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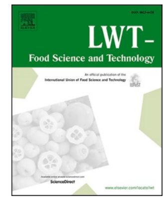

Transcriptome and metabolome analysis reveals the potential mechanism of tuber dynamic development in yam (*Dioscorea polystachya* Turcz.)

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ABSTRACT

Yam (*Dioscorea* spp.) is a multispecies and multipurpose tuber crop. To clarify the mechanism of tuber development, we performed a time-course phenotypic, cytological, physiological, metabolomic, and transcriptomic analysis of yam tubers. The results showed that the tuber weight increased with the accumulation of starch, and sucrose metabolism was also active during tuber development. Meanwhile, abscisic acid (ABA) levels were positively correlated with tuber weight, while gibberellin (GA) was negatively. Metabonomic analysis showed that 400 metabolites accumulated during tuber development, which played an important role in the regulation of tuber growth and development, flavor and medicinal ingredients. A total of 743 differentially expressed genes (DEGs) were assigned to 21 KEGG pathways such as starch and sucrose metabolism, plant hormone signal transduction pathway and flavonoid pathway by comparative transcriptome. Comprehensive transcriptome and metabolome analysis revealed the DEGs and differentially accumulated metabolites (DAMs) of plant hormone signal transduction pathway, starch and sucrose metabolism, and flavonoid synthesis pathway. In conclusion, the DAMs and DEGs involved in plant hormone signal transduction pathway, starch and sucrose metabolism pathway and flavonoid metabolism pathway play an important role in the regulation of tuber development. This study provides theoretical basis and practical guidance in molecular breeding and quality improvement of yam.

1. Introduction

Yam (*Dioscorea polystachya* Turcz.) is an edible and pharmaceutical food in China that comprise abundant starch, protein, and medicinal ingredients such as allantoin, flavonoids, and diosgenin (Lebot et al., 2019; Shan et al., 2020). Plant tubers are a kind of underground metamorphic stem that is formed by the expansion of the end of the underground stem. The stem is metamorphic, suitable for storing nutrients, is rich in nutrients, and is also an economically important organ that acts a vital role in plant adaptation and reproduction. Tuber development includes tuber morphogenesis, accumulation of starch, storage protein, and other secondary substances (Aksenova et al., 2012), which are affected by genotype, environment, phytohormones, enzymes, and many other factors (Liang et al., 2011).

Yam tubers are an excellent source of phenols, flavonoids, diosgenin and other medicinal ingredients. Flavonoids are vital secondary metabolites in plants with substantial benefits to human health defeating liver injury, cancer, vascular disease, and oxidative stress (Boots et al., 2008; Calderon-Montano et al., 2011; Kreft, 2016; Palazzolo et al., 2012). They are a class of phenolic compounds, and their metabolism and regulatory mechanisms are one of the hotspots in plant research, which have been revealed in tea, *Artemisia annua*, and *Sophora flavescens* (Falcone Ferreyra et al., 2012; Li et al., 2021). The biosynthesis of flavonoids derived from the phenylpropane pathway is a particularly convoluted process, catalyzed by a variety of enzymes, and changing any one of the enzymes will change its final product (Lepiniec et al., 2006; Pelletier et al., 1999; Wu et al., 2015). In addition, phytohormones, transcription factors, and other factors affect the synthesis of flavonoids (Wu et al., 2021).

Phytohormones are considered the key factors of tuber development.

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They can interact with environmental signals, which participate in regulating the growth and expansion of underground storage organs and also take a crucial part in regulating the starch synthesis and accumulation (Kim & Kim, 2005; Sarkar, 2008). High levels of ABA increase the activity of starch synthase in tubers and improve starch content during bulb development of *Tulipa edulis* (Miao et al., 2016). Many genes and proteins rich in plant hormone signaling pathway are closely related to plant tuber formation (Aksenova et al., 2012; Dutt et al., 2017; Hannapel et al., 2004). The *ABF* (*ABA RESPONSIVE ELEMENT BINDING FACTOR*) gene, the response element of ABA signal transduction, can regulate the increase in ABA levels and the decrease in GA levels, thereby promoting the rapid development of tubers (Muniz Garcia et al., 2014). *ARF6* (*AUXIN RESPONSE FACTOR6*) has a high expression level in the early stage of potato stolon expansion and is downregulated in the later stage of tuber development (Faivre-Rampant et al., 2004). Auxin, as an induction factor of tuber formation, is beneficial to plant growth before tuber formation. The expression of *TMS1*, an auxin synthesis gene, promotes auxin synthesis and inhibits carbohydrate consumption, thereby promoting tuber morphogenesis (Kolachevskaya et al., 2015; Roumeliotis et al., 2012). Starch is an essential polysaccharide in storage organs, and its synthesis and accumulation also affect the growth and quality of tubers. The weight of starch accounts for 70%–90% of the dry weight of yam tubers, which is a critical component that affects the yield and nutrition of yam tubers (Otegbayo et al., 2018; Riley, 2014). Starch accumulation occurs in the cytoplasm and is regulated by *SuS* (*SUCROSE SYNTHASE*), *AGP* (*ADENOSINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE*), *GBSS* (*GRANULE-BOUND STARCH SYNTHASE*), *SBE* (*STARCH BRANCHING ENZYME*), and other genes (Aksenova et al., 2012; Greene & Hannah, 1998; Tanaka et al., 2004).

Although yam has high edible and medicinal value and considerable economic benefits, it has been regarded as an orphan crop for a long time and has rarely attracted the attention of researchers (Tamiru et al., 2017). To date, most of the research on the yam has focused on the classification of germplasm resources, analysis of genetic diversity, extraction of specific components, and medicinal effects (Cao et al., 2020, 2021; Dong et al., 2012, 2013; Lu et al., 2012). Currently, metabolomics and transcriptomics analyses have been used extensively to explore the mechanisms of plant development and the accumulation of metabolites (Xue et al., 2020; Zhao et al., 2020; Zhou et al., 2020). Recent studies have focused on the exploration of medicinal ingredients by metabolomics analyses (Li et al., 2019). Based on the transcriptome analysis, Li et al. (2020) found that the development of microtubers in yam tissue culture was closely related to the metabolism of starch and sucrose, and ABA positively regulated tuber formation and identified the regulatory genes of plant hormones. Zhou et al. (2020) examined the mRNAs and small RNAs in the tuber of *D. opposita* cv. Guihuai 16, and identified the genes and transcription factors related to expansin of tuber. In the present study, to explore the developmental mechanism of yam tubers and the regulatory network of starch and flavonoid biosynthesis, the Landrace “Ruichang” yam was used as the material, and the dynamic development of yam tubers was detected by integrating the phenotype, cytology, physiology, transcriptome and metabolomics data. The results of this article will provide a new perspective for realizing the development mechanism of yam tubers and potential targets for molecular breeding in yam.

2. Materials and methods

2.1. Plant materials

The Landrace “Ruichang” yam (*D. polystachya* Turcz.) were planted in an experimental field (29°67′18.3″ N, 115°66′70.5″E) in Ruichang city, Jiangxi Province in China in year 2019. The plants were supported by bamboo poles, with a single row with a row spacing of 65 cm, plant spacing of 25 cm, furrow depth of 40 cm, and furrow width of 20 cm.

2.2. Sample collection and phenotype analysis

Underground tubers with uniform size without disease, insect or injury were gathered at 90, 110, 130, 150, 170, 190, 210, and 230 days after planting (DAP). The diameter, length, and weight of tubers were determined after rinsing with distilled water. Then, all samples were immediately frozen with liquid nitrogen and finally stored at −80 °C for later analysis.

2.3. Paraffin sectioning and scanning electron microscopy (SEM) analysis

The fresh tubers were peeled and cut into small pieces and transferred to a formaldehyde-acetate-ethanol fixation solution (FFA) for paraffin sectioning, fixed at 4 °C with 3.7% FAA fixation solution for one week, dehydrated with 50%, 70%, 85%, 95%, and 100% ethanol, cleaned with ethanol and xylene, waxed, embedded, and finally cut into thin sections of 3–4 μm in thickness. All sections were stained with I-KI, then restained with Fast Green, and finally observed and photographed under a microscope at Olympus BX53 (Olympus Corporation, Tokyo, Japan). In addition, other tuber samples were ground into powder, transferred to a centrifuge tube, repeatedly washed with 0.1 mol/L NaOH more than three times, and dried at 40 °C for starch granule observation with SEM.

2.4. Measurement of carbohydrate and flavonoid content and enzyme activity

The soluble sugars (sucrose, glucose, and fructose) were extracted as previously described (Zhao & Oosterhuis, 1998). That is, 100 mg of fresh samples was extracted three times at 80 °C for 30 min in 3 ml of 80% ethanol (v/v) and then centrifuged at 4200 rpm for 15 min. The supernatant was taken and brought up to 10 mL with 80% ethanol (v/w) for the subsequent analysis. The contents of sucrose, fructose, and glucose were determined by resorcinol method, enzyme colorimetric method and hexokinase method respectively (Hendrix, 1993). The extraction and determination of starch were carried out with perchloric acid and anthrone sulfate according to previous suggestions (Gao et al., 2012; Morris, 1948). The activities of soluble starch synthetase (SSS), AGP, GBSS and SBE were determined by assay kit (Suzhou Comin Biotechnology Co., Ltd., Suzhou China). Flavonoid content, phenylalanine ammonia-lyase (PAL), and 4-coumarate coenzyme A ligaseis (4CL) activity were also determined by kit (Beijing Solarbio Science and Technology Co., Ltd., Beijing China).

2.5. Analysis of phytohormones in yam tubers

The levels of ABA, GA, JA, indole-3-acetic acid (IAA), zeatin riboside (ZR), and brassinolide (BR) in tubers were determined with an enzyme-linked immunosorbent assay kit (ELISA, School of Agriculture and Biotechnology, China Agricultural University) as previously described (Maldiney et al., 1986) and repeated three times.

2.6. Metabolomic analysis

Metabolomic analysis was performed for five stages of rapid growth yam tubers (150, 170, 190, 210, and 230 DAP, T1-T5), with three repetitions of each stage. The data generated by High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) were processed, integrated and peaks corrected by SCIEX OS Version 1.4 (Sciex, Framingham, MA, USA). The peak area was used to calculate the relative content of the corresponding substance. The annotation of metabolites was performed according to the following databases: KEGG, Human Metabolome (HMDB, http://www.hmdb.ca/), and Lipidmaps (http://www.lipidmaps.org/). The DAMs were identified at VIP >1 with $\log_2{}^{(\text{fold change})} \geq 1.2$ or p value < 0.05, and mapped to the KEGG database to identify the metabolic pathways participated in the

development of yam tubers.

2.7. RNA extraction, isoform sequencing (Iso-Seq), and illumina sequencing

Extraction of total RNA from five yam tissues (bulbil, leaf, root, stem, and flower) and five developmental stages (150, 170, 190, 210, and 230 DAP, T1-T5) was performed according to the protocol of RNAprep Pure Plant Kit (Tiangen Biotechnology Co., Ltd., Beijing, China) with three biological replicates. RNA quality was assessed using agarose gels, a NanoDropTM 2000 Spectrophotometer (Thermo Scientific, Shanghai, China), and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California, USA). Total RNA from different tissue samples was mixed equally, and the Iso-Seq library was constructed according to the protocol of Clontech SMARTer PCR cDNA Synthesis Kit (Clontech Laboratories, Inc., mountain view, CA, USA). For each tuber total RNA sample, an Illumina sequencing cDNA library was constructed by the NEBNext® UltraTM RNA Library Prep Kit for Illumina (New England Biolabs (Beijing) LTD), and RNA-seq was performed on the HiSeqTM 2500 platform. SMRTlink 4.0 software was used to work with the primary Iso-Seq data to acquire the read sequences, and circular consistency sequences (CCSs) were obtained after correction. The sequences were divided into nonfull-length sequences and full-length sequences according to the 5′ primers, 3′ primers, and polyA tails. The cluster consensus sequence was obtained by clustering the full-length sequence and polishing full-length consensus sequences for subsequent analysis. The PacBio data were calibrated with RNA-Seq data using LoRDEC software and CD-HIT 4.6.142 to remove redundant transcripts as the gene reference sequence.

The sequence annotation of gene function was performed with reference to the databases as follows: NR (National Center for Biotechnology Information (NCBI) nonredundant protein sequences), Pfam (http://pfam.xfam.org/), NT (NCBI nonredundant nucleotide sequences), KO (Kyoto Encyclopedia for Genes and Genomes (KEGG) Ortholog database), GO (Gene Ontology), Swiss-Prot (http://www.expasy.ch/sprot), and KOG/COG (http://www.ncbi.nlm.nih.gov/COG/). The raw reads of RNA-seq in fastq format were manufactured by an in-house Perl script. The raw data was stripped of linker sequences, low-quality reads, and reads containing polyNs finally mapped to gene reference sequences.

2.8. Transcriptomic data analysis and quantitative real-time PCR (qRT-PCR) analysis

The gene expression was calculated as the values of fragments per kilobase of transcript per million mapped reads (FPKM). The raw count data were screened for significant DEGs by differential expression sequencing (DESeq2) method at the absolute value of $\log_2{}^{(\text{fold change})} >$ 1.2 and p value < 0.05.

To further identify gene clusters related to tuber development, the FPKM from 15 samples were used to conduct weighted correlation network analysis (WGCNA) by the R package WGCNA (Langfelder & Horvath, 2008). The weighted expression correlation of genes was calculated, and a hierarchical clustering analysis was conducted to construct a gene coexpression network. The genes with highly correlated expression were identified as a gene module, represented by branches and different colors of the cluster tree. Then, correlation analysis between gene modules and phenotypes was performed to screen modules related to the development of yam tubers. Finally, the genes in the screened modules were enriched using gene set enrichment analysis (GSEA) software, and critical regulatory networks and candidate genes took part in the development of yam tubers were identified.

To validate the RNA-seq data, reverse transcription of total RNA from each tuber to cDNA was performed by the PrimeScript RT reagent Kit with gDNA Eraser (Takara, Beijing, China). A total of 16 key candidate genes selected from the DEGs in the sucrose and starch metabolic pathways, flavonoid metabolic pathways, and phytohormone signal transduction pathway were carried out for qRT-PCR, and the yam actin gene was used as the internal reference gene (Gong et al., 2016). The sequence design of primers for each validation gene was carried out by primer5 software, as shown in table S1. The qRT-PCR was operated by the SYBR Premix Ex Taq™ Kit (Takara, China) manufacturer's protocol with reaction conditions of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 15 s of 40 cycles. The relative expression levels of qRT-PCR were calculated with reference to the previous the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

2.9. Statistical analysis

Image J (version 1.52 V) software was used to count the cell area, starch particle diameter and area in paraffin section and starch particle scanning electron microscope. SPSS 25.0 software was used to analyze the difference significance ($P < 0.05$) and correlation of the data, and graphpad prism 5.0 software was used to graphics. The origin 9.0 software was used for logistic regression analysis to determine the dynamic models, the maximum rate of change (Vm), start time for the rapid accumulation phase (t1), termination time for the rapid accumulation phase (t2), duration of the rapid accumulation phase (T), Maximum accumulation rate (Vm) and occurrence time of Vm (tm) of tuber weight and starch content.

3. Results

3.1. Phenotypic changes during yam tuber development

To understand the development process of yam tubers, we continuously observed the whole process of yam tuber development (Fig. 1, A). We found that tubers could be observed at 90 DAP, and the growth of tubers was slow from 90 to 130 DAP. Then, the tubers entered the rapid growth and expansion period, and the diameter of tubers increased significantly. The weight of tubers increased considerably from 150 to 210 DAP. The length of tubers showed a steady and continuous elongation trend over the whole growth period (Fig. 1, B). In addition, the starch grains formed in the tubers were smaller in the early stage of development and became larger step by step with the growth of the tuber (Fig. 1, C). Moreover, starch grains were not observed before 90 DAP, but an enormous number of starch grains accumulated from 130 DAP in the tuber cells. The starch grains were oval with a smooth surface, with a diameter of 13–21 μm and an area of 150–400 μm^2 at 130 DAP.

Meanwhile, the starch content was characterized by an increasing trend during tuber development, which was a rapid accumulation period from 130 to 210 DAP. There was no significant change from 90 to 110 DAP and 210 to 230 DAP (Fig. 1, D). The initial time of the rapid increase in starch content was 128.9 DAP, while the termination time was 186.2 DAP, the duration of the rapid increase was 57 d, and the maximum rate was 2.48 mg $g^{-1}\cdot d^{-1}$ (Table S2). The sucrose concentration of tubers increased sharply from 90 to 110 DPA, followed by a gradual decline, and finally increased gradually after 210 DAP (Fig. 1, D). The concentration of fructose increased greatly from 90 DAP to 110 DAP, then decreased gradually and finally stabilized (Fig. 1, D). In addition, the glucose concentration was similar to the concentration of fructose, while the glucose concentration after 190 DAP was not detectable (Fig. 1, D).

3.2. Metabolomic analysis of yam tubers at different developmental stages

Three hundred and ninety-five metabolites were identified at the metabolomic analysis of T1, T2, T3, T4, and T5 stages of yam tubers, which can be divided into 45 categories, and 12 categories contained more than ten metabolites (Table S3 and Fig. 2, A). The top five categories were "Amino Acid and Its Derivatives," "Nucleotide and Its

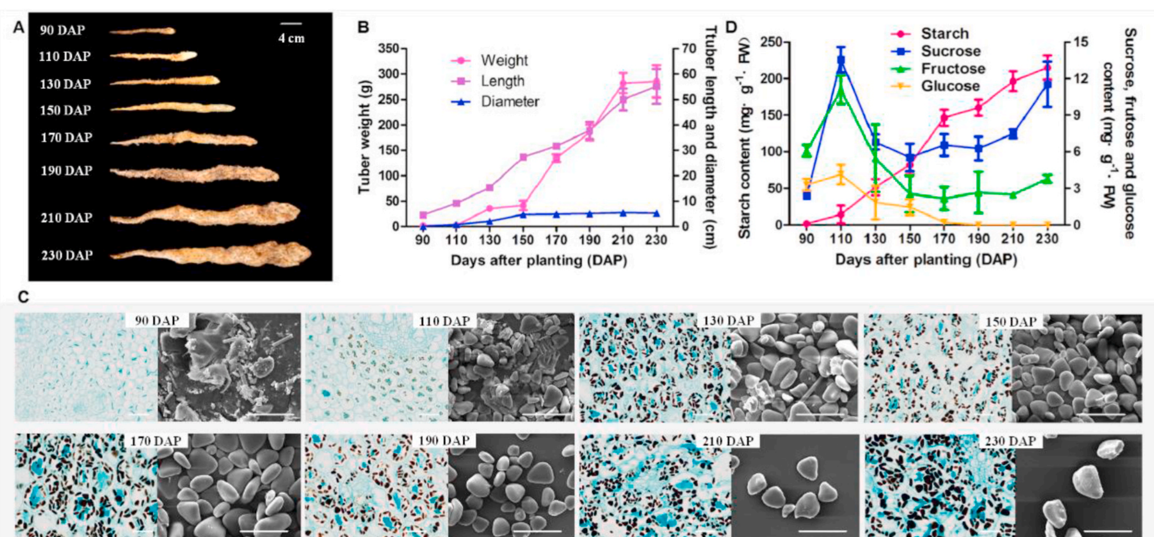


Fig. 1. Morphological characterization of yam tubers at different developmental stages: (A) Morphological analysis of yam tubers development, Bar = 4 cm, (B) Dynamic changes in weight, length and diameter of yam tubers at different developmental stages, (C) The paraffin section diagram and the starch grain structure scanning diagram of tubers at different development stages, Bar = 50 μm, (D) Carbohydrate content of yam tubers at different developmental stages.

Derivates," "Organic Acid and Its Derivatives," "Carbohydrates," and "Lipids and lipid-like molecules," with 62, 41, 36, 26, and 19 metabolites, respectively. A total of 75 DAMs were identified in four pairwise comparisons (Fig. 2, B), which were divided into 25 categories (Fig. 2, C). In the T1 stage, we identified more than 30 metabolites, including "Nucleotide And Its Derivates," "Amino Acid And Its Derivatives," "Polyamine," "Alkaloids," "Phenols," and "Phytohormones,". However, 37 metabolites were identified at T5 stage, including "Amino Acid And Its Derivatives," "Carbohydrates," "Nucleotide And Its Derivatives," "Organic Acid And Its Derivatives," "Flavanone," and "Sugar Alcohols,". The iP9G, erucic acid, galactinol, coniferin, and sucrose were found at T1 and T5 stages. Moreover, the metabolic pathways were mainly involved in the biosynthesis of secondary metabolites, ABC transporters, biosynthesis of amino acids, and carbon fixation in photosynthetic organisms, and 12 metabolites were obtained in the biosynthesis of the secondary metabolites pathway (Fig. 2, D). In addition, the DAMs of sucrose and trehalose had the highest accumulation in the T5 stage, and the DAMs of fustin, pinocembrin and afzelechinhave high accumulation in the T1 stage, while Pinocembrin and afzelechin had high accumulation in the T5 stage. (Fig. 2C and D).

3.3. Transcriptome analysis of yam tubers at different developmental stages

Transcriptome analysis showed that more than 43,000,000 clean reads were obtained (Table S4), a total of 21,776 genes (FPKM >0.1) were expressed in five stages, and the genes specifically expressed in T1, T2, T3, T4 and T5 stages were 260, 35, 29, 19 and 93 respectively (Fig. 3, A). Furthermore, there were significant differences between the two stages for the DEGs ($p < 0.05$, FC ≥ 1.2); the minimum DEGs (720) were in the T3 vs. T2 stage, with 371 upregulated and 349 downregulated genes, while the maximum (3845) were in the T5 stage vs. T4 stage, with 1353 upregulated and 2492 downregulated genes (Fig. 3, B). Further, the results of the16 DEGs verified by qRT-PCR were consistent with their sequencing results (Fig. 3, C). To determine the main active pathway of yam tuber development, a KEGG enrichment analysis of the DEGs was conducted. A total of 1312 genes were enriched to the known KEGG metabolic pathway, including phenylpropane biosynthesis, starch and sucrose metabolism, plant hormone signal transduction, plant pathogen interaction, flavonoid biosynthesis, and amino sugar and nucleotide sugar metabolism (Fig. 3, D). In addition, phenylpropanoid biosynthesis and flavonoid biosynthesis were significantly enriched in the T1-T5 stage, starch and sucrose metabolism in the T1-T4 stage, and plant hormone signal transduction in the T2-T5 stage (Fig. 3, E).

3.4. WGCNA and GSEA

To identify the potential regulatory network related to the development of yam tubers, WGCNA was conducted to identify the modules with high gene coexpression. To avoid false results, low-expression genes (FPKM <1) were eliminated, and a total of 12310 genes were used for WGCNA (Fig. 4, A). The dynamic hierarchical tree cutting showed that 13 modules were detected to have similar gene expression patterns. The number of genes identified in the turquoise module was 7611 at most, and the brown and salmon modules were 53 at least (Fig. 4, B). The turquoise module was negatively correlated with the weight, length, starch content, and sucrose content of yam tubers by Pearson correlation of phenotypic traits and expression patterns of each module (Fig. 4, B). Additionally, the blue module was correlated with sucrose content, and the pink module was correlated with fructose content. Thus, the turquoise module is related to the growth and development of yam tubers, and the turquoise module was selected for further analysis. To define the potential function of genes in the turquoise module, enrichment analysis of KEGG pathways was conducted by GSEA v4.1.0 software obtained from the Broad Institute (www.broadinstitute.org/gsea), and we obtained 17 KEGG pathways in 10 categories, of which 11 were negatively correlated, and six were positively correlated ($p < 0.05$ and FDR <0.25). Moreover, the mitogen activated protein kinase (MAPK) signaling pathway, ribosome biogenesis in eukaryotes, arginine biosynthesis, oxidative phosphorylation, biosynthesis of amino acids, glycerolipid metabolism, and pantothenate and CoA biosynthesis were not significant or not enriched in differential gene enrichment analysis (Fig. 4, C).

3.5. Changes in phytohormones during yam tuber development

Changes in phytohormones during the development of yam tubers were detected, and the gibberellin content was relatively high in the early stage of tuber formation and development of Chinese yam but gradually decreased with the growth period and tended to be stable after 130 DAP (Fig. 5, A). However, the ABA content showed the opposite trend, being low in the early stage and increasing rapidly after 130 DAP

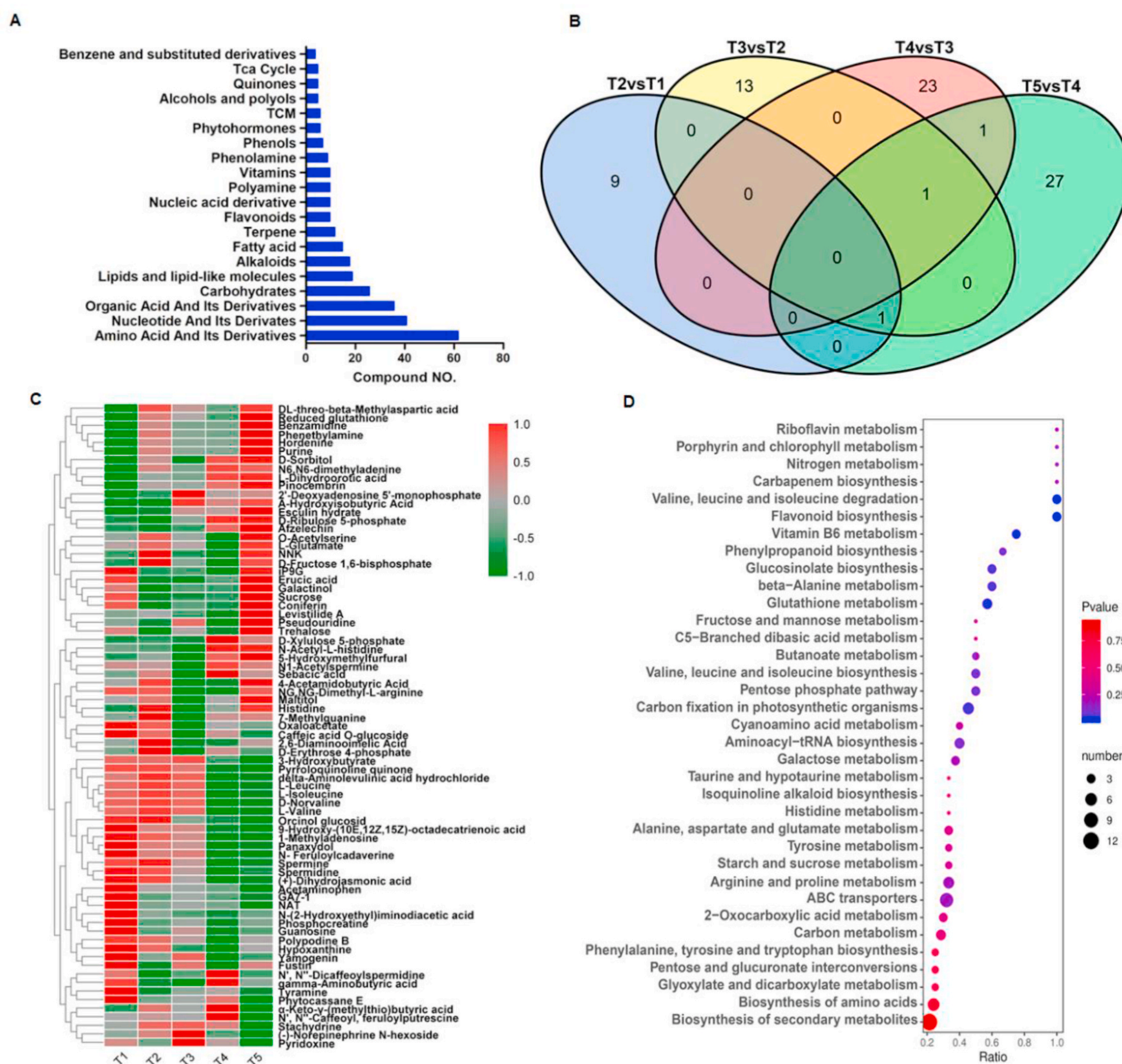


Fig. 2. Metabolic analysis of the dynamic pattern of yam tuber development: (A) Top 20 categories of metabolites identified by metabolome during yam tuber development, (B) Venn diagram of DAMs by pair-wise comparisons, (C) The heatmap of 75 DAMs in yam tubers development (differential metabolites marked with red (up-regulated) and green (down regulated) in yam tubers), (D) KEGG enrichment pathway of 75 DAMs. The number of DAMs is represented by the size of the circle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 5, A). The JA was lower in the early stage of tuber formation and development but increased after 130 DAP (Fig. 5, A). The IAA level first decreased and then increased, and the lowest appeared at 150 DAP, which was significantly lower than other periods (Fig. 5, A). The ZR content increased first and then decreased at 90–170 DAP, and then increased slightly at 190 DAP, the highest peak appeared at 130 DAP, which was 5.18 ng g^{-1}, and was significantly higher than other periods (Fig. 5, A). Similar to ZR, the peak of BR level also appeared at 130 DAP (Fig. 5, A).

Meanwhile, the phytohormone signal transduction pathway during the development of yam tuber was analyzed. Twenty-six genes were detected in the ABA signaling pathway, containing two downregulated *PYL* genes, 13 *PP2C* genes (eight downregulated and five upregulated), ten *SNPK2* genes (nine downregulated and one upregulated), and one downregulated *ABF* gene (Fig. 5, B). Nine genes encoding *PP2C* genes were highly expressed (Table S5). The expression levels of *SnRK2_2*, *SnRK2_3*, *SnRK2_4*, *SnRK2_5*, *SnRK2_6*, *SnRK2_7*, *SnRK2_8*, *SnRK2_9*, and *SnRK2_10* were higher than those of the other genes (Table S4). In the GA signal transduction pathway, four *GID1* and two *DELLA* genes were detected, their expression was downregulated, and the expression levels of *GID1_2* and *GID1_4* were relatively high (Fig. 5, C). In the JA signal transduction pathway, one *JAR1* gene, 25 *JAZ* genes, and ten *MYC2* genes were identified. *JAR1* was highly expressed at T2, and all *JAZ* and *MYC2* genes were downregulated. Interestingly, compared with other homologous genes, only the expression levels of *JAZ_8*, *JAZ_9*, *JAZ_10*, and *JAZ_15* were relatively low, and the expression levels of *MYC2_6* and *MYC2_10* were relatively low (Fig. 5, D). Thirty-one genes were identified in the auxin signal transduction pathway, encoding *AUX1*, *T1R1*, *AUX/IAA*, *ARF*, *CH3* and *SAUR*, and most of the genes were highly expressed in T1 (Fig. 5, E). Fourteen genes are involved in encoding the brassinolide (BR) signal transduction pathway, encoding *BRI*, *BAK1*, *BSK*, *BZR1/2* and *TCH4*, respectively (Fig. 5, F). Sixteen genes encoding *ETR*, *MPK6*, *EIN2*, *EIN3*, *EBF1_2* and *ERF1* were identified in ethylene signaling pathway (Fig. 5, G). Thirteen genes encoding *GRE1* (2), *ARR-B* (5) and *ARR-A* (6) were identified in the cytokinin (CTK) signal transduction pathway (Fig. 5, H). In addition, nine genes encoding *PR1*, *TGA* and *PR1* were identified in the SA signal transduction pathway. Only one gene encoding *TGA* was highly expressed in T2, and the other genes were highly expressed in T1 (Fig. 5, I).

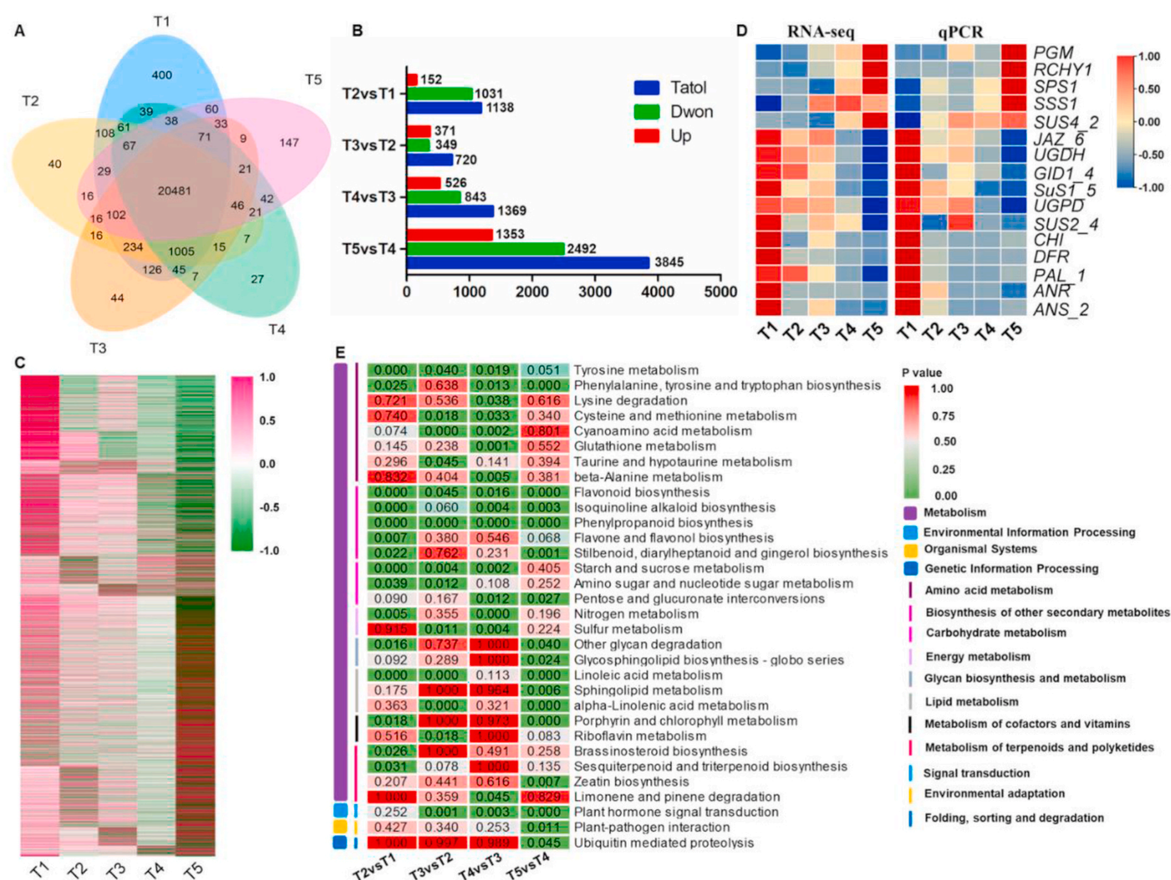


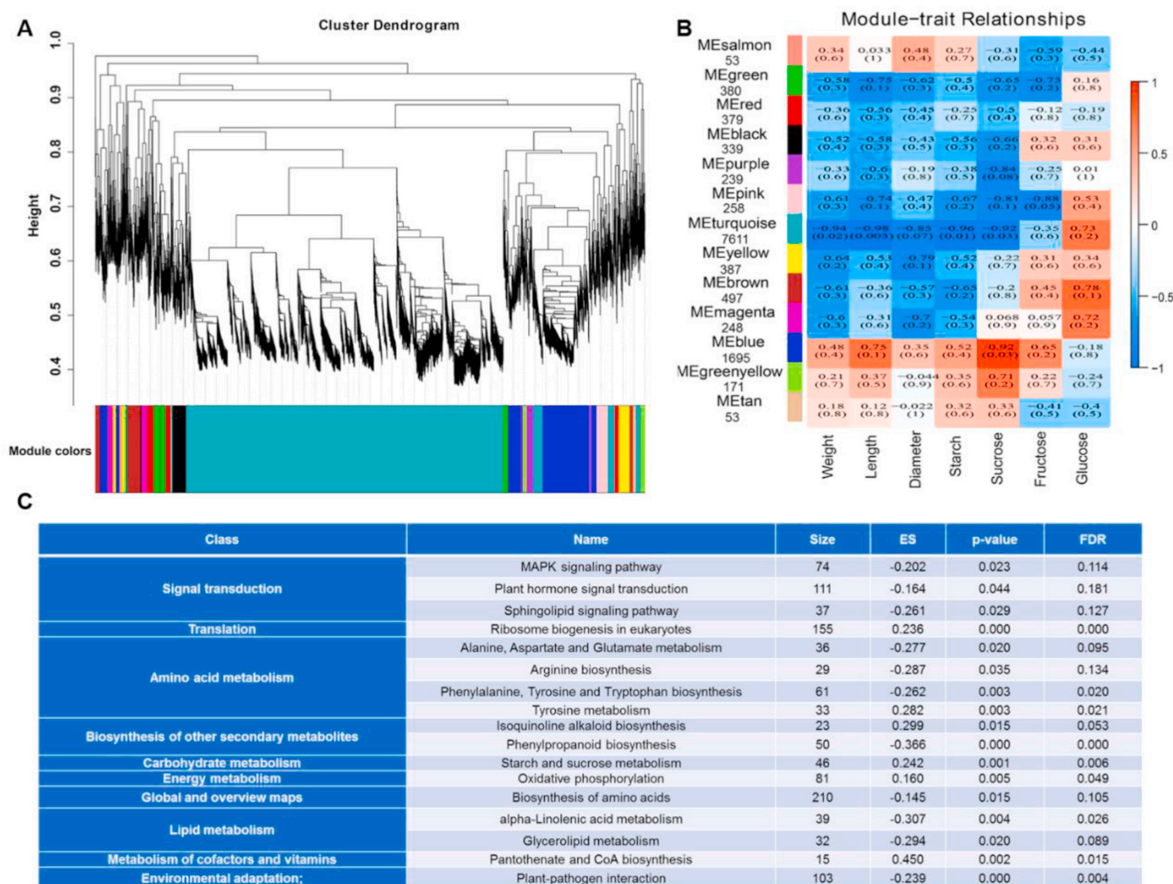
Fig. 3. Transcriptome analysis of yam tubers at different developmental stages: (A) Venn diagram for gene expression analysis in different samples of yam tubers, (B) Numbers of DEGs in the pair-wise comparisons, (C) Heat map of the DEGs during tuber development, (D) Validation of transcriptome data by qRT-PCR, (E) Heat map showing the *P*-value significance of enriched KEGG pathways of the DEGs in different stages of tuber development.

3.6. Gene regulatory network associated with sucrose and starch metabolism

The activity of AGP showed a trend of first increasing, then decreasing, and finally increasing, with a peak at 170 DAP, which was significantly higher than that at other developmental stages (Fig. 6, A). The enzyme activity of SBE was extremely low before 110 DAP, increased significantly at 110- 170DAP, and had no significant change after 170 DAP (Fig. 6, A). The activity of GBSS showed a trend of first decline, then rise and then decline throughout the development period (Fig. 6, A). The activity of SSS decreased first and then increased at 90–170 DAP and 170–230 DAP, with a peak value at 170 DAP (Fig. 6, A).

Seven metabolites of sucrose and starch metabolic pathways were identified by the metabolomic analysis, which was fructose, glucose-1-phosphate (G-1-P), sucrose, glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), trehalose and trehalose-6-phosphate (T-6-P), and sucrose and trehalose were DAMs (Fig. 6). Moreover, both KEGG and GSEA of transcriptome sequencing were enriched in sucrose and starch metabolic pathways, with 77 genes enriched by KEGG analysis and 46 genes enriched by GSEA. A total of 111 genes were obtained after the union (Table S6), most of which were upregulated in the process of rapid starch accumulation. In addition, multiple enzymes involved in the metabolism of sucrose and starch have multiple homologous genes; for example, *SuSs* has 17 homologous genes, upregulated at the T1 stage except *SuS4_1* and *SuS4_2* (Fig. 6, B). Furthermore, the accumulation of fructose, a sucrose metabolite, was positively correlated with the *SuS1_1*, *SuS1_2*, *SuS1_3*, *SuS1_4*, *SuS1_5*, *SuS2_3*, *SuS2_6*, *SuS2_7*, *SuS2_8*, and *SuS2_9* genes, respectively. Similarly, INV is one of the key enzymes in the hydrolysis of sucrose. Four genes were predicted to encode INV, and the expression of *INV_1* and *INV_2* genes is positively correlated with the accumulation of fructose and glucose (Fig. 6, D). Two genes encoding the GPI enzyme were identified, and the expression pattern of the *GPI_2* gene was significantly positively correlated with the accumulation of F-6-P and G-6-P (Fig. 6, B). The accumulation of G-1-P was significantly negatively correlated with the *AGP3_1* gene encoding its breakdown.

The expression patterns of the five genes encoding *GBSS* and the three genes encoding *SBE* were similar, and the expression levels were higher in the T1-T3 stages and decreased in the T4 and T5 stages. In particular, the *SBE2* and *GBSS12* genes showed high expression levels, and their FPKM values were >1000 and > 700, respectively (Table S6). In addition, one *α-Amy* and five *β-Amy* genes encoding amylase and α-Amy genes showed high expression levels at T4, and the *β-Amy* gene showed high expression levels at T1 and T3 (Fig. 6, B). However, the activity changes of AGP, SSS, SBE and GBSS enzymes measured have no significant correlation with the expression of genes regulating these enzymes. In addition, *TPS* genes and *TPP* genes involved in regulating trehalose biosynthesis have also been observed in different developmental stages of tubers (Fig. 6, C). The expression of *TPS6_1*, *TPS6_2*, and *TPS7_2* genes was positively correlated with the accumulation of T-6-P, and the *TPS7_3* gene was positively correlated with the accumulation of trehalose (Fig. 6, C). To understand the regulatory network of starch biosynthesis in yam tuber, the correlation test between DEGs and DAMs identified in sucrose and starch metabolic pathways was conducted (Fig. 6, E). The results showed that 24 DEGs and 6 DAMs showed high correlation coefficient values ($r > 0.8$, $P < 0.05$). Most notably, trehalos-6p was correlated with 18 DEGs, including 4 positive correlations and 14 negative correlations.



Class	Name	Size	ES	p-value	FDR
Signal transduction	MAPK signaling pathway	74	-0.202	0.023	0.114
	Plant hormone signal transduction	111	-0.164	0.044	0.181
	Sphingolipid signaling pathway	37	-0.261	0.029	0.127
Translation	Ribosome biogenesis in eukaryotes	155	0.236	0.000	0.000
Amino acid metabolism	Alanine, Aspartate and Glutamate metabolism	36	-0.277	0.020	0.095
	Arginine biosynthesis	29	-0.287	0.035	0.134
	Phenylalanine, Tyrosine and Tryptophan biosynthesis	61	-0.262	0.003	0.020
	Tyrosine metabolism	33	0.282	0.003	0.021
Biosynthesis of other secondary metabolites	Isoquinoline alkaloid biosynthesis	23	0.299	0.015	0.053
	Phenylpropanoid biosynthesis	50	-0.366	0.000	0.000
Carbohydrate metabolism	Starch and sucrose metabolism	46	0.242	0.001	0.006
Energy metabolism	Oxidative phosphorylation	81	0.160	0.005	0.049
Global and overview maps	Biosynthesis of amino acids	210	-0.145	0.015	0.105
Lipid metabolism	alpha-Linolenic acid metabolism	39	-0.307	0.004	0.026
	Glycerolipid metabolism	32	-0.294	0.020	0.089
Metabolism of cofactors and vitamins	Pantothenate and CoA biosynthesis	15	0.450	0.002	0.015
Environmental adaptation;	Plant-pathogen interaction	103	-0.239	0.000	0.004

Fig. 4. Analysis of WGCNA and GSEA during tuber development in yam: (A) Clustering dendrogram of 12310 DEGs, the color rows provide a simple visual comparison of module assignments based on the dynamic hybrid branch cutting method, (B) The number of genes contained in each module, and the correlation coefficient between phenotypic traits and module eigengenes presented with a color scale with red and blue representing positive and negative correlations, respectively. (C) Genes in enriched pathways of gene set enrichment analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.7. *Gene regulatory network associated with flavonoid metabolites*

The peak of flavonoid content in yam tubers appeared at the T1 stage, which was 1.8 mg/g, and showed a downward trend with the development of tubers (Fig. 7, A). The 4CL activity showed an upward trend and the peak value was at T5 stage, while thePAL activity showed a pattern of first increasing and then decreasing, and the peak value appeared at T4 stage (Fig. 7, B). Furthermore, a total of 34 DEGs and three metabolites of afzelechin, fustin and pinocembrin were mapped into the flavonoid biosynthesis pathway (Fig. 7, C). The key genes encoding the enzymes PAL, 4CL, chalcone synthase (CHS), *trans*-cinnamate 4-monooxygenase (CYP73A), chalcone isomerase (CHI), naringenin 3-dioxygenase (F3H), bifunctional dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS) in this pathway were identified (Table S7). Among these genes, six genes encoding *PAL* were identified and were highly expressed at T1, except *PAL_4* at the T2 stage. Meanwhile, correlation analysis showed that PAL activity was negatively correlated with *PAL_1*, *PAL_2*, *PAL_3* and *PAL_5* genes (Fig. 7, D). Five genes encoding 4CL enzymes, nine genes encoding *CHS*, one gene encoding *CHI*, and five genes encoding *CYP75B* were identified at the T1 stage. The genes *CYP75B* and *CHI* are the genes encoding the metabolite fustin and pinemabrin synthetases, respectively, but there is no correlation between them. The gene regulating afzelechin synthetase was not identified in this study. The *CHI*, *DFR*, *PAL_1*, *ANR*, and *ANS_2* genes of the flavonoid synthesis pathway were selected for RT-qPCR verification, which was consistent with the transcriptome data (Fig. 3, D). Furthermore, the correlation of network analysis showed that metabolites afzelechin and pinocembrin and there is a high correlation between 19 DEGs (Fig. 7, E).

4. Discussion

4.1. *Accumulation of metabolites promotes the tuber development of yam*

The formation and development of plant tubers is a complicated process, including tuber morphogenesis and nutrient accumulation, which are affected by genotype, environment, enzymes, and many other related factors (Aksenova et al., 2012; Liang et al., 2011; Yoshida et al., 2000). In this study, the phenotypic analysis showed that the tuber development of yam showed an S-shaped curve, with growth peaks ranging from 130 to 210 DAP (Fig. 1B and Table S2). Meanwhile, the starch accumulation also showed an S-shaped curve, and the rapid accumulation period was before the rapid growth period of tubers (Fig. 1D and Table S2). In addition, correlation analysis showed that tuber weight, length and diameter were positively correlated with starch content, and paraffin sections showed that tubers grew rapidly after starch accumulation (Fig. S1 and Fig. 1C). This is consistent with previous reports that starch is a signifcant contributor to the increase in tuber diameter and weight, and its accumulation can also be used as a marker of tuber development and expansion (Sturm et al., 1999). Moreover, sucrose, a product of plant photosynthesis, is synthesized in the leaves and transferred to the tubers, where it is decomposed into fructose and glucose and further synthesized into starch in the tubers, resulting in tubers expanding in volume and increasing in weight (Sturm

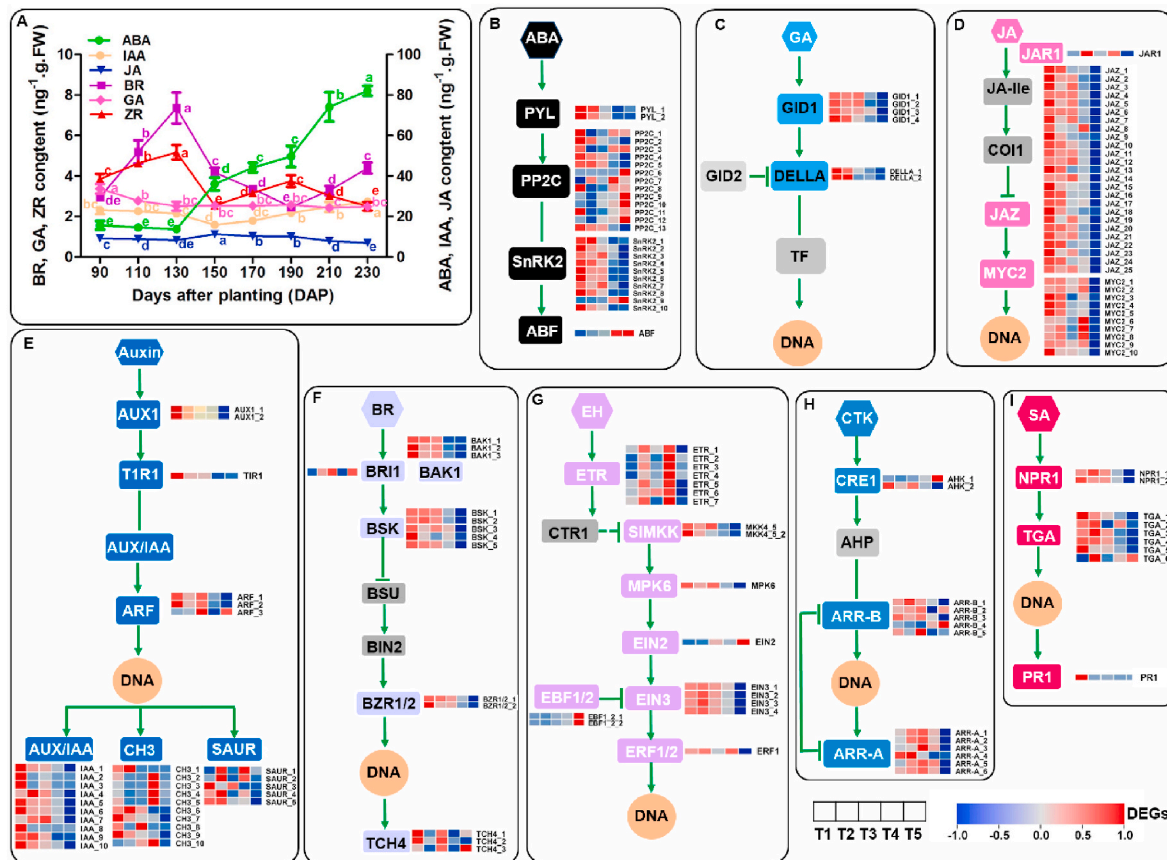


Fig. 5. Analysis of phytohormones during tuber development in yam: (A) The contents of ABA, GA_3, JA, IAA, ZR and BR, and signal transduction pathways for (B) ABA, (C) GA, (D) JA, (E) Auxin, (F) BR, (G) EH, (H) CTK, and (I) SA in yam tubers at different developmental stages. The line graph represent ± SE of three repeated. The letters indicate significant differences based on one-way ANOVA, $P < 0.05$.

et al., 1999; Zamski & Barnea, 1996). Here, sucrose content decreased significantly when starch began to accumulate (110 DAP) and increase when starch accumulation slowed down (210 DAP). A recent study showed that sucrose takes a crucial part in the corm development and starch synthesis in the *Gladiolus hybridus* (Li et al., 2021).

In addition, metabolomic analysis showed that a large number of metabolites were accumulated during the rapid development of yam. In this study, a total of 395 metabolites with 75 DAMs were detected, involving amino acids and their derivatives, carbohydrates, polyamines, organic acids and their derivatives, flavanones, phenolic substances, and alkaloids (Table S3). "Amino Acid And Its Derivatives," "Nucleotide And Its Derivates," are indispensable components of organisms and play an important role in protein synthesis, genetic variation and growth and development. At the same time, amino acid and organic acid compounds often determine the flavor of yam tubers. Additionally, it is well known that carbohydrate is the main substance of yam tubers (Boots et al., 2008; Calderon-Montano et al., 2011; Palazzolo et al., 2012; Sturm et al., 1999). At T1 stage, "Nucleotide And Its Derivates," "Amino Acid And Its Derivatives," "Polyamine," "Alkaloids," "Phenols," and "Phytohormones," were identified, which might act a significant regulatory role in growth and development of yam tubers. However, "Amino Acid And Its Derivatives," "Carbohydrates," "Nucleotide And Its Derivatives," "Organic Acid And Its Derivatives," "Flavanone," and "Sugar Alcohols," were identified at T5 stage, which might ultimately determine the quality, flavor and medicinal components of yam tubers.

4.2. Vital candidate genes involved in the tuber development were efficiently identified by combining comparative transcriptome analysis and WGCNA

A total of 5494 DEGs were identified by transcriptome analysis, and KEGG analysis showed that tuber development was regulated by multiple signaling pathways (Fig. 3B and D). However, DEG analysis often ignores key genes with little change in gene expression level. Genes with similar expression patterns are generally considered to be involved in similar biological processes, and WGCNA is an important tool for studying gene sets with similar expression patterns (Zhao et al., 2010). Therefore, WGCNA was performed to identify high gene co-expression modules to identify potential regulatory networks associated with yam tuber development. The results showed that the genes in turquoise module were closely related to the weight, length and starch content of tuber. GSEA analysis of the genes in the module identified 17 signaling pathways related to tuber development. Phytohormone signaling pathways, starch and sucrose metabolic pathways, and flavonoid metabolism are among the signaling pathways identified by both GSEA and KEGG analysis. Moreover, the mitogen activated protein kinase (MAPK) signaling pathway, ribosome biogenesis in eukaryotes, arginine biosynthesis, oxidative phosphorylation, biosynthesis of amino acids, glycerolipid metabolism, and pantothenate and CoA biosynthesis were not significant or not enriched in differential gene enrichment analysis (Fig. 4 C). This suggests that the two analyses can complement each other so as not to miss key signaling pathways and key genes. The genes of plant hormone signal transduction pathway, starch and sucrose metabolism and flavonoid-synthesis pathway identified by the two methods were pooled to analyze the candidate genes involved in regulating the development of yam tuber.

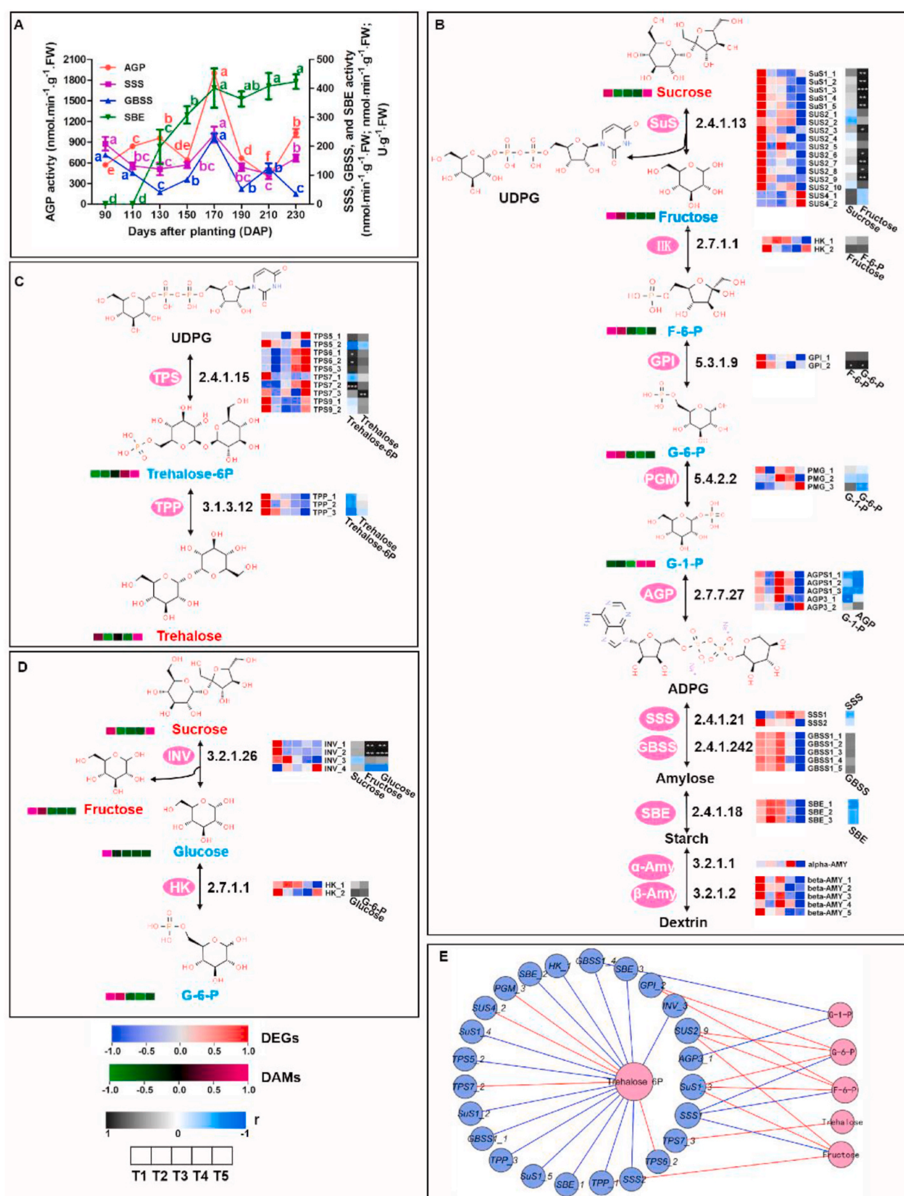


Fig. 6. Sucrose and starch metabolic pathways during tubers development in yam: (A) AGP, SSS, GBSS and SBE activity, (B) Starch metabolic pathways, (C) Trehalose metabolic pathways, (D) Glucose-6-phosphate sucrose metabolic pathway , (E) Coexpression networks of DEGs, and DAMs involved in sucrose and starch metabolic pathways (coefficient >0.8, P < 0.05). The line graph represent ±SE of three repeated. The letters indicate significant differences based on one-way ANOVA, P < 0.05. Pink and gray indicate significantly and insignificantly enriched genes, respectively. The red, blue, and black fonts indicate accumulated differential metabolites, accumulated undifferentiated metabolites, and undetected metabolites, respectively. The "*", "**" and "***" represent significant differences at levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$ according to Pearson's correlation coefficient, respectively. The DEGs are marked with pink circles, and the DAMs are marked with blue circles, the size of the circle represents the degree of connectivity, the larger the circle. The red and blue lines indicate positive and negative correlations, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.3. Phytohormones act a significant role in regulating tuber development of the yam

Phytohormones act a significant role in the growth and development of root and tuber crops, and ABA, GA, and auxin have been found to regulate the tuber swelling of the yam (Gong et al., 2016; Kolachevskaya et al., 2019; Wang et al., 2006). High levels of endogenous ABA can promote potato tuber formation (Xu et al., 1998). Also in the current study, tuber weight was positively correlated with endogenous ABA, indicating that it can promote the growth and development of yam tubers. Moreover, a total of 153 genes involved in plant hormone signal transduction pathways were detected by KEGG and GSEA, comprising ABA, GA, JA, CTK, auxin, BR, SA, and EH pathways (Table S5). Two ABA receptor genes (*PYL_1* and *PYL_2*) had high expression levels at the T1 stage, indicating that they were involved in regulating the development of yam tubers, consistent with the changing trend of the tuber expansion stage of mustard *Brassica juncea* var. *tumida* Tsen et Lee (Sun et al., 2012). The combination of ABA and PYR1 can release *SnPK2* in the *PP2C–SnPK2* complex through phosphorylation and dephosphorylation, thereby activating ABA-responsive gene expression (Park et al., 2009). In potatoes, upregulation of *ABF1* expression can inhibit tuber formation, while *ABF4* can regulate the gene expression of ABA and GA metabolism and promote tuber formation (Muniz Garcia et al., 2014). In this study, the expression level of the *ABF* gene was low in the early stage of tuber development and high in the late growth stage, which also indicated that the *ABF* gene acted a negative role in tuber development. Four *GID1* homologous genes and two *DELLA* homologous genes were detected in the GA pathway, which was highly expressed during the rapid growth period (T1 and T2). Similar to this study, three *GID1* homologous genes were also identified in *Arabidopsis*, which were expressed in most tissues during development, and GA treatment could reduce the transcriptional abundance of the three *GID1* genes, indicating that they have feedback regulation (Griffiths et al., 2006). Additionally, JA also plays a significant regulatory role in the growth and development of tuber crops, and JA-Me increases significantly during the rapid growth of tubers. In potatoes, the JA content rises significantly during the initial tuber formation period (Vreugdenhil & Struik, 1989), and the *JMT* gene can dramatically increase potato yield (Sohn et al., 2010). In this study, one *JAR1*, 25 *JAZ* family genes, and ten *MYC2* family genes were all highly expressed at the T1 stage, which indicated that these

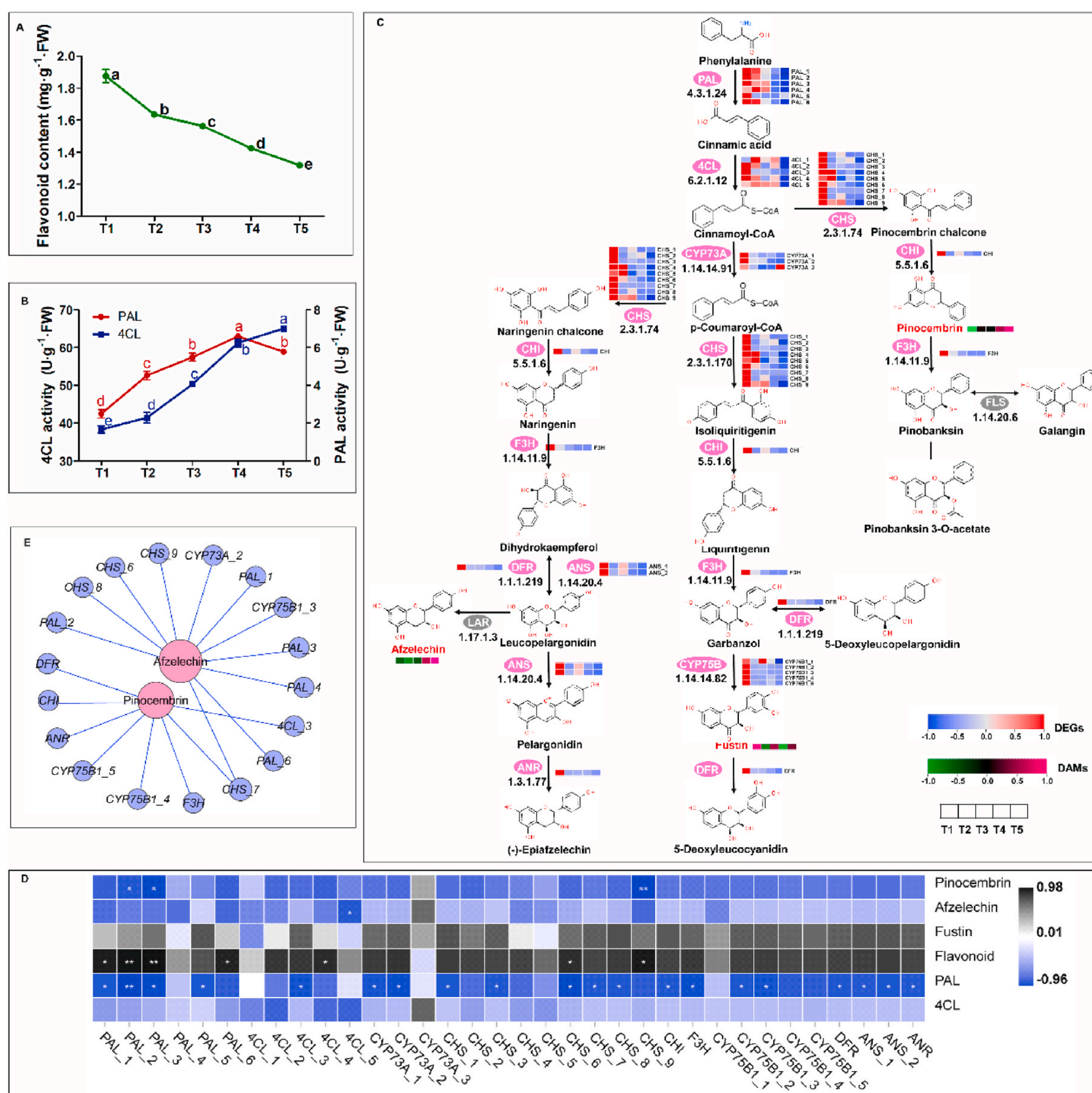


Fig. 7. Flavonoid metabolic pathways during tuber development in yam: (A) flavonoid content, (B) The PAL and 4CL activity, (C) Flavonoid metabolic pathways, Pink and gray indicate significantly and insignificantly enriched genes, respectively. The red, blue, and black fonts indicate accumulated differential metabolites, accumulated undifferentiated metabolites, and undetected metabolites, respectively, (D) Correlation heat map of DEGs, DAMs and enzyme activities in flavonoid metabolic pathways, (E) Coexpression networks of DEGs, and DAMs involved in flavonoid metabolic pathways (coefficient >0.8, $P < 0.05$). The line graph represent ±SE of three repeated. The letters indicate significant differences based on one-way ANOVA, $P < 0.05$. The "*" and "**" represent significant differences at levels of $P < 0.05$ and $P < 0.01$ according to Pearson's correlation coefficient, respectively. The DEGs are marked with pink circles, and the DAMs are marked with blue circles, the size of the circle represents the degree of connectivity, the larger the circle. The red and blue lines indicate positive and negative correlations, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

genes also play a significant role in regulating the development of yam tubers.

4.4. The DEGs and DAMs identified in starch and sucrose metabolic pathway

Moreover, sucrose and starch metabolic pathways were enriched by both KEGG and GSEA, which shows that they paly a crucial role in the development of yam tubers. Corm development and starch accumulation of gladiolus were inhibited in *GhSUS2* gene-silenced plants and participated in the antagonism of GA and ABA to regulate corm development (Li et al., 2021). *SuS* is a multigene family in plants; five *SuS1* genes and ten *SuS2* genes had high expression levels at the T1 stage, and two *SuS4* genes had high expression levels at the T5 stage (Fig. 6). Although there was no correlation between the 17 *SuS*s genes and sucrose accumulation, the accumulation of fructose, its metabolite, was positively correlated with the *SuS1_1*, *SuS1_2*, *SuS1_3*, *SuS1_4*, *SuS1_5*, *SuS2_3*, *SuS2_6*, *SuS2_7*, *SuS2_8*, and *SuS2_9* genes, respectively (Fig. 6, B). Meanwhile, the expression levels of *SuS1_2*, *SuS1_3*, *SuS1_4*, *SuS1_5*, *SuS2_3*, *SuS2_4*, *SuS2_6*, *SuS2_7*, *SuS2_8*, and *SuS2_9* genes were negatively correlated with the content of starch (Fig. S2). It is speculated that these genes play important regulatory roles in sucrose decomposition, fructose and starch synthesis in yam tuber. However, the expression levels of *SuS2_1*, *SuS2_2*, *SuS2_4*, and *SuS2_10* genes were not correlated with sucrose, fructose, and starch content, but they were highly expressed in the rapid development stage of the tuber and were much higher than other genes, suggesting that they also played an important role in the regulation of sucrose decomposition and fructose synthesis. *SuS4_1* and *SuS4_2* genes were highly expressed in the T5 stage of yam tuber development, suggesting that they played a key role in sucrose

metabolism in the later stage of tuber development (Fig. 6, B). The expression of the *GPI_2* gene is positively correlated with the accumulation of metabolites F-6-P and G-6-P, which indicates that the *GPI_2* gene plays an important regulatory role in their transformation (Fig. 6, B). AGP is the initial critical regulatory and rate-limiting enzyme in plant starch synthesis, and its activity affects starch synthesis. AGP consists of two small α subunits and two large β subunits, encoded by different genes, and they play different roles in starch synthesis (Ohdan et al., 2005; Okita et al., 1990; Radchuk et al., 2009). In this study, three *AGPS1* genes encoding the small subunit and two *AGP3* genes encoding the large subunit were identified. At the expression level, *AGPS1_1* and *AGPS1_3* genes were significantly higher than *AGPS1_2* gene, *AGP3_2* gene was higher than *AGP3_1* gene, and *AGP3_1* was negatively correlated with G-1-P accumulation. These results indicated that *AGPS1_1*, *AGPS1_3*, and *AGP3_2* genes played important regulatory roles in starch accumulation in yam tubers, while the *AGP3_1* gene may negatively regulate the decomposition of G1P (Fig. 6 and Table S6). There was no correlation between AGP enzyme activity and its coding gene, which may be due to the synergistic regulation of AGP by multiple genes. Similar to AGP, SSS, GBSS and SBE enzymes are co-encoded by multiple genes (Fig. 6, B). Additionally, previous studies have shown that sucrose content is regulated by trehalose 6 phosphate (T6P), the synthesis of the latter is also affected by changes in sucrose content (Lunn et al., 2006), and the increase in T6P is also related to the enhancement of starch synthesis (Debast et al., 2011). Similarly, T6P was highly correlated with 18 DEGs in sucrose and starch metabolic pathways, with four positive and 14 negative correlations in this study, indicating that T6P is a key metabolite in sucrose and starch metabolic network of yam tuber and is involved in regulating tuber development (Fig. 6, E).

4.5. The DEGs and DAMs identified in flavonoids biosynthesis pathway

In the current study, afzelechin (3,5,7,4′-tetrahydroxyflavane), fustin (2,3-dihydrofisetin), and pinocembrin (5,7-dihydroxyflavanone) and 34 DEGs were identified in the flavonoid metabolic pathway. The biosynthesis of flavonoids comes from the phenylpropanoid pathway. Phenylalanine is catalyzed by PAL, C4H, and 4CL enzymes to produce coumaroyl-CoA, which is then transferred to the flavonoid metabolism pathway. In this study, PAL and 4CL activities were negatively correlated with flavonoid content (Fig. S3), and it has been previously reported that these enzyme activities can significantly affect flavonoid accumulation (Xu et al., 2019). In addition, *PAL_1*, *PAL_2*, *PAL_3*, and *PAL_5* genes were negatively correlated with PAL activity, suggesting that they may negatively regulate PAL activity. CHS is an essential enzyme in the biosynthetic pathway of secondary flavonoid metabolites and takes a key part in the accumulation of flavonols. The expression of *CHS_6* and *CHS_9* genes was positively correlated with the accumulation of flavonoids (Fig. 7, D). Moreover, downregulated expression of *F3H* and *FLS* genes could reduce the content of rutin and quercetin-3-rutin, upregulating the expression of *CYP73B*, *DFR*, and *ANS*, can increase the content of flavonoids and anthocyanins (Bovy et al., 2007; Wang et al., 2018). In this study, although there was no correlation between the metabolite fusin and CYP73B gene regulating its synthesis, both of them had high accumulation or expression at T1 stage (Fig. 7, C). The *CHI* gene has no correlation with pinocembrin, which may be due to its regulation of the activity of catalyzing naringenin synthetase. Although metabolome analysis identified afzelechin, the gene regulating its biosynthetase was not identified. The expression of 4CL_*4* gene was also positively correlated with flavonoid content, indicating that it plays a key role in flavonoid synthesis. Candidate genes involved in flavonoid accumulation, such as *CYP73A*, *F3H*, *DFR*, and *ANS* were also identified in this study. In addition, the correlation network analysis of DAMs and DEGs in the biosynthesis pathway of flavonoids showed that afzelechin and pinocembrin were highly correlated with 11 and 8 DEGs, respectively, indicating that they were key metabolites in the biosynthesis of yam flavonoids (Fig. 7, E).

5. Conclusions

The mechanism of tuber development was studied by time-course phenotypic, cytological, physiological, transcriptomic, and metabolomic analyses of yam. Phytohormones play vital roles during yam tuber development, especially, and the ABA content is significantly increased, while the GA content is decreased rapidly during the growth of yam tubers. Related candidate genes were detected, including *PYL*, *ABF*, and *GID1*. Starch is an important substance or potential inducer of yam tuber development and yield increase, and sucrose and T6P play a significant role in yam tuber development and starch synthesis. The *SuS2_1*, *SuS2_2*, *SuS2_4*, and *SuS2_10* genes are important candidate genes that regulate sucrose metabolism during the rapid growth of yam tubers. In addition, the metabolomic analysis showed that yam tubers are rich in amino acids, organic acids, phenols, and flavonoids, and some candidate genes regulating flavonoid synthesis were detected combined with the transcriptome. The findings will provide a new perspective for further revealing the molecular mechanism of tuber development and metabolite accumulation and can provide a theoretical basis for molecular breeding and quality improvement of yam.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Tianxu Cao: Methodology, Investigation, Formal analysis, Writing – original draft. **Shenglin Wang:** Formal analysis, Writing – original draft. **Asjad Ali:** Writing – review & editing. **Nan Shan:** Methodology. **Jingyu Sun:** Formal analysis. **Xin Chen:** Supervision. **Putao Wang:** Supervision, Formal analysis. **Qianglong Zhu:** Writing – review & editing. **Yao Xiao:** Formal analysis. **Sha Luo:** Writing – review & editing. **Qinghong Zhou:** Project administration, Methodology, Formal analysis, Writing – review & editing. **Yingjin Huang:** Methodology, Project administration, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lwt.2023.114764.

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