Auxin response factor 6A regulates photosynthesis, sugar accumulation, and fruit development in tomato

Yujin Yuan¹, Xin Xu¹, Zehao Gong¹, Yuwei Tang¹, Mengbo Wu¹, Fang Yan¹, Xiaolan Zhang¹, Qian Zhang², Fengqing Yang², Xiaowei Hu¹, Qichen Yang³, Yingqing Luo¹, Lihua Mei¹, Wenfa Zhang¹, Cai-Zhong Jiang⁴, Wangjin Lu⁶, Zhengguo Li¹ and Wei Deng¹

Abstract
Auxin response factors (ARFs) are involved in auxin-mediated transcriptional regulation in plants. In this study, we performed functional characterization of SlARF6A in tomato. SlARF6A is located in the nucleus and exhibits transcriptional activator activity. Overexpression of SlARF6A increased chlorophyll contents in the fruits and leaves of tomato plants, whereas downregulation of SlARF6A decreased chlorophyll contents compared with those of wild-type (WT) plants. Analysis of chloroplasts using transmission electron microscopy indicated increased sizes of chloroplasts in SlARF6A-overexpressing plants and decreased numbers of chloroplasts in SlARF6A-downregulated plants. Overexpression of SlARF6A increased the photosynthesis rate and accumulation of starch and soluble sugars, whereas knockdown of SlARF6A resulted in opposite phenotypes in tomato leaves and fruits. RNA-sequence analysis showed that regulation of SlARF6A expression altered the expression of genes involved in chlorophyll metabolism, photosynthesis and sugar metabolism. SlARF6A directly bound to the promoters of SIGLK1, CAB, and RbcS genes and positively regulated the expression of these genes. Overexpression of SlARF6A also inhibited fruit ripening and ethylene production, whereas downregulation of SlARF6A increased fruit ripening and ethylene production. SlARF6A directly bound to the SAMS1 promoter and negatively regulated SAMS1 expression. Taken together, these results expand our understanding of ARFs with regard to photosynthesis, sugar accumulation and fruit development and provide a potential target for genetic engineering to improve fruit nutrition in horticulture crops.

Introduction
Tomato is the world’s second largest vegetable crop rich in nutrients¹. Tomato fruit development includes three stages². The first stage is characterized by an increase in cell number and starch accumulation, followed by cell enlargement with starch degradation and soluble sugar accumulation in the second stage³. Fruit ripening is the last stage, associated with the accumulation of soluble sugars, carotenoids, organic acids, and volatile organic compounds in fruits⁴. The chlorophyll accumulation and photosynthetic activity of green fruits influence the nutritional components and flavor of ripening tomato fruits⁵. Some genes have been reported to affect chlorophyll accumulation, chloroplast development and fruit quality. As negative regulators, DE-ETIOLATED 1/high pigment 2 (DET1/hp2) and UV-DAMAGED DNA-BINDING PROTEIN 1/high pigment 1 (DDB1/hp1) are involved in chloroplast formation and chlorophyll accumulation in tomato fruits⁶. The tomato GOLDEN2-LIKE transcription factors SIGLK1 and SIGLK2 play an important role in chloroplast formation and chlorophyll accumulation⁷.

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Evidence suggests that the *SLGK2* gene is predominantly expressed in fruits and that the latitudinal gradient of *SLGK2* expression influences the production of unevenly colored tomato fruits. Overexpression of the *APRR2-LIKE* gene, the closest homolog of *SLGK2*, increased the size and number of chloroplasts and enhanced chlorophyll accumulation in green tomato fruits. TKN2 and TKN4, two Class I KNOTTED1-LIKE HOMEBOX (KNOX) proteins, act as transcriptional activators of *SLGK2* and *APRR2-LIKE* genes to promote chloroplast development in tomato fruits. *BEL1-LIKE HOMEODOMAIN11* (SIBEL11) also plays an important role in chlorophyll synthesis and chloroplast development in tomato fruits.

The ripening of tomato is mainly regulated by the ethylene pathway and many transcription factors. In the ethylene biosynthetic pathway, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) catalyze the conversion of SAM to ACC and of ACC to ethylene, respectively. The MADS box gene *RIPENING INHIBITOR (RIN)* controls the early phase of ripening and ethylene production via transcriptional regulation of ACSs and ACOs. The other ripening regulators affecting ethylene pathway and many transcription factors have indicated that ARFs are involved in many tomato developmental processes, an indication of the ubiquitous expression in all tissues tested. The GUS staining in the transgenic tomato plants was detected in leaves, stems, buds, flowers, and fruits at different developmental stages, an indication of the ubiquitous expression of *SIARF6A* in all tissues tested. The GUS staining was weak in the early fruits at 2 and 4 days post anthesis (DPA) but became strong at 8, 30 and 45 DPA (Fig. 1a).

**Results**

**Sequence and expression analysis of *SIARF6A* gene and subcellular localization and transcriptional activity of *SIARF6A* protein**

The *SIARF6A* gene has an open reading frame of 2608 bp encoding a putative protein of 869 amino acids. Amino acid sequence analysis revealed that, like SIARF7 and SIARF8, which have typical conserved ARF domains, SIARF6A protein also contained B3-DNA, ARF, and AUX/IAA binding domains (Fig. S2), indicating possible functional similarity among them.

To determine the expression pattern of *SIARF6A* in planta, a transcriptional fusion was constructed between the *SIARF6A* promoter and the GUS reporter gene. GUS staining in the transgenic tomato plants was detected in leaves, stems, buds, flowers, and fruits at different developmental stages, an indication of the ubiquitous expression of *SIARF6A* in all tissues tested. The GUS staining was weak in the early fruits at 2 and 4 days post anthesis (DPA) but became strong at 8, 30 and 45 DPA (Fig. 1a), suggesting possible roles of *SIARF6A* in the development of tomato fruits.

To examine its subcellular localization in plants, SIARF6A was fused to GFP and transferred into tobacco protoplasts. Fluorescence microscopy analysis revealed that SIARF6A was specifically localized in the nuclei (Fig. 1b). A GAL4-responsive reporter system in yeast was employed to reveal the transcriptional activity of SIARF6A. SIARF6A was fused to GAL4-BD (DNA binding domain) to form a pGBK7-SIARF6A fusion plasmid and subsequently transformed into yeast. Yeast transformants harboring the pGBK7-SIARF6A construct grew well in the medium lacking Trp, His, and Ade (SD-W/H/A), while the yeasts transformed with pGBK7 vector alone (negative control) could not (Fig. 1c). Assessing transcriptional activity revealed that SIARF6A is a transcriptional activator.
**SlARF6A** is involved in chlorophyll accumulation and chloroplast development in tomato

To elucidate the physiological significance of the **SlARF6A** gene in fruit development, upregulated and downregulated transgenic lines corresponding to independent transformation events were generated in tomato plants. qRT-PCR was used to evaluate the expression level of **SlARF6A** in all transgenic lines. Compared with the...
level in the wild type (WT), the expression level of \( \text{SIARF6A} \) was decreased in RNAi 2 and 6 plants (Fig. 2a) but increased in OE-4 and 6 plants (Fig. 2a).

It is noteworthy that altered \( \text{SIARF6A} \) expression led to a dramatic change in chlorophyll accumulation in transgenic lines. Compared with WT plants, the OE-SIARF6A plants had dark-green fruits at the green fruit stage, whereas the RNAi-SIARF6A plants had light-green fruits (Fig. 2b). The impact of altered \( \text{SIARF6A} \) expression on chlorophyll accumulation was analyzed by measuring the chlorophyll content in fruits and leaves. The SIARF6A overexpression lines possessed greater accumulation of chlorophyll in the fruits at immature green, mature green, breaker, and orange stages, whereas the RNAi lines had lower chlorophyll accumulation in the fruits at immature green and mature green stages than the WT plants (Fig. 2c). In leaves, the upregulated and downregulated SIARF6A transgenic lines possessed higher and lower chlorophyll levels, respectively, than the WT plants (Fig. 2d). Then, chlorophyll autofluorescence in the pericarp was detected using confocal laser scanning microscopy. OE-SIARF6A plants had stronger chlorophyll autofluorescence, while the RNAi-SIARF6A lines had weaker chlorophyll autofluorescence in both epicarp and endocarp tissues compared with that of the WT plants (Fig. 3a). Then, the chloroplasts were observed using a transmission electron microscope (TEM). The growth of individual chloroplasts in OE-SIARF6A fruits was obviously promoted, with a significant increase in size and length (Fig. 3b). However, the number of chloroplasts per cell in OE-SIARF6A fruits was the same as that in the WT plants. For the RNAi-SIARF6A lines, the number of chloroplasts per cell was obviously decreased, but the size of individual chloroplasts was not changed (Fig. 3c–e).

\( \text{SIARF6A} \) positively affects photosynthesis and photosynthesize accumulation in tomato

The dark-green phenotype and associated increased chlorophyll content may potentially lead to enhanced photosynthetic performance in tomato plants. The
photosynthetic performance in leaves and fruits of SlARF6A transgenic lines was measured. In both leaves and green fruits, the photochemical potential was elevated in OE-SlARF6A lines, whereas the value was decreased in RNAi-SlARF6A plants compared with the WT plants (Fig. 4a, b). The effective photochemical quantum yield of PSII in OE-SlARF6A lines was higher than that of the WT plants in both leaves and fruits, while the values for RNAi-
Fig. 4 Photochemical potential of SIARF6A transgenic plants and accumulation of photosynthetic substances in transgenic fruits. 

a Photochemical potential in leaves.  

b Photochemical potential in fruits.  
c Effective photochemical quantum yield of PS II in leaves.  
d Effective photochemical quantum yield of PS II in fruits.  
e–h demonstrate the contents of starch (e), glucose (f), fructose (g), and sucrose (h) in transgenic plants, respectively. The data represent the mean ± SD of three biological replicates. ** and *** indicate significant differences between the transgenic and WT plants at $P<0.05$ and $P<0.01$, respectively, as determined by t-test.
SIARF6A plants were lower than that for the WT plants in both leaves and fruits (Fig. 4c, d). Thus, the SIARF6A gene positively affects photosynthesis in the fruits and leaves of tomato plants.

Sugars are the major products of photosynthesis, so it is essential to evaluate whether the altered chlorophyll level and photosynthetic performance in SIARF6A plants result in altered sugar accumulation. As shown in Fig. 4e, starch levels decreased rapidly throughout fruit development in the transgenic and WT plants. The starch content in OE-SIARF6A fruits was much higher than that in WT fruits at green fruit stages, whereas the starch content in RNAi-SIARF6A fruits was much lower than that in the WT fruits at green stages (Fig. 4e). These data demonstrated that the SIARF6A gene positively affects starch accumulation during green fruit development.

It is well established that starch degradation is the dominant source of soluble sugars in fruits. The contents of fructose, glucose and sucrose were analyzed in SIARF6A transgenic plants. The levels of glucose, fructose and sucrose were significantly higher in the OE-SIARF6A fruits than in the WT fruits, particularly at the orange and red fruit stages (Fig. 4f–h). Compared with the WT fruits, the RNAi-SIARF6A fruits exhibited obviously decreased contents in glucose, fructose and sucrose (Fig. 4f–h). Our data indicated that the SIARF6A gene positively affects the levels of glucose, fructose and sucrose during fruit development.

**SIARF6A is involved in fruit ripening and ethylene production in tomato**

The SIARF6A transgenic plants also exhibited different ripening of fruits than the WT plants. Downregulation of SIARF6A accelerated fruit ripening, with the breaker stage occurring 5 days sooner than that in the WT plants (Fig. 2b), while overexpression delayed the breaker stage by 5 days compared with that of the WT plants (Fig. 2b). The assessment of color change via measurement of the evolution of hue angle values further confirmed the difference between the SIARF6A transgenic lines and WT plants throughout the ripening process (Fig. 5a). The ethylene production was measured using a GC method. When compared with that of the WT plants, the ethylene production of RNAi-SIARF6A plants showed a dramatic induction of ~2-fold and 4-fold at the breaker stage and remained at high levels for 2 and 3 days after the breaker stage, while that of overexpressed lines was inhibited at the breaker stage and remained at low levels for 5 days after the breaker stage compared with the levels in the WT plants (Fig. 5b).

**Regulation of SIARF6A expression alters the expression of genes involved in chlorophyll metabolism, photosynthesis and sugar metabolism**

To investigate the molecular mechanism of chlorophyll accumulation, photosynthesis and fruit ripening in SIARF6A transgenic plants, RNA-sequencing (RNA-Seq)
was conducted to analyze the differentially expressed genes (DEGs) in OE-SlARF6A and RNAi-SlARF6A plants. Under the criterion of a false discovery rate (FDR) < 0.05, 591 upregulated and 508 downregulated DEGs were identified in 4 DPA ovaries of RNAi-SlARF6A plants, and 254 upregulated and 424 downregulated DEGs were identified in 35 DPA fruits of OE-SlARF6A plants (Table S1). GO function and pathway enrichment analyses showed that knockdown of SlARF6A affected multiple metabolic pathways, including those of porphyrin and chlorophyll metabolism, photosynthesis, photosynthesis-antenna proteins, carbon fixation, starch and sucrose metabolism, fructose and mannose metabolism, and plant hormone signal transduction (Fig. 6a, Table S2). Overexpression of SlARF6A also affected metabolic pathways, including those of photosynthesis, photosynthesis-antenna proteins, carbon fixation, starch and sucrose metabolism, fructose and mannose metabolism, and plant hormone signal transduction (Fig. 6b, Table S3). The expression of two genes encoding chlorophyll A/B binding protein (CAB1 and CAB2) (Solyc02g070950 and Solyc02g071010) was induced in OE-SlARF6A plants. The expression of a gene encoding ribulose bisphosphate carboxylase small chain (RbcS) (Solyc02g085950) was upregulated in OE-SlARF6A plants. Moreover, the expression of a gene encoding SAM synthetase 1 (SAMS1) (Solyc12g099000), which is involved in ethylene biosynthesis, was induced in RNAi-SlARF6A plants. Analysis of the RNA-Seq data also showed that among tomato ARF family genes, only SlARF6A was

![Fig. 6 RNA-Seq analysis of SlARF6A transgenic plants.](image)

- **a** Functional categories of differentially expressed genes (DEGs) between WT and RNAi-SlARF6A plants.
- **b** Functional categories of differentially expressed genes (DEGs) between WT and OE-SlARF6A plants.
- **c** Transcript levels of the genes identified from the RNA-Seq analysis were validated by qRT-PCR in the RNAi-SlARF6A plants (c) and OE-SlARF6A plants (d). The solid line indicates relative expression levels in the WT. The data represent the mean ± SD of four biological replicates.
SIARF6A directly targets the SAMS1 promoter and negatively regulates SAMS1 expression

SAMS1 is the key enzyme catalyzing the synthesis of SAM in the ethylene biosynthesis pathway. Motif analysis showed that the SAMS1 promoter contains the conserved ARF binding site, the TGTCTC box. The transient expression assays showed that the LUC/REN ratios were significantly decreased compared with that of the control, suggesting that SIARF6A negatively regulates the expression of SAMS1 genes (Fig. 8a, b). ChIP-qPCR was carried out to confirm the binding of SIARF6A with the SAMS1 promoter in vivo, and the results showed that the promoter sequences containing the TGTCTC of SAMS1 were significantly enriched compared with those with the negative control anti-IgG (Fig. 8c).

The direct binding of SIARF6A protein to the SAMS1 promoter was further verified by EMSA. The results indicated that the SIARF6A protein directly bound to the TGTCTC motif in the SAMS1 promoter (Fig. 8d). Taken together, SIARF6A can target the SAMS1 promoter and negatively regulate the expression of SAMS1 genes. The data demonstrate that SIARF6A plays an important role in ethylene production and fruit ripening.

Discussion

In this study, we functionally characterized the transcription factor SIARF6A in tomato. However, there are two very similar SIARF6 genes in the tomato genome, namely, SIARF6A and SIARF6B. We also examined the function of SIARF6B using genetic approaches and found no obvious phenotypes in the transgenic RNAi and overexpression tomato plants (data not shown). This may be related to the fact that SIARF6B lacks the AUX/IAA domain in the C-terminus of the protein (Fig. S1).

SIARF6A regulates photosynthesis in tomato

Previous studies reported that chlorophyll accumulation increased in Arabidopsis roots when they were detached from shoots, which was repressed by auxin treatment. Mutant analyses showed that auxin inhibits the accumulation of chlorophyll through the function of IAA14, ARF7, and ARF19 in Arabidopsis. In tomato, SIARF4 plays an important role as an inhibitor in chlorophyll biosynthesis and sugar accumulation via transcriptional inhibition of SIGLKL expression in tomato.

In this study, overexpression of SIARF6A resulted in enhanced chlorophyll accumulation and chloroplast development, whereas downregulation of SIARF6A decreased chlorophyll accumulation and chloroplast number in tomato (Fig. 3). These results demonstrate that SIARF6A positively regulates chlorophyll accumulation and chloroplast number in tomato. Our study also showed that SIARF6A directly targeted the SIGLKL promoter and positively regulated SIGLKL expression (Fig. 7). Nguyen et al. (2014)
reported that overexpression of *SlGLK1* and *SlGLK2* produced dark-green fruits and increased chlorophyll accumulation and chloroplast development\(^8\). The fact that the phenotypes of *SlGLK1* overexpression plants resembled those described in the OE-*SlARF6A* plants further suggests that *SlARF6A* positively regulates *SlGLK1* to improve chlorophyll accumulation and chloroplast development in tomato leaves and fruits.

Although *SlGLK1* and *SlGLK2* have similar functions, *SlGLK1* functions largely in leaves, while *SlGLK2* functions in fruits\(^8\). However, *SlGLK2* does not account for the chlorophyll phenotypes in OE and RNAi-*SlARF6A* plants because the ‘Micro-Tom’ variety possesses two null alleles of *SlGLK2\(^39\). In our study, downregulation of *SlARF6A* reduced *SlGLK1* expression and chlorophyll accumulation, whereas overexpression of *SlARF6A* increased *SlGLK1* expression and chlorophyll accumulation in leaves and fruits of tomato plants (Figs. 2 and 3). The data demonstrate that *SlGLK1* may be involved in chlorophyll accumulation not only in tomato leaves but also in fruits. Further study is needed to elucidate the important role of *SlGLK1* in tomato fruit using CRISPR/Cas9 technologies.

The chlorophyll a/b-binding proteins (CABs) are the apoproteins of the light-harvesting complex of photosystem II (PSII). CABs are normally complexed with xanthophylls and chlorophyll, functioning as the antenna
complex, and are involved in photosynthetic electron transport\textsuperscript{10}\textsuperscript{10}. Meng et al. (2018) reported that SlBEL11 directly acted on the promoter of CABs to suppress their transcription\textsuperscript{10}. Silencing of SlBEL11 increased the expression of CAB genes, resulting in enhanced chlorophyll accumulation and stability in thylakoid membranes of chloroplasts in green tomato fruit\textsuperscript{10}. In our study, SlARF6A targeted the promoter of CABs, which positively regulated chlorophyll accumulation, chloroplast development and photosynthesis in tomato (Figs. 2, 3, 4 and 6). Our data further demonstrate important roles of CABs in chloroplast activity and photosynthesis in tomato.

Rubisco, a key enzyme in the fixation of CO\textsubscript{2}, is the rate-limiting factor in the photosynthesis pathway under conditions of saturating light and atmospheric CO\textsubscript{2}\textsuperscript{41}. The RbcL and RbcS genes encode two subunits that form the Rubisco enzyme\textsuperscript{42}. The RbcL and RbcS genes are localized to the chloroplasts and to the nucleus, respectively\textsuperscript{43}. Our study showed that overexpression of SlARF6A increased the expression of the RbcS gene. Moreover, SlARF6A directly targeted the RbcS promoter and positively regulated RbcS expression (Fig. 7). In addition, SlARF6A positively affected photosynthesis in the fruits and leaves of tomato plants (Fig. 4). Our study demonstrates that SlARF6A has important roles in photosynthesis via the direct regulation of the RbcS gene in tomato.

Interestingly, RNA-Seq data showed that the expression levels of SlARF4 and SlARF10 genes were not altered in RNAi-SlARF6A and OE-SlARF6A plants, suggesting that SlARF6A may act on chlorophyll accumulation...
between SlARF6A and the promoters of tomato. However, the EMSA failed to detect any binding even though auxin-responsive motifs were detected in the expression of AGPase.

In this study, AGPase catalyzes the first regulatory step in starch biosynthesis, converting glucose-1-phosphate and ATP into ADP-glucose. This critical catalytic reaction is also a limiting step during starch biosynthesis in potato (Solanum tuberosum) tubers. Knockdown of SlARF4 increases the expression of AGPase genes and starch content.

Evidence suggests that sucrose induces the expression of AGPase genes in leaves and fruits. In this study, overexpression of SlARF6A led to increased sucrose content in tomato fruits, while the RNAi-SlARF6A fruits displayed decreased sucrose accumulation.

Overexpression of SlARF6A also resulted in increased glucose and fructose content, which was likely due to the increased starch content degraded into increased contents of soluble sugars in tomato fruits. Our results are consistent with the notion that incipient starch content determines soluble sugars in the process of fruit development. Our study also provides a valuable method to improve the nutritional value of tomato fruits via regulation of SlARF6A expression.

**SlARF6A regulates photosynthesis accumulation in tomato**

Downregulation of SlARF4 increased the photosynthesis rate and enhanced the accumulation of starch, glucose and fructose in tomato fruits. In this study, the increased chlorophyll accumulation and photosynthesis rate in OE-SlARF6A plants resulted in the increased contents of starch and soluble sugars in fruits.

Starch is a dominant factor in the nutrients and flavor of fruits. AGPase catalyzes the first regulatory step in starch synthesis, converting glucose-1-phosphate and ATP into ADP-glucose. This critical catalytic reaction is also a limiting step during starch biosynthesis in potato (Solanum tuberosum) tubers. Knockdown of SlARF4 increases the expression of AGPase genes and starch content.

In this study, SlARF6A was positively correlated with the expression of AGPase genes, suggesting the important role of AGPase genes in starch biosynthesis in tomato. However, the EMSA failed to detect any binding between SlARF6A and the promoters of AGPase genes, even though auxin-responsive motifs were detected in the promoters of AGPase S1 and AGPase S2 genes.

Evidence suggests that sucrose induces the expression of AGPase genes in leaves and fruits. In this study, overexpression of SlARF6A led to increased sucrose content in tomato fruits, while the RNAi-SlARF6A fruits displayed decreased sucrose accumulation.

The altered accumulation of starch in OE-SlARF6A and RNAi-SlARF6A lines may be explained by the altered expression of AGPase genes influenced by sucrose in tomato. Overexpression of SlARF6A also resulted in increased glucose and fructose content, which was likely due to the increased starch content degraded into increased contents of soluble sugars in tomato fruits. Our results are consistent with the notion that incipient starch content determines soluble sugars in the process of fruit development. Our study also provides a valuable method to improve the nutritional value of tomato fruits via regulation of SlARF6A expression.

**SlARF6A is involved in ethylene production and fruit ripening in tomato**

The tomato ARF2A gene was reported to positively regulate fruit ripening. Overexpression of ARF2A in tomato resulted in blotchy ripening, and silencing of ARF2A led to retarded fruit ripening. Overexpression of ARF2A in tomato promoted early production of ethylene and expression of ethylene biosynthesis and receptor genes. In this study, SlARF6A negatively regulated fruit ripening and ethylene biosynthesis in tomato fruit (Fig. 5).

S-adenosyl-L-methionine (SAM), synthesized by SAM synthetase from ATP and methionine, is a substrate for ethylene biosynthesis (Roje, 2006). SAM is converted to ACC by the ACS enzyme, and ACC is then converted to ethylene by ACO.

The level of SAM is tightly controlled to integrate developmental signals into the hormonal control of plant development. In Arabidopsis, overexpression of SAMS1 increases the SAM and ethylene levels, whereas sam1/2 mutants show the opposite phenotype in seedlings.

Similarly, in tomato plants, overexpression of SAMS1 results in higher concentrations of ACC and ethylene compared with those in WT plants. These data indicate the important role of the SAMS1 gene in ethylene biosynthesis in plants. In this study, SlARF6A directly targeted the SAMS1 promoter and negatively regulated SAMS1 expression (Fig. 8).

The regulatory mechanism by which SlARF6A affects fruit ripening and ethylene production in tomato fruits can be explained by the interaction between SlARF6A and the SAMS1 promoter.

It is interesting that ethylene and auxin interact with each other to control some plant developmental processes. For example, ethylene controls root growth through regulation of auxin biosynthesis, transport and signaling, while the formation of hypocotyl apical hooks is also regulated in a similar fashion in Arabidopsis.

In tomato, knockdown of IAA3 results in both auxin and ethylene phenotypes, suggesting that IAA3 might be the molecular connection between ethylene and auxin. Liu et al. (2018) reported that the ethylene response factor SIERFB3 integrated ethylene and auxin signaling through direct regulation of the Aux/IAA27 gene in tomato. Our results indicate that SlARF6A negatively regulates ethylene biosynthesis and that the interaction of SlARF6A and SAMS1 represents an important integrative hub mediating ethylene-auxin cross-talk in tomato.

In summary, our results demonstrate that SlARF6A regulates chlorophyll level and chloroplast development by directly binding to the promoters of the SIGK1, CAB1, and CAB2 genes. SlARF6A also directly targets the RbcS gene promoter, activating RbcS expression and increasing the photosynthetic rate. The increased chlorophyll accumulation and chloroplast activity improve photosynthesis, resulting in the increased accumulation of starch and soluble sugars in tomato. In addition, SlARF6A can act directly on the promoter of SAMS1 and negatively regulate its expression, thereby influencing ethylene.
production and fruit ripening. The present study provides new insight into the link between auxin signaling, chloroplast activity, and ethylene biosynthesis during tomato fruit development. Our data also provide an effective way to improve fruit nutrition of horticulture crops via regulation of chlorophyll accumulation and photosynthetic activity.

**Materials and methods**

**Plant materials and growth conditions**

Tomato plants (*Solanum lycopersicum* ‘Micro-Tom’) were used in this study. ‘Micro-Tom’ is a popular variety because of its fast turnaround time and easy transformation. The plants were grown on soil under standard greenhouse conditions with a 14-h-day/10-h night cycle, 25 °C/20 °C day/night temperature, 60% relative humidity and 250 mol m^−2^ s^−1^ intense light. Transgenic seeds of T1, T2 and T3 generations were screened by sterilizing, rinsing in sterile water, and then transfer into Magenta vessels containing 40 mL of 1/2-strength Murashige and Skoog medium with R3 vitamin (100 mg L^−1^ kanamycin, 0.5 mg L^−1^ thiamine, 0.5 mg L^−1^ pyridoxine and 0.25 mg L^−1^ nicotinic acid), 0.8% (w/v) agar, and 1.5% (w/v) sucrose, pH 5.9.

**Plasmid construction and generation of transgenic plants**

DNA fragments, the *SIARF6A* (Solyc12g006340) promoter, the full-length *SIARF6A* coding sequence and a partial *SIARF6A* coding sequence were amplified using tomato genomic DNA or cDNA. The PCR primers used for amplification are detailed in Supplementary Table S3. The *SIARF6A* promoter sequence was cloned into a pLP100 vector containing the GUS reporter gene. To obtain overexpressed *SIARF6A* vector, the ORF sequence of *SIARF6A* was cloned into plant binary vector pLP100 in the sense orientation under the transcriptional control of a cauliflower mosaic virus (CaMV) 35S promoter. For construction of the RNAi vector, the 200 bp sequences of *SIARF6A* were amplified and inserted in pCAMIBA2301 under control of the (CaMV) 35S promoter and a nopaline synthase terminator. The resulting transgenic plant was obtained by *Agrobacterium tumefaciens*-mediated transformation according to Jones et al. (2002)\(^{13}\). All experiments were performed using homoyzogous lines from the T3 generation.

**qRT-PCR analysis**

Tomato total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), and qRT-PCR was carried out using All-in-One™ qPCR Mix (GeneCopoea, Rockville, MD, USA) with a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) according to Zhang et al. (2015)\(^{32}\). The relative expression levels of genes were calculated from ΔΔCt values using ubiquitin gene expression as an internal control. The primer sequences used for qRT-PCR are listed in Supplementary Table S3.

**GUS staining and analysis**

Tissues from *SIARF6A* promoter-GUS plants were collected and submerged in GUS staining solution (0.1 M sodium phosphate buffer, pH 7.2, 10 mM EDTA). After being infiltrated with GUS staining solution under vacuum for 15 min twice, tissues were incubated in the solution at 37 °C overnight. Then, the samples were washed via a graded ethanol series and observed under a light microscope.

**Subcellular localization and transcriptional activation activity of *SIARF6A***

The ORF sequence of *SIARF6A* was cloned into a PCX-DG vector to generate the *SIARF6A-GFP* fusion expression vector. Specific primer sequences are listed in Supplementary Table S1. Suspension-cultured tobacco (*Nicotiana tabacum* cv. Bright Yellow-2) cells were used to obtain protoplasts that were transfected with the *SIARF6A-GFP* fusion expression vector. Transformation assays were carried out according to the procedures described by Chaabouni et al. (2009)\(^{60}\).

The ORF sequence of *SIARF6A* was amplified and fused to the GAL4 DNA-binding (DB) domain to obtain the pGBKT7-*SIARF6A* fusion construct (DB-*SIARF6A*). The pGBK7-*SIARF6A* vectors were transformed into Y2H Gold yeast cells and cultivated on plates in minimal medium without tryptophan (SD-Trp) or without tryptophan, histidine, and adenine (SD-Trp/His/Ade). The transcriptional activation activity was analyzed based on the growth status and α-galactosidase (α-gal) activity.

**Chlorophyll analysis and chlorophyll fluorescence parameter measurements**

For chlorophyll content determination, the fruits at different developmental stages and leaf tissues were collected and examined based on the methods described by Powell et al. (2012)\(^{39}\). To determine chlorophyll autofluorescence, pericarp was peeled off tomato fruits and observed with a TCS SP2 laser confocal microscope (Leica, Germany). For transmission electron microscopy, pericarp tissues were examined with a FEI Tecnai T12 twin transmission electron microscope according to the method described by Nguyen et al. (2014)\(^8\).

For measurements of photosynthesis rates, the green mature fruits and leaves were measured via a Pam-2500 pulse-amplitude modulation fluorometer (Heinz Walz, Effeltrich, Germany). The chlorophyll fluorescence parameter was measured based on the method described by Maury et al. (1996)\(^{64}\).
**Extraction and assay of metabolites**

For sugar extraction, 1 g of fruit tissue was collected and ground under liquid nitrogen. Subsequently, 10 mL of 80% (v/v) ethanol was used for extraction three times at 80°C for 30 min. After centrifugation, samples were completely evaporated under vacuum and then dissolved in 4 mL of distilled water. Using the dissolved samples, HPLC was carried out to determine the content of sucrose, fructose and glucose. Starch content determination was performed using fruit pellets. Four milliliters of 0.2 M KOH was used to dissolve the pellet by incubating the sample in a boiling water bath for 30 min. Then, 1.48 mL of 1 M acetic acid (pH 4.5) with 7 units of amyloglucosidase was employed to hydrolyze each sample for 45 min. Finally, 10 mL of distilled water was adopted to dissolve the sample, and then the dissolved sample was used for starch content measurement.

For metabolite measurement, HPLC analysis was conducted using an Agilent 1260 Series liquid chromatograph system (Agilent Technologies, Palo Alto, CA, USA) with a vacuum degasser, an autosampler, a binary pump, and a diode array detector (DAD) controlled by Agilent ChemStation software. A precolumn (Waters XBridge BEH Amide column, 3.9 × 5 mm i.d., 3.5 μm) and a Waters XBridge Amide column (4.6 × 150 mm i.d., 3.5 μm) were used for analysis. The separation was performed via an isocratic solvent system with solvent A (0.2% triethylamine water solution) and solvent B (acetonitrile), while the mobile phase was maintained at 75% B for 15 min for elution. The column temperature was maintained at 38 °C, and the solvent flow rate was 0.6 mL/min. Meanwhile, the injection volume was 10 μL for each sample. With a drift tube temperature at 80 °C, the detection system for HPLC was an ELSD 2000, and air was used as the carrier gas with a flow rate of 2.2 L/min. Finally, the contents of glucose, fructose, sucrose and starch in tomato fruits were determined based on the methods described by Geigenberger et al. (1996)62.

**RNA-Seq analysis**

The ovaries (4 DPA) of WT and RNAi-SlARF6A plants and the mature green fruits (35 DPA) of WT and OE-SlARF6A plants were collected for RNA-Seq analysis. Total RNA was isolated using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). RNA-Seq was carried out at the Shanghai Majorbio Biopharm Technology Co., Ltd., as described by Zhang et al. (2015)32. The Illumina HiSeq™ 2000 platform was used according to the manufacturer’s instructions. All clean reads were aligned to the tomato genome (http://solgenomics.net/organism/Solanum_lycopersicum/genome) using TopHat (http://tophat.cbcb.umd). Transcript abundance was normalized by the fragments per kilobase of exon per million mapped reads (FRKM) method using Cuffdiff software (http://cole-trapnell-lab.github.io/cufflinks/). A false discovery rate (FDR) of less than 0.05 was used as the threshold for differentially expressed genes (DEGs). GO functional enrichment and KEGG pathway analysis were carried out using goatools (https://github.com/tanghaibao/goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do). Pathway enrichment was analyzed using the Benjamini and Hochberg correction method with FDR < 0.05.

**Promoter analysis and dual-luciferase transient expression assay**

For promoter analysis, PLACE signal scan search software (http://www.dna.affrc.go.jp/PLACE/signalscan.html) was used to analyze the motifs of target genes. A dual-luciferase transient expression assay for SlARF6A was carried out using tobacco leaves (Nicotiana benthamiana). For effector vector construction, the full coding sequence of SlARF6A was amplified and then cloned into the pGreenII 62-SK vector. For reporter vector construction, the promoters of SIGLK1, CAB, RbcS, and SAMSI genes were cloned into a pGreenII 08000-LUC vector (Hellens et al., 2005)63. The primer sequences used for the vector construct are shown in Supplementary Table S3. A dual-luciferase assay kit (Promega, USA) was employed to measure the activities of LUC and REN luciferase according to the manufacturer’s instructions via a Luminoskan Ascent microplate luminometer (Thermo Fisher Scientific, USA). For each pair of vectors, six biological repeats were performed.

**Protein expression and EMSA**

The nucleotide sequence of the putative DNA-binding domain of SlARF6A (from 1 to 978 bp) was amplified and fused to that of the glutathione S-transferase (GST) tag in a pGEX-4T-1 bacterial expression vector (GE Healthcare Life Science, China) and expressed using Escherichia coli strain BM Rosetta (DE3). Isopropyl-β-D-thiogalactopyranoside (1 mM) was used to induce recombinant protein expression, and a GST-Tagged Protein Purification Kit (Clontech, USA) was used to purify the protein. Purified recombinant proteins and biotin-labeled fragments of the target promoters were used to conduct EMSA with a LightShift Chemiluminescent EMSA kit (Thermo Fisher Scientific, USA) based on the method described in detail by Han et al. (2016)64. The Pierce Biotin 3’ End DNA Labeling Kit (Thermo Fisher Scientific, USA) was employed to label the probe containing the TGTCTC sequence with biotin. The unlabeled same sequence was used in the assay as a competitor. To generate the mutant probe, the TGTCTC DNA fragment was changed to AAAAA. Biotin-labeled DNA was assayed via a ChemiDoc™ MP Imaging System (Bio-Rad, USA) based on the manufacturer’s procedures. All primers for the EMSA are listed in Supplementary Table S3.
Chromatin immunoprecipitation

A ChiP-qPCR assay was carried out based on the method described in detail by Qin et al. (2012). All primer sequences used in this analysis are listed in Table S3.

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Author contributions

W.D., Y.Y., X.C., C.J. and Z.G. contributed to the experimental design and data analysis; F.Y., Y.T., Z.X. and X.H. contributed to chlorophyll analysis; Q.Z. and F.Y. contributed to sugar measurement; and W.Z., L.M. and Y.L. contributed to RNA-Seq data analysis. All authors edited the combined manuscript. W.D., Y.Y. and C.J. finalized the article.

Author details

1. Key Laboratory of Plant Hormones and Development Regulation of Chongqing, School of Life Sciences, Chongqing University, 401331 Chongqing, China. 2. School of Chemistry and Chemical Engineering, Chongqing University, 400044 Chongqing, China. 3. College of Basic Science, Tianjin Agricultural University, 300384 Tianjin, China. 4. Department of Plant Sciences, University of California, Davis, CA 95616, USA. 5. Crops Pathology and Genetics Research Unit, United States Department of Agriculture, Agricultural Research Service, Davis, CA 95616, USA. 6. State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources/Guangdong Provincial Key Laboratory of Postharvest Science of Fruits and Vegetables, College of Horticulture, South China Agricultural University, 510642 Guangzhou, China

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary Information

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References

1. Kleer, H. J. & Giovannoni, J. J. Genetics and control of tomato fruit ripening and quality attributes. Annu. Rev. Genet. 45, 41–59 (2011).
2. Ho, L. C. & Hewitt, J. D. Fruit development. In: Atherton, J. G. & Rudich, J. (eds) The Tomato Crop. 201–240 (Chapman and Hall, London, 1986).
3. Schaffer, A. A. & Petekov, M. Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. Plant Physiol. 113, 739–746 (1997).
4. Nadakuduti, S. S., Holdsworth, W. L., Klein, C. L. & Barry, C. S. KNOX genes influence a gradient of fruit chloroplast development through regulation of GOLDEN2-LIKE expression in tomato. Plant Cell 78, 1022–1033 (2014).
5. Koltin, I. et al. Transcriptional profiling of high pigment 2 (dpd) tomato mutant links early fruit plastid biogenesis with its overproduction of photynthetins. Plant Physiol. 145, 389–401 (2007).
6. Rohmann, J. et al. Combined transcription factor profiling, microarray analysis and metabolite profiling reveals the transcriptional control of metabolic shifts occurring during tomato fruit development. Plant J. 68, 999–1013 (2011).
7. Waters, M. T., Moylan, E. C. & Langdale, J. A. GLK transcription factors regulate chloroplast development in a cell-autonomous manner. Plant J. 56, 432–444 (2008).
8. Nguyen, C. V. et al. Tomato Golden 2-like (GLK) transcription factors reveal molecular gradients functioning during fruit development and ripening. Plant Cell 26, 585–601 (2014).
9. Pan, Y. et al. Network inference analysis identifies an APR2-like gene linked to pigment accumulation in tomato and pepper fruits. Plant Physiol. 161, 1476–1485 (2013).
10. Meng, L. H. et al. BEL1-LIKE HOMEDOMAIN1 regulates chloroplast development and chlorophyll synthesis in tomato fruit. Plant Cell 94, 1126–1140 (2018).
11. Li, S. et al. The RN-MC fusion of MADS-box transcription factors has transcriptional activity and modulates expression of many ripening genes. Plant Physiol. 176, 891–909 (2018).
12. Gao, Y. et al. A NAC transcription factor, NOR-like1, is a new positive regulator of tomato fruit ripening. Hort. Sci. 5, 75 (2018).
13. Roje, S. S-Adenosyl-L-methionine: beyond the universal methyl group donor. Physiochemistry 67, 1686–1698 (2006).
14. Barry, C., Llop-Tous, M. I. & Grierson, D. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol. 123, 979–986 (2000).
15. Manning, K. et al. A naturally occurring epimutigenic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat. Genet. 38, 948–952 (2006).
16. Ihtkin, M. et al. TOMATO AGAMOUS-LIKE 1 is a component of the fruit ripening regulatory network. Plant J. 60, 1081–1095 (2009).
17. Chung, M. Y. et al. A tomato (Solanum lycopersicum) APETALA2/ERF gene, SIAP2a, is a negative regulator of fruit ripening. Plant J. 64, 936–947 (2010).
18. Korkova, R. et al. Transcriptome and metabolite profiling show that APETALA2a is a major regulator of tomato fruit ripening. Plant Cell 23, 923–941 (2011).
19. Bemer, M. et al. The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. Plant Cell 24, 4437–4451 (2012).
20. Gao, Y. et al. Diversity and redundancy of the ripening regulatory networks revealed by the FRUITFULL and the new CRP/GRF19 CRR and NOR mutants. Hortic. Res. 6, 39 (2019).
21. Liu, M. et al. The chimeric repressor version of an Ethylene Response Factor (ERF) family member, Sf-ERF3, shows contrasting effects on tomato fruit ripening. New Phytol. 203, 206–218 (2014a).
22. De Jong, M., Wolters-Ants, M., Feron, R., Mariani, C. & Vriezen, W. H. The Solanum lycopersicum auxin response factor 7 (SLARF7) regulates auxin signaling during tomato fruit set and development. Plant J. 57, 160–170 (2009).
23. Devogelaere, F. et al. A genomics approach to understanding the role of auxin in apple (Malus x domestica) fruit growth control. BMC Plant Biol. 12, 7 (2012).
24. Ulmasov, T., Hagen, G. & Guilfoyle, T. Dimerization and DNA binding of auxin response factors. Plant J. 19, 309–319 (1999).
25. Guilfoyle, T. J. & Hagen, G. Auxin response factors. Curr. Opin. Plant Biol. 10, 453–460 (2007).
26. Guilfoyle, T. J. & Hagen, G. Getting a grasp on domain III/IV responsible for Auxin Response Factor–IAA protein interactions. Plant Sci. 190, 82–88 (2012).
27. Zouine, M. et al. Characterization of the tomato ARF gene family uncovers a multi-levels post-transcriptional regulation including alternative splicing. PLoS ONE 9, e88203 (2014).
28. Wang, Y. et al. Diversification, phylogeny and evolution of auxin response factor (ARF) family: insights gained from analyzing maize ARF genes. Mol. Biol. Rep. 39, 2401–2415 (2012).
29. Guan, X. X. et al. Temporal and spatial distribution of auxin response genes during tomato flower abscission. J. Plant Growth Regul. 33, 17–327 (2013).
30. Liu, X. et al. AUXIN RESPONSE FACTOR 3 integrates the functions of AGAMOUS and APETALA2 in floral meristem determination. Plant J. 80, 629–641 (2014a).
31. Okurushumova, W., Smimova, T., Marcos, D., Zayed, Y. & Berleth, T. Irrepressible MONOPTEROS/ARF5 promotes de novo shoot formation. New Phytol. 204, 556–566 (2014).
32. Zhang, X. L. et al. Auxin response gene SARF3 plays multiple roles in tomato development and is involved in the formation of epidermal cells and trichomes. Plant Cell Physiol. 56, 2110–2124 (2015).
33. Jones, B. et al. Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. Plant Cell 32, 603–613 (2002).
34. Sagar, M. et al. SI-ARF4, an auxin response factor involved in the control of sugar metabolism during tomato fruit development. Plant Physiol. 161, 1362–1374 (2013).
35. Yuan, Y. J. et al. SIARF10, an auxin response factor, is involved in chlorophyll and sugar accumulation during tomato fruit development. J. Exp. Bot. 69, 5507–5518 (2018).

36. Liu, N. et al. Down-regulation of AUXIN RESPONSE FACTORS 6 and 8 by microRNA 167 leads to floral development defects and female sterility in tomato. J. Exp. Bot. 65, 2507–2520 (2014).

37. Krogan, N. T., Churumová, W., Marcos, D., Caragea, A. E. & Berleth, T. Deletion of MP/ARFS domains III and IV reveals a requirement for Aux/IAA regulation in Arabidopsis leaf vascular patterning. New Phytol. 194, 391–401 (2011).

38. Kobayashi, K. et al. Regulation of root greening by light and auxin/cytokinin signaling in Arabidopsis. Plant Cell 24, 1081–1105 (2012).

39. Powell, A. L. T. et al. Uniform ripening encodes a Golden 2-like transcription factor regulating tomato fruit chloroplast development. Science 336, 1711–1715 (2012).

40. Spreitzer, M. E. & Salvucci, R. J. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Ann. Rev. Plant Biol. 53, 449–475 (2002).

41. Patel, M. & Berry, J. O. Rubisco gene expression in C4 plants. J. Exp. Bot. 59, 1625–1634 (2008).

42. Spreitzer, M. E. & Salvucci, R. J. Rubisco: structure, regulatory interactions, and possibilities for a better enzyme. Ann. Rev. Plant Biol. 53, 449–475 (2002).

43. Sasanuma, T. Characterization of the rbcS multigene family in wheat: sub-family classification, determination of chromosomal location and evolutionary analysis. Mol. Gen. Genet. 265, 161–171 (2001).

44. Stark, D. M., Timmerman, K. P., Barry, G. F., Press, J. & Kishore, G. M. Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. Science 258, 287–292 (1992).

45. Yin, Y. G. et al. Salinity induces carbohydrate accumulation and sugar-regulated starch biosynthetic genes in tomato (Solanum lycopersicum L. cv/Micro-Tom) fruits in an ABA- and osmotic stress-independent manner. J. Exp. Bot. 61, 563–574 (2009).

46. Tisschen, A. et al. Starch synthesis in potato tubers is regulated by post-translational redox-modification of ADP-glucose pyrophosphorylase. A noble regulatory mechanism linking starch synthesis to the sucrose supply. Plant Cell 14, 2191–2213 (2002).

47. Sauter, M., Moffatt, B., Saechao, M. C., Hell, R. & Wirtz, M. Methionine salvage and S-adenosylmethionine: essential links between sulfur, ethylene and polyamine biosynthesis. Biochim. Biophys. Acta 1584, 145–154 (2013).

48. Schaffer, A. A. et al. ADP glucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a Lycopersicon hirsutum-derived introgression encoding for the large subunit. Plant Sci. 152, 135–144 (2000).

49. Baxter, C. J. et al. Fruit carbohydrate metabolism in an introgression of tomato with increased fruit soluble solids. Plant Cell Physiol. 46, 425–437 (2005).

50. Breitel, D. A. et al. AUXIN RESPONSE FACTOR 2 intersects hormonal signals in the regulation of tomato fruit ripening. Plant. Sci. Genet. 12, e1005903 (2016).

51. Wang, K. L., Li, H. & Ecker, J. R. Ethylene biosynthesis and signaling networks. Plant Cell 14, S131–S151 (2002).

52. Yang, S. F. & Hoffman, N. E. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35, 155–189 (1984).

53. Van de Poel, B. et al. Targeted systems biology profiling of tomato fruit reveals coordination of the Yang cycle and a distinct regulation of ethylene biosynthesis during post-climacteric ripening. Plant Physiol. 160, 1498–1514 (2012b).

54. Mao, D. et al. FERONIA receptor kinase interacts with S-adenosylmethionine synthetase and suppresses S-adenosylmethionine production and ethylene biosynthesis in Arabidopsis. Plant Cell Environ. 38, 2566–2574 (2015).

55. Gong, B. et al. Overexpression of S-adenosyl-l-methionine synthetase increased tomato tolerance to alkalai stress through polyamine metabolism. Plant Biotechnol. J. 12, 694–708 (2014).

56. Ruzicka, K. et al. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. Plant Cell 19, 2197–2212 (2007).

57. Stepanova, A. N., Yun, J., Likhacheva, A. V. & Alonso, J. M. Multi-level interactions between ethylene and auxin in Arabidopsis roots. Plant Cell 19, 2169–2185 (2007).

58. Muday, G. K., Rahman, A. & Binder, B. M. Auxin and ethylene collaborators or competitors? Trends Plant. Sci. 17, 181–195 (2012).

59. Liu, M. et al. The tomato Ethylene Response Factor SI-ERF B3 integrates ethylene and auxin signaling via direct regulation of Sl-Aux/IAA27. New Phytol. 219, 631–640 (2018).

60. Chaibouni, S. et al. Sl-IAA3, a tomato Aux/IAA at the crossroads of auxin and ethylene signaling involved in differential growth. J. Exp. Bot. 60, 1349–1362 (2009).

61. Maury, P., Mojajad, F., Berger, M. & Planchon, C. Photocatalytic response to drought acclimation in two sunflower genotypes. Physiol. Plant. 98, 57–66 (1996).

62. Geigenberger, P., Lerchi, J., Sittt, M. & Sonnewald, U. Phloem-specific expression of pyrophosphatase inhibits long-distance transport of carbohydrates and amino acids in tobacco plants. Plant Cell Environ. 19, 43–55 (1996).

63. Hellens, R. P. et al. Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. Plant. Methods 1, 13 (2005).

64. Han, Y. C. et al. Banana transcription factor MaERF11 recruits histone deacetylase MahDA1 and represses the expression of MaACO1 and expansins during fruit ripening. Plant Physiol. 171, 1070–1084 (2016).

65. Qin, G., Wang, Y., Cao, B., Wang, W. & Tian, S. Unraveling the regulatory network of the MADS box transcription factor RIN in fruit ripening. Plant J. 70, 243–255 (2012).