

Transcriptome Analysis of Lateral Roots Development in *Aconitum Carmichaelii*

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Abstract

Background: Fuzi is a processed product of the lateral root of *Aconitum carmichaelii* Debx, a plant of the Ranunculaceae, and has been used to treat various diseases. This study used Illumina HiSeq High-throughput platform to sequence, assemble and annotate, screen development related genes, transcription pathways and functional enrichment in true root, lateral roots and “bridge”, and analyzed their correlations with the formation and development of lateral roots of *A. carmichaelii*, which can reveal the process and regulation mechanism of lateral roots growth and maturation of *A. carmichaelii*.

Results: By sequencing, a total of 66.13Gb clean data and 28,982 unigenes with function annotation were finally obtained, with N50 of 1,627 bp, and 12,833 genes were assigned to 130 specific metabolic pathways by Kyoto Encyclopedia of Genes and Genomes (KEGG), then 2,599 were significantly differentially expressed. The KEGG analysis of the DEGs revealed that it was mainly enriched in starch and sucrose metabolism, ribosome, carbon metabolism, plant hormone signal transduction which play an important role in the expansion of lateral roots. The DEGs and pathways indicated that there was little differences between true root and “bridge”, while a big difference between them and lateral roots. The DEGs of auxin, cytokinin and other pathways may be conducive to the formation of lateral roots, which explained the development mechanism of lateral roots from a molecular point of view.

Conclusions: This study provides a reference for the study of cultivation and management of lateral roots of *A. carmichaelii*.

Background

Aconitum carmichaelii, an important traditional Chinese medicine, belongs to Ranunculaceae, it is an annual to perennial herb ^[1-2]. It has a cultivation history of nearly a thousand years in Mianyang in Sichuan ^[3-5]. Its medicinal part is the true root which is called "Chuanwu" after processing for dispersing cold and relieving pain. Lateral roots are also used as medicine and called “FuZi” ^[6-9]. *A. carmichaelii* mainly distributed in Asia, Europe and North America and is the most widely distributed species of *A. carmichaelii* in China.

Most dicotyledons have straight roots, which are composed of true roots and lateral roots, such as *Arabidopsis*, *Aconitum* etc ^[10-11]. Some studies have shown that the embryo of dicotyledons first forms the radicle during the development process, and only one true root is formed after the seed germination ^[12-13]. The lateral roots are the main roots and nodes, which usually includes the initiation of lateral roots occurrence, the construction of lateral roots primordium and the exposure of lateral roots ^[14-15]. There is a true root and one or several lateral roots in *A. carmichaelii*, and FuZi is a special structure, which is attached to the root of the plant and consists of a terminal bud, axillary bud and tuberous adventitious roots ^[16]. The lateral roots are oboval, 2-4cm long, 1-1.6cm thick and dark brown in outer skin ^[6]. In March of the second year, the part on the abaxial side below the first node of axillary bud formed the

adventitious root primordial that is directly connected with the bud telogen cambium. At the same time, the first node of axillary buds extended horizontally to form a "bridge" connecting the true root ^[17] (Fig. 1).

Traditional physiological development studies have shown that the lateral root of *A. carmichaelii* is actually a renewal bud, mainly develop from the first node of the stem base of the plant, and the true root decay in winter, then the lateral root develops into a new plant in the next year ^[18]. In the process of practical cultivation, it was found that in the process of stem withering in autumn and winter, the multi stem nodes buried under the soil can grow into lateral roots, which shows that the efficiency of asexual reproduction is very high ^[19–20].

Compared with the aboveground part, the morphological and anatomical characteristics of root are relatively simple, and it only plays a "secondary role" in function. There are few studies on the development process of lateral roots, especially the formation mechanism of lateral roots ^[21]. At present, researches on the mechanism of lateral roots development mainly stay in *Arabidopsis thaliana* and some monocotyledons such as rice and wheat ^[22]. As an important medicinal part of *A. carmichaelii*, FuZi has little research on its development process and influencing factors, and its molecular biological mechanism is even less clear. In this study, the true root-"bridge"-lateral root of *A. carmichaelii* were selected to compare the transcriptome and molecular expression activities of related genes, and to reveal the development regulation mechanism of lateral roots of *A. carmichaelii*.

Results

Library sequencing and assembly

A total of 66.13Gb clean data was obtained from 9 transcriptome libraries of *A. carmichaelii*'s true root, lateral roots and "bridge". There were 77,202,226 reads in true root, 70,043,540 reads in "bridge" and 73,419,580 reads in lateral roots. The Q30 of each sample was 93.21% or higher and GC content of 45.22%. A total of 28,185 Unigenes were obtained after assembly, with a total length of 42,159,509. The N50 of Unigenes was 1,627 bp, among which 15,651 Unigenes were more than 1kb in length.

Based on the data of 9 samples, the genes whose expression threshold was greater than or equal to 0.1 were screened by union method, and compared according to the same sample mixing tank. The results showed that 16,478 genes were expressed in true root, 16,743 in "bridge" and 17,552 in lateral root (Fig.2). Among them, 14,915 genes were expressed in all tissues, and 1,197 genes were expressed only in lateral roots. There are many genes that the other parts don't have in the lateral roots, There are many genes in the lateral roots that don't exist in the true root and "bridge", indicating that the lateral roots have different growth and development mechanisms.

Functional annotation and enrichment analysis of expressed genes

To obtain a comprehensive annotation of *A. carmichaelii* transcriptome, 36,203 full-length transcripts was annotated by searching against seven protein databases and a total of 28,185 transcripts were

annotated. In addition, 8018 unannotated unigenes might represent novel *A. carmichaeli* genes

BLASTx similarity analysis against the Nr database demonstrated that the *A. carmichaeli* full-length transcripts were similar to several plant species (Table 2). Among them, 12,477 (45.96%) transcripts showed significant homology with that of *Aquilegia coerulea* and 1215 (4.48%) and 724 (2.67%) transcripts had high similarity with sequences of *Macleaya cordata* and *Nelumbo nucifera*, respectively.

Based on the results of Nr annotation, 13,932 unigenes were assigned to 44 functional groups in GO database, and most of the unigenes showed more than one functional group, and we totally detected 107,745 hits followed by 6986 unigenes and 6977 unigenes, 8093 unigenes in the remaining three main functional categories, i.e., 'cellular component', 'biological process' and 'molecular function', respectively (Fig.3). The dominant subgroups were 'oxidation-reduction process', 'translation' and 'transmembrane transport' which were annotated genes 2770, 1465, 1103 respectively in the group of biological processes. Among cellular component functions, 6474, 2494, 2347 annotated genes were classified into 'the integral component of membrane', 'nucleus', and 'cytosol respectively'. In the group of molecular function, 'ATP binding', 'metal ion binding', 'structural constituent of ribosome' were the principal GO-terms comprising of 3296, 1725, 1629 annotated genes respectively. These functional categories are important activities in plants and participate in the biosynthesis of metabolites. A total of 10,735 unigenes that annotated by the COG database were functionally classified into 25 molecular families. the five largest categories were "Translation, ribosomal structure and biogenesis" (1650,15.54%), "Posttranslational modification, protein turnover, chaperones" (1183,11.14%), "General function prediction only" (1173,11.05%), "Carbohydrate transport and metabolism" (1078,10.15%) and "Energy production and conversion" (861~8.11%). KEGG pathway enrichment analysis is helpful for functional genes identification, understanding the functions of genes in the biosynthetic pathways and annotated a total of 12,104 unigenes and assigned them to five main categories and 131 biological pathways. The largest pathway was the "Ribosome" pathways containing 1207 transcripts. Moreover, a number of transcripts were assigned to other significant pathways, such as biosynthesis of amino acids and carbon metabolism.

Enrichment analysis of metabolic pathways of differentially expressed genes

To investigate and understand the variation of transcript abundance and expression patterns of genes, we carried out a comparative analysis of the differential genes of true root (A), "bridge" (B), lateral root (C) of *A. carmichaelii* (A vs. B, A vs. C and B vs. C) and the results were displayed in Table 3. Moreover, true root and lateral root had the most specifically expressed differential genes (1468), while lateral root and "bridge" had fewer differential genes (1248) and fewer differences between true root and "bridge". 81 genes were differentially expressed in all comparison groups, suggesting that there was a larger biological differences between true root and lateral root of *A. carmichaelii* and these genes may play an important role in the metabolism of different root of *A. carmichaelii*.

To obtain insight into the functional categories of the DEGs between true root and lateral root, the GO enrichment analysis was performed using Goatools (Fisher exact test, P-value \leq 0.05). a total of 646 DEGs

were annotated into GO database, The most enriched GO category among these DEGs was 'catalytic activity' (GO: 0005488, 399 DEGs), followed by 'metabolic process' (GO:0008152, 370 DEGs), 'cellular process' (GO: 0009987, 334 DEGs), 'binding'(GO: 0005488, 287 DEGs). 'cells' (GO: 0005623, 257 DEGs), 'cell part' (GO:0044464, 254 DEGs). Thus, the growth and development of between true root and lateral root in *A. carmichaelii* is complex and various

Through KEGG enrichment search, we found that there were 31 transcriptome differential genes between true root and "bridge", which were distributed in 23 metabolic pathways. These metabolic pathways can be classified into three categories, the most is metabolism, and followed by environmental signal processing; There are 498 transcriptome differential genes between true root and lateral roots, which are distributed in 100 metabolic pathways. These metabolic pathways can be classified into five categories, the most is metabolic pathway, and followed by genetic information processing. There are 331 transcriptome differential genes between "bridge" and lateral roots, which are mainly distributed in 77 metabolic pathways. These metabolic pathways can be classified into four categories, the most of which are metabolic pathway. The pathways that displayed significant changes between true root and lateral root were identified using the KEGG database. A total of 12 KEGG pathways were significantly enriched (Table 4), among which the 'Starch and sucrose metabolism', 'Ribosome', 'Carbon metabolism', 'Phenylpropanoid biosynthesis', and 'Plant hormone signal transduction' pathways were the most highly represented. The largest number of DEGs were in the 'Starch and sucrose metabolism' category (ko00500), indicating that starch and sucrose play an important role in the growth and development of lateral roots. The 'plant hormone signal transduction' pathway (ko04075) exhibited the 15 DEGs, indicating that plant hormones play important roles in the growth and development of roots in *A. carmichaelii*.

Statistics of SNPs and SSRs

Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphisms (SNP) are important marker types for screening transcriptome sequence differences among true root, lateral root and "bridge" of *A. carmichaelii*. In this study, 3 838 SSR markers were obtained from single gene sequence structure analysis of 9 transcriptome libraries, and most of them were single base repeats (2038 genes), and then three base repeats (1080 genes) and two base repeats (517 genes) were followed. The results showed that there were 676,573 SNPs in the transcriptome library of three true root samples, 661 848 SNPs in three "bridge" samples and 673 537 SNPs in three lateral root samples.

Transcription factors TF prediction

The Unigene annotated in this study was compared with PlantTFDB (plant transcription factor database) and AnimalTFDB (animal transcription factor database) databases to predict the transcription factor and the family information. A total of 1910 expressed TFs belonging to 211 TF families were identified from the transcriptome dataset (Fig.5). Among them, the most abundant TF family was The Cys2His2 (C2H2)-type zinc-finger protein (ZFP) family, which is one of the largest class of plant TFs and have been extensively studied and have been shown to play important roles in plant development and

environmental stress responses by transcriptional regulation. The expression pattern analysis of the nine transcripts indicated that TFs regulating the growth and development of roots in *A. carmichaelii* displayed high expression in true root and lateral roots.

Discussion

Using FDR less than 0.05 and FC (fold change) greater than or equal to 2 as the standard, we found that there were little differences between true root and “bridge” (Fig. 1), while there was a big difference between them and lateral roots. It could be inferred that the “bridge” may be an extension of the true root of *A. carmichaelii*. In the screening process of traditional quality, the evaluation criterias that “excellent shape and high quality” are the shape of *A. carmichaelii* is superior to that with proper internode, less angle, and inferior to that of the shape with irregular shape and wrinkle” and “the shape of *A. carmichaelii* is proper internode, less angle, thin top” [23]. Through the analysis of transcriptome differences, we found that the “bridge” and true root have a high similarity. But the lateral roots with renewal bud is often used as inferior but not effectively used (Fig. 1, D). This will be a breakthrough point to inspire new quality standards, and it needs to be further studied and verified.

Among the metabolic pathways of true root and “bridge”, “carbon metabolism” (ko01200) is the most abundant differential genes and there are three differential genes in this pathway. In the metabolic pathway of true root and lateral roots, the most abundant differential genes are “starch and sucrose metabolism” (ko00500). Starch is the storage form of important carbohydrate nutrients during the growth, development and reproduction of higher plants. Generally, the accumulation of substances in the storage organs of plants reaches its peak in autumn, and then begins to decrease in the spring of the following year, reflecting that it provides substances and energy for the activities of the buds on the storage organs. Starch is the main storage substance of *A. carmichaelii*, but the starch accumulated in the lateral roots of *A. carmichaelii* reaches the highest peak in summer [17, 24–25]. According to KEGG database, a total of 270 genes were annotated in the Starch and sucrose metabolism pathway (ko00500). These genes have the highest expression in lateral roots, followed by bridges, and finally roots. There are 26 DEGs between true root and lateral root—including 18 up-regulated genes and 8 down-regulated genes, and there are 19 DEGs between the “bridge” and lateral root—including 9 up-regulated genes and 10 down-regulated genes (Fig. 4). Compared with true root, starch synthase (EC:2.4.1.21, *c432375.graph_c0*) and cellulase (EC:3.2.1.4, *c423764.graph_c2*, *c419143.graph_c1*) was up-regulated in lateral roots. And polygalacturonase (EC: 3.2.1.1, *c419754.graph_c0*, *c420224.graph_c0*) was down regulated. Compared with “bridge”, hexokinase (EC: 2.7.1.1, *c413999.graph_c2*) is up-regulated in lateral roots, and sucrose-phosphate synthase (EC: 2.4.1.14, *c434389.graph_c0*) was down regulated. The reason may be that carbohydrates provided by the true-root for growth and development in the early stage of development, and then these carbohydrates are converted into starch and stored in the lateral roots in large quantities, causing the lateral roots of *A. carmichaelii* to expand. The artificial cultivation of *A. carmichaelii* is imperative, and starch-related genes are artificially used to promote the expansion of lateral roots and improve the quality of *A. carmichaelii*. In spring and summer, immature lateral roots can quickly

accumulate large amounts of starch in the true root to provide material and energy for later growth and development. As a result, lateral roots are earlier independent of the true root in terms of material and energy supply, and then the next generation of *A. carmichaelii* be produced on the mature lateral root, which further shows that the lateral roots is a very special renewal bud.

Plant root system not only plays a role in fixing plants, absorbing water and nutrition, but also has the function of synthesizing plant hormones and essential substances [22, 26]. In the process of lateral roots growth and development, plant hormone is one of the most popular and important factors. At present, six kinds of plant hormones have been found to play different roles in the development of lateral roots [27], including auxin [28–30], cytokinin (CTK) [31–34], gibberellin [35–39], Ethylene [40–42], abscisic acid (ABA) [43–44] and brassinosteroids (BR) [45–47]. Auxin can increase the number of lateral roots and promote the formation of lateral roots. Cytokinin and abscisic acid play a negative regulatory role in the formation of lateral roots [48], while brassinolide positively regulates the formation of lateral roots [49], and other hormones such as ethylene plays in a double-edged way [50–51]. Based on the comparative transcriptomic analysis of the true root, “bridge” and lateral roots of *A. carmichaelii*, it was found that the regulatory hormones related to the growth and development of lateral roots were mainly enriched in the plant hormone signal transduction pathway (*ko04075*). Auxin influx carrier (Aux1) two-component response regulator ARR-B and abscisic acid receptor *PYR/PYL* were up-regulated genes, while histidine-containing phosphotransfer protein (*AHP*) was down-regulated genes (Fig. 5).

Four sequences (*c420910.graph_c0*; *c426192.graph_c0*; *c415294.graph_c0*; *c419888.graph_c0*) were found in *AUX1* gene, where the expression of auxin influx carrier (*c429056.graph_c0*) was the highest. During the growth and development of Arabidopsis lateral roots, Aux1 binds to and activates transport inhibitor response 1 receptor (*TIR1*), leading to ubiquitin mediated proteolysis of *Aux / IAA* protein, and auxin response factor binding to *Aux1* is activated, promoting cell division and initiating the occurrence of lateral roots [52–56]. It may be similar to the lateral roots development of *A. carmichaelii*. CTK is generally considered as a negative regulator of lateral root formation. In this pathway, five sequences (*c422916.graph_c2*; *c422916.graph_c1*; *c436898.graph_c0*; *c435355.graph_c0*; *c429056.graph_c0*) of the cytokinin receptor (*CRE1*) have been found the highest expression. CTK are bound with *CRE1* receptors, after the receptors recognize the CTK, they transfer the phosphate groups through *AHP*. *ARR* and *BRR* participate in competitive phosphorylation reaction, phosphorylated *ARR* interacts with various effector factors and mediates the response of CTK to regulate the development of lateral roots [57–60]. In *ABA* signaling pathway, a total of 8 sequences (*c432849.graph_c0*; *c423931.graph_c0*; *c422059.graph_c0*; *c414097.graph_c0*; *c419860.graph_c2*; *c419164.graph_c0*; *c412687.graph_c1*; *c415984.graph_c0*) were found on *PYR / PYL* receptor, of which the expression level of abscisic acid receptor *PYR/PYL* (*c415984.graph_c0*) was the highest. In this pathway, abscisic acid was mediated by *PYR / PYL* receptor protein, and then activated serine/threonine-protein kinase (SnRk2) to inhibit the development of lateral roots (Fig. 5).

Conclusion

In this study, the regulation and molecular mechanism of plant hormones in the occurrence and development of *A. carmichaelii*'s lateral roots were discussed. During the development of lateral roots, transcriptome analysis showed that the growth and development regulating hormones of lateral roots were mainly enriched in plant hormone signal transduction pathways, among which auxin, cytokinin, abscisic acid and other hormones played an important role, which was similar to the regulatory mechanism of other dicotyledonous plants such as *Arabidopsis thaliana*. Further studies are needed to find the possible mechanisms of the lateral roots development as noted in the present study. To a certain extent, it provided a basics for the target species with good root characteristics. Besides, the marker candidates around genes will become one of the new research directions.

Materials And Methods

Plant materials

The plant materials of *A. carmichaelii* (Fig.1) with consistent genetic background and growth were collected in Mianyang, Sichuan, China (N31°53'E104°04" in October 2020 and were randomly divided into three groups. There wasn't necessary to obtain permission for collecting these samples. Each plant was divided into three different tissues (true root, "bridge", lateral root), and each tissue obtained three biological replicate samples. (The true roots were A1, A2 and A3; The "bridges" were B1, B2, B3; The lateral roots were C1, C2, C3). The plant materials was identified by Jihai Gao and stored at - 80 °C in the State Bank of Chinese Drug Germplasm Resources.

Library construction and sequencing

The total RNA of each sample was extracted by Trizol Kit (Burlington Co. Canada), isolated and purified by Oligo (dT) magnetic beads. The mRNA was decomposed into short gene fragments and then reverse transcribed into cDNA strand and nine transcriptome libraries of FuZi were established. The concentration and insert size of the library were detected by Qubit2.0, Agilent 2100 and Q-PCR method, respectively. Then sequencing was performed on the Illumina novaseq 6000 platform with double-ended strategy.

High throughput sequencing, assembly and functional annotation of transcriptome

The Transcriptome Library of FuZi was sequenced by using Illumina Hiseq2500 High-throughput sequencing platform. After intercepting the sequencing primer and filtering the low-quality data of the raw sequence, the clean reads were obtained. The long segment Contig was assembled by Trinity software, and obtained the long fragment set Component (Unigene) .

Blast software was used to compare Unigene sequences with the NCBI non-redundant protein sequence (NR), Swiss-Prot protein (Swiss-Prot), Gene Ontology (GO), Clusters of Orthologous Group (COG), eukaryotic Orthologous Groups (KOG), eggNOG4.5, Kyoto Encyclopaedia of Genes and Genomes (KEGG)

and other databases, while KOBAS2.0 was used to get the KEGG Orthology result of Unigene in KEGG, and got the annotation information of Unigene.

Gene expression and differential enrichment analysis

TransDecoder software was used to predict the coding region of Unigene and its corresponding amino acid sequence. The SSR Analysis of Unigene with more than 1kb was performed by MISA software. STAR software was used to compare the reads and unigene sequences of each sample, and the Single Nucleotide Polymorphism (SNP) was identified by the SNP recognition process of GATK for RNA-Seq. The Bowtie software was used to compare the sequenced Reads with Unigene library. According to the comparison results, the expression level was estimated combined with RSEM. FPKM value was used to represent the expression abundance of corresponding Unigene.

Quantitative real-time PCR validation analysis

The expression levels of these transcripts were checked in true root, lateral roots and “bridge” of *A. carmichaeli* by quantitative real-time PCR (qRT-PCR) in order to see if they are specifically up-regulated. To verify the accuracy and reproducibility of the RNA-Seq data, and further confirm the pattern of differential gene expression, The quantitative reactions were performed on a BioRad CFX 1,000 Real-Time PCR System, using SYBR® Premix Ex Taq™ (Perfect Real Time) (TaKaRa). In this study, we did some quantitative analysis of 16 genes (Table 1) which was related to the the mechanism of rue root, lateral roots and “bridge” development. Q-PCR was performed under the following conditions: 95 °C for 20 s; forty cycles of 95 °C for 15 s, and 55 - 60 °C for 30 s. The relative expression of each gene was calculated according to the comparative cycle threshold (ct) method.

Abbreviations

A: The true roots; B:The “bridges”; C:The lateral roots;Aux1:Auxin influx carrier ; AHP:histidine-containing phosphotransfer peotein; CRE:the cytokinin receptor; ABA:abscisic acid; BR:brassinosteroids; SSR:Simple Sequence Repeat; SNP:Single Nucleotide Polymorphisms; TF:Transcription factors; qPCR:Quantitative polymerase chain reaction; C2H2:The Cys2His2; ZFP:zinc-finger protein.

Declarations

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Authors' contributions

SZ, LZ planned the experiments, YF, YY interpreted the results, LZ, CP and JG made the write up and LZ statistically analyzed the data and made illustrations. All authors have read and approved the manuscript.

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Availability of data and material

Raw data for the transcriptomics experiment are available on the the BGI Database (<https://db.cngb.org/search/project/>) as series CNP0001721. All data analyzed during this study are included within this published article and any supplementary material. Raw data is available upon request from the corresponding author. The experimental research, including the collection of plant material, comply with the national and international guidelines.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1 Primers used in quantitative real-time PCR

unigene ID	Primer sequence(forward) 5'-3'	Primer sequence(reverse) 5'-3'
c75304.graph_c0	CCCACTTCGGAGAGAACTATGGGC	TGCGTCCTCGTTCTCGTTCTCATTC
c81496.graph_c0	CTGTTCCCAGTTCAGTGTCATGGCT	TGCCAAGAGGAAGTTGACAAGGCAT
c86619.graph_c1	CCGTAATCATACTCAACCCCGTGG	TCCCGGTTTCATTGAAGGGAAGCAA
c69909.graph_c0	TCGGATGATGAGGACGAGGAAGAGA	TGGCTTCCTCATCCAGATTGGCTTC
c72189.graph_c0	ATCCTCCACCTTGGGCACATCTCTA	CGGGTTGTGTTTTGATTCTGCATCG
c73748.graph_c0	TGTTCGAGACGGAGGAGATCGAGAA	CACTTTTTTCACCTCCTCCTCGTCCC
c70275.graph_c0	AACCATGGTTTTACACCCCCAGTAC	CAGTTGGGGGTTATCGAAACGACGA
c73411.graph_c0	CGGACTTGGCCCTTGGTAGAGTAAA	GCCAAACCAGTGATCTCCCCTTCTT
c71781.graph_c0	GTCGGCTCCAACATAAGTGCAGCT	ACAAAGATGCACGTGTACTIONCGTCGT

Tab 2 Unigene information annotated in different databases

Anno_Database	Annotated_Number	Annotated_ DEGs Number		
		A/B	B/C	A/C
COG_	10735	29	428	610
GO_	13932	32	518	646
KEGG_	12104	23	355	491
KOG_	16980	37	499	734
Pfam_	21307	63	932	1170
Swissprot_	18367	65	911	1136
eggNOG_	26267	71	1067	1348
NR	27203	71	1098	1377
All_	28185	71	1104	1384

Tab 3 Number of upregulated and downregulated transcripts for three transcriptomic comparison

DEG_Set	All_DEG	up-regulated	down-regulated
A vs B	81	44	37
A vs C	1468	647	821
B vs C	1248	596	652

Tab 4 Significantly enriched gene pathways involving differentially expressed genes (DEGs) between the true root and lateral root

Pathway	DEGs	All genes	Pvalue	Pathway ID
Starch and sucrose metabolism	26	270	1.31E-05	ko00500
Ribosome	22	1207	0.999996	ko03010
Carbon metabolism	21	642	0.815279	ko01200
Phenylpropanoid biosynthesis	16	126	2.36E-05	ko00940
Plant hormone signal transduction	15	178	0.003472	ko04075
Biosynthesis of amino acids	14	521	0.94521	ko01230
Amino sugar and nucleotide sugar metabolism	13	181	0.022071	ko00520
Cysteine and methionine metabolism	12	190	0.062222	ko00270
Tyrosine metabolism	11	78	0.000176	ko00350
Glycerophospholipid metabolism	11	94	0.000905	ko00564
Carbon fixation in photosynthetic organisms	11	199	0.144343	ko00710
Glycerolipid metabolism	10	88	0.001924	ko00561

Figures

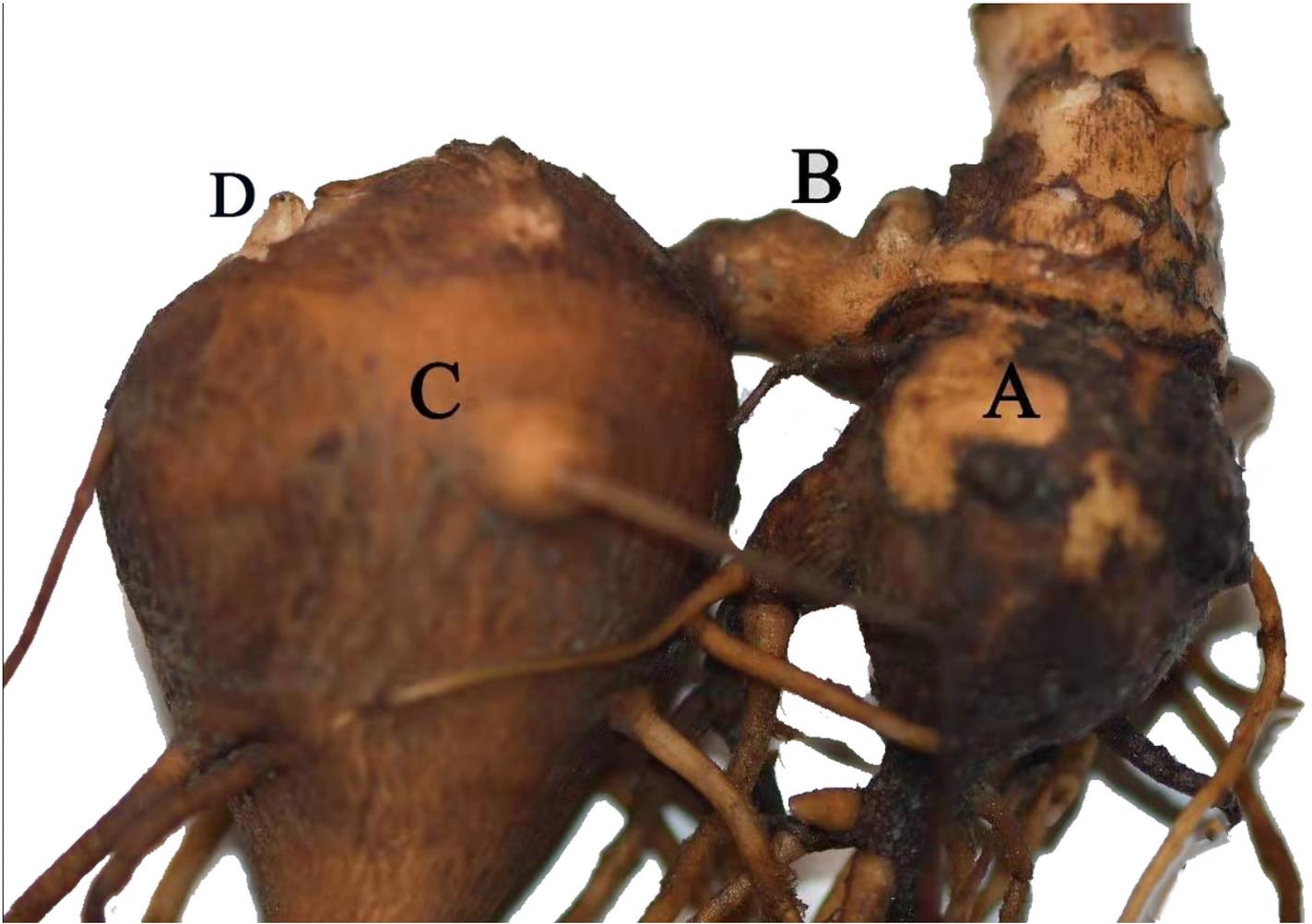


Figure 1

Schematic diagram of trueroot - "bridge"-lateral root of *A. carmichaelii* A: Trueroot B: "bridge" C: Lateral root D: Renewal bud

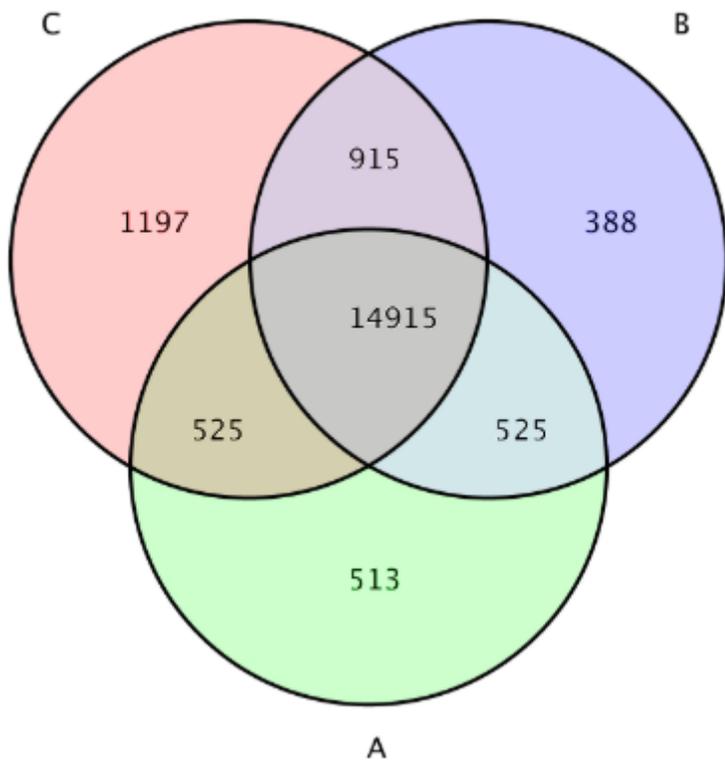


Figure 2

Venn Diagram of gene expression in different tissues A: Trueroot B: "bridge" C: Lateral root

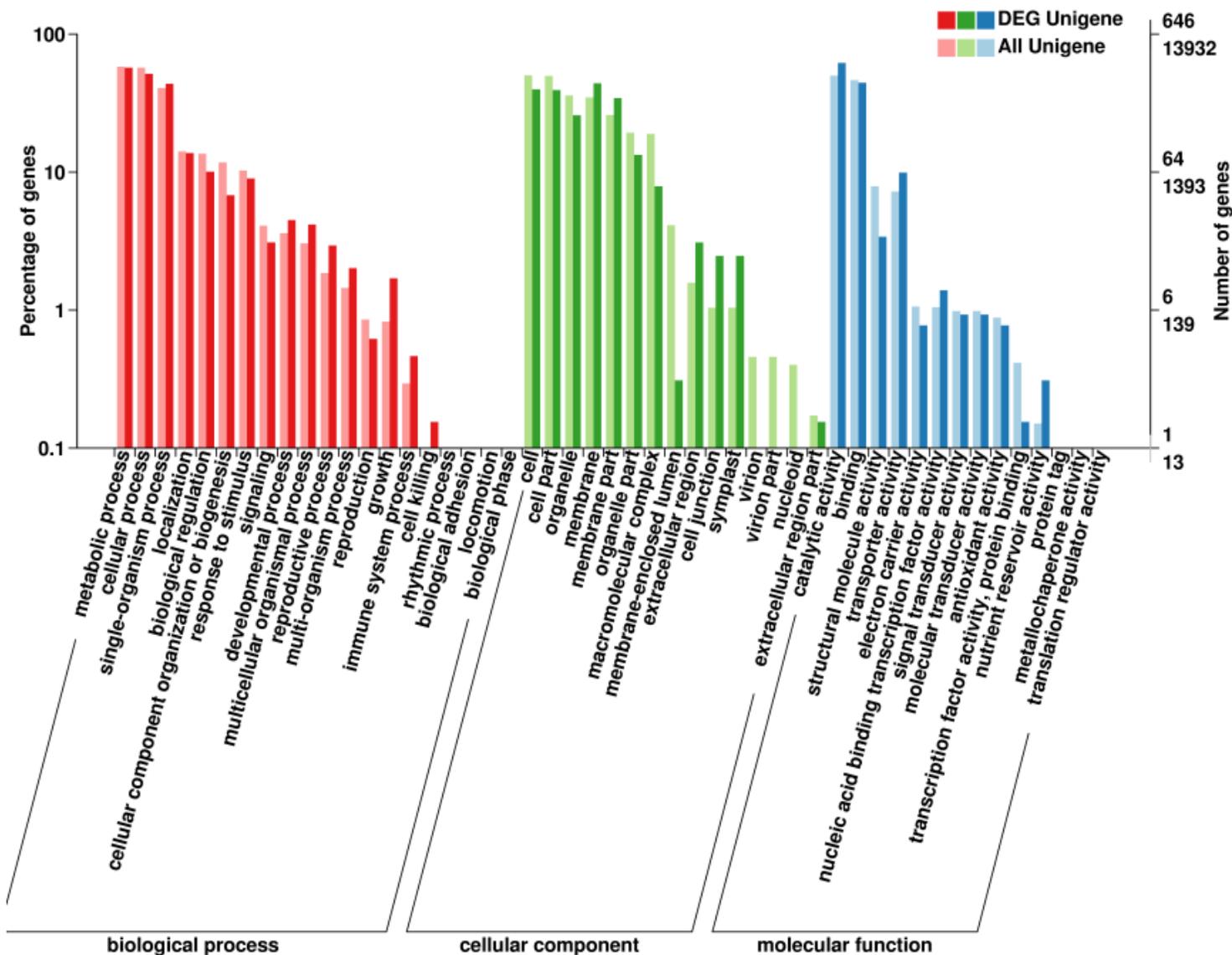


Figure 3

Unigene of true root and lateral root of *A. carmichaelii* and GO classification of DEG

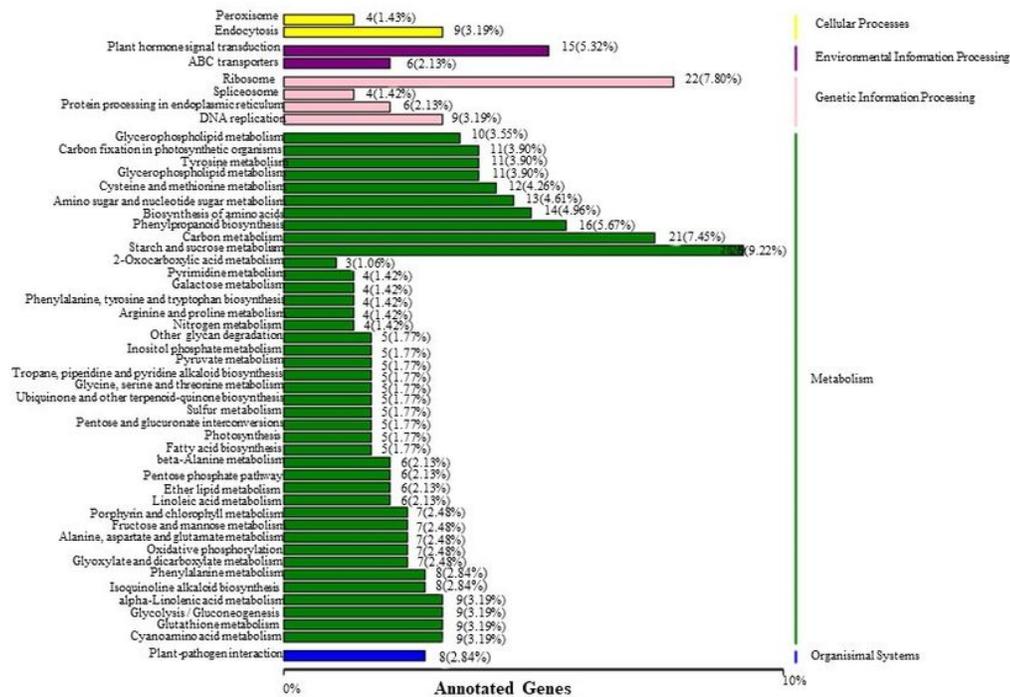


Figure 4

KEGG enrichment and classification of differentially expressed genes in true root and lateral root of *A. carmichaelii*

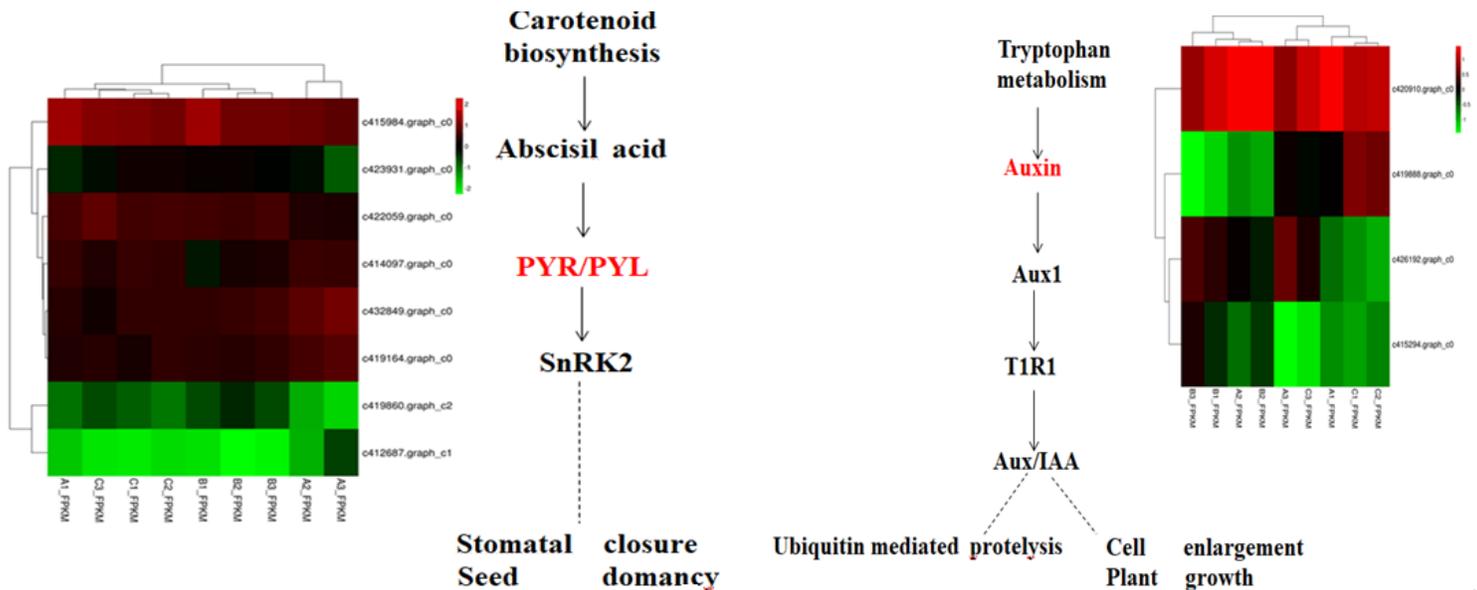


Figure 5

Heat map of CRE1/B-ARR/Aux1 and PYR/PYL differentially expressed genes

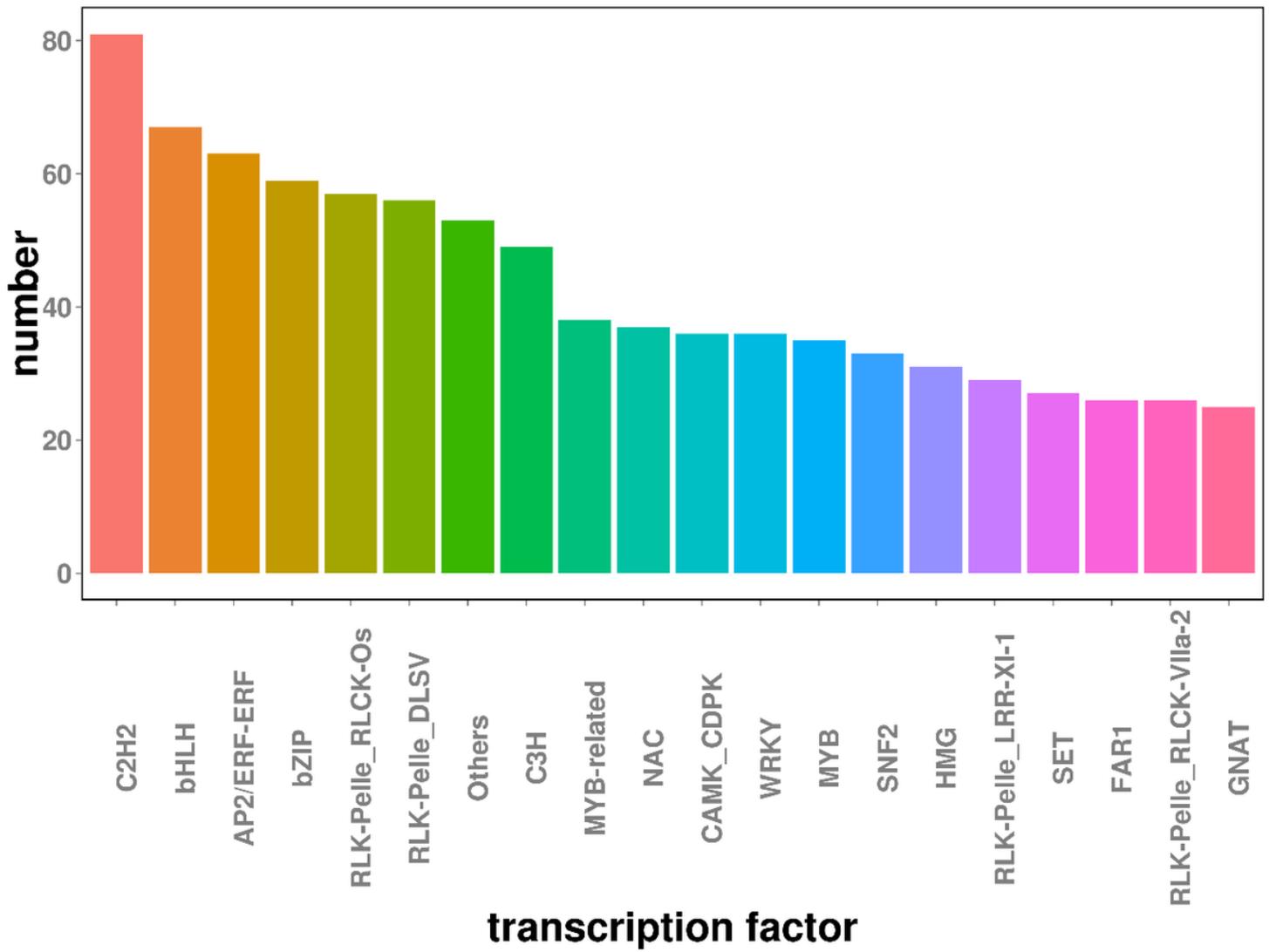


Figure 6

Differentially expressed transcription factors (TFs)