Article

VvSUN may act in the auxin pathway to regulate fruit shape in grape

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Abstract

Fruit shape is an essential agronomic feature in many crops. We identified and functionally characterized an auxin pathway-related gene, VvSUN. VvSUN, which belongs to the SUN/IQ67-DOMAIN (IQD) family, localizes to the plasma membrane and chloroplast and may be involved in controlling fruit shape through auxin. It is highly expressed in the ovary, and the expression level 1 week before the anthesis stage is positively correlated with the fruit shape index. Functional analyses illustrated that VvSUN gene overexpression in tomato and tobacco plants changed fruit/pod shape. The VvSUN promoter directly bound to VvARF6 in yeast and activated β-glucuronidase (GUS) activity by indole-3-acetic acid (IAA) treatments in grapevine leaves, indicating that VvSUN functions are in coordination with auxin. Further analysis of 35S::VvSUN transgenic tomato ovaries showed that the fruit shape changes caused by VvSUN were predominantly caused by variations in cell number in longitudinal directions by regulating endogenous auxin levels via polar transport and/or auxin signal transduction process variations. Moreover, enrichment of the 35S::VvSUN transgenic tomato differentially expressed genes was found in a variety of biological processes, including primary metabolic process, transmembrane transport, calcium ion binding, cytoskeletal protein binding, tubulin binding, and microtubule-based movement. Using weighted gene co-expression network analysis (WGCNA), we confirmed that this plant hormone signal transduction may play a crucial role in controlling fruit shape through auxin. It is highly expressed in the ovary, and the expression level 1 week before the anthesis stage is positively correlated with the fruit shape index. Functional analyses illustrated that VvSUN acts as a hub gene, altering cellular auxin levels and the plant hormone signal transduction pathway, which plays a role in cell division patterns, leading to anisotropic growth of the ovary and, ultimately, an elongated fruit shape.

Introduction

Fruits are the most valuable produce of horticultural crops. The size and shape of the fruit are crucial selection features in the course of developing new cultivars in the breeding process [1]. Wild fruits are usually small and round. Cultivars with varying fruit shapes and sizes have emerged as a result of gradual selective breeding and domestication [2]. Inheritance studies reveal that these traits are quite complex and are determined by multiple loci [3]. Researchers have undertaken comprehensive investigations on fruit shape and size as a vital criterion in the breeding of new cultivars to fulfill particular market demands, and a number of advancements have been made as a result of their efforts [4]. In recent decades, we have witnessed the cloning of several major quantitative trait loci (QTLs) related to fruit/grain size or shape in tomato [4, 5], papaya [5], cucumber [6], melon [7], peach [8], watermelon [9], cucurbits [10], rice [11, 12], and so on.

QTLs identified in tomato are perhaps the best characterized for any fruit species; the ovate and sun loci influence elongated shapes, whereas the locule number (lc) and fasciated (fas) loci both modify locule number, and both influence the shape [13]. Of these QTLs, sun was identified as the primary locus influencing the elongated shape of the tomato fruit, explaining up to 58% of the phenotypic variation. As speculated by Xiao et al. [14], the origin of the locus was a consequence of a unique 24.7-kb gene duplication activity facilitated by the long terminal repeat retrotransposon rider. Fine mapping and cloning indicate the SUN gene as an affiliate of the IQ67-domain-containing family [14]. The plant-specific SUN/IQ67-DOMAIN (IQD) family has been identified as modulating the shape of fruits/grains among a variety of plant species. Fine mapping of a large F2 population of cucumber led to the identification of a putative gene, CsSUN, which is a homologous SUN gene for tomato fruit shape. Gene expression analysis has indicated that the long fruit expresses much more CsSUN as opposed to the round fruit [15, 16]. OsSUN-14, a homologous gene of CsSUN, could play a role in the development of melon fruit shape [16]. In watermelon, a 159-bp deletion mutation in the ClF51 gene, which encodes the IQD protein, is crucial for determining the shape of the fruits [9]. OsIQD14 has been identified as a critical component in modulating microtubule reconfigurations in rice hull cells and, consequently, grain shape [12]. These findings suggested that the SUN/IQD-induced fruit shape may be modulated via a conserved mechanism [12]. Overexpression of SUN in tomato resulted in highly elongated parthenocarpic fruits as well as twisted leaf and stem axes. Additionally, the extent of elongation is positively linked to the level of SUN gene expression [4, 14]. Despite the fact that SUN has no substantial impact on fruit weight, it does
influence tomato fruit morphology by enhancing longitudinal cell division and attenuating transverse fruit cell division [4].

Auxin performs an instrumental modulatory function in the regulation of cell division and expansion and cell identity establishment [17]. Microtubule dynamics were suggested to be influenced by auxin and to have roles in controlling post-embryonic division orientation [18] or cell shape [12]. Recent studies showed that IQD proteins may be involved in controlling cell shape or cell division by modulating auxin-mediated microtubule behavior [18]. Although plant phenotypes associated with elevated SUN expression levels indicated a role for auxin in controlling fruit shape [13], auxin level did not change dramatically in SUN as opposed to wild-type fruit. Further study found that SUN is linked to Ca^{2+} signaling and alters auxin signal transduction gene expression, demonstrating that SUN might influence fruit/ovary shape by modulating the auxin-associated gene expression level in the early phase of ovary formation [19].

Grape (Vitis L.) is amongst the most frequently produced fruit crops and it is characterized by a broad range of fruit sizes and shapes. Wild germplasm and wine grapes are usually circular or nearly circular. Modern domesticated table grapes have more diverse shapes, including heart-shaped, ovoid, oval, circular, narrow ellipsoid, nearly circular, obvoid, broad ellipsoid, and cylindrical [20]. The improvement of people's living conditions has resulted in a greater emphasis on grape quality, both in the flavor and the aesthetic aspects. The cultivation and sale of fruit varieties with unusual fruit shapes have the potential to significantly increase economic advantages. Thus, the promotion of berry quality features, as well as the discovery of the genetic pathways that influence them, have gained considerable attention. To date, numerous QTLs and potential genes associated with berry weight and size have been genetically studied in grapevine [20]. However, research on berry shape mainly focuses on physiological aspects [1]; the genetic mechanism of this diversity remains unknown, although berries have been reported to exhibit a wide range of phenotypic diversity in shape.

In this investigation, we discovered that VvSUN is a protein that has an IQ domain, localizes to the plasma membrane and chloroplast, and is highly expressed in the ovary 1 week before the anthesis stage. More importantly, the expression level is positively correlated with the fruit shape index (FSI). VvSUN overexpression in tomato and tobacco led to an elongated fruit/pod shape. We further showed that VvARF6 interacts with the promoter of VvSUN in yeast and that VvSUN activity was triggered by indole-3-acetic acid (IAA) treatment in vitro. Moreover, the IAA level and auxin-related genes were significantly altered in 35S::VvSUN transgenic tomato lines. Combined weighted gene co-expression network analysis (WGCNA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis confirmed that the plant hormone signal transduction pathway may have an important role in controlling fruit shape. Our results offer a novel perspective on the function performed by VvSUN in modulating the elongated fruit shape in the auxin pathway during the early phases of fruit growth.

**Results**

**Phylogenetic and transcriptional profiling analysis of VvSUNs**

A BLASTP search of the Vitis vinifera genome using 33 Solanum lycopersicum SUN protein sequences as a query identified a total of 25 putative SUN genes (VvSUNs). Twenty-five genes were named VvSUN1–VvSUN25 according to their physical locations on the chromosomes. The phylogenetic tree was constructed with predicted S. lycopersicum SUNs, VvSUNs and other species’ SUN protein sequences using a neighbor-joining algorithm in MEGA11 with 1000 bootstrap replicates (Fig. 1A). VvSUN13 (LOC100253695), VvSUN14 (LOC100265924), and VvSUN18 (LOC100256816) were classified into the same subgroup as Slin1, indicating that these genes might share similar functions. Slin1 was identified as SUN, and its role in fruit shape control has been widely studied [14, 21]. Further study through PlantDGD (http://pdgd.njau.edu.cn:8080) online software revealed that VvSUN13 and VvSUN14 are a duplicate gene pair [22], both of them located on chromosome 8.

Expression profiling of VvSUNs related to flowers and berries were analyzed by using the previously published grape (V. vinifera cv. ‘Corvinathe’) RNA-sequencing data from NCBI (accession number GSE36128) [23]. VvSUN13 showed high expression in young and well-developed inflorescences, the pericarp at mid-ripening stage, and berry skin at veraison stage, while VvSUN14 was expressed constantly from young inflorescences to flowering flowers and also in berries (skin, pericarp, and flesh) at post-fruit-set stage (Fig. 1B). These duplicate gene pairs that have diverged in their expression level indicated that their function might alter during evolution. VvSUN18 is not present in Fig. 1B because of the lack of corresponding expression data. VvSUN13, VvSUN14, and VvSUN18 were considered as the candidate genes in controlling fruit shape for further research.

**Morphological assay and expression analysis of VvSUN**

Xiao et al. [14] discovered that the SUN transcription factor plays a role in the modulation of tomato fruit shape. Mature fruits harvested from six different grape cultivars were utilized to study the potential relationship between the level of VvSUN gene expression and fruit morphology (Fig. 2A), measure their longitudinal diameter and length (Supplementary Data Fig. S1A and B), and derive the FSI (length/diameter ratio) (Fig. 2B). The FSIs of ‘Gold Finger’ (GF), ‘Minicure Finger’ (MF), and 8-6-1 (‘Beni Pizzutello’ seedling) were much higher than those of ‘Shine-Muscat’ (SM), ‘Kourgan Rose’ (KR), and ‘Houman’ (HM) (Fig. 2B).

Different cultivars were used to assess the levels of VvSUN13, VvSUN14, and VvSUN18 mRNA transcripts in their ovary and young fruit growth phases at various periods (Fig. 2C, Supplementary Data Fig. S1C and D). VvSUN13 showed consistency in expression patterns among all the cultivars. It gradually increased in all cultivars 2 weeks before the anthesis (WBA) stage and reached the highest expression level at the 1 WBA stage; after that, the transcript levels were sharply decreased 3 days before the anthesis (DBA) stage and remained low until 2 weeks after the anthesis (WAA) stage. Moreover, the expression of VvSUN in GF, MF, and 8-6-1 at 1 WBA was much higher than in other cultivars. However, both VvSUN14 and VvSUN18 showed no direct relationship between the levels of VvSUN gene expression and fruit morphology (Supplementary Data Fig. S1C and D). Moreover, sequence comparisons revealed that VvSUN13 was most closely related to Slin1, with 43.57% identity for the gene as the corresponding grapevine SUN ortholog, which may also be involved in controlling fruit shape. Since VvSUN13 was first studied as VvSUN by Zhang et al. [24], we also refer to VvSUN13 as VvSUN in the present article.

In accordance with these parameters measured above, the correlation coefficients between fruit shape parameters and the relative expression of VvSUN were calculated. The VvSUN
expression level at the 1 WBA stage was positively correlated with the longitudinal length of the fruit and showed the highest correlation to FSI (0.87) (Fig. 2D).

We additionally analyzed the expression profiles by fusing the VvSUN promoter region to the β-glucuronidase (GUS) reporter (Supplementary Data Fig. S2). GUS staining was visible in the ovaries, anthers, stigmas, and young petals (Fig. 2E–I). These findings indicate that although VvSUN is predominantly expressed in the young ovaries, it may also function in other tissues, mostly those in which many cell divisions occur.

A conserved domain search confirmed that VvSUN protein has the IQ67 domain, a conserved core section of 67 amino acids that are involved in the recruitment of calmodulin or function as a Ca\textsuperscript{2+} sensor. There are two distinct categories of IQ67 domains: one is the Ca\textsuperscript{2+}-independent IQ motif, the IQ motif ([I/L]VQxxxxRxRxxxxK or IQxxxRGxxxxR); the other one is the Ca\textsuperscript{2+}-dependent IQ motifs, the 1-8-14 motifs (1-4 [FILVW]x6[FILVW]x5[FILVW]) and 1-5-10 (1-4 [FILVW]x3[FILV]x4[FILVW]) [25]. The IQ67 domain of VvSUN contains two IQ motifs (amino acid residues 109–123 and 135–145), three 1-5-10 motifs, and two 1-8-14 motifs (Supplementary Data Fig. S3).

Our results revealed that the elongated fruit shape was positively correlated with the high expression level of VvSUN at the 1 WBA stage, but what induced the VvSUN expression level variation is still unknown. To subsequently examine the genetic mechanisms for these differences in expression, we separately cloned and sequenced the cDNA and promoter sequences (from −1833 bp to ATG) of the VvSUN gene from these six grape cultivars. Sequence alignment analysis showed no direct link between the SNPs and fruit shape (Supplementary Data Figs S4 and S5), indicating that the VvSUN coding sequences and −1833 bp upstream cannot explain expression variation.

**Overexpression of VvSUN leads to altered plant architecture**

To verify the role of the VvSUN gene in the control of fruit shape, transgenic tomato lines were generated in which the VvSUN gene was overexpressed under the control of the cauliflower mosaic virus (CaMV) 35S promoter. From the five independent T\textsubscript{2} generations of transgenic tomato lines that were produced, two VvSUN overexpression lines (lines 4 and 5) with the highest expression were selected for subsequent investigation (Supplementary Data Fig. S6A, Fig. 3A). The ovaries and fruits were obtained from the control and two overexpressing lines, and their longitudinal length, diameter, and shape index were determined (Fig. 3B and C, Supplementary Data Fig. S6B and C). The differences in length, width, and shape index were firstly observed in 1 WBA ovaries, and it was found that each genotype’s FSI did not alter much with the variation in days post-anthesis (DPA) and that it remained constant from 1 WBA till the mature stage (Fig. 3C). This shows that the shape of the fruit has already been decided during the early stages of ovary development.

On the sliced paraffin section three distinct regions were identified: the pericarp, the columella, and the placenta (Supplementary Data Fig. S7). Cells in these regions steadily increased in size from 1 WBA to 5 DPA. Nonetheless, at any stage of development, the cell size and shape of transgenic tomatoes were comparable to those of the control (Fig. 3D). We further checked the cell numbers (number/mm\textsuperscript{2}) in the pericarp both in longitudinal section and cross-section (Fig. 3E, Supplementary Data Fig. S8A), as well as cell shapes at 1 WBA stage, and the results showed
no significant difference between transgenic tomatoes and control (Supplementary Data Fig. S8B–G), which implies that the elongated fruit morphology of lines 4 and 5 was mostly attributable to the creation of more cells in the longitudinal axis as a result of the increased rate of cell division.

To further validate the function of VuSUN for elongated fruits, we also introduced 35S::VuSUN into tobacco (K326). Twelve putative transgenic lines were chosen on a medium that contained 30 mg l⁻¹ hygromycin and confirmed by qRT-PCR (Supplementary Data Fig. S9A). Significant differences in pod morphology were discovered in tobacco plants that constitutively expressed VuSUN, with transgenic pods exhibiting a longer pod length, shorter pod width, and a higher pod shape index compared with pods from control plants (Fig. 3F and G, Supplementary Data Fig. S9). Interestingly, we also noticed that the transgenic tobacco plants had longer leaf rachises and an increased leaf shape index compared with the control plants. Cell forms and sizes in the lower epidermis leaves of transgenic tobacco were very similar to that in the wild type (Supplementary Data Fig. S5). Therefore, these findings also indicate that longer leaves contain more cells.

The VuSUN promoter interacts with VuARF6 in yeast and exhibits stronger auxin-induced activity

Numerous IQD genes in Arabidopsis are potential targets of ARF5, an early auxin-responsive factor, and the AtIQD15 expression level is elevated following exogenous auxin application [17]. Interestingly, in silico analysis of the cis elements present in the −1833-bp promoter sequence of the VuSUN genes revealed numerous motifs (Supplementary Data Table S1), including an ARFAT element that functions as an ARF binding site. Our previous research found that VuARF6 (LOC100242923) was activated by exogenous plant hormone treatment (not published data). To determine whether VuARF6 interacts with the VuSUN promoter, we examined the interactions between VuARF6 and the VuSUN promoter using a yeast one-hybrid (Y1H) assay. The Y1H results demonstrated that the VuARF6 protein interacted with the VuSUN promoter fragment, confirming that the VuARF6 protein recognizes the cis element in the VuSUN promoter in yeast (Fig. 4A).

For the purpose of determining whether the VuSUN gene could be triggered by auxin, we transiently transformed the VuSUN promoter into grape leaves and measured the GUS activity in
leaves treated with 0, 10, 50, and 100 mg/l of auxin. Compared with the mock control (35S::GUS), GUS activity mediated by the VvSUN promoter was significantly increased when exogenous auxin was applied, and the highest GUS activity was achieved at 50 mg/l IAA treatment (Fig. 4B and C).

**Subcellular localization of VvSUN**

We also transiently generated the GFP-VvSUN fusion protein controlled by the 35S promoter in Nicotiana benthamiana leaves and recorded its cell localization utilizing confocal laser scanning microscopy to establish the subcellular location where VvSUN operates. By overlapping the fluorescence of GFP and chlorophyll, strong fluorescence of the GFP-VvSUN fusion protein was identified in the plasma membrane and chloroplast (Fig. 4D).

**The VvSUN gene affects auxin pathways**

Previous studies on IQD family proteins hypothesized possible links to auxin pathways [14], and the fruit shape phenotype of SUN-overexpressing plants is comparable to the shape of auxin mutants [4, 26]. In this study, auxin response factor VvARF6 interacted with the VvSUN promoter and induced GUS activity under different IAA treatments. To determine whether the VvSUN gene can regulate fruit shape by modulating auxin, UHPLC–MS/MS analysis was carried out to detect and quantify auxin and auxin-related compounds, such as IAA precursors (indole-3-acetamide, IPYA), free auxin (IAA), and IAA conjugates (indole-3-acetic acid-aspartate, IAA-Asp) in 35S::VvSUN line 5 and control at 1 WBA, anthesis and 5 DPA stages. VvSUN overexpression contributed to elevation in the levels of IPYA and IAA in all stages (Fig. 5A and B). IAA-Asp
Gene expression profiles associated with VvSUN

To evaluate the mechanisms through which VvSUN modulates fruit shape, we determined the differentially expressed genes (DEGs) in pairwise comparisons of 35S::VvSUN transgenic tomato ovary/fruits and control tomato at different stages (1 WBA, anthesis, and 5 DPA stages). Analysis between VvSUN_1WBA versus control_1WBA, VvSUN_Anthesis versus control_Anthesis, and VvSUN_5DPA versus control_5DPA showed that 2971, 2118, and 2785 genes were upregulated, respectively, and that 3128, 1927, and 2919 genes were downregulated, respectively, at three different stages (Supplementary Data Figs S10 and S11). Gene ontology (GO) term enrichment (P ≤ 0.05) analysis illustrated that these DEGs were predominantly implicated in organic substance metabolism, primary metabolic process, oxidoreductase activity, catalytic activity, metabolic process, ribosome, and so on. In addition, genes related to transmembrane transport, calcium ion binding, cytoskeletal protein binding, tubulin binding, and microtubule-based movement were also enriched (Fig. 6A).

Interestingly, we found that the expression of transmembrane transport pathway (GO:0055085)-related genes was significantly changed among the three stages (Supplementary Data Fig. S12; gene_ID is listed in Supplementary Data Table S4).

We further clustered the DEGs on the basis of the log2-fold change in 35S::VvSUN and control utilizing the K-mean cluster. There were four patterns detected in the time-series gene expression profiles, which were then displayed utilizing a multigene line plot (Fig. 6B). The same DEG subclusters in two different genotypes showed different expression patterns and were considered the main genes regulated by the VvSUN gene. In subclusters 1 and 4, there were 317 and 217 DEGs, respectively, that exhibited
The VvSUN gene affects auxin pathways. Comparative analysis of (A) IAA precursors (IPYA), (B) free auxin (IAA), and (C) IAA conjugates (IAA-ASP) changes between the 35S::VvSUN transgenic tomato and control at different fruit development stages. Each value represents the mean ± standard deviation of three repetitions. FW, fresh weight. (D) Simplified diagram of IAA metabolism. (E) Heat map for each class indicates the expression changes of different auxin-related genes. The color legend represents the log2-fold change of the VvSUN/control ratio. A positive value means that the gene is significantly up-regulated in the transgenic plants and a negative value means that the gene is significantly down-regulated in the transgenic plants.

WGCNA analysis identified highly connected ‘hubs’ and associated specific modules with genetic and fruit shape traits

In order to reveal gene networks associated with fruit shapes in 35S::VvSUN transgenic tomatoes, The gene expression profiles of all these 22510 genes were analyzed to identify gene co-expression modules using the R package WGCNA. Here, 11 co-expression modules were identified, among which the ‘green’, ‘lightcyan’, ‘darkred’, and ‘pink’ modules were not only significantly associated with fruit shape but also with auxin-related compounds (Supplementary Data Fig. S14), indicating that auxin-related genes may be correlated with the control of fruit shape. Specifically, the longitudinal diameter was positively correlated with the expression of genes in the ‘green’, ‘darkred’, and ‘pink’ modules (Supplementary Data Fig. S14), with a coefficient of 0.77 (P = 2e-04), 0.83 (P = 2e-05), and 0.6 (P = 0.008), respectively. Genes clustered in above modules were picked out according to the gene significance (GS) values (GS > coefficient values of the trait) and P values (P.GS < P values of the trait) for further KEGG pathway analysis. The results showed that these genes were significantly enriched in several pathways (Supplementary Data Table S5). Interestingly, the plant hormone signal transduction pathway was a jointly owned pathway by longitudinal diameter, transverse diameter, and FSI trait modules (Fig. 7A). Then, genes involved in the processes of the plant hormone signal transduction pathway in each trait module were selected to construct a gene network by Cytoscape. As seen in Supplementary Data Fig. S15, out of the 41 hormone signal transduction pathway genes, 14 were plant hormone-related genes and among them around 50% were auxin-related genes according to the annotation of genes. Therefore, it is conceivable that the mechanisms underlying plant hormone signal transduction serve as the primary hub for interactions with other pathways implicated in controlling the elongated fruit morphology that arises from VvSUN overexpression in the plant.

Discussion

VvSUN regulates fruit shape

The shape of the fruit is among the most distinguishing characteristics of the table grape. Nonetheless, only a few gene-oriented research reports have concentrated on the discovery of genes relevant for grape berry morphology, despite the fact that a vast spectrum of phenotypic diversity in berry shape has been reported. In this study, we cloned a grape VvSUN gene that encodes an
Figure 6. Identification of DEGs in 35S:VvSUN transgenic tomato. (A) GO term enrichment analysis of the DEGs of ovary/fruit in 35S:VvSUN transgenic tomato at 1 WBA, anthesis, and 5 DPA stages. *P < .05. (B) Positive values were significantly higher in 35S:VvSUN transgenic tomato. Negative values were significantly higher in the control. Cluster analysis results were visualized using a multigene line plot. The expression patterns of the DEGs were divided into groups based on the developmental time points utilizing K-means clustering. (C) KEGG enrichment analysis of the DEGs in 35S:VvSUN transgenic tomato, showing contrasting expression trends in comparison with the control tomato.

IQD-like protein. The phylogenetic tree showed that VvSUN is one of the closest homologs to tomato SUN (Fig. 1A). Genes clustered in the same subclade are more likely to have similar functions [12]. Earlier research reports have demonstrated that upregulation of SUN contributed to the development of fruit that was very elongated and generally seedless [4]. Using anatomical findings, it was discovered that SUN has a significant influence on the morphology of the fruit before its anthesis, but it is after anthesis that SUN’s most striking influence on shape is evident, which is likely due to the changes in cell division rates in the longitudinal direction, leading to a cell number increase along the proximal-distal axis [4]. In our results, the expression levels of VvSUN were much higher in elongated grape cultivars than in round or near-round types at the 1 WBA stage (Fig. 2C). Moreover, the VvSUN expression level at the 1 WBA stage was positively correlated with the longitudinal length of the mature berry and also showed the highest correlation to FSI (0.87) (Fig. 2D). Overexpression of VvSUN driven by the 35S promoter in tomatoes led to an increase in the FSI in tomatoes but showed little or no impact on cell size and form (Fig. 3A–E, Supplementary Data Figs S6–S8). The function of VvSUN was also validated in transgenic tobacco, which showed a significant increase in the pod shape index as well as in the leaf shape index. Moreover, the cell form and sizes in lower epidermis leaves of transgenic tobacco were similar to those of the wild type (Fig. 3F and G, Supplementary Data Fig. S9). Given the difference in fruit types between tomato and tobacco, it is likely that the basic function of VvSUN in regulating the FSI by changing cell division is likely conserved. Furthermore, to our knowledge, VvSUN has not previously been recognized as a gene that regulates the morphology of fruits. Hence, we infer that...
Figure 7. The pathway correlation network and proposed model of fruit shape control. (A) Key pathways in the control of fruit shape by WGCNA and KEGG analysis were characterized and identified, then significantly enriched pathways were used to construct a correlation network by Cytoscape. (B) Proposed model of how VvSUN regulates fruit shape. VvSUN is situated in the plasma membrane and exogenous auxin may induce expression of VvARF6, then VvARF6 activates cis-elements in VvSUN promoters to induce gene expression. The increased expression of VvSUN stimulates endogenous auxin accumulation and polar transport and/or auxin signal transduction process variations. Therefore, we suppose that VvSUN may not only respond to exogenous auxin treatment but also modulate the elongated fruit shape in the plant hormone signal transduction pathway during the early phases of fruit growth.

VvSUN is a novel gene that modulates fruit shape by changing the cell division rate.

Our data also show that the differences in the levels and timing of VvSUN expression were positively correlated with the fruit shape phenotype (Fig. 2C and D). However, sequence analyses revealed that there were no consensus sequence diversities in the coding regions and −1833 bp upstream of the VvSUN gene between the elongated and the round grape cultivars (Supplementary Data Figs. S4 and S5), indicating that the VvSUN coding sequences and −1833 bp upstream cannot be the reason for expression variation. Gene regulation in multicellular eukaryotes is complex, with many layers of regulation [27], including mutations in coding sequences or promoter regions [14, 28], long-range control by distant repressors or enhancers, alteration of epigenetic states, coordinated expression of genes [29], and regulation by transcription factors, including microRNAs (miRNAs), small interfering RNAs (siRNAs), messenger RNAs (mRNAs), and non-coding RNAs [30]. In this case, it is a big challenge and needs further effort in order to decipher how variation in regulatory mechanisms eventually results in changes in VvSUN gene expression profiles.

Mechanisms by which VvSUN controls fruit shape

The plant hormone auxin performs a fundamental function in the modulation of cell expansion, cell division, and cell identity establishment [18, 31]. Earlier research reports illustrated that IQ domain-containing proteins belong to a calmodulin-binding protein family and play a role in the regulation of fruit shape by modulating auxin signal transduction [12, 32]. In this study, we also showed that the VvSUN promoter interacts with grape VvARF6 in yeast (Fig. 4A), and GUS activity driven by the VvSUN promoter was significantly increased when exogenous auxin was applied (Fig. 4B and C). More importantly, ectopic overexpression of VvSUN in tomatoes not only enhanced endogenous IAA content but also remarkably influenced the expression of auxin-associated genes, especially those implicated in polar transport and signal transduction (Fig. 5E). In addition, the combination of clustering, WGCNA, and KEGG enrichment analysis demonstrated a substantial enrichment of the DEGs in 35S::VvSUN transgenic tomatoes in the plant hormone signal transduction pathway in pairwise comparisons with control (Figs 6B and C and 7A). It has been shown that auxin signaling plays an important role in apple size [33] and the inhibition of polar auxin transport in
tobacco (Nicotiana tabacum) changes the orientation of cell division [34, 35]. Recent studies have shown that multiple IQD genes are candidate ARFs targets and are transcriptionally regulated by auxin signaling [12, 32]. Multiple studies have demonstrated that active auxin levels and distributions are closely regulated by auxin signaling [12, 32]. Multiple studies have demonstrated that active auxin levels and distributions are closely regulated by auxin signaling [12, 32].

Recently, several lines of evidence have shown that auxin can influence microtubule dynamics [36], and genetically controlled microtubule depolymerization in embryos leads to the disruption of asymmetric divisions [18]. Most IQD members co-localize with microtubules, the cell nucleus, or membranes, which are implicated in the transduction of Ca\textsuperscript{2+} signals into cell responses via the modulation of a variety of target proteins [17, 37]. As a result of the elevation in cytosolic Ca\textsuperscript{2+} concentrations caused by auxin treatment, the activity of the IQD is regulated posttranslationally via stimulation of the Ca\textsuperscript{2+} CaM signaling pathway [38]. Ca\textsuperscript{2+} CaM, on the other hand, has an effect on auxin production by directly interfacing with components of the auxin transport and signaling mechanism, including PINOID (PID) or small auxin upregulated RNA 19 (SAUR19) [38]. A conserved domain search confirmed that the VvSUN protein contains a Ca\textsuperscript{2+}-dependent IQ motif (Supplementary Data Fig S3), and we detected that VvSUN was localized in the plasma membrane and chloroplast (Fig 4D). The transcriptome analysis of VvSUN overexpression in tomatoes revealed that DEGs were enriched in calcium ion binding, cytoskeletal protein binding, tubulin binding, and microtubule-based movement pathways (Fig 6A). Moreover, the transmembrane transport pathway (GO:0055085) was activated in 3SS:VvSUN transgenic tomato among the three stages using pairwise comparisons with control (Supplementary Data Fig S12). The findings presented in this work lead us to postulate that VvSUN is situated in the plasma membrane and functions as a hub gene to translocate cellular auxin and calcium signaling (Fig 7B). This, in turn, alters the pattern of cell division, contributing to the anisotropic expansion of the ovary and the presence of a fruit with an elongated morphology.

**Materials and methods**

**Plant materials and growing conditions**

Grape plants (V. vinifera L.) of cv. ‘Minicure Finger’ (MF), ‘Kourgan Rose’ (KR), 8-6-1 (‘Beni Pizzutello’ seeder), and V. vinifera × Vitis labrusca cv. ‘Goldfinger’ (GF), ‘Houman’ (HM), and ‘Shine-Muscato’ (SM) from the Tang Shan Vineyard (College of Horticulture, Nanjing Agricultural University, Nanjing, China) were sampled during the 2020 growing season. Ovary and fruit samples were collected at 4 WBA, 3 WBA, 2 WBA, 1 WBA, 3 DBA, anthesis (when 50% of the caps were off), 3 days post anthesis (3 DPA), 1 WAA, and 2 WAA. The samples were promptly frozen in liquid nitrogen and kept at a temperature of 80°C till RNA extraction was done.

Tomato (S. lycopersicum Mill. cv. ‘Micro Tom’) and tobacco (N. tabacum L. ‘K326’) plants were cultivated in a normal greenhouse environment at Nanjing Agricultural University. The following were the conditions under which the culture chamber was set up: 16-hour day/8-hour night cycle at constant 25°C, 60% humidity, and 250 μmol m\textsuperscript{-2} s\textsuperscript{-1} luminous intensity [39].

**Morphological analysis of grapes and transgenic plants**

The fruits of grapes (the six different cultivars mentioned above) and tomato, and the tobacco pod samples were measured for fruit/pod diameter and length by using a Vernier caliper (Mitutoyo, Kawasaki, Japan) at 7, 9, 15, 20, 36, 40 DPA. The ovaries and young fruits of tomato at 1 WBA, anthesis, and 5 DPA stages were measured with a stereo microscope (SZX10, Olympus, Japan).

Histological examination of the fruits at various growth stages was accomplished by the use of paraffin segmentation. After fixing the obtained fruit samples in formaldehyde-acetic acid-ethanol (FAA) for at least 24 hours, they were washed in 50% ethanol for 10 minutes before being dried and embedded in paraffin in accordance with the conventional techniques reported by Godoy et al. [40].

The VvSUN expression level and phenotypic correlation coefficients were analyzed and visualized using online software (http://www.cloudtutu.com/).

**Identification and phylogenetic analysis of VvSUN genes**

The IQ67 domain of SISUN [22] was utilized to categorize members of this family in grapes. Systematic BLAST screenings were conducted on all sequence data in genome databases for grape (version 2.0) and its annotation (http://ftpensemblgenomes.org/pub/plants/release-46/gtf/vitis_vinifera/) based on the domain retrieved from the SOL Genomics Network (SGN, http://solgenomics.net) as initial queries. The identified protein sequences were subsequently validated for IQ67 domain components in the SMART databases (http://smart.embl-heidelberg.de/) and NCBI Conserved Domains database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). All the protein sequences containing errors or lacking the IQ67 domain were removed from the study.

A phylogenetic tree was constructed utilizing the neighbor-joining (NJ) technique in the MEGA (Molecular Evolutionary Genetics Analysis) program version 11.0, and edge support was evaluated by applying 1000 bootstrap replicates [41]. The SUN sequences of additional species, such as cucumber (Cucumis sativus) and watermelon, were acquired from GuGenDB (http://cucurbitgenomics.org/) and the sequences of AtIQDs were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/). This study comprised sequences with the following GenBank accession codes: V. vinifera, VvSUN1 (LOC100267340), VvSUN2 (LOC100260828), VvSUN3 (LOC100260747), VvSUN4 (LOC100254717), VvSUN5 (LOC100854442), VvSUN6 (LOC100254187), VvSUN7 (LOC100243111), VvSUN8 (LOC100240779), VvSUN9 (LOC100244856), VvSUN10 (LOC100266234), VvSUN11 (LOC100247418), VvSUN12 (LOC100256590), VvSUN13 (LOC100263595), VvSUN14 (LOC100259524), VvSUN15 (LOC100266890), VvSUN16 (LOC100241183), VvSUN17 (LOC100241471), VvSUN18 (LOC100256816), VvSUN19 (LOC100245132), VvSUN20 (LOC100254639), VvSUN21 (LOC104882151), VvSUN22 (LOC100255609), VvSUN23 (LOC100245291), VvSUN24 (LOC100263965), VvSUN25 (LOC100246947); Arabidopsis thaliana, AtIQD15 (AEE78534.1), AtIQD16 (AEE82912.1), AtIQD17 (AEE81939.1), AtIQD18 (AEE27391.1); C. sativus, CsSUN2 (XP_011659956.1); Citrullus lanatus, (Csa1G575000), C. lanatus, CisUN8 (Cla011257); and S. lycopersicum, SISUN1-SISUN33 [21].

**Expression investigations**

The ‘TriZol’ reagent (Thermo Fisher Scientific, USA) was employed to isolate total RNA in compliance with the guidelines.
stipulated by the manufacturer. From each sample, 1 μg of total RNA was obtained and subjected to treatment with RNase-free DNase (Vazyme, Nanjing, China) to eliminate any remaining genomic DNA, followed by conversion to cDNA with the aid of the First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). Subsequently, we conducted qRT–PCR utilizing SYBR Premix ExTaq (Takara Biotech, Japan) on a Quant Studio™ 5 System (Thermo Fisher Scientific, USA). Normalization of the targeted gene was carried out by using the VvActin and SlActin genes. Next, the ΔΔCt method was employed to compute gene expression levels. As depicted in the corresponding figures, all qPCR tests for each biological replicate were carried out with three technical replicates. Supplementary Data Table S2 displays the primer sequences that were utilized for qRT–PCR.

**Isolation of the VvSUN and VvSUN promoter**

Information on DNA and protein sequences was obtained from the NCBI database. The procedures reported by Zheng et al. [42] were utilized for DNA extraction, isolation of total RNA, first-strand cDNA synthesis, and DNase I treatment. The VvSUN (LOC100253695) cDNAs were isolated from different grape cultivars during the pre-bloom phase by RT–PCR based on primers VvSUN-F/VvSUN-R (Supplementary Data Table S2) before cloning them into the pEASY®-Blunt Cloning Vector (TransGen). The VvSUN promoter (−1833 bp to ATG) was amplified by primers VvSUNpro-F/R from grape DNA, followed by cloning into the pEASY®-Blunt Cloning Vector (TransGen). Several clones were randomly chosen and verified by sequencing. The DNAMAN program (Lynnon Biosoft, San Ramon, CA, USA) was employed to assess sequence alignment. Supplementary Data Table S2 gives a complete list of all the primer pairs that were utilized.

**Generation of VvSUN overexpression transgenic lines**

Amplification of the VvSUN gene’s coding sequence was accomplished utilizing PCR, followed by cloning of the resulting fragment into the pEASY®-Blunt Cloning Vector and subsequently into the pYH4215 vector utilizing a One Step Cloning Kit (Vazyme, Nanjing, China). A 35S::VvSUN cDNA construction was transformed into ‘Micro Tom’ tomato and tobacco via Agrobacterium-induced transformation (strain EHA105) in accordance with the procedures reported by De Jong et al. [43]. In half-strength MS media that contained hygromycin (30 mg l⁻¹), potential transgenic lines were chosen, and their existence was subsequently verified by RT–qPCR, PCR, and GUS staining. Supplementary Data Table S2 shows the primers that were utilized in the PCR and RT–qPCR experiments. The T₀ generation of the transgenic tomato and the T₁ generation of the transgenic tobacco lines were chosen for physiologic experiments and molecular analyses.

**Yeast one-hybrid assay**

The −1833 bp fragment (upstream from the start codon) obtained from the VvSUN promoter was amplified from grape genomic DNA and subsequently cloned into the pAbAi vector (Clontech) for the YIH assay. Co-transformation of recombinant plasmid pGADT7-VvARF6 and pAbAi-VvSUN-pro into yeast strain Y1HGold (Clontech) was performed in accordance with the guide-lines provided by the manufacturer. Additionally, transfection of the pGADT7 vector into baits was performed, which served as a negative control. SD/−Ura drop-out medium was used to culture the transformants. After a selection of colonies was made and diluted in sterile ddH₂O attained an OD₆₀₀ density of 0.5, 3 μl of suspension was spotted on SD/−Ura/−Leu drop-out containing AbA antibiotic at 30°C. Supplementary Data Table S2 provides detailed information on the primers that were utilized in this investigation.

**GUS activity analysis**

The promoter of VvSUN was ligated into the pBI121-GUS vector before infusion into GV3101 to allow temporary expression in grape leaves. Subsequently, the grape leaves were grown in an incubator for 24 hours in darkness following vacuum infiltration and then treated with increasing dosages of 10, 50, and 100 mg/l of IAA 48 hours later. The positive and negative controls used in the experiment included the CaMV35S–GUS vector and the water treatment, respectively GUS activity experiments were performed as previously described [44].

**Subcellular localization of VvSUN**

The VvSUN subcellular localization was determined by the PCR amplification of its full-length cDNA utilizing the primers VvSUN-GFP-F/R with incorporated Ncol and SpeI restriction regions and subsequent cloning into the pClone007 Blunt Simple vector (TSINGKE, China). Cloning of the full-length VvSUN cDNA into the pCAMBIA1302 vector was done after being verified by sequencing to generate the plasmid that would express the VvSUN-GFP fusion protein when driven by the 35S promoter. pCAMBIA1302-GFP was used as the control vector. The plasmids were added to Agrobacterium tumefaciens strain GV3101 following the protocol of Zheng et al. [42]. A Zeiss confocal scanning microscope (LSM700) was utilized to monitor the expression of the VvSUN-GFP fusion protein and chlorophyll signals in the infiltrated N. benthamiana leaves 3 days after inoculation.

**Quantitative analysis of endogenous IAA and IAA-related compounds**

IAA, IAA-Asp, and IPYA used as the standards were procured from Sigma–Aldrich (USA). Endogenous IAA and IAA-related compounds were extracted from ovaries and young fruits of tomatoes by using liquid–liquid extraction and determined by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) following a published protocol [45]. Data were analyzed using MassHunter Workstation software (Agilent, CA, USA) and the final result was expressed in nanograms per gram FW.

**RNA-seq data analysis**

We extracted total RNA from three biologically separate pools of the wild tomato (control) and 3SS::VvSUN line 5 ovaries at 1 WBA, anthesis, and 5 DPA as described above in Expression investigations. The Stranded mRNA-seq kit (Vazyme, Nanjing, China) was utilized to create RNA-seq libraries. Next, the illumina Novaseq platform (HiSeqTM2500/4000) was utilized for sequence analyses in Vazyme (China). A Trimmomatic (v0.33) was employed to screen the raw reads by eliminating the low-quality and adapter sequences. Mapping of the clean reads to the tomato genome was conducted by using STAR (v2.5.2b) [46]. DESeq (Padj<0.05, v1.10.1) was employed to analyze the transcript assembly and expression levels of genes [47]. The DEGs affected by the genotype and developmental phase were clustered utilizing K-means in R [48]. Co-expression networks were created by using the WGCNA (v1.29) package in R and Cytoscape software (v3.9.1) [49]. The expression data used for WGCNA analysis comprised a total of 22510 identified genes in the present study from the 18 datasets, and the trait data included longitudinal diameter, transverse diameter,
FSI, IAA, IAA-ASP, IPYA, and fruit development stage. Clustering of the genes identified from K-means and WGCNA were performed using GO (GOseq, v1.22) [50] and KEGG (KOBAS, v2.0) [51] enrichment analyses. The annotation file was downloaded from the tomato database (ftp://ftp.ensemblgenomes.org/21/pub/plants/release-47/fasta/solanum_lycopersicum/).

Based on the gene's annotations, transmembrane transport pathway genes and auxin-related genes implicated in its metabolic activities, signal transduction, and polar transport were identified from DEGs. Excel was utilized to perform pairwise comparisons of the expression values between the 35S::VsSUN and control at each developmental stage and the findings were subjected to log2 transformation.

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Author contributions

H.Z. and J.M.T designed the study. H.Z., Y.D., and H.L.N conducted the related experiments and data analysis and wrote the manuscript. J.L., X.Y., Y.G.Z., B.H., W.W., and L.Y.H. participated in the experiments. L.N.Y. and J.M.T. reviewed and revised the manuscript.

Data availability

The RNA sequencing datasets generated in this study have been deposited in the National Genomics Data Center with the accession number PRJCA009128 (https://ngdc.cncb.ac.cn/). Other data supporting our findings are available in the manuscript file or from the corresponding author upon request.

Conflict of interest

The authors declare no competing interests.

Supplementary data

Supplementary data is available at Horticulture Research online.

References

1. Wycislo AP, Clark JR Karcher DE. Fruit shape analysis of Vitis using digital photography. HortScience. 2008;43:677–80.
2. Zhang C, Cui LF, Fang J. Genome-wide association study of the candidate genes for grape berry shape-related traits. BMC Plant Biol. 2022;22:1–19.
3. Lipman Z, Tanksley SD. Dissecting the genetic pathway to extreme fruit size in tomato using a cross between the small-fruited wild species Lycopersicon pimpinellifolium and L. esculentum var. Giant Heirloom. Genetics. 2001;158:413–22.
4. Wu S, Xiao H, Cabrera A et al. SUN regulates vegetative and reproductive organ shape by changing cell division patterns. Plant Physiol. 2011;157:1175–86.
5. Blas AL, Yu Q, Veatch OJ et al. Genetic mapping of quantitative trait loci controlling fruit size and shape in papaya. Mol Breed. 2012;29:457–66.
6. Wang Y, Bo K, Gu X et al. Molecularly tagged genes and quantitative trait loci in cucumber with recommendations for QTL nomenclature. Hortic Res. 2020;7:3.
7. Pérez C, Hagen L, Giovinazzo N et al. Genetic control of fruit shape acts prior to anthesis in melon (Cucumis melo L.). Mol Gen Genomics. 2002;266:933–41.
8. Cirilli M, Baccichet I, Chiozzotto R et al. Genetic and phenotypic analyses reveal major quantitative loci associated to fruit size and shape traits in a non-flat peach collection (P. persica L. Batsch). Hortic Res. 2021;8:232.
9. Dou J, Zhao S, Lu X et al. Genetic mapping reveals a candidate gene (CIIF51) for fruit shape in watermelon (Citrus lanatus L.). Theor Appl Genet. 2018;131:947–58.
10. Jin B, Kim J, Jung J et al. Characterization of IQ domain gene homologs as common candidate genes for elongated fruit shape in cucurbits. Hortic Sci Technol. 2018;36:85–97.
11. Wang S, Wu K, Yuan Q et al. Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet. 2012;44:950–4.
12. Yang BJ, Wendrich JR, De Rybel B et al. Rice microtubule-associated protein IQ67-DOMAIN14 regulates grain shape by modulating microtubule cytoskeleton dynamics. Plant Biotechnol J. 2020;18:1141–52.
13. Wang YP, Clevenger JP, Illa-Berenguer E et al. A comparison of sun, ovate, f58.1 and auxin application on tomato fruit shape and gene expression. Plant Cell Physiol. 2019;60:1067–81.
14. Xiao H, Jiang N, Schaffner E et al. A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. Science. 2008;319:1527–30.
15. Colle M, Weng Y, Kang Y et al. Variation in cucumber (Cucumis sativus L.) fruit size and shape results from multiple components acting pre-anthesis and post-pollination. Planta. 2017;246:641–58.
16. Pan Y, Liang X, Gao M et al. Round fruit shape in W7239 cucumber is controlled by two interacting quantitative trait loci with one putatively encoding a tomato SUN homolog. Theor Appl Genet. 2017;130:573–86.
17. Bürstenbinder K, Mitra D Quegwer J. Functions of IQD proteins as hubs in cellular calcium and auxin signaling: a toolbox for shape formation and tissue-specification in plants? Plant Signal Behav. 2017;12:1692–708.
18. Vaddepalli P, de Zeeuw T, Strauss S et al. Auxin-dependent control of cytoskeleton and cell shape regulates division orientation in the Arabidopsis embryo. Curr Biol. 2021;31:4946–4955 e4.
19. Wang Y, Clevenger JP, Illa-Berenguer E et al. A comparison of sun, ovate, f58.1 and auxin application on tomato fruit shape and gene expression. Plant Cell Physiol. 2019;60:1067–81.
20. Wang H, Yan A, Sun L et al. Novel stable QTLs identification for berry quality traits based on high-density genetic linkage map construction in table grape. BMC Plant Biol. 2020;20:1–15.
21. Huang Z, Van Houten J, Gonzalez G et al. Genome-wide identification, phylogeny and expression analysis of SUN, OFP and YABBY gene family in tomato. Mol Gen Genomics. 2013;288:111–29.
22. Qiao X, Li Q, Yin H et al. Gene duplication and evolution in recurring polyploidization-diploidization cycles in plants. Genome Biol. 2019;20:1–23.
23. Fasoli M, Dal Santo S, Zenoni S et al. The grapevine expression atlas reveals a deep transcriptome shift driving the entire plant into a maturation program. Plant Cell. 2012;24:3489–505.

24. Zhang Y, Yuan Y, Gao S et al. Cloning of VvSUN gene in grape (Vitis L.) and a preliminary study on the function of controlling fruit shape. Acta Bot Boreali-Occiden Sin. 2017;37:1271–7.

25. Abel S, Savchenko T, Levy M. Genome-wide comparative analysis of the IQD gene families in Arabidopsis thaliana and Oryza sativa. BMC Evol Biol. 2005;5:1–25.

26. Hu J, Israeli A, Ori N et al. The interaction between DELLA and ARF/IAA mediates crosstalk between gibberellin and auxin signaling to control fruit initiation in tomato. Plant Cell. 2018;30:1710–28.

27. Munsky B, Neuert G, Van Oudenaarden A. Using gene expression noise to understand gene regulation. Science. 2012;336:183–7.

28. Frary A, Nesbitt TC, Frary A et al. fw2.2: a quantitative trait locus key to the evolution of tomato fruit size. Science. 2000;289:85–8.

29. Dekker J. Gene regulation in the third dimension. Science. 2008;319:1793–4.

30. Nilsen TW. Mechanisms of microRNA-mediated gene regulation in animal cells. Trends Genet. 2007;23:243–9.

31. Petersson SV, Johansson AI, Kowalczyk M et al. An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. Plant Cell. 2009;21:1659–68.

32. Wendrich JR, Yang BJ, Mijnhout P et al. IQD proteins integrate auxin and calcium signaling to regulate microtubule dynamics during Arabidopsis development. BioRxiv. 2018;275560.

33. Bu H, Yu W, Yuan H et al. Endogenous auxin content contributes to larger size of apple fruit. Front Plant Sci. 2020;11:592540.

34. Petrásek J, Mravec J, Bouchard R et al. PIN proteins perform a rate-limiting function in cellular auxin efflux. Science. 2006;312:914–8.

35. Mravec J, Skůpa P, Bailly A et al. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature. 2009;459:1136–40.

36. Chen X, Grandont L, Li H et al. Inhibition of cell expansion by rapid ABP1-mediated auxin effect on microtubules. Nature. 2014;516:90–3.

37. Abel S, Bürstenbinder KMüller J. The emerging function of IQD proteins as scaffolds in cellular signaling and trafficking. Plant Signal Behav. 2013;8:e24369.

38. Vanneste S, Friml J. Calcium: the missing link in auxin action. Plants (Basel). 2013;2:650–75.

39. Zheng H, Kawabata S. Identification and validation of new alleles of FALSIFLORA and COMPOUND INFLORESCENCE genes controlling the number of branches in tomato inflorescence. Int J Mol Sci. 2017;18:1572.

40. Godoy F, Kühn N, Muñoz M et al. The role of auxin during early berry development in grapevine as revealed by transcript profiling from pollination to fruit set. Hortic Res. 2021;8:140.

41. Tamura K, Dudley J, Nei M et al. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24:1596–9.

42. Zheng H, Yu X, Yuan Y et al. The VviMYB80 gene is abnormally expressed in Vitis vinifera L. cv. ‘Zhong Shan Hong’ and its expression in tobacco driven by the 35S promoter causes male sterility. Plant Cell Physiol. 2016;57:540–57.

43. De Jong M, Wolters-Arts M, Feron R et al. The Solanum lycopersicum auxin response factor 7 (SIARF7) regulates auxin signaling during tomato fruit set and development. Plant J. 2009;57:160–70.

44. Kosugi S, Ohashi Y, Nakajima K et al. An improved assay for β-glucuronidase (GUS) in transformed cells: methanol almost suppresses a putative endogenous GUS activity. Plant Sci. 1990;70:133–40.

45. Wu X, Prior RL. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. J Agric Food Chem. 2005;53:2589–99.

46. Dobin A, Davis CA, Schlesinger F et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29:15–21.

47. Trapnell CR, Goff L, Pertea G et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and cufflinks. Nat Protoc. 2012;7:562–78.

48. Wang L, Feng Z, Wang X et al. DEGseq: an R package for identifying differentially expressed genes from RNA-seq data. Bioinformatics. 2010;26:136–8.

49. Kuang JF, Wu CJ, Guo YF et al. Deciphering transcriptional regulators of banana fruit ripening by regulatory network analysis. Plant Biotechnol J. 2021;19:477–89.

50. Young M, Wakefield MJ, Smyth GK et al. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. 2010;11:R14-4.

51. Shen S, Park JW, Lu ZX et al. rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. Proc Natl Acad Sci USA. 2014;111:5593–601.