone 2'-hydroxylase (CYP81E). Terpenoid biosynthesis-related genes were also found in the two datasets, such as ent-kaurene oxidase (GA3), ent-kaurenoic acid monooxygenase (KAO), gibberellin 2beta-dioxygenase (GA2ox), geranyllinalool synthase (GES), dimethylnonatriene synthase (CYP82G1), farnesyl-diphosphate farnesyltransferase (FDFT1), (-)-germacrene D synthase (GERD), and lupeol synthase 2 (LUP2), while some anthocyanin biosynthesis genes were principally found for *A. mongholicus*, such as anthocyanidin 3-*O*-glucosyltransferase (BZ1), anthocyanidin 3-*O*-glucoside 5-*O*-glucosyltransferase (UGT75C1), and anthocyanidin 3-*O*-glucoside 2'''-O-xylosyltransferase (UGT79B1).

According to the *KEGG* enrichment analysis of DEGs, several enriched pathways were involved in metabolic pathways of important active ingredient, such as flavone and flavonol biosynthesis (Cluster-8005.109983, Cluster-8005.80858, Cluster-8005.116286), isoflavonoid biosynthesis (Cluster-8005.109983, Cluster-8005.162143, Cluster-8005.19345, Cluster-8005.80858, Cluster-8005.123867). We performed the qPCR analysis to validate the gene expression profiles of several genes related to

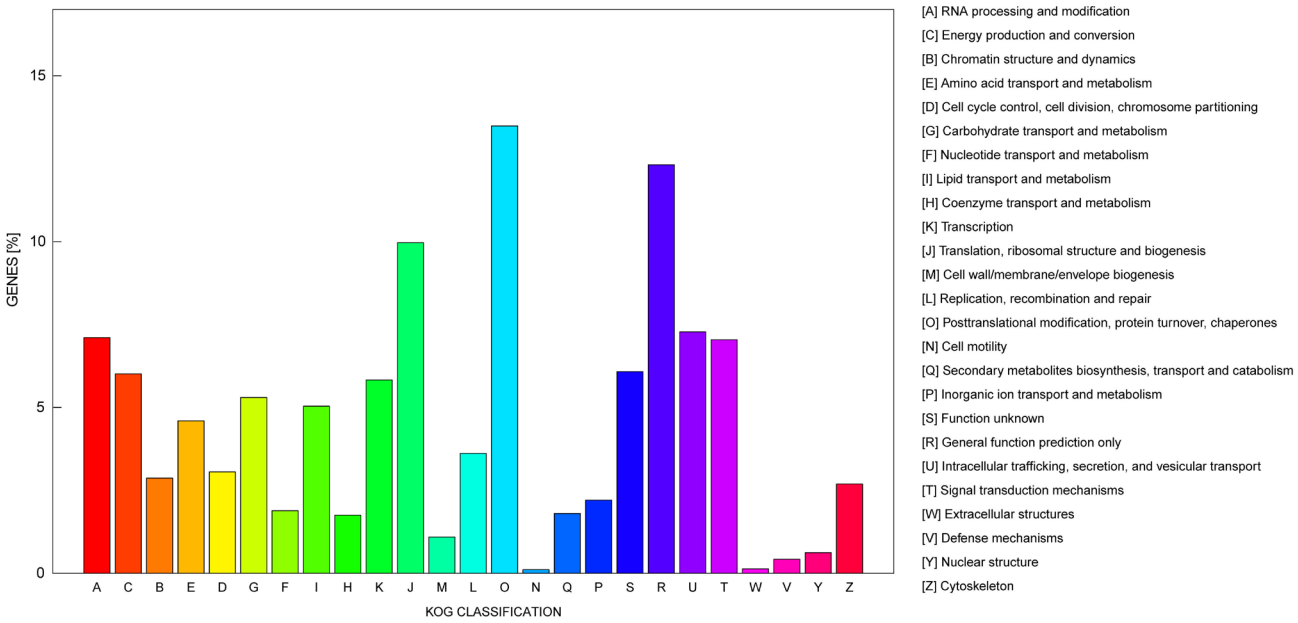

Fig. 2. Functional annotation and classification of unigenes. The annotations from the *KOG* database were used to predict the functions of all unigenes in *Radix astragali* based on sequence homology. A total of 53 088 unigenes were classified into 25 groups.

starch and sucrose metabolism, flavonoids biosynthesis (Fig. 4). Beta-glucosidase (*BGL-1*, *BGL-2*) are two major subfamilies of glycoside hydrolase and play key roles in the pathway of sucrose metabolism. The results showed that *BGL-1*and *BGL-2* were significantly upregulated in *A. mongholicus*. Sucrose synthase (*SUS-1*, *SUS-2*) was also identified as important genes related to sucrose metabolism, and the qPCR results demonstrated different subfamilies exhibited opposite expression patterns. Genes of *IF7MAT*, *F3H*, *CYP71D9*, and *CYP75B1* play essential roles in the regulation of flavonoids biosynthesis; our results indicated that *IF7MAT* and *CYP71D9* were upregulated in *A. mongholicus*, otherwise *F3H* and *CYP75B1* were downregulated. These results indicated that these genes related to some secondary metabolites expressed at different levels were linked to different active ingredient quantities in the medicinal roots of *A. mongholicus* and *A. membranaceus*.

Transcription factors (TFs) have important functions in regulating the biosynthesis process of metabolites; thus, transcription factors were identified across two different datasets (Fig. 5). A total of 5 227 and 5 101 TFs were identified in *A. mongholicus* and *A. membranaceus*, respectively. For *A. mongholicus*, MYB, Orphans, WRKY, bHLH, C3H, and C2H2 were the top six transcription factor categories, with more than 347 unigenes. For *A. membranaceus*, MYB, Orphans, bHLH, WRKY, AP2-EREBP, and C3H were the top six transcription factor categories, with more than 363 unigenes. Three TF categories had twice as many unigenes in *A. mongholicus* than in *A. membranaceus*, *i.e.*, SOH1 (4:2), LIM (18:5), and GRF (19:5), while MED7 had more unigenes in *A. membranaceus* (6:12). The different numbers of TF unigenes in the two different species suggested significant divergence in the metabolic regulation of Chinese traditional herbs, as medicinal plants have unique utilization characteristics.

Discussion

Transcriptomics is an effective and useful method for providing valuable genetic background information and revealing gene expression profiles. It has been frequently utilized to analyze important expression models between related resources or under different environments in plants, such as peanut, poplar, and tea (Chen *et al*. 2020, Wu *et al*. 2021, Xue *et al*. 2021). Especially for traditional Chinese herbs, the literature containing transcriptome research has shown rapid growth in recent years and continues to increase. It is important to explore the biosynthesis of key active ingredients in common medicinal plants using transcriptome analysis. In this study, two germplasm resources with the same medicinal usage for several years in China belonging to the genus *Astragalus* L., *A. mongholicus* and *A. membranaceus*, were subjected to transcriptome sequencing and comparison analysis. The roots of *Radix astragali* were utilized as the medicinal tissue; thus, the expression patterns of important putative genes in the roots that may be related to the biosynthesis or regulation of crucial medicinal active ingredients or secondary metabolites, were identified. Our purpose was to determine the diversity between the two resources and to examine the molecular mechanism responsible for their different active metabolites to guide their practical application.

The biologically active ingredients of *Radix astragali* include astragalosides (mainly triterpenoid saponins), flavonoids, polysaccharides, alkaloids, organic acids,

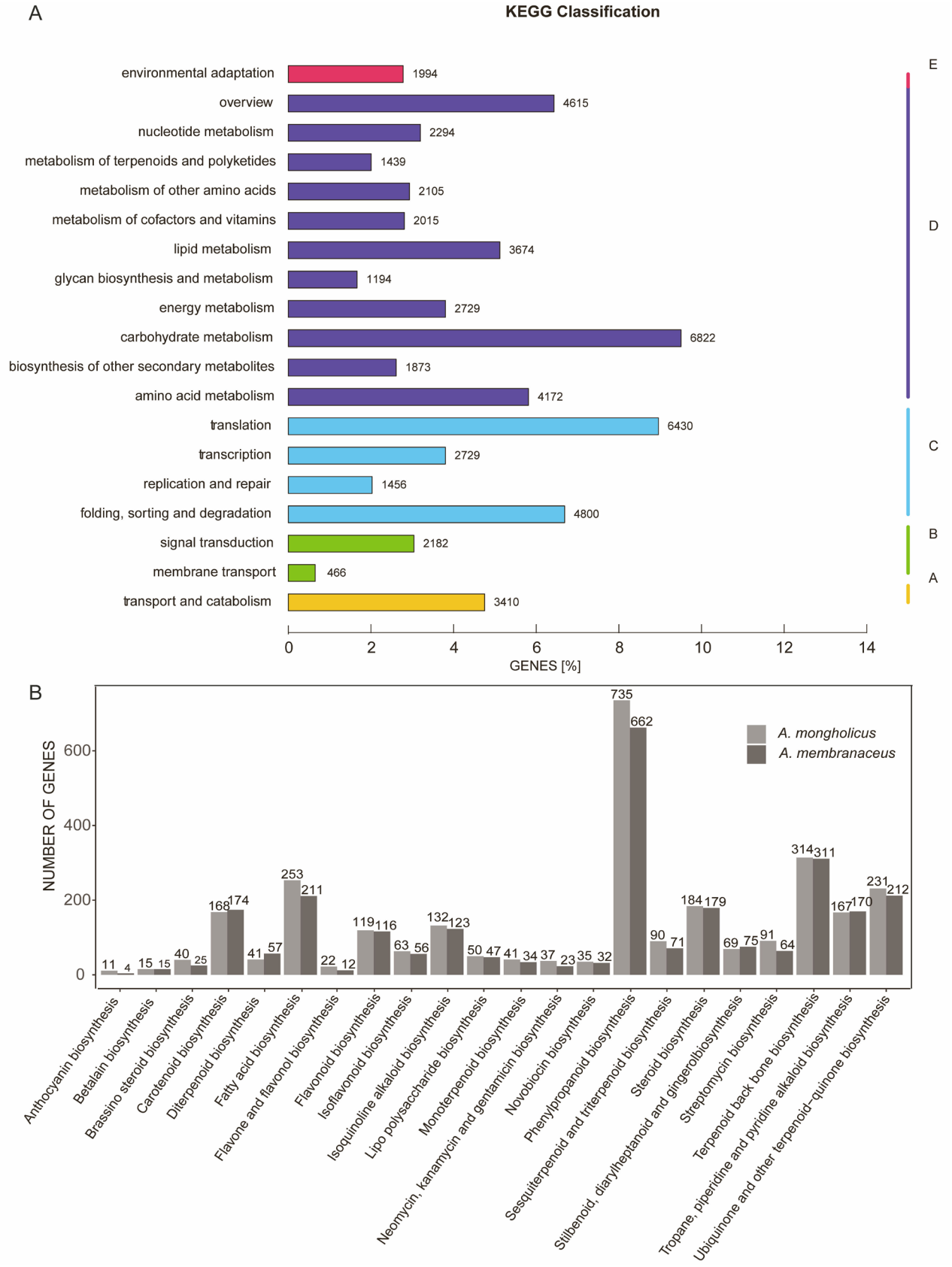

Fig. 3. Summary of metabolic pathway assignments of the high quality DEGs of *Radix astragali* based on *KEGG* (*A*). A total of 204 and 184 DEGs were related to biosynthesis of secondary metabolites in *A. mongholicus* and *A. membranaceus*, respectively (*B*).

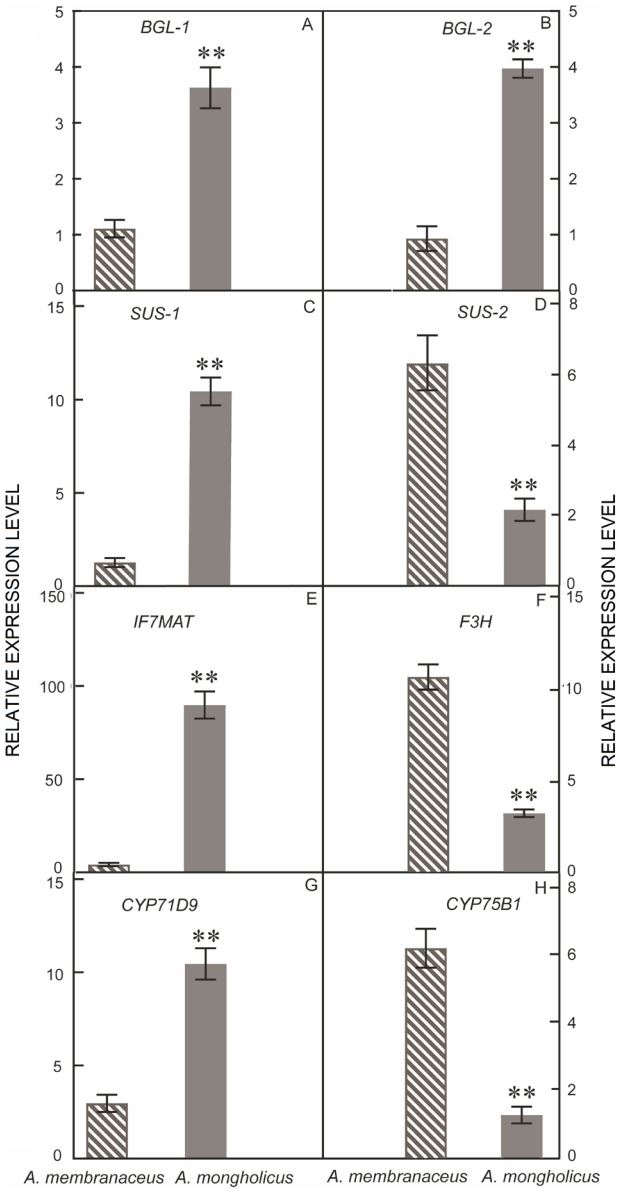

Fig. 4. The relative expression (qPCR analysis) of several representative DEGs of *A. mongholicus* and *A. membranaceus.* Means ± SEs, $n = 4$, ** - significant differences at $P < 0.01$.

and microelements (Zhang *et al.* 2021). Among these active ingredients, ten MVA pathway-related genes were quantified with RT-qPCR to analyze the accumulation of astragalosides (Kim *et al.* 2014). A global transcriptome study on *A. mongholicus* was conducted and there were identified triterpene saponin and isoflavonoid biosynthesis-related genes in different tissues, and the *AmCAS* gene was cloned as part of astragaloside metabolism (Chen *et al.* 2015). A full-length transcriptome of *A. membranaceus* was performed to explore genes involved in the biosynthesis of astragalosides, calycosin and calycosin-7-O-β-D-glucoside (Li *et al.* 2017). Here, we aimed to identify and compare putative genes that may encode enzymes involved in the metabolism of steroids, carotenoids, flavonoids, sesquiterpenoids, diterpenoids, triterpenoids, pyridine alkaloids, and anthocyanins between *A. mongholicus* and *A. membranaceus*. Our work offers a comprehensive understanding of the profile of putative enzyme unigenes between the two species. Fully understanding the molecular background underlying the variation in the abundance of valuable active ingredients with medicinal properties between *A. mongholicus* and *A. membranaceus* requires further research, while the key genes found here will be a benefit for future studies on biosynthesis pathways.

Most of the medicinal ingredients of *Radix astragali* have been found to be secondary metabolites, whose metabolic process depends on not only key encoding genes but also temporarily and spatially regulated genes. Transcription factors (TFs) play an important regulatory role in the transcription and expression pathways of active ingredient biosynthesis (Davies and Schwinn 2003, Thakur *et al.* 2020). The categories of plant species-specific TFs are recognized by the different DNA-binding domains, such as MYB, WRKY, NAC, and so on. And many TFs are related to the biosynthesis regulation pathways of terpenoid and flavonoids. Interestingly, several key TFs, including MYB, C3H, AP2-EREBP, C2H2 expressed differently in the two species were found. Thus, the recognition and identification of special TFs is critical for further study on gene regulation. In our analysis of the genes related to TFs between *A. mongholicus* and *A. membranaceus*, different expression patterns were identified. Eighty TF families were found among these unigenes, and MYB, Orphans, WRKY, bHLH, C3H, AP2-EREBP, C2H2, HB, Bzip, and NAC were the top ten groups in the two species. MYB genes, which employ a highly conserved domain, are reported to largely exist in plants and comprise one of the largest groups of TFs. Many biochemical and molecular regulatory functions of MYB genes have been found, including gene expression regulation, abiotic stress resistance, and secondary metabolism, including that for anthocyanins, lignins, and flavonoids (Du *et al.* 2009, Wei *et al.* 2021, Abubakar *et al.* 2022). In the present study, 347 and 363 MYB unigenes were identified in *A. mongholicus* and *A. membranaceus*, respectively. Much more work remains to be done to identify the MYB genes from these two species and determine their regulatory patterns. WRKY genes are TFs that are involved in plant resistance to biotic and abiotic stress based on their regulatory function in secondary metabolism (Meraj *et al.* 2020). Overexpression of MdWRKY11 in apple increased flavonoid and anthocyanin content (Wang *et al.* 2018). We identified 255 and 234 WRKY unigenes in *A. mongholicus* and *A. membranaceus*, respectively. This result may be related to the different characteristics of secondary metabolites in the two species. Fourteen families, Tify, TCP, SWI/SNF-BAF60b, SBP, HMG, C2C2-YABBY, ABI3VP1, MADS, IWS1, AUX/IAA, SOH1, LIM, GRF, and MED7, showed significant differences in expression abundance between the two resources; however, verification of their regulatory function based on external circumstances and secondary metabolism requires further research.

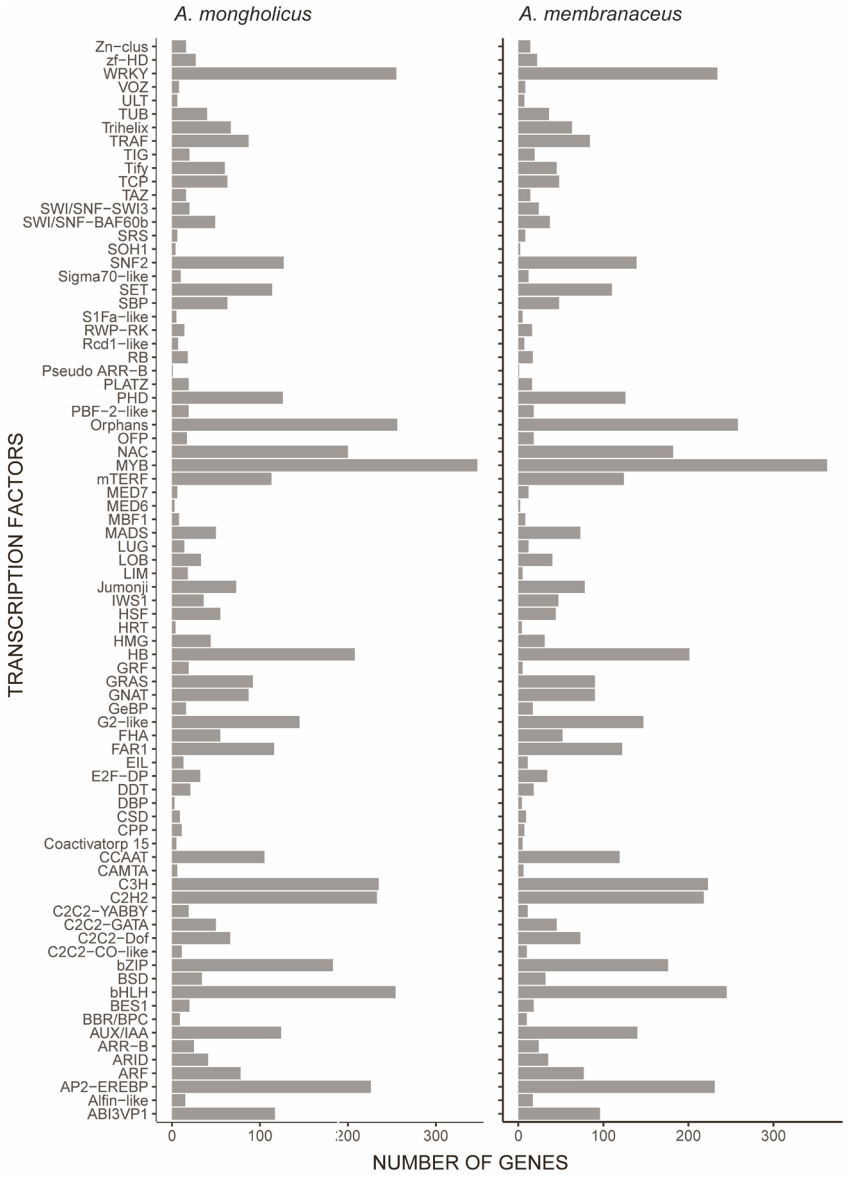

Fig. 5. Annotation of transcription factors recognized from the unigenes of *A. mongholicus* and *A. membranaceus*.

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