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Original Article

Transcriptome profiling identifies tissue-specific genes regulating sugar metabolism in *Actinidia valvata* Dunn

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Abstract

Actinidia valvata Dunn is a fruit tree that originated in China and is well recognized for its nutrient, health, and medicinal value. With the aim of identifying the nutrients involved, this study conducted a comprehensive transcriptome analysis of the stem, leaf, and fruit of *A. valvata* using the Illumina HiSeq platform. RNA-Seq analysis produced 39.03 GB of clean data and further identified a total of 50,928 unigenes with an average length of 1,236.40 bp. Functional annotation of these unigenes was carried out by comparing them with several databases. This resulted in the incorporation of annotated information regarding 32,202 unigenes, which constituted 63.23% of the total. Moreover, 11,809 unigenes participated in 129 unique metabolic pathways. Comparative analysis of gene expression across various tissues led to the identification of differentially expressed genes (DEGs). Among these, 26 DEGs were closely associated with sugar metabolism pathways. Moreover, correlation analysis between total sugar content and expression of the DEGs implied in sugar metabolism proved that the identified key genes *SUS*, *INV*, *SPS*, *HK*, *malZ*, and *GPI* have strong functions in sugar metabolism in *A. valvata*. Our findings reveal that sucrose synthase (*SUS*) and invertase (*INV*) are critical drivers of sugar accumulation in fruits, providing targets for breeding sweeter kiwifruit varieties.

Keywords: Kiwi berry, *A. valvata*, Total phenolic, *SUS*, *INV*, *SPS*, DEGs, Sugar metabolism

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Introduction

Actinidia valvata Dunn (*A. valvata*), commonly known as the kiwi berry or Chinese gooseberry, is a species in the Actinidia genus and Actinidiaceae family, which encompasses around 76 species primarily found in temperate regions. First described in 1911, *A. valvata* is known for its distinctive white pith stems and unique flower structure, which features two to three sepals and five to nine petals.(Van-Dijk et al., 2018). *A. valvata*, is mostly distributed in the valleys and jungles of low-mountain regions of China. The fruit is glabrous fruit with soft edible skin, and carbohydrate rich. The accumulation and metabolism of sugar in *A. valvata* entail the breakdown of starch into dissolved sugars, which mainly contribute to its total sweetness and taste quality (Hutton et al., 2000). The fruit possesses a rich mixture of sugars that accumulate throughout growth and maturation, such as monosaccharides that include fructose, glucose, and galactose. The sugars obtained determine the flavor of the fruit and its acceptance by the consumer (Nagar et al., 2022).

Mature *A. valvata* vines produce orange fruit with a good taste and palatability. Research on kiwifruit covers several areas of study: germplasm resource analysis (Cochavi et al., 2021), cultivation techniques (Kwon et al., 2012), variety selection (Sang et al., 2017), genomic studies on traits like flesh color (Lin et al., 2022), and disease resistance (Lago et al., 2019). Sugar metabolism studies have primarily focused on other Actinidia species, such as *A. arguta* (Grabherr et al., 2011) and A. chinensis (Lago et al., 2019), with findings highlighting their role in flavor formation and consumer preferences. However, the genes related to sugar metabolism have not yet been explored in *A. valvata*. This study fills this gap using transcriptome sequencing to produce a transcriptional library for *A. valvata*. Genes related to sugar metabolism pathways were identified using bioinformatics analysis and real time fluorescence quantitative PCR (qRT-PCR) (Van-Dijk et al., 2018). Furthermore, the total sugar content in different organs was measured, and correlation analysis was conducted to identify key genes re- lated to sugar metabolism. This study provides valuable insights into the genetic basis of sugar metabolism in A. valvata, which will ultimately con- tribute to the understanding of fruit sweetness, flavor, and quality. Additionally, this study lays the groundwork for future molecular assisted breeding approaches focused on enhancing desirable fruit traits.

Material and Methods

Experimental materials

The experimental materials included samples of *A. valvata*, which were collected from the kiwifruit germplasm resource nursery at the Zhengzhou Institute of Fruit Trees, Chinese Academy of Agricultural Sciences, Zhengzhou, China (113°42’E, 34°48’N). Three types of samples were collected at 90 days post pollination from three biological replicates (i.e., three different branches of the same plant). Immediately after collection, the samples were flash frozen in liquid nitrogen and transported to the laboratory for RNA extraction. The remaining samples were stored at -80°C for future use.

RNA extraction and quality testing

Total RNA was isolated from three biological samples of fresh tissues using the RNeasy Plant Mini Kit (Cat. No. 74904), according to the manufacturer's instructions. RNA quantity and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific), and RNA integrity was determined by electrophoresis on a 1% agarose gel. Samples with an A260/A280 ratio between 1.8 and 2.1 and an A260/A230 ratio greater than 2.0 were considered to be of good quality for further applications.

Library preparation, sequencing, and assembly

RNA libraries were prepared and sequenced using the Illumina HiSeq platform.

Quality control of raw reads was performed using FastQC v0.11.9, and adapter trimming and quality filtering were conducted using Trimmomatic v0.39. Gene expression levels were quantified using featureCounts v2.0.1. Transcriptome assembly was performed using Trinity v2.11.0 (Altschul et al., 1997) with default parameters. The assembled transcripts were clustered and deduplicated to generate unigenes. Quality metrics such as N_{50} and average contig length were evaluated to assess assembly completeness.

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Genes annotation

Unigenes were annotated by BLASTx alignment (E-value < 1e-5) against multiple databases: Nr (Apweiler et al., 2004), SwissProt (Ashburner et al., 2000) , GO (Tatusov et al., 2000), COG (Koonin et al., 2004) , KOG (Huerta-Cepas et al., 2016), KEGG (Huerta-Cepas et al., 2016), and eggNOG v4.5 (Kanehisa et al., 2004). HMMER v3.3.2 (Eddy 1992) was used to identify conserved protein domains by searching against the Pfam (v35.0) (Yue et al., 2022) database (E-value < 1e-10). KEGG orthologs were refined using KOBAS 2.0 (Xie et al., 2011).

Differential gene expression

Differentially expressed genes (DEGs) between tissues were identified using the DESeq2 package in R, with thresholds of $|\log2FoldChange| \geq 1$ and adjusted *p*-value (FDR) < 0.01. GO and KEGG enrichment analyses were performed for DEGs using the clusterProfiler package in R. Sugar metabolism-related pathways were specifically examined to identify key candidate genes.

Determination of total sugar content

Total sugar content was measured using the phenol-sulfuric acid method as described by Xu et al. (2009), with minor modifications.1 g of each tissue sample was homogenized in dH_2O (1:21 w/v) and extracted by ultrasound for 62 minutes. Extracts were treated with ethanol precipitation and reconstituted in water.

To 2 mL of sample solution, 1 mL of 5% phenol was added, followed by 5 mL of concentrated sulfuric acid. After incubating the reaction mixture in a boiling water bath for 20 min and cooling in ice water, absorbance was measured at 490 nm using a UV-Vis spectrophotometer. Glucose was used as the standard to generate a calibration curve. Sugar concentrations in each organ type were calculated from the standard curve and expressed as mg/g fresh weight. The sugar content of the different organs was determined as follows:

$$\mathbf{M} = \frac{\mathbf{c} \times \mathbf{v}}{\mathbf{m}}$$

where:

M is the total sugar content,

c is the concentration of the sample solution,

v is the volume of the sample solution,

m is the mass of the sample powder.

RNA-seq expression validation by qRT-PCR

Total RNA was extracted and reverse transcribed into cDNA using a commercial kit. Primers for the candidate genes were designed using Premier software (5.0) using actin (TGCATGAGCGATCAAGTTTCAAG, TGTCCCATGTCTGGTTGATGACT) from Actinidia as the internal reference gene (Zhang et al., 2023). The synthesized sequences are presented in Table.

Quantitative real-time PCR (qRT-PCR) was performed using SYBR ® Green Master Mix (Cat. No. 4309155) on a StepOnePlus™ Real Time PCR System (Thermo Fisher Scientific). Primer design used the gene sequences retrieved from the *A. valvata* transcriptome and was carried out using Primer3 (version 2.5.0). The PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Melting curves were analyzed between 60°C and 95°C to confirm primer specificity. Primers of desired genes, such as AvHexose Transporter and AvSucrose Synthase, are listed in Table 1.

Primers for qRT-PCR were designed using Primer3 software. Each primer was tested for specificity through a BLAST search of the *A.valvata* transcriptome to ensure that they were unique. The primer sequences are shown in Table 1. Five genes related to sugar metabolism were selected for validation: AvSUS, AvHT, AvIN, and AvAMY and AvGPI. To make the analysis more reliable, multiplex qRT-PCR with TaqMan probes was considered but not implemented because the probe sequences were not available in this study.

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Table-1. Sequences of the primers used in the qRT-PCR experiment.

Gene ID	Gene Name	Forward Primer	Reverse Primer
c156101.graph c0	*Glucose-6-phosphate isomerase (GPI)*	GTGCCCGACCATTCTCCCTT	GGAGTTAGGGTTGTATTTGGATGT
c167150.graph c0	*Beta-fructofuranosidase (IN)*	TTCACCTTTGACCCAATAGC	AACCTATCTGATCCCTTACTCCTA
c167938.graph c0	*Alpha-amylase (AMY)*	GCCATACTGCGTATCTGTGA	GAAGCACCAAGACCTCAACA
c169677.graph c0	*Sucrose synthase (SUS)*	AACCACAGGTCATCGCCTCCAC	AAGCCGAGATGAAGAAGATG
c174255.graph c0	*Betaine-aldehyde dehydrogenase (betB)*	TTGATTGTATCTGAAGGGTCT	CGGTTCTCATCCTGGTAAAG
c178025.graph c0	*Sucrose-phosphate synthase (SPS)*	CTGGTCACTCACTTGGTAGGGATA	TGGCTCGTAGTTTCCGTTCT
c185025.graph c0	*AvHexose Transporter*	ATGGTGACCTGCTGAACTGA	TCAGGTCGATGTGTTCCACA
c186025.graph c0	*AvSucrose Synthase*	TCCGATGTCTTGAGGAGACA	AGCAGTCCACACGTTGTTGA

Results

Transcriptome sequences and data output

Raw data were produced by the sequencing platform but were subjected to quality control checks to ensure that the data generated would be accurate and reliable for analysis. Thereafter, 39.03 Gb of clean data were achieved after quality control. Quality testing showed that percent bases had a Q30 score for the different samples tested. This is not less than 94.20%. The GC content ranged from 45.96% to 46.84%, both of which were within the limits considered acceptable (Table 2).

Table-2. The read statistics of the data output and quality control.

Sample	Read Number	Base Number	GC Content
stem01	22,376,428	6,680,233,370	46.24%
stem02	23,456,555	7,008,542,932	45.96%
leaf01	20,002,182	5,973,781,100	46.15%
leaf02	19,620,622	5,860,403,812	46.42%
fruit01	23,577,600	7,023,571,096	46.58%
fruit02	21,655,645	6,479,081,590	46.84%

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De novo assembly

The sequencing data generated were used for assemblages to align the in- formation appropriately. Trinity, a high throughput transcriptome sequencing assembly software, was dedicated to accomplishing this task. Thus, 50,928 unigenes and 120,239 transcripts were identified (Table 3). Overall, the N50 lengths were 1727 bp for unigenes and 1691 bp for transcripts, while the average lengths were 1123.72 bp and 1236.4 bp, respectively. The Unigenes and Transcripts were, therefore, further length distributed. It was observed that 17,581 unigenes (34.52% of the total) and 27,323 transcripts (22.72%) were within the range of 300-500 bp, which depicted a great in- tegrity of assembly. For the 500-1000 bp range, there were 13,586 unigenes (26.68%) and 35,573 transcripts (29.59%). Sequences between 1000-2000 bp yielded 11,721 unigenes (23.01%) and 37,070 transcripts (30.83%). For higher end sequence sizes above 2000 bp, 8,040 unigenes accounted for 15.79% and 20,273 transcripts constituted 16.86%. Most of the assembled Unigenes and Transcripts had a length range of 300–1000, which depicted the quality and integrity of the assembly process.

Table-3. Statistics of assembly transcripts and unigenes.

Length	Unigenes	Transcripts
300-500	17,581 (34.52%)	27,323 (22.72%)
500-1000	13,586 (26.68%)	35,573 (29.59%)
1000-2000	11,721 (23.01%)	37,070 (30.83%)
2000+	8,040 (15.79%)	20,273 (16.86%)
Total Number	50,928	120,239
Total Length (bp)	57,228,653	148,663,600
N50 Length	1,727	1,691
Mean Length (bp)	1123.72	1236.4

Gene annotation results

To obtain transcript annotation information, we compared unigenes against widely used biological databases using the BLAST (Baisc Local Alignment Search Tool (BLAST) software. The acquired annotations totaled 32,202, accounting for 63.23% of the total transcripts. Specifically, the number of annotations mapped to the COG, GO, KEGG, KOG, Pfam, SwissProt, eggNOG, and NR databases was 10,555, 19,222, 12,755, 17,971, 21,740, 20,788, 30,554, and 31,860, respectively. These are 32.78%, 59.69%, 39.61%, 55.81%, 67.51%, 64.55%, 94.88%, and 98.94% of the total annotations. Among them, the database with the greatest number of annotated Unigenes was that of NR, and the database with the least number was that of COG (see Table 4; Table S1).

Table-4. Unigenes annotation statistics across eight databases.

Annotated Database	Annotated Number	Percentage (%)
COG	10,555	32.78%
GO	19,222	59.69%
KEGG	12,755	39.61%
KOG	17,971	55.81%
Pfam	21,740	67.51%
Swissprot	20,788	64.55%

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NR database annotation results

The NR database, it is a non-redundant protein database maintained by NCBI and we have compared 31,860 annotated Unigenes with diverse known species. The result showed that the two species had the highest similarity between A. valvata and Vitis vinifera, 4,444 Unigenes were matched with 16.2%. This was followed by Quercus suber at 1,284 or 4.7%, Oryza sativa at 1,016 or 3.7%, Juglans regia with 832 or 3%, Olea europaea at 811 or 3%, Coffea canephora with 772 or 2.8%, Sesamum indicum at 650 or 2.4%, Nelumbo nucifera with 606 or 2.2%, and Hevea brasiliensis at 522 or 1.9%. We attribute this high homology between the Actinidia gene and the grape to their characteristics as deciduous vine fruit trees (Figure 1).

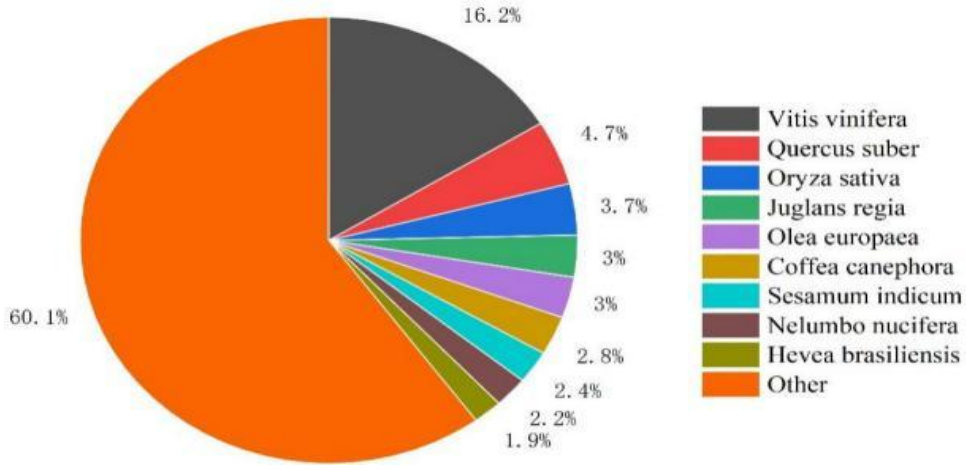

Figure-1. NR database annotation results of all unigenes.

KOG database annotation results

By comparing the retrieved Unigenes with the KOG database, altogether 17,971 genes were annotated, which accounted for 55.81% of all the annotated genes. Annotation results are shown in Figure 2. These Unigenes were divided into 25 functional classifications of the classification from the KOG system (Huerta-Cepas et al., 2016). Of these, most or 3,813 of the annotated genes 19.05 percent of all annotated genes had only general function prediction. The largest of the remaining categories were posttranscriptional modifications, protein turnover, and chaperones, with 2,093 genes or 10.46 percent. Other large and important categories included signal transduction mechanisms with 1,575 genes, or 7.87 percent; translation, ribosome structure, and biogenesis with 1,384 genes, or 6.91 percent; and carbohydrate transport and metabolism with 1,079 genes or 5.39 percent.

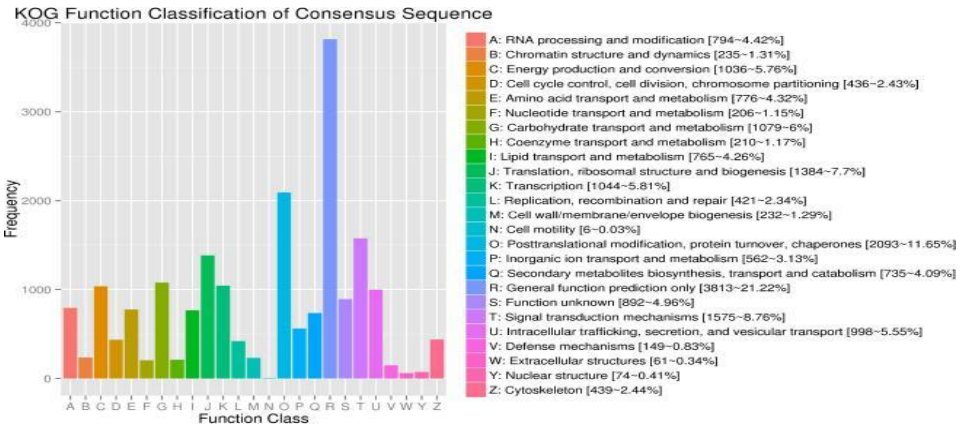

Figure-2. KOG function classification of all unigenes.

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GO database annotation results

The annotated Unigenes were 19,222 (59.69%) that fell under the three major categories: cellular component, molecular function, and biological process (Tatusov et al., 2000)(Fig 3).

The most proliferated subcategory of cellular component was cell followed by cell part, and then by membrane with annotations on 9,529, 9,473, and 7,246, respectively. Molecular functions were divided into 16 groups. Catalytic activity, binding, and transporter activity appeared most frequently, with 9,904, 9,252, and 1,371 annotations, respectively. There were 21 groups of biological processes, primarily annotated for the categories of metabolic, cellular, and single-organism processes, with 10,376, 9,537, and 6,824 annotations, respectively.

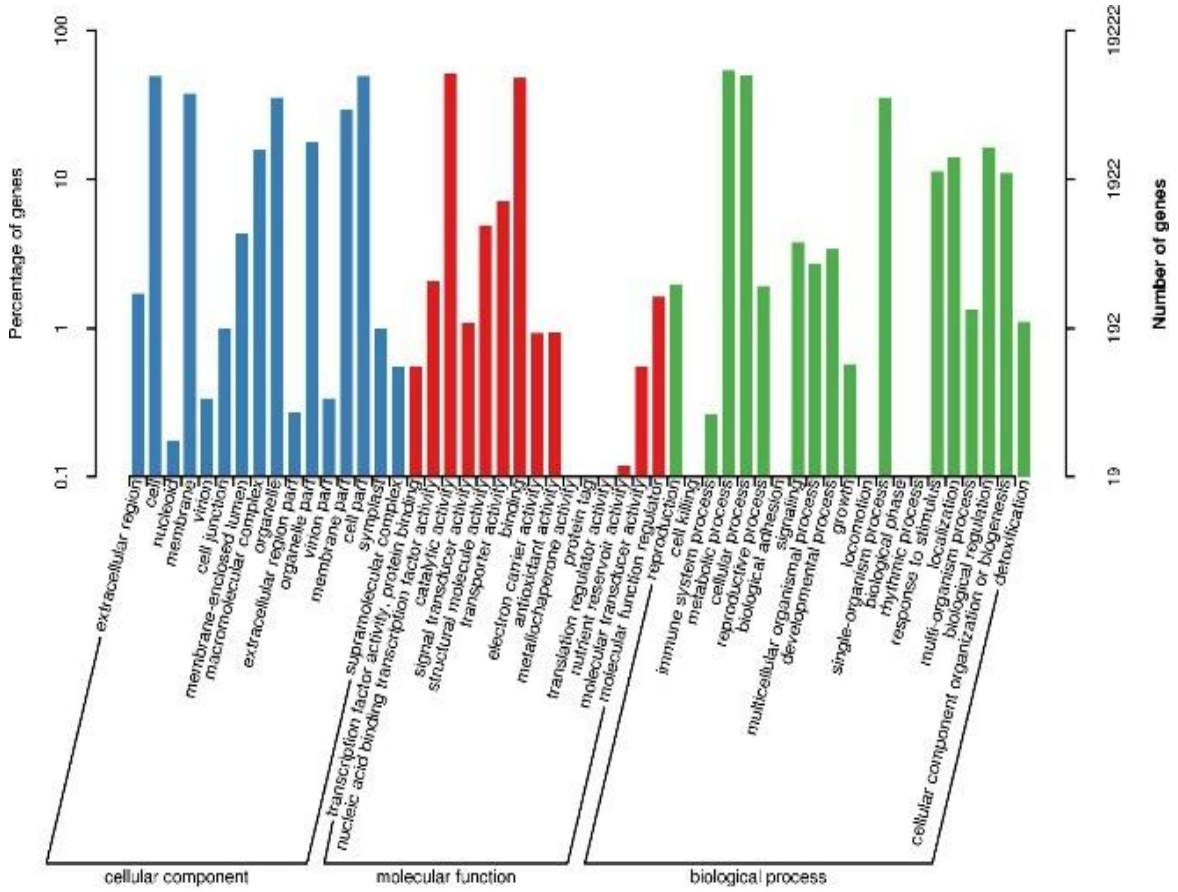

Figure-3. GO Function annotation of all unigenes.

KEGG database annotation results

In total, 11,809 Unigenes were annotated across 130 pathways in the KEGG database (Finn et al., 2014) Table 5. The most annotated pathways include Ribosome (752), Carbon metabolism (610), and Biosynthesis of amino acids (438). Other more notable pathways from large annotation counts include Protein processing in the endoplasmic reticulum (406), spliceosomes (311), starch and sucrose metabolism (302), and oxidative phosphorylation (295). The pathway most annotated with 289 annotations was RNA transport, followed by Glycolysis/Gluconeogenesis (278), plant hormone signal transduction (266), Endocytosis (265), Purine metabolism (228), Ubiquitinmediated proteolysis (220), and Pyruvate metabolism (216).

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Table-5. Path annotation in transcriptome Unigenes.

Pathway	Pathway ID	Gene Number	Percentage (%)
Ribosome	ko03010	752	6.07
Carbon metabolism	Ko01200	610	4.92
Biosynthesis of amino acids	Ko01230	438	3.54
Protein processing in endoplasmic reticulum	Ko04141	406	3.28
Spliceosome	Ko03040	311	2.51
Starch and sucrose metabolism	Ko00500	302	2.44
Oxidative phosphorylation	Ko00190	295	2.38
RNA transport	Ko03013	289	2.33
Glycolysis/ Gluconeogenesis	Ko00010	278	2.24
Plant hormone signal transduction	Ko04075	266	2.15
Endocytosis	Ko04144	265	2.14
Purine metabolism	Ko00230	228	1.84
Ubiquitin mediated proteolysis	Ko04120	220	1.78
Pyruvate metabolism	Ko00620	216	1.74
Others	—	7,511	60.64

Differential gene expression analysis

Three organs of A. valvata Unigenes were analyzed for differential gene expression. The screening criteria included an FPKM value, a False Discovery Rate (*P* - value) 0.01, and a Fold Change ≥ 2. The data are presented in Table 6. Between stems and leaves, 6,500 genes showed differential expression, including 3,418 up-regulated and 3,082 down-regulated. In stems compared to fruit, 7,300 genes showed differential expression, with 2,933 up-regulated and 4,367 down-regulated. In leaves compared to fruit, 6,016 genes were differentially expressed, with 2,879 up-regulated and 3,137 down-regulated.

Further analysis of metabolic pathways depicted active gene expression in the following pathways by more than twofold in stem vs. leaf, stem vs. fruit, and leaf vs. fruit: In stem vs. leaf, 1,191 up-regulated and 1,271 down-regulated genes were identified.

In stem vs. fruit, 1,342 up-regulated and 1,646 down-regulated genes were detected.

In leaf vs. fruit, 1,339 up-regulated and 910 down-regulated genes were detected (Table 7).

Table-6. Differentially expressed genes in comparison of stem, leaf, and fruit.

DEG Set	All DEG	Up-Regulated	Down-Regulated
Stem vs Leaf	6,500	3,418	3,082
Stem vs Fruit	7,300	2,933	4,367
Leaf vs Fruit	6,016	2,879	3,137

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Table-7. Pathway enrichment analysis of DEGs in comparison of stem, leaf, and fruit.

DEG Set	All Pathways	All DEG	Up-Regulated	Down-Regulated
Stem vs Leaf	125	2,462	1,191	1,271
Stem vs Fruit	127	2,988	1,342	1,646
Leaf vs Fruit	121	2,249	1,339	910

Screening of sugar metabolism pathways

The present study aimed at characterizing sugar metabolism during fruit development in *A. valvata*, with special emphasis on dynamic changes in sugar accumulation in three important tissues, namely stem, leaf, and fruit. The tissues were selected to provide a comparative carbohydrate profile analysis about the CAR metabolism at the various fruit developmental stages. The major metabolic pathways participating in the synthesis and degradation of carbohydrates in *A. valvata* include starch and sucrose metabolism, fructose and mannose metabolism, pentose and glucuronate interconversions, galactose metabolism, and glycolysis/gluconeogenesis, among others. These pathways are essential for the biosynthesis of sugars and energy for fruit growth, ripening, and also in the formation of secondary metabolites. The KEGG pathway classification results (Table 8) showed that, in the starch and sucrose metabolism pathway, 302 unigenes were enriched; in the galactose metabolism pathway, 99 unigenes; in the fructose and mannose metabolism pathways, 114 unigenes; in the pentose and glucuronate interconversion pathway, 110 unigenes; and in the glycolysis/gluconeogenesis pathway, 278 unigenes (Table S2). These results thus thoroughly explain the main pathways associated with carbohydrate metabolism of A. valvata. Differential expression analysis between stem, leaf, and fruit tissues identified several significant genes related to glucose metabolism, which also displayed high transcript levels (Table S3). Such genes are responsible for the biosynthesis and degradation of sugars through tightly regulated enzymic reactions. Sugar synthesis or degradation is therefore directly dependent upon gene expression levels encoding the respective enzymes and results in the net sugar content of the fruit. Overall, the biosynthetic capacity of the fruit and the efficiency of the degradation and recycling processes influence sugar contents. These pathways are finely regulated by enzymes, where the gene expression levels of these enzymes play a key role in determining the sugar composition and quality of the fruit. The study thus underscores the complex regulation of sugar metabolism in A. valvata and lays down a basis for future study directed toward enhancement in fruit quality through metabolic engineering and selective breeding.

Table-8. Gene number and DEGs in part of sugar metabolism-related pathways (KEGG enrichment analysis).

Pathway	Gene Number of Pathway	Stem vs Leaf	Stem vs Fruit	Leaf vs Fruit
Starch and sucrose metabolism	302	83	104	78
Fructose and mannose metabolism	114	24	28	24
Galactose metabolism	99	25	22	26
Pentose and glucuronate	110	28	38	24
Glycolysis/Gluconeogenesis	278	52	75	63

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Validation of transcriptome data by qRT-PCR

To confirm the reliability of the transcriptome data, six genes that were significantly expressed GPI, SUS, betB, SPS, INV, and AMY were randomly chosen for further validation. Relative expression levels of these genes were determined by qRT-PCR experiments and compared with their corresponding FPKM values in the transcriptome dataset. The results indicated that the expression patterns identified in the transcriptome data were highly consistent with the trends of relative expression levels measured through qRT- PCR, validating the reliability and accuracy of the transcriptome data (Fig. 4).

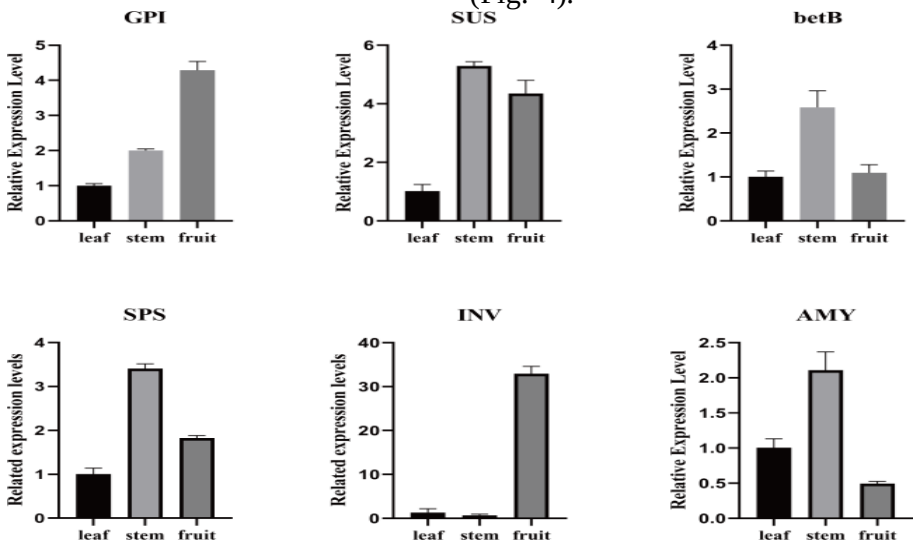

Figure-4. The expression of genes associated with sugar metabolism in different tissues of *A. valvata*.

Screening of genes related to sugar metabolism in *A. valvata*

The total sugar content in stems, leaves, and fruits of *A. valvata* were 265, 165 and 328 mg/g fresh weight, respectively. The differences of the total sugar content between different organs are significant. Sugar was present in the descending order in all the parts: fruit , stem , leaf. Fruit sugars reflect a higher accumulation than stem, with the pattern following normal enrichment of sugars in the tissue of fruits (Van-Dijk et al., 2018). This discovery provides a good basis for the identification of differentially expressed genes related to sugar metabolism and accumulation.

Analysis of differentially expressed genes in sugar metabolism path- way of *A. valvata*

It comprehensively analyzed the five metabolic pathways linked to sugar metabolism in *A. valvata.* This study identified starch and sucrose metabolism, galactose metabolism, and fructose and mannose metabolism as key pathways. These pathways have 302, 99, and 114 unigenes, respectively, that were enriched. These unigenes' annotations show a strong correlation with the sugar metabolism of A. valvata , further establishing their role in the growth, development, and metabolic processes of plants.

Further screening of pivotal genes related to sugar metabolism through FPKM values is shown in Table S4. Twenty-six annotated genes were identified, mostly enriched in the sucrose, glucose, and fructose synthesis pathways. Sucrose, glucose, and fructose have been previously reported to be critical contributors to sugar content in fruits. Gene annotation data were integrated with literature on sugar metabolism to map the sugar metabolism pathway in *A. valvata* (Figure 5).

Sucrose synthase (SUS), a key enzyme in sugar metabolism, catalyzes the synthesis of sucrose from UDP-glucose and simultaneously breaks down sucrose into UDP-glucose. This study identified three SUS-encoding genes, predominantly expressed in stems and fruits but with low expression in leaves. Sucrose phosphate synthase (SPS), another critical enzyme in sucrose synthesis, catalyzes the production of sucrose from fructose-6P. The SPS-regulated genes appeared to have high expression levels in leaves while being low in stems and fruits.

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Second, the breaking down of sucrose through invertase breaks it up into D-glucose and D-fructose, with glucose being key to its fruit sugar accumulation. Glucose is also derived from starch that is broken into glucose following the action of α-amylase, BAM as well. Fructose interconverts with fructose-6 phosphate (fructose-6P) through the action of fructokinase (FK) and hexokinase (HK), whereas glucose is synthesized from glucose-6 phosphate via hexokinase activity. The expression levels of the differentially expressed genes involved in sugar metabolism in A. valvata were significantly correlated with sugar content. Notably, *SUS* c138347.graph c0, *HK* c168593.graph c0, *HK* c158367.graph c0, *GPI* cfigure175461.graph c1, and *GPI* c156101.graph c0 were positively correlated with sugar content. Conversely, *SPS* c172403.graph c0, *malZ* c157824.graph c2, *malZ* c157824.graph c0, *INV* c168053.graph c0, INV c167099.graph c0, BAM c174606.graph c0, BAM c171387.graph c0, BAM c171350.graph c0, *BAM* c163643.graph c0, and BAM c161122.graph c0 were negatively correlated (Figure 6). These findings suggest that these enzymes represent key genes in the sugar metabolism of *A. valvata*, with their expression levels closely linked to sugar content. Fruit tissue exhibited the highest sugar content (328.82 mg/g), correlating with upregulated SUS and HK genes.

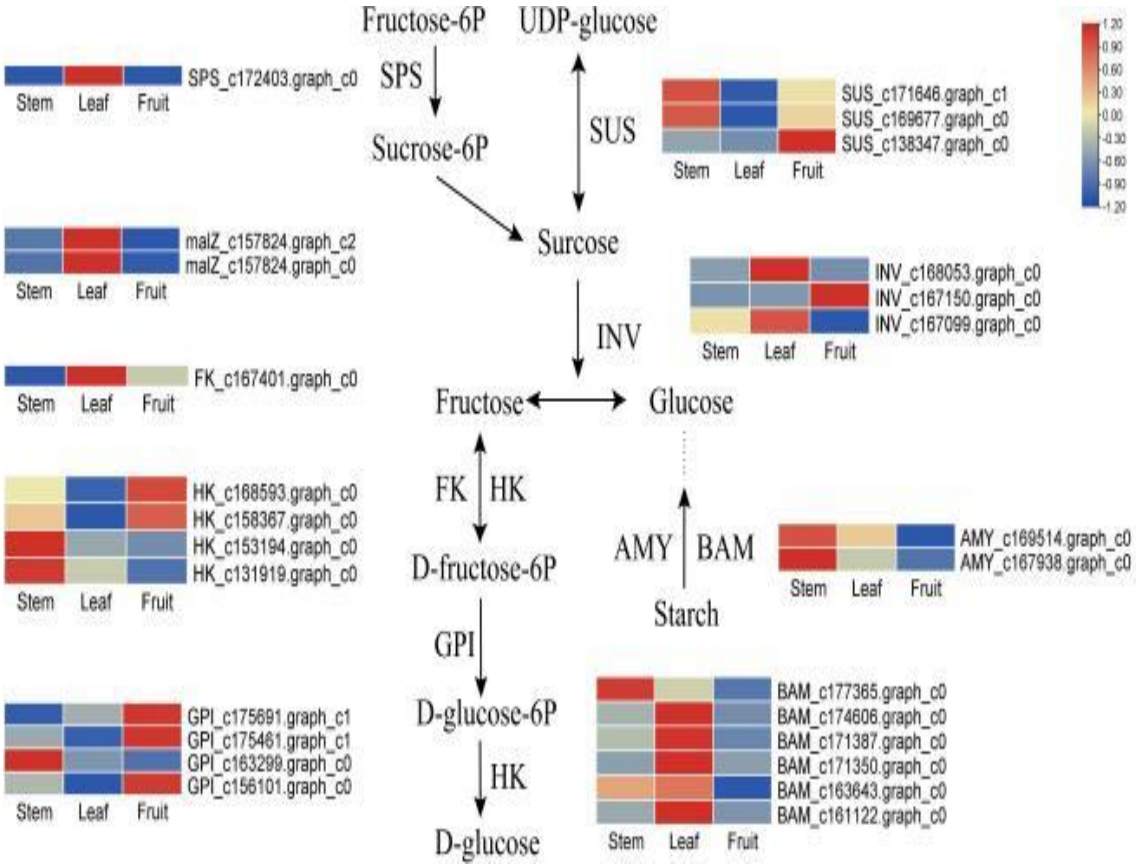

Figure- 5. Analysis of DEGs in sugar metabolism pathway.

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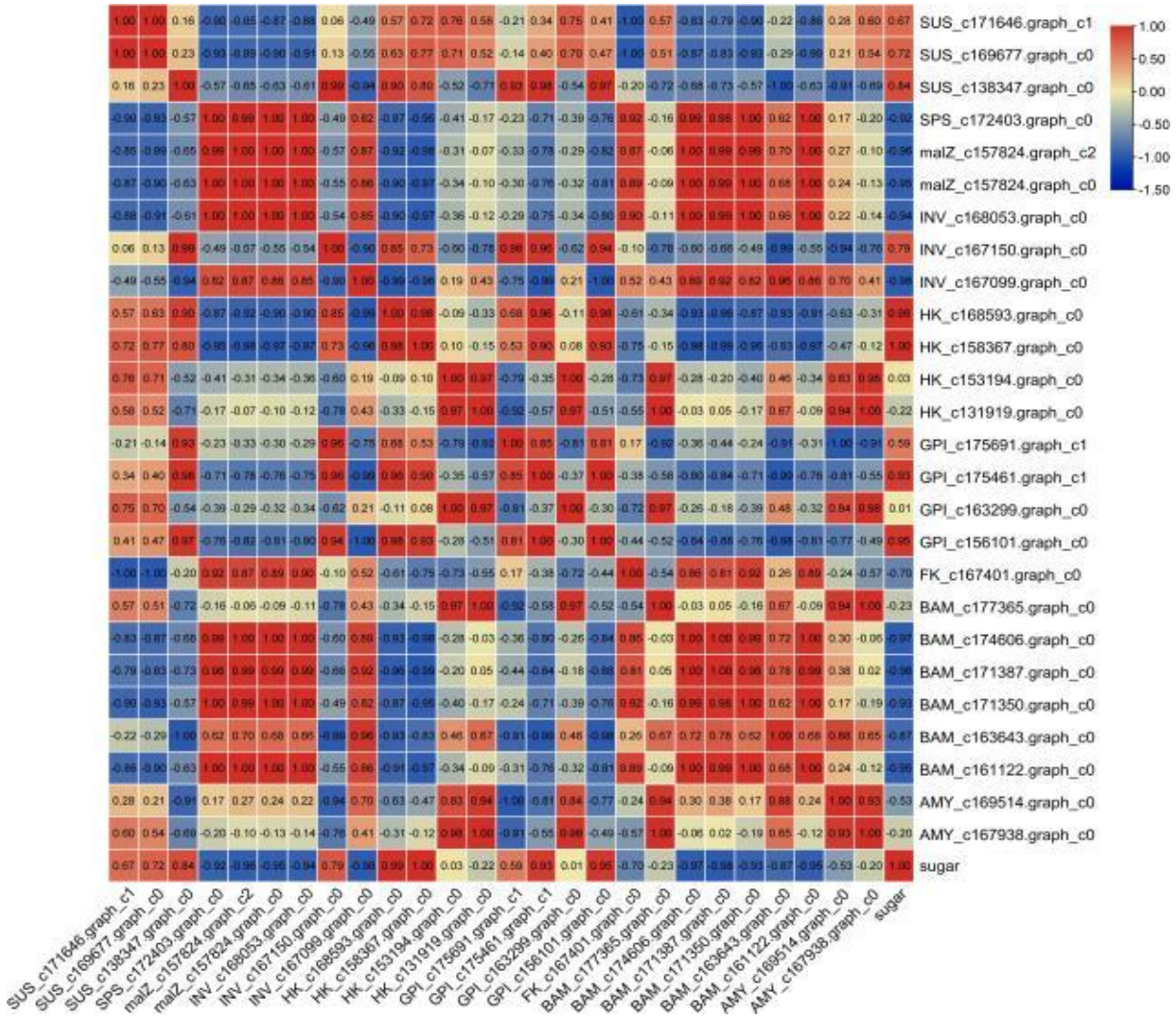

Figure-6. Correlation analysis of total sugar content and DEGs of sugar metabolism pathway in different organs of *A. valvata*

Discussion

A. valvata is an integral, wild plant resource unique to China and has medicinal and edible value. Notably, the roots of the plant are effective in the treatment of digestive tract tumors. Current studies on *A. valvata* have been limited to germplasm resources (Cochavi et al., 2021), cultivation and management techniques (Kwon et al., 2012), as well as variety selection (Sang et al., 2017). However, studies regarding genes associated with sugar metabolism in *A. valvata* are rare.

Transcriptome sequencing was performed to investigate the key genes in *A. valvata*, representing the stem, leaf, and fruit tissues. As a result, we obtained 39.03 Gb of clean data, and through assembly, generated 50,928 unigenes. Of these unigenes, we conducted GO, KEGG, and NR annotations, and 32,202 unigenes were available for annotation. Specifically, 19,222 unigenes were annotated in the GO database, 11,809 in the KEGG database, and 31,860 in the NR database. Further investigation on DEGs in KEGG metabolic pathways revealed 26 annotated genes generally associ- ated with the synthesis pathways of sucrose, glucose, and fructose, consistent with previous findings. While transcriptome data identified DEGs, future proteomic studies are needed to confirm enzyme activity.

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Sucrose, fructose, and glucose are essential constituents of fruit sugar, directly affecting fruit quality. Mapping relevant metabolic pathways identified several key genes, including *Sucrose Phosphate Synthase* (*SPS*), *Sucrose Synthase* (*SUS*), *invertase* (*INV*), *fructokinase* (*FK*), and *hexokinase* (*HK*). These genes regulate sucrose, fructose, and glucose metabolism in fruit tis- sues. The accumulation of carbohydrates in fruits is primarily driven by photosynthesis in the leaves (Xie et al., 2011), and these carbohydrates are transported to the fruit in the form of sucrose and sorbitol via the phloem. Sucrose, galactose, fructose, and glucose are distributed across various parts of the fruit through protein-mediated transport and enzymatic reactions, contributing to fruit sweetness.

Among the key genes, sucrose synthase (SUS c171646.graph c1) demonstrated intense expression in fruit, corresponding to sugar content measurements. Therefore, it can be assumed that the key gene controlling sucrose synthesis in A. valvata fruit is SUS c171646.graph c1. Similarly, Sucrose Phosphate Synthase (SPS), responsible for fructose sucrose synthesis, was highly negatively correlated with sugar content; its lower expression levels in fruits indicated its function in sugar regulation. Notably, Invertase (INV), which breaks down sucrose into fructose and glucose, is also negatively correlated with sugar content, but with higher expression in leaves than in fruits according to observed trends. Fructokinase (FK) and hexokinase (HK) are enzymes involved in the phosphorylation of fructose and glucose, respectively. In this analysis, HK c168533.graph c0 and HK c158367.graph c0 correlated positively with sugar content, whereas FK c167401 showed a higher expression profile in fruit tissues, contrary to previous reports (Yue et al., 2022) showing differences in gene expression between fruit types. Moreover, starch breaks down into glucose due to the action of α-amylase (AMY) and β-amylase (BAM), which are negatively correlated with sugar content in fruit, consistent with results in potatoes (Zhang et al., 2023). This emphasizes the vital role of AMY and BAM in glucose synthesis in *A. valvata* fruit. Contrary to findings in *A. chinensis* (Zhang et al., 2023), SPS in *A. valvata* showed negative correlation with sugar content, suggesting species-specific regulatory mechanisms.

In summary the key genes included SUS c138347.graph c0, HK c168533.graph c0, HK c158367.graph c0, GPI c175461.graph c1, and GPI c156101.graph c0, which were positively correlated with sugar content. In contrast, genes such as SPS c172403.graph c0, malZ c157824.graph c2, INV c168053.graph c0, and BAM c174606.graph c0 were negatively correlated. These important genes involved in the sugar metabolism of A. valvata provide insights into its sugar metabolism pathways.

This study, performed on the transcriptome sequencing of sugar metabolism pathways in *A. valvata*, provides a critical foundational step to improve its edible quality and moves a step closer to functional studies on the genes involved in its metabolic process.

Conclusion

This study provides a comprehensive transcriptomic exploration of *A. valvata*, elucidating the molecular mechanisms underlying sugar metabolism across its stem, leaf, and fruit tissues. Differential expression analysis identified tissue-specific gene regulation, with 26 key differentially expressed genes (DEGs)—including *SUS*, *INV*, *SPS*, *HK*, *malZ*, and *GPI*—strongly correlated with total sugar content. Notably, sucrose synthase (*SUS*) and invertase (*INV*) exhibited pronounced expression in fruits and stems, aligning with their roles in sucrose synthesis and degradation, while sucrose phosphate synthase (*SPS*) was upregulated in leaves. The validation of transcriptome data via qRT-PCR confirmed the reliability of expression patterns for pivotal genes like *GPI* and *SUS*. Furthermore, the high sugar accumulation in fruits, compared to stems and leaves, underscores the fruit's metabolic priority for sugar biosynthesis. These findings highlight the intricate regulatory network governing sugar metabolism in *A. valvata* and pinpoint candidate genes for enhancing fruit quality through targeted breeding or metabolic engineering. This work establishes a foundational resource for future studies on *A. valvata*'s nutritional and medicinal traits, offering insights into optimizing sugar-related pathways for agricultural and biotechnological applications. Further functional characterization of the identified DEGs and their regulatory mechanisms will advance our understanding of carbohydrate dynamics in this economically valuable species.

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Contribution of Authors

Han S, Dong J, Sajjad M & Fang Y: Conceived and planned the study.

Wang Y, Han S, Ali MSY & Zheng S: Contributed to drafting the manuscript.

Wang Y, Han S & Zheng S: Performed the experiments, analyzed the data, prepared figures and/or tables.

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