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Comprehensive analysis of transcriptome and metabolome identified the key gene networks regulating fruit length in melon

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Abstract

Background Melon is an ideal crop model for studying fruit development. Fruit shape is an important quality trait, and fruit length is a key indicator affecting fruit shape. However, studies on the genes regulating melon fruit length are still limited.

Results In this study, we investigated the gene network regulating fruit morphology in melons utilizing transcriptome profile and a co-expression pattern-based approach. Four co-expression modules/gene networks highly correlated with changes in endogenous plant hormone levels at different developmental stages were identified. We pinpointed 11 key genes associated with cell development, 4 genes related to microtubule development, and 16 genes involved in the auxin (IAA, indole-3-acetic acid) pathway. These genes were identified as module hubs, and their expression level correlated with phenotypic variation. Through rigorous screening methods, we enhanced the likelihood that these genes are genuine candidates in the regulation of the fruit morphology network. These genes play a significant role in controlling fruit length, providing crucial insights into the molecular mechanisms underlying melon fruit development.

Conclusions Our findings revealed candidate genes that regulate melon fruit length, helping in the understanding of the molecular mechanisms underlying melon fruit development. These genes will be valuable for implementing marker-assisted breeding strategies.

Keywords Melon, Fruit length, Transcriptome, Metabolome, Gene networks

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Introduction

Melon (*Cucumis melo* L.), a species of the Cucurbitaceae family, has garnered significant economic value, with a production exceeding 30 million metric tons (FAO Stat 2021). Fruit length and fruit weight of melon ranges from 5 to 200 cm, and 0.1 to 10 kg, respectively. Therefore, melon is an ideal model crop for studying fruit development. The differences in number, size, density, and voids of the internal fruit cells determine difference in fruit shapes, and can be observed during fruit set, growing and ripening [1]. The ovary shape is closely related to the fruit shape, which is mainly controlled by genetic factors and the environment [2–4]. The growth phase of fruit, including both cell division and expansion, is the longest stage of fruit growth, which determines the final size of fruit [5–8].

Plant hormones such as auxin (IAA), cytokinin (CTK), ethylene (ETH) and gibberellin (GA), regulate fruit growth through affecting the division and expansion of fruit cells, and ultimately determine the size and shape of fruit [9–11]. Previous studies have shown that IAA and GA work together to influence the cell development of Cucurbitaceae fruits, thereby controlling their size. IAA is one of the key hormones involved in plant fruit development. It plays a crucial role in fruit initiation and size by regulating cell division and cell expansion [12]. In tomato, IAA's regulatory role assigned to plant tissues and cells leads to fruit setting. Multiple genes are involved in IAA signaling and fruit development. *SlARF9* regulates fruit size through negatively controlling cell division in tomato [13], *CsYUC10b* is associated with fruit curvature in cucumber, and the IAA transcription repressor *SlIAA17* leads to larger tomato [9, 14, 15]. CTK can affect fruit shape by regulating cell division [16–18]. Studies have found that GA regulates IAA maximal distribution and DELLA protein in plant growth and development [19]. Additional genes related to ETH also play a key role in fruit development [20]. For example, members of the NAC family as *NOR-like1* and *CmNAC-NOR* correlate with fruit ripeness, whereas members of *SISHN1* correlate with fruit weight [21, 22]. The AP2/ERF genes *LeERF2*, *CmERF1*, and *CmERF2* are also involved in fruit ripening. *LeERF2* has been shown to be involved in ETH biosynthesis [23]. *CmERF1* promoted the development of fruits and leaves, and took part in various hormone pathways in melon [24]. Members of the ARF transcription factor family are involved in IAA regulation, and *CmARF* and *CsARF14* are involved in the regulation of fruit shape [25]. In summary, in addition to IAA, CTK, GA and ETH, other transcription factors also have an impact on determining fruit shape.

CmbHLH and other members of the bHLH transcription factor family help melons ripen early. Tomatoes that overexpress *SIPRE2* have a slightly bigger fruit diameter

but a smaller overall size [26]. *CsFUL1* belongs to the MADS-box transcription factor family inhibiting IAA transport, thereby affecting cucumber fruit length [27]. *BZR1.7* and *BES1.8* from the BES1/BZR1 transcription factors, regulate fruit shape in tomato [28]. *SUN* and *OVATE* modulate fruit elongation, while *LC* and *FAS* regulate ventricular number and flattened fruit shape, where the expression level of *CsSUN* correlates with the concentration of specific hormones, influencing fruit shape development. *OVATE* encodes a protein of the OFP family that negatively regulates downstream genes, including *SIOFP20*, *OVATE*, and *CmFSI8/CmOFP13*, these genes also being associated with fruit elongation [29–34]. *FAS* encodes a protein of the YABY family, which regulates organ polarity, and *CsCRC* and *CmCRC* are associated with fruit shape through the IAA pathway [35, 36]. In addition to these gene families, the interaction between *CsTRM5*, a member of the TRM family, also affects fruit shape [37]. In Cucurbitaceae, especially pumpkin, the increase in cell number by cell proliferation is responsible for fruit size [38]. In horticultural crops like cucumber, and melon, the cell division timing and cell expansion also impact the difference in fruit size [39]. In summary, the regulation of fruit morphology is a complex process, involving a large number of transcription factors and gene families.

However, despite the importance of melon fruit shape, an in-depth study of the gene networks and molecular mechanisms controlling melon fruit length is still limited. The precision of quantitative biology combined with the progress in omic technologies opens the door to understanding the development of melon fruit [40]. We are able to navigate the domains of metabolic networks and gene expression patterns due to this integrated methodology, which gives us an in-depth understanding of the underlying regulatory mechanisms [41]. This study combined the differentially expressed genes (DEGs) from the transcriptome data with the weighted gene co-expression network analysis (WGCNA) to determine the gene network involved in fruit length regulation of melon. Furthermore, the integrated analysis with metabolomic data provided insights into the gene networks and metabolites involved in fruit maturing development. Our study helps to clarify the genetic mechanism that shapes the fruit morphology of melon.

Materials and methods

Plant material

The long-fruited cultivated melon accession '551' and the short-fruited melon accession 'B22' were cultivated in Xinxiang planting base of Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences in 2023. Melon ovaries and fruits with uniform size and no apparent effect at 6 days before pollination (6 DBP), the

day of pollination (DP), 6 days after pollination (DAP), 10 DAP, 20 DAP, 30 DAP, and 40 DAP were selected as experimental materials. Three randomly chosen fruits were collected for each sample, with three replicates for each sample. Immediately after harvesting, the length, width, and weight of the fruits were phenotyped. Subsequently, the fruits were divided longitudinally into two halves, and flesh samples were homogenized and sampled in liquid nitrogen and stored at -80°C . All fruit samples were utilized for subsequent metabolomic and transcriptomic analyses.

Cell size and number analysis

In order to observe the changes of cell size and cell volume, we conducted paraffin sections detection of melon fruit tissue [42]. For fruits at different developmental stages, $1.0\text{ cm} \times 1.5\text{ cm} \times 0.5\text{ cm}$ sections were excised along both the longitudinal and transverse directions using a sterilized scalpel. Samples were swiftly fixed and preserved in FAA (Formalin-acetic acid-alcohol). After dehydration in the ethanol series, samples were sliced using a microtome by infiltrating xylene and embedded in paraffin by conventional methods. Analysis was performed using three biological replicates.

Fruit hormonal analysis

The quantitative analysis of endogenous plant hormones, including IAA, CTK, GA, abscisic acid (ABA), ethylene (ETH), strigolactone (SL), was conducted by Wuhan Meitweir Biological Technology Co., Ltd. (Wuhan, China) as previously described [43]. The quantification of plant hormones by LC-MS using internal standards was described in the Supplemental Table 1.

RNA-seq data analysis

Total RNA was extracted by the RNeasy Pure Kit (Bio-man, Beijing, China). RNA-seq and bioinformatics analyses were conducted by Berry Genomics Co., Ltd. (Beijing, China). The reference genome was obtained from the Cucurbit Genomics Database for melon (<http://cucurbitgenomics.org/>), and gene annotations were extracted from the genome. Hisat2 [44] and FeatureCounts [45] were applied for gene expression and quantification, calculating Fragments Per Kilobase of transcript per Million mapped reads (FPKM). DEGs analysis was carried out using EdgeR, with the criteria $|\log_2\text{FoldChange}| > 1$, and $P\text{-value} < 0.05$ [46–48]. The enrichment analysis was performed using the topG and KOBAS software packages, respectively [49]. Subsequently, hierarchical clustering analysis of all DEGs was handled by R language Pheatmap (1.0.8) package [50]. Each data point represents the average of three biological replicates.

Correlation analysis of transcriptomic and metabolomic data

WGCNA in R was employed to simplify genes into co-expression modules using default parameters [51, 52]. Initially, FPKM expression data for 29,980 genes were utilized, and after $\log_2$ normalization, genes with an absolute median deviation greater than 0 were selected, resulting in 18,387 retained genes for subsequent analysis. The WGCNA package (Version: 1.72) was then used to calculate the soft-thresholding power for constructing a co-expression gene network, aiming to make it more similarly to a scale-free network. Subsequently, the dynamic tree-cutting algorithm was applied to identify gene functional modules, grouping genes with similar expression patterns into a module. Finally, the similarity between modules was calculated and merged. The WGCNA modules were subjected to an associate analysis with hormone metabolism. The parameters were set as follows: minClusterSize: 50; cutHeight: 0.3 with the default values. Using Cytoscape, genes from each module were exported with default parameters [53].

Real-Time Quantitative PCR

We designed qRT-PCR primers (Supplemental Table 2) and using Primer Premier 6 and synthesized cDNA of different fruit stages of '551' and 'B22' using the Bioman reverse transcription kit (Bioman, China). qRT-PCR analysis was conducted on the LightCycler[®] 96 according to the instructions provided by Amp[™] SYBR[®] Green qPCR Mix (Bioman Technology Co., Ltd, Beijing), *CmACTIN* was used as an internal control. The reaction conditions were set as follows: initial denaturation at 95°C for 3 min, followed by 45 cycles of 95°C for 10 s, 60°C for 10 s, and 72°C for 10 s; and a final step of 95°C for 10 s, 65°C for 60 s, and 97°C for 1 s. The relative gene expression was calculated using the $2^{-\Delta\Delta\text{CT}}$ method [54].

Statistical analysis

A minimum of three biological replicates were used per experiment. Results are provided as means $\pm$ SD using Origin 2021 and GraphPad Prism 8.

Results

Observation of the phenotypic development of melon fruit

To investigate the changes in melon fruit length during development, we selected two melon accessions with significant differences in fruit shape and conducted extensive experiments and metabolic analyses at 6 DBP and DP, 6, 10, 20, 30, 40 DAP. The appearance of the two different melon accessions at different stages is illustrated in Fig. 1a. At 6 DBP, the fruit length and fruit shape index of '551' and 'B22' already exhibited differences, and these differences gradually increased as the fruit developed. During fruit development, from 6 DBP to 20 DAP, fruit

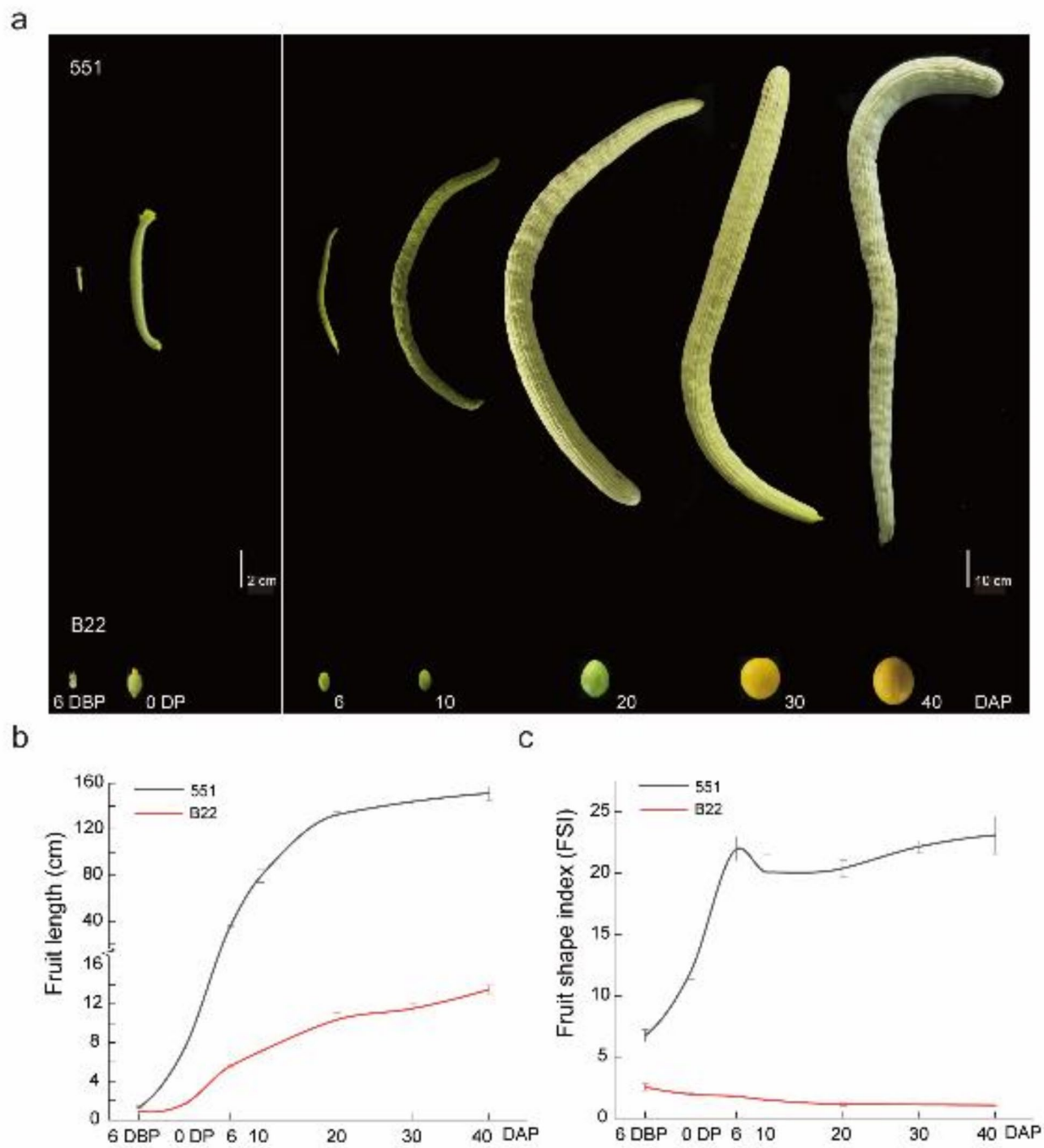


Fig. 1 Morphological characteristics of '551' and 'B22' melon fruits. **(a)** Appearance of '551' and 'B22' melon fruits at different stages (6 DBP, DP, 6, 10, 20, 30 and 40 DAP). **(b, c)** Fruit length and fruit shape index of '551' and 'B22' melon fruits at different stages. Each stage for these two melon accessions was evaluated with three biological replicates

length gradually increased with considerable variation. The differences between accessions also became more pronounced. Subsequently, the changes in fruit length from 20 DAP to 40 DAP were relatively small, and the differences between accessions were also minor (Fig. 1b). In terms of fruit morphology, the fruit shape index of

accession '551' increased significantly from 6 DBP to 10 DAP, showing substantial variation. However, the changes in fruit shape index from 10 DAP to 40 DAP were also relatively small, and the fruit shape index (FSI) of 'B22' remained stable (Fig. 1c, Supplemental Table 3). There were significant increases and changes in fruit

length and fruit shape index during the early growth stages of fruit development from 6 DBP to 20 DAP. However, fruit growth and morphological changes slowed down, correlating with fruit maturation during the later growth stages from 20 DAP to 40 DAP. Due to the huge phenotypic differences between the two melon accessions, we investigated the reasons through cell morphological observations.

Cellular differences of fruits at different developmental stages

To investigate cellular-level differences during melon fruit development, the cellular slice observations of two distinct melon accessions were conducted at 6 DBP, DP, 6, 10, 20, 30 and 40 DAP. The differences in cell size between the two accessions at different stages are shown in Fig. 2a. The average cell area and cell length of '551' were smaller than those of 'B22', while it is opposite in cell density and cell count at 6 DBP, 10 and 20 DAP. Conversely, the average cell area and cell length of '551' were larger than those of accession 'B22'; while the cell density of '551' and cell count were smaller than those of 'B22' at DP, 6 and 30 DAP (Fig. 2b, Supplemental Table 4). This suggests that in the early stages of fruit formation, '551' had a higher rate of cell division and cell density than 'B22'; and cell length and cell area rapidly decreased as fruit develops, resulting in the huge difference in fruit shape. We suspect that the differences in cell morphology in melons are due to differences in the content or composition of hormones during fruit development.

Metabolomic analysis of melon revealed the dynamic changes of plant hormones during fruit development

The expression levels of nine major classes of plant hormones were investigated in the fruits of melon accessions '551' and 'B22' at 6 DBP, 0 DP, 6, 10, 20, 30 and 40 DAP stages. Significant differences in the contents of plant hormones were observed (Fig. 3a). Combining the Principal Component Analysis (PCA) analysis of plant hormones and metabolome revealed distinct intergroup differences between the fruit of the two melon accessions (Fig. 3b). To further compare the differential core hormones between the two melon accessions in the early development (6 DBP and DP), mid-development (6, 10, and 20 DAP), and late development (20, 30, and 40 DAP), a differential analysis of internal developmental stages was conducted. These results indicated that 20, 23, and 6 differentially expressed plant hormones were observed in early development (Fig. 3c), mid-development (Fig. 3d), and late development (Fig. 3e), respectively.

The content of endogenous hormones IAA, IAA-Asp, IAA-Glu, Zeatin (cZ, tZ), and GA24 in '551' was consistently higher than those in 'B22' throughout the entire period of fruit development (Fig. 4). Specifically, the IAA content of endogenous hormone in '551' was 17.73, 18.79, 15.43, 7.30, 1.67, 1.68 and 1.09 ng/g at 6 DBP, DP, 6, 10, 20, 30 and 40 DAP, respectively, which were decreased by 1.93, 5.96, 2.08, 4.03, 2.83, 4.94 and 3.21 times in 'B22', respectively. These results suggested that IAA plays a key role in melon elongation during fruit development.

In contrast, the content of endogenous hormones trans-Zeatin Riboside (tZR), trans-Zeatin Riboside-5'-monophosphate (tZRMP), and iP riboside 5'-monophosphate (iPRMP) in '551' were consistently lower than

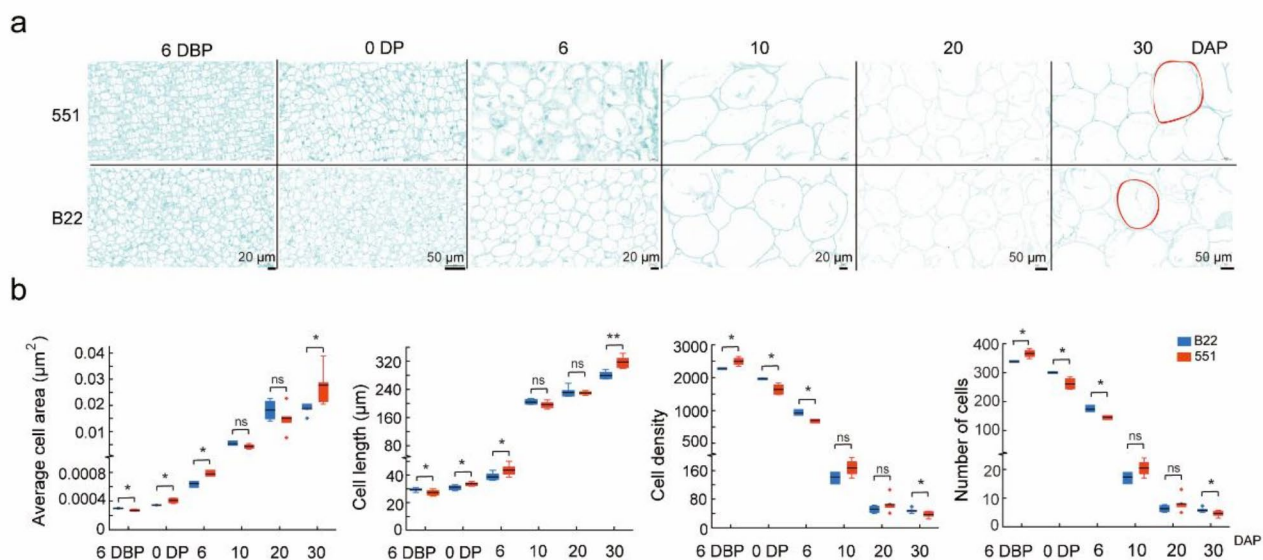
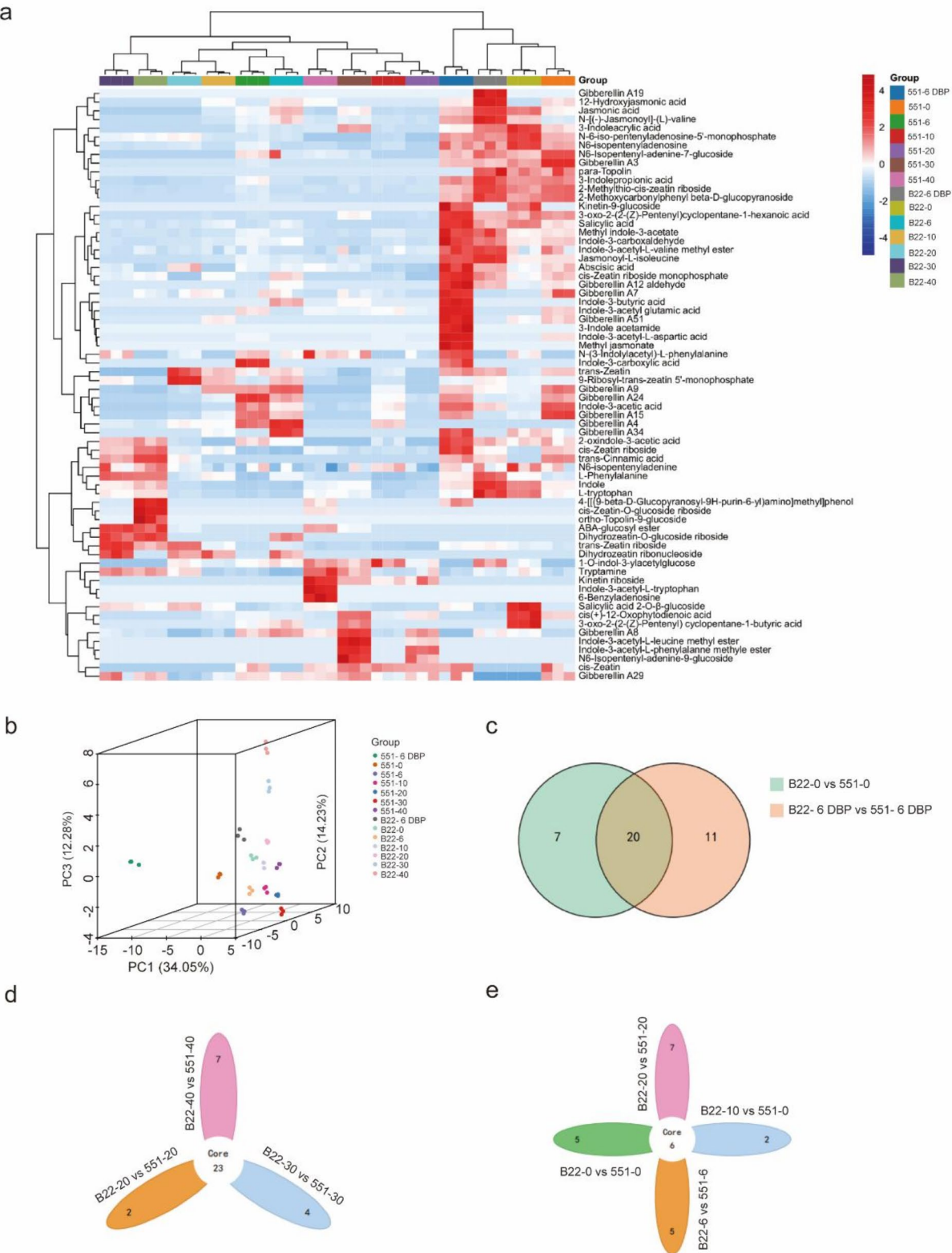


Fig. 2 Cellular characteristics of '551' and 'B22' melon fruits. (a) Cellular morphology of '551' and 'B22' melon fruits at 6 DBP, DP, 6, 10, 20, 30 and 40 DAP. (b) Average cell area (μm^2), cell length (μm), cell density, and cell number of '551' and 'B22' fruits at different developmental stages



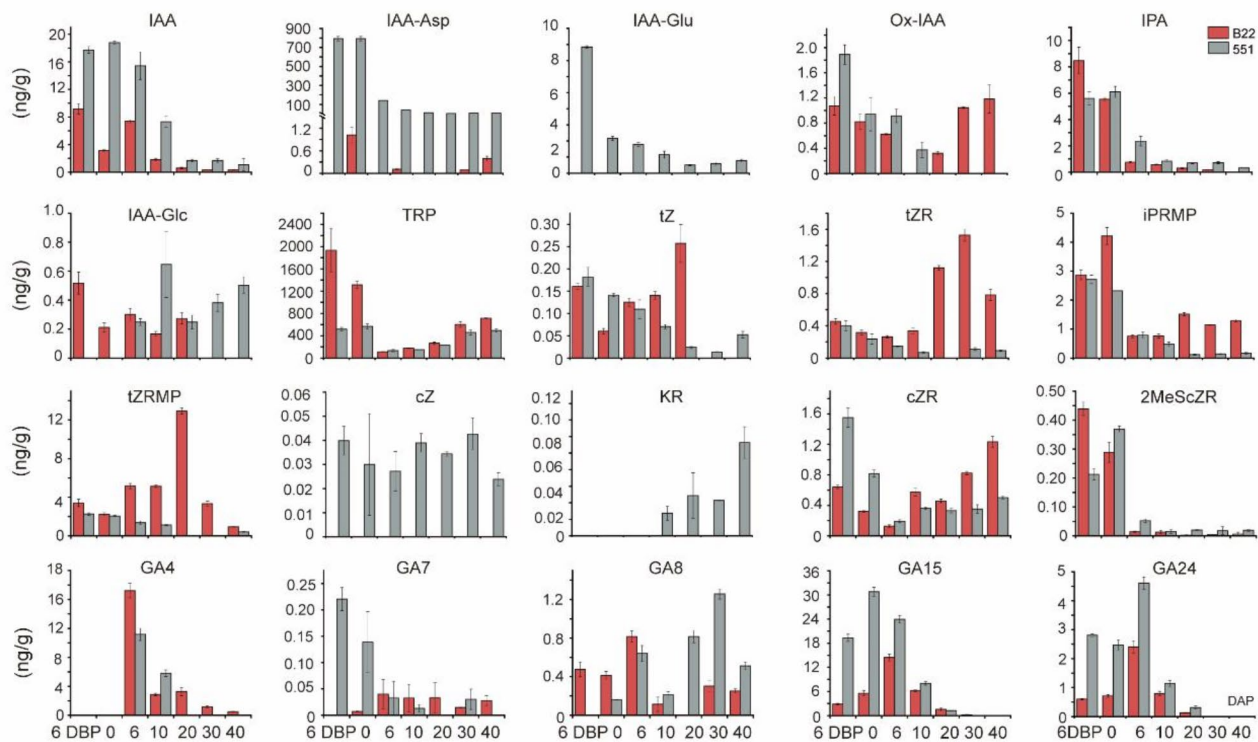


Fig. 4 Detection of 20 differentially produced metabolites in melon fruit at 6 DBP, DP, 6, 10, 20, 30 and 40 DAP. The red column indicated '551' accession, and the grey column indicated 'B22' accession

those in 'B22' during the entire developmental period (Fig. 4, Supplemental Table 5). These results showed that fruit development is primarily regulated by endogenous hormones such as auxin, cytokinins, and gibberellins during the entire development period, jasmonates and salicylic acid also play a role in the special developmental process.

Identification of DEGs associated with fruit development in melon

To investigate the DEGs of fruits, we conducted transcriptome analysis at seven developmental stages for '551' and 'B22'. The PCA results indicated that during the early stages, the differences between the two accessions were minimal, but the differences between the accessions became more pronounced as the fruits development, especially at 20, 30 and 40 DAP (Fig. 5a). We identified 13,876 genes with significant expression differences (Fig. 5b), and the number of DEGs was higher at 20, 30 and 40 DAP compared to other stages (Fig. 5c, Supplemental Table 6). Enrichment analysis of differential genes revealed that at 6 DBP, DEGs were mainly enriched in pathways such as plant hormone signal transduction, linoleic acid metabolism, cutin, suberine, and wax biosynthesis. DEGs of DP stage were focused on plant hormone signal pathway, phenylalanine metabolism, and flavonoid biosynthesis. At 6 DAP, DEGs were mainly enriched

in plant hormone signal transduction, phenylalanine metabolism, and diterpenoid biosynthesis. At 10 DAP, DEGs were mainly enriched in plant hormone signal transduction, biosynthesis of secondary metabolites, and photosynthesis-antenna proteins. At 20 DAP, DEGs were mainly enriched in plant hormone signal transduction, amino sugar and nucleotide sugar metabolism, and metabolic pathways. At 30 DAP, DEGs were mainly enriched in pathways such as carotenoid biosynthesis, plant hormone signal transduction. At 40 DAP, DEGs were mainly enriched in pathways such as mismatch repair, DNA replication, and phenylpropanoid biosynthesis (Fig. 5d). Totally, genes involved in plant hormone signal transduction played a significant role during fruit development in melon.

Identification of co-expressed gene networks and key candidate genes

To detect key genes associated with hormone expression, this study conducted Weighted Gene Co-expression Network Analysis (WGCNA). Totally, 11 different gene modules were identified based on the co-expression patterns of individual genes from the FPKM data. These gene modules were labeled with different colors and presented in the form of a clustering diagram and a network heatmap (Fig. 6a). Among the 11 co-expression gene networks, four modules showed a significant and

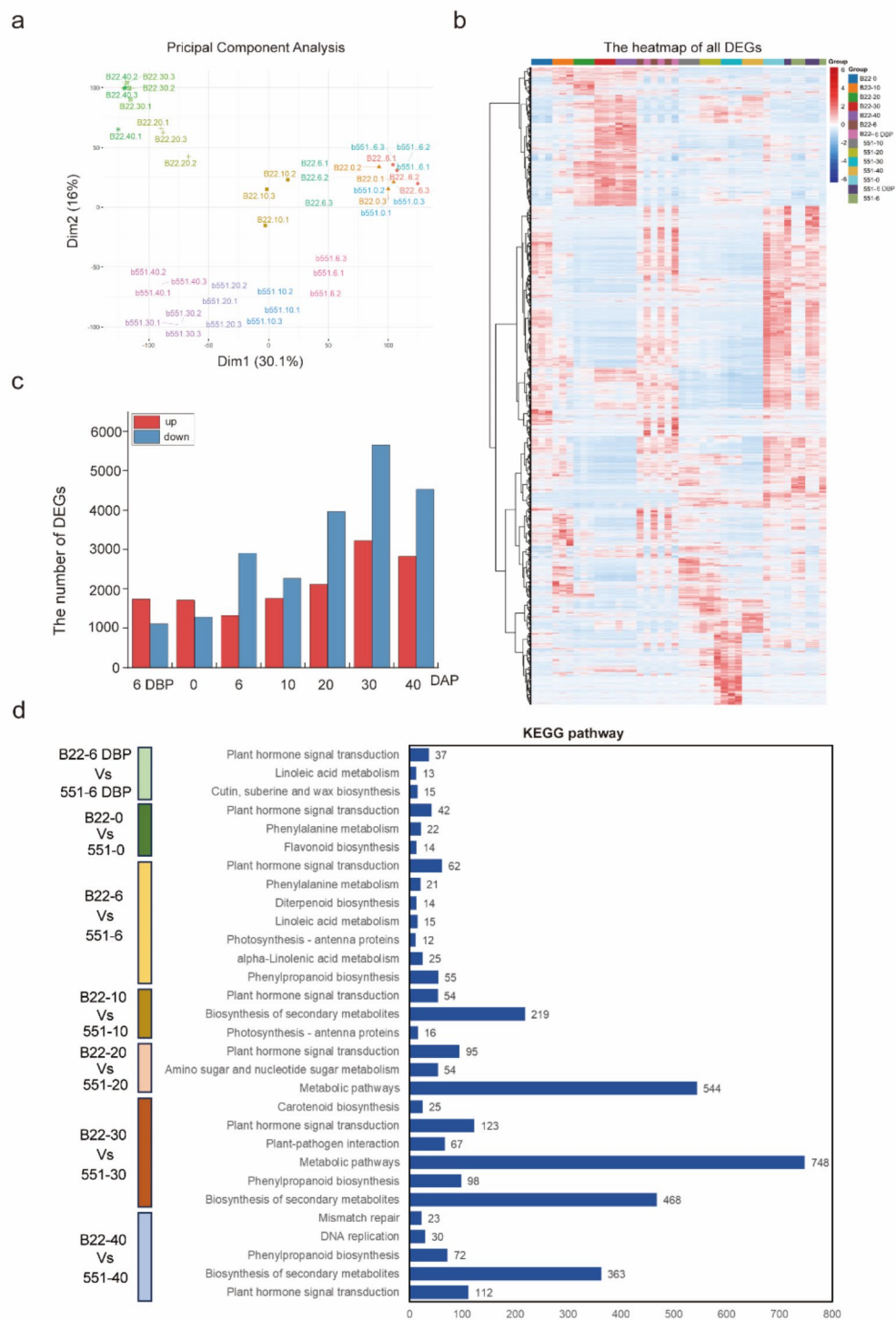


Fig. 5 Transcriptome analysis results during melon fruit development. **(a)** The PCA of the transcriptomes in ‘B22’ and ‘551’. **(b)** Hierarchical clustering heatmap of DEGs in ‘B22’ and ‘551’. **(c)** Number of DEGs in ‘B22’ and ‘551’. **(d)** KEGG annotation of ‘B22’ and ‘551’ (each bar represents the number of DEGs)

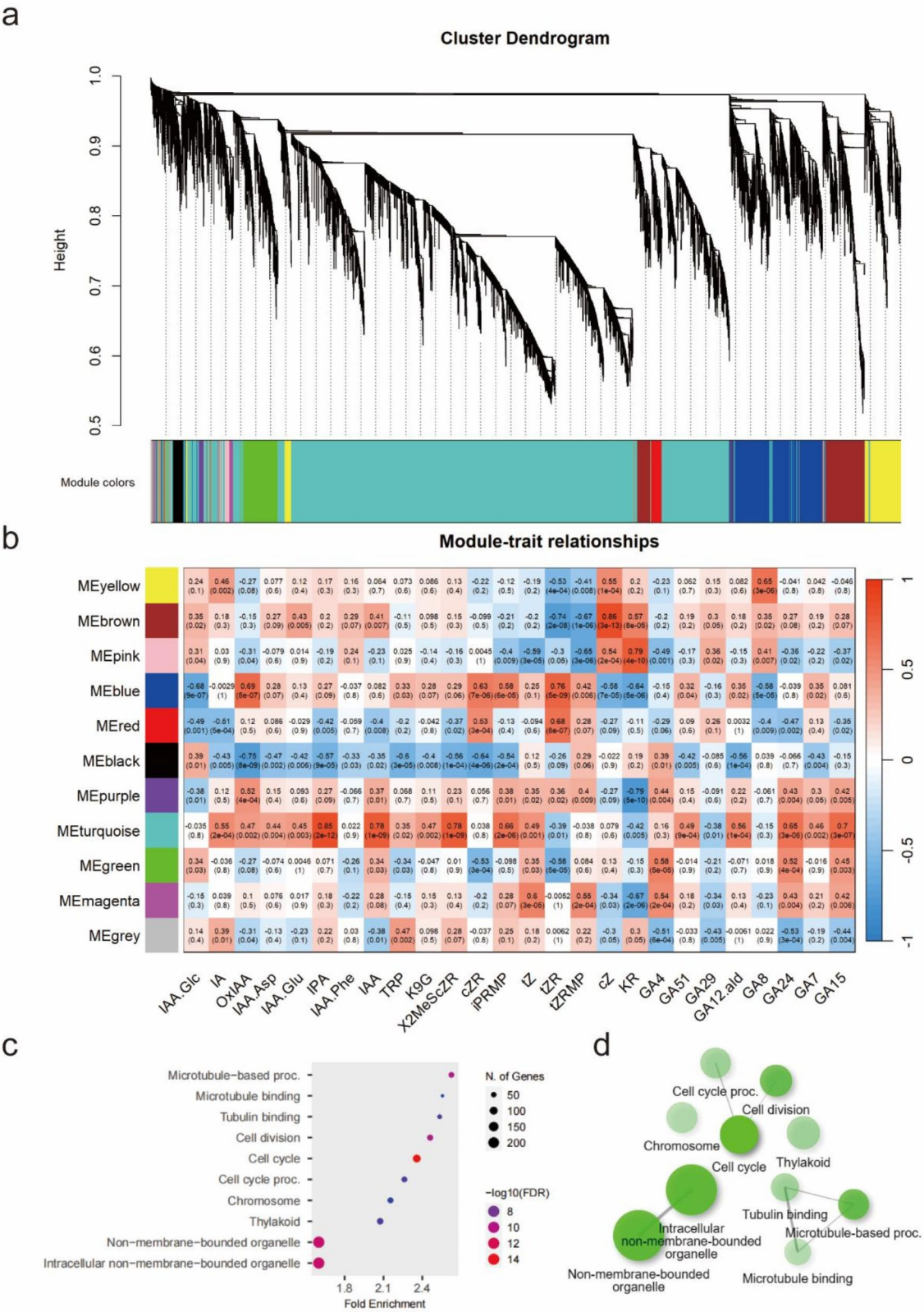


Fig. 6 Gene networks and key candidate genes regulating differential metabolites during melon fruit development identified by WGCNA. **(a)** Hierarchical clustering of 11 modules of co-expressed genes. Each leaf on the tree corresponds to an individual gene. **(b)** Module-trait associations based on Pearson correlation. The color key from blue to red represents r^2 values ranging from -1 to 1 . **(c)** Gene Ontology (GO) enrichment analysis of genes selected from 48 modules. **(d)** Key candidate genes in each network are highlighted in red within the module and annotated with gene descriptors

high correlation with auxins, cytokinins, and gibberellins (Fig. 6b). The ME turquoise and MEblack modules were associated with auxin, ME turquoise mainly positively correlated with IPA and IAA, while E black mainly negatively correlated with Ox-IAA. The ME turquoise, ME blue, and ME pink modules were associated with cytokinin-related hormones, ME turquoise and ME blue mainly positively correlated with 2MeScZR and tZR respectively, while ME pink mainly negatively correlated with tZRMP. The ME turquoise module was associated with gibberellin-related hormones, and mainly, showed a positive correlation with GA51, GA-ald, GA24, GA7 and GA15. To further identify candidate genes with significant contributions to the gene network, 4,264 genes were extracted from the 48 modules. GO enrichment showed genes mainly about cell cycle, cell division, cell cycle process, microtubule-based process, tubulin binding, and microtubule binding (Fig. 6c). The cell division and cell cycle process were interrelated with cell development,

while the microtubule-based process, tubulin binding, and microtubule binding were associated with microtubule development (Fig. 6d). These results indicated that genes related to auxins, cytokinins, and gibberellins regulating cell and microtubule development would be the key genes affected fruit elongation.

Identification of genes related to cell division, microtubule development and IAA transduction

Cell number and cell size influence fruit morphology; therefore, genes associated with cell development also play a role in fruit formation. Among the genes related to cell development, we selected 17 genes located at the core of the module based on their importance within the module (Fig. 7), revealing that these genes occupy central positions. Quantitative analysis of the 17 genes related to cell development showed that differences in expression were concentrated at 6 DBP and 20 DAP. Microtubules play a critical role in determining cell morphology by

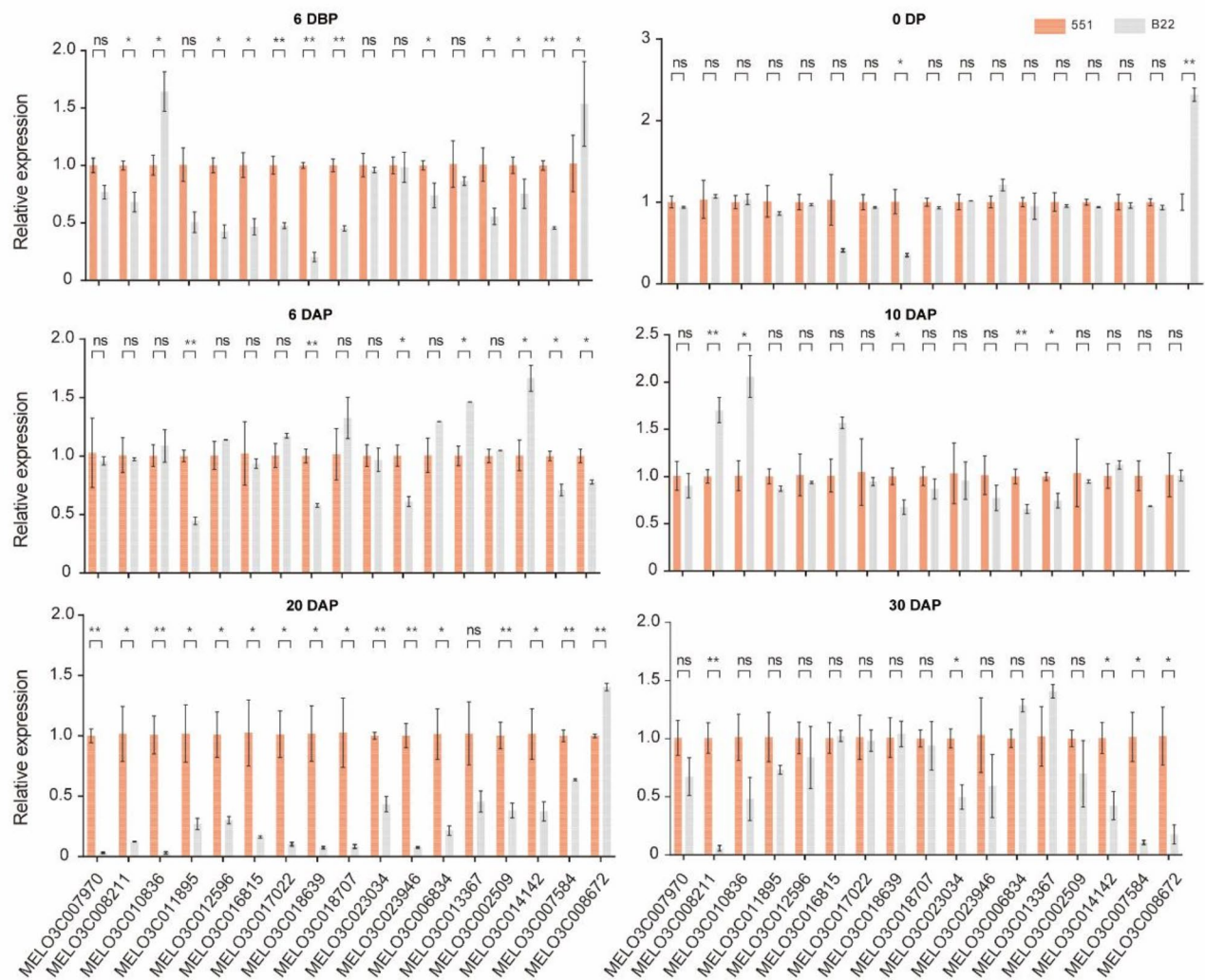


Fig. 7 The expression pattern of genes related to cell expansion

regulating the dynamics of cell shape and structure. Thus, genes related to the development of microtubule will impact fruit morphology.

qRT-PCR determination of candidate genes related to cell expansion of melon fruit. *CmACTIN* was used as an internal control, and three biological replicates were calculated, error bars indicate the standard error of the mean. The different colors denote different samples.

Among genes related to cell development, we selected 6 DEGs (*MELO3C013878*, *MELO3C013605*, *MELO3C013582*, *MELO3C008211*, *MELO3C018639*, *MELO3C023034*) with distinct expression trends from the transcriptome data that promoted subsequent analysis and key gene exploration. All of the 6 genes are mainly expressed in the early fruit developmental stages of melon (Fig. 8a), and the expression pattern is consistent with the RNA-seq data. The expression level of the six genes was similar at 0 DP in 'B22' and '551'. The expression level of *MELO3C013878*, *MELO3C013605* and *MELO3C023034* was lower at early developmental stages in '551' than that in 'B22'. These results suggested that those genes may play a similar role in the early stage of fruit development, but not in the late stage. (Fig. 8b, Supplemental Table 7).

Genes correlated with changes in endogenous plant hormone content were determined by combining metabolomic and transcriptome analyses, and the quantitative expression correlated with phenotypic variation. To confirm the relationship among the key genes in regulating melon fruit elongation, joint analysis with co-expressed transcriptome analysis was performed (Fig. 9). *MELO3C013875* and *MELO3C013606* may interact with genes which regulated cell development, microtubule development and auxin transduction, and jointly regulate melon fruit growth, which being the most likely candidate genes in regulating the melon fruit elongation. To confirm the expression of *MELO3C013875* and *MELO3C013606* in fruits at different stages, the quantitative PCR (qPCR) were conducted in diverse fruit developmental stages. The results demonstrated that both genes are highly expressed in melon fruit tissues at 6, 10, 20 and 30 stages, suggesting expression levels strongly related to phenotypic variance in fruit length. These findings support that *MELO3C013875* and *MELO3C013606* take part in fruit elongation.

Genes on plant hormone signal transduction pathways

Through transcriptome analysis, we observed a significant enrichment in the plant hormone pathways, and genes in this pathway increased expression during fruit development. We extracted 16 DEGs related to IAA signal transduction and illustrated the expression patterns of these genes (Fig. 10, Supplemental Tables 8 and 9). According to the pathway diagram, genes associated with IAA, such as *AUX1* and small auxin upregulated

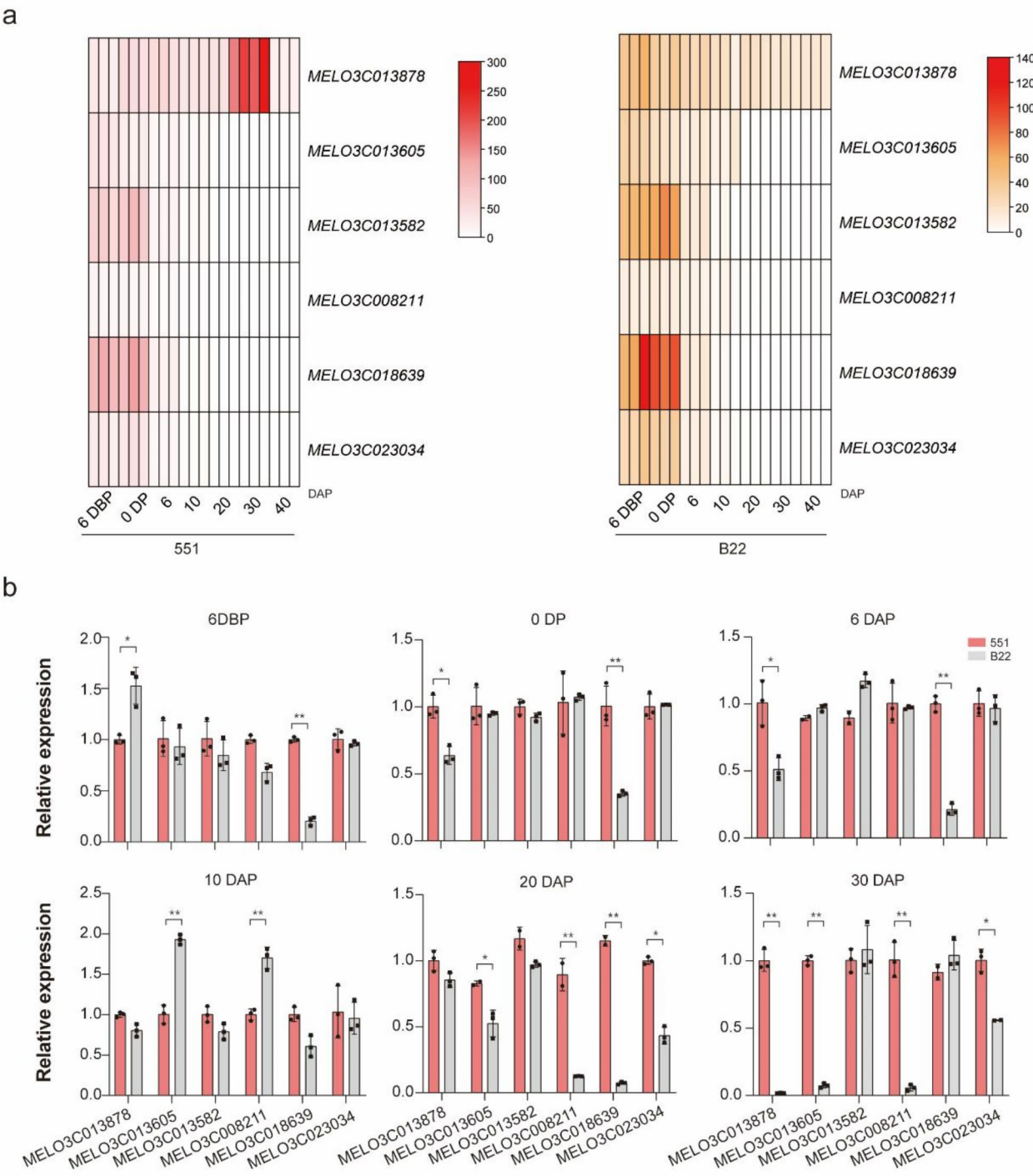
RNAs (*SAUR*), were predominantly expressed in the early stages of fruit development. Genes related to *auxin/indole-3-acetic acid* (*AUX/IAA*) and *Gretchen Hagen 3* (*GH3*) were mainly expressed in the early and middle stages, while genes associated with ARF were expressed throughout fruit development. Based on their importance within the modules, we selected two genes located at the core of the modules as key candidate genes. *MELO3C008672* belongs to the black module, positioned at the core of this module, while *MELO3C013367* belongs to the turquoise module and occupies a central position. Quantitative analysis of the two IAA-related genes revealed that *MELO3C008672* was differentially expressed at all stages, whereas *MELO3C013367* showed differential expression at 6 and 10 DAP.

Discussion

Fruit growth and development constitute a series biological processes affected by external factors, plant hormones, and gene regulatory elements. The plant hormone regulatory pathways play a crucial role in the metabolic components of fruit development, with IAA, CTK, and GA playing essential roles in fruit length morphogenesis. In this work, we employed WGCNA, integrating transcriptome and metabolome data to identify the complex genetic factors underlying plant hormone variations during melon fruit development.

The results in our study indicated that, in the early stages of fruit development, there were morphological differences between the '551' and 'B22' accessions. At pollination day, '551' exhibited an elongated fruit shape, while 'B22' showed an elliptical shape. Studies have suggested a strong correlation between ovary shape and fruit shape in melons, a trait predominantly controlled by genetic factors and minimally influenced by the environment [2, 4]. In the middle stage of melon development, fruit length increased rapidly. In the late stage, 'B22' fruit morphology stabilized, while '551' fruit length continued to increase. Overall, fruit development followed an S-shaped curve, consistent with previous research [6]. Fruit growth initiates during a highly proliferative cell division phase, and fruits with a higher cell count during the fruit-setting period tend to be larger [56]. The internal cell count in '551' fruits exceeded that in 'B22' at 6 DAP, and the internal cell length in '551' was greater than in 'B22' at 40 DAP, contributing to the longer fruit of '551'.

Plant hormones directly or indirectly affect cell division and expansion during fruit development, regulating fruit growth and ultimately determining fruit size and shape [9–11]. The primary hormones involved include IAA, CTK, and GA. Previous studies have revealed the involvement of hormone-related genes in regulating cucumber fruit length [57]. In this study, by measuring endogenous hormones in fruits at different



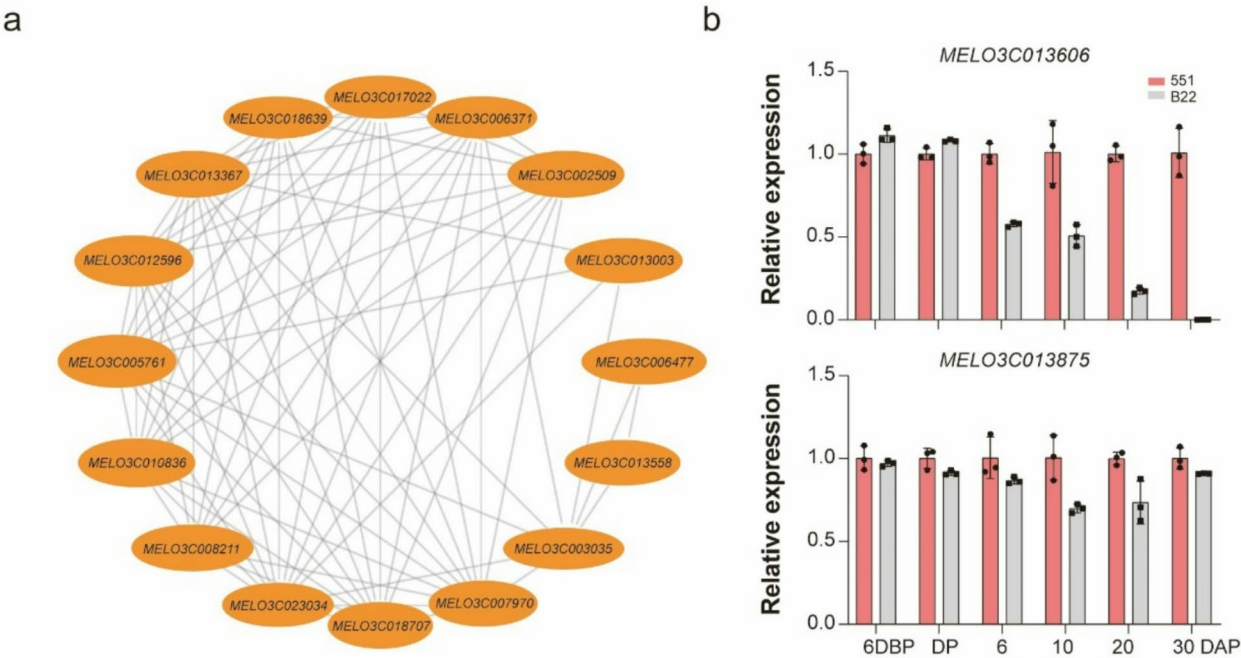


Fig. 9 Gene co-expression network diagram and expression pattern. **(a)** The network consisted of the most highly correlated genes in the orange module. In this network, the overlap of topological exceeds 0.08, with a coefficient above 0.6 and the value of R-squared was higher than 0.4. **(b)** qRT-PCR expression level detection of two genes at different developmental stages of melon fruit. *CmACTIN* was used as an internal control, and three biological replicates were calculated, error bar indicates the standard error of the mean value. The different colors denote different samples

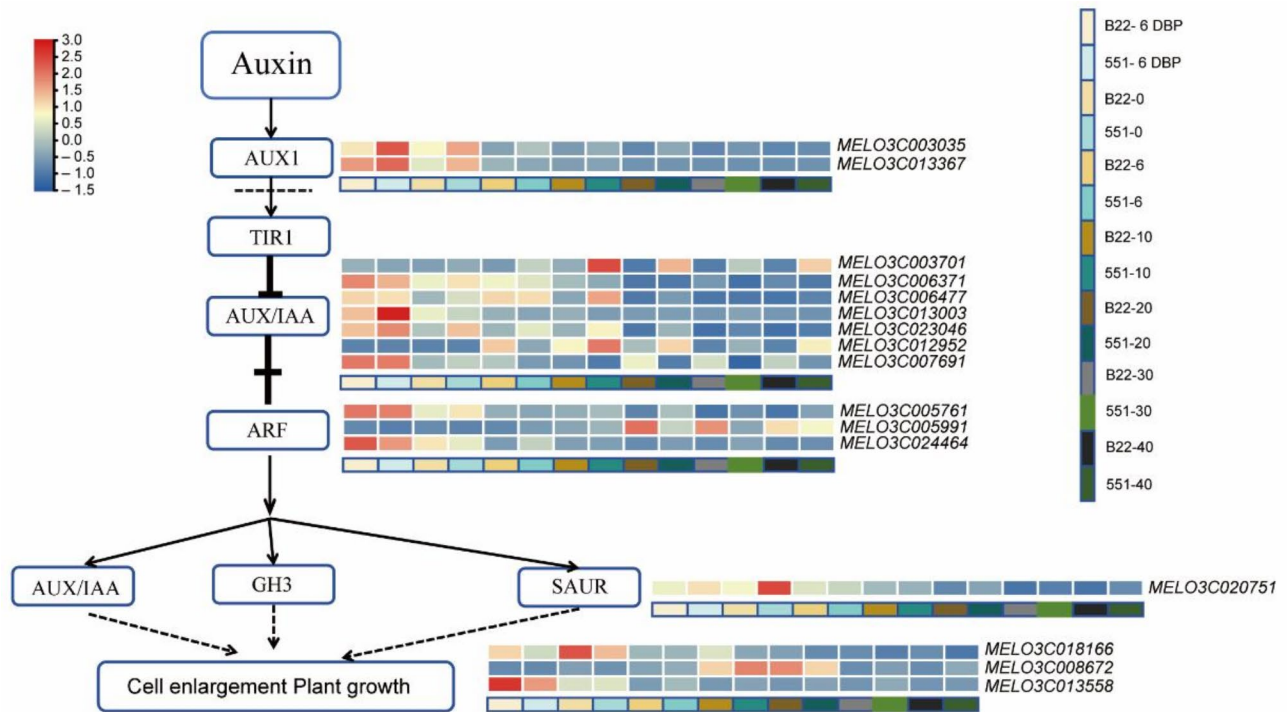


Fig. 10 Diagram of the IAA signal pathway. The plant hormone signal transduction pathways were modified from the Kanehisa laboratory [55]. DEGs were selected in heat map. Red color indicates significantly up-regulated

developmental stages of '551' and 'B22', we identified differences in some hormones, primarily IAA, CTK, and jasmonic acid, in the early and middle stages of fruit development. In the late stage, the main differences were observed in IAA. In *Arabidopsis*, IAA promotes the development of the microtubule formation layer [58]. Metabolomic analysis in this study revealed differential metabolites in the IAA pathway, including IA, IAA, IAA-Asp, IAA-Glc, IAA-Glu, IAA-Phe, IPA, and OxIAA. These substances participate in the IAA synthesis pathway, leading to differences in IAA content between accessions and resulting in morphological differences [59, 60]. Multiple genes are involved in IAA signal transduction and fruit development. For instance, *SLARF9* negatively controls cell division to regulate fruit size [13, 61], *CsYUC10b* is associated with fruit curvature [14, 15], and the IAA transcription repressor *SIL1A17* can lead to enlarged tomato fruits [9]. While *CmGH3-5*, *CmGH3-6* and *CmGH3-7* have been found to participate in IAA synthesis as enzymes and can interact with IAA response factors, being involved in the development of melon fruit [62, 63]. In this study we identified two genes related to IAA signal transduction, *MELO3C008672* and *MELO3C013367*, belonging to the *GH3* gene family and the *AUX1* gene family, respectively, involved in melon fruit elongation, which is consistent with previous findings. In grapes, the expression level of *GH3* increased with grape development, significantly reducing the content of free IAA in fruits, effectively promoting fruit ripening, a phenomenon also reported in tomatoes and other crops [64–66]. Quantitative analysis of *GH3* genes in this study revealed that at 6 DBP, 0 DP, and 20 DAP, the expression levels of *GH3* genes in 'B22' were higher than that in '551'. This difference might be one of the reasons for the lower IAA content in 'B22' compared to '551', ultimately leading to the differences in fruit shape. Treating with IAA directly promotes microtubule development, and some mutants in IAA transport and response show defects in microtubule formation [67–70]. The role of internal flow carriers in microtubule development using the *aux1/lax* quadruple mutant, revealed fewer vascular bundles and larger gaps between them. The *AUX1* gene identified in this study is related to genes involved in the cell cycle and microtubule development, all within the same color module [68, 71, 72]. There are interactions between *AUX1* and these genes, collectively regulating changes in cell morphology and subsequently affecting fruit shape.

In this study, we observed morphological differences between '551' and 'B22', particularly in the early stages, emphasizing the strong genetic control over fruit shape. The middle and late stages of melon development exhibited a typical S-shaped growth curve, with '551' displaying prolonged cell division, resulting in increased cell

numbers and larger fruit. Our analysis focused on the interaction of plant hormones, such as IAA, CTK, and GA, highlighted their direct and indirect effects on cell division and expansion, thereby determining fruit size and shape. Additionally, the study identified key genes associated with cell microtubule proteins and cell cycle proteins, elucidating their differential expression patterns during critical stages of fruit development and underscoring the role of IAA in regulating fruit size. In conclusion, this research provided a comprehensive understanding of the compound interactions among genetic factors, plant hormones, and environmental cues influencing melon fruit development. The integration of different omic datasets contributed to a holistic comprehension of the molecular mechanisms governing fruit size and morphology, paving the way for further research in crop improvement and agricultural practices.

Conclusions

In this study, we employed transcriptome profiling to investigate the gene network regulating fruit morphology in melons, utilizing a co-expression pattern-based approach. We identified four co-expression modules/gene networks highly correlated with changes in endogenous plant hormone levels at different developmental stages. Within these networks, we pinpointed 11 genes associated with cell development, 4 genes related to microtubule development, and crucial genes regulating IAA signal transduction pathway. These genes were weighted as module hubs, and the expression levels correlated with phenotypic variation. Through screening methods, we enhanced the likelihood and confidence that these genes are genuine candidates in the regulation of the fruit morphology network. These genes likely play a significant role in controlling fruit length during melon fruit development, providing crucial insights into the molecular mechanisms underlying melon fruit development.

Abbreviations

ABA	Abcisic Acid
Aux/IAA	Auxin/Indole-3-Acetic Acid
DAMs	Differentially Accumulated Metabolites
DAP	Day After Pollination
DBP	Day Before Pollination
DEG	Differentially Expressed Genes
DP	The Day of Pollination
ETH	Ethylene
FAA	Formalin-Acetic Acid-Alcohol
FPKM	Fragments Per Kilobase of exon model per Million mapped fragments
GA	Gibberellin
GH3	Gretchen Hagen 3
GO	Gene Ontology
IAA	Auxin, indole-3-acetic acid
JA	Jasmonic Acid
PCA	Principal Component Analysis
SA	Salicylic Acid
SL	Strigolactone

tZR	trans-Zeatin Riboside
tZRP	trans-Zeatin Riboside-5'-monophosphate
WGNA	Weighted Gene Co-expression Network Analysis
iP	isopentenyladenine
iPRMP	iP riboside 5'-monophosphate

Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-025-06332-0>.

Supplementary Material 1

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Author contributions

G.Z. and L.T.: Planned and designed the research. L.T. and Q.W.: analyzed the data, prepared all the figures and wrote the original draft. Y.H.: Performed the material collection. J.Z., W.K. and K.H.: Conducted fieldwork. Y.X., G.Z. and L.T.: Acquired funding. Jordi, Marta, G.Z. and L.T.: Edited and approved the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets generated and analyzed during this study are available in the SRA repository of NCBI, with the accession number PRJNA1186691 (<http://www.ncbi.nlm.nih.gov/bioproject/1186691>). All data generated and analyzed during this study are included in this manuscript.

Declarations

Ethics approval

The plant materials were collected from Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences. The experiments and the methods in this study were carried out in compliance with local and national regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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