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The BES1/BZR1 transcriptional factor SlBES2 regulates photosynthetic apparatus in tomato fruit

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Abstract

Background Fruit photosynthetic apparatus development comprises a series of biological processes which is essential in determining fruit development and quality formation. However, the understanding of the regulation of fruit photosynthetic apparatus development remains poor.

Results In this study, we identified a transcriptional factor SlBES2, the closest homolog of BES1 and BZR1 in tomato BES1 family, is highly expressed in fruit at mature green (MG) stage and exhibited transcriptional inhibition activity. Down-regulation of *SlBES2* resulted in fruits showing paler fruit than wild type at MG stage, in contrast, SlBES2-overexpressing tomato lines bore deeper green fruits. Notably, chlorophyll content and number of thylakoids per chloroplast in fruit was substantially increased in SlBES2-overexpressing lines, while markedly decreased in SlBES2-suppressing lines. Comparative transcriptome analysis revealed that multiple genes of the photosystem, chloroplast development and chlorophyll metabolism pathways were regulated by SlBES2. Further verification revealed that SlBES2 can significantly repress the transcriptional activity of *SlNYC1* and *Green-Flesh*, and physically interact with protein SlHY5.

Conclusions Collectively, this study demonstrated that SlBES2 plays an important role in regulating fruit photosynthetic apparatus development through either transcriptional repression of genes involved in chlorophyll breakdown, or posttranscriptional regulation of proteins associated with plant photomorphogenesis and chloroplast development. Our findings add a new actor to the complex mechanisms underlying photosynthetic apparatus during fruit development.

Keywords BES1 transcriptional factor, Fruit development, Photosynthetic apparatus, Tomato

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Background

Tomato is one of the most planted and consumed horticultural crops worldwide, and it is also a model plant for studying fruit development and ripening. Tomato fruits undergo a series of complex changes from development to ripening, which also endows fruits with unique flavor, nutrition and texture. Tomato is a typical climacteric fruit, and the process of fruit maturation and ripening is commonly divided into five stages: immature, mature green (MG), breaker, pink ripening, and red ripening. During MG stage, a period that fruit generally achieve physiological maturity, fruit cells have fully expanded, compound accumulation has been completed, fruit color is varying from dark green to light green. Meanwhile, some transcription factors have been found playing an important role in the regulation of this process. GOLDEN 2-LIKE transcription factors (GLKs) are essential for coregulating fruit photosynthesis and chloroplast development, and a loss-of-function of GLK2 in tomato is responsible for the uniform ripening phenotype [1, 2]. ARABIDOPSIS PSEUDO RESPONSE REGULATOR2-LIKE (APRR2-Like), a homolog of tomato GLK2, is also critical in the regulation of chloroplast development and chlorophyll accumulation in unripe tomato fruit, and overexpressing APRR2-Like in tomato resulted in highly dark green fruit color at MG stage, which is quite similar to the phenotype of GLK2-overexpressing tomato [3]. Besides, loss-of-function of ELONGATED HYPOCOTYL 5 (HY5) in tomato, a master transcription regulator of light signaling pathway, can also impair chlorophyll accumulation in unripe tomato fruit and pigments composition in ripe fruit [4].

The transcriptional factors BRI1-EMS-SUPPRESSOR 1 (BES1) and BRASSINAZOLE-RESISTANT 1 (BZR1) are two core members of BES1 family that mediate brassinosteroid (BR) signaling pathway in plant [5]. BES1 and BZR1 also play important roles in integrating multiple phytohormones signaling pathways to regulate plant growth, development and stress response [6–11]. Moreover, they have been found involved in light morphology [12–14]. Besides, BES1 can also regulate petal morphogenesis in chrysanthemum [15]. So far, the regulatory mechanism of BES1 and BZR1 has been well studied in many plant species, but other members of BES1 family were rarely reported. One common explanation is that BES1 family members were supposed to be function redundant and indispensable in regulating plant growth and development [16, 17], which was concluded based on the findings in Arabidopsis only. As for the role of BES1 during fruit development and maturation is still unknown.

In tomato, a total of nine BES1 family members were identified, of which SlBES2 (Solyc02g063010), SlBES5 (Solyc04g079980) and SlBES9 (Solyc12g089040) are highly homologous to Arabidopsis AtBES1 (At1g19350) and AtBZR1 (At1g75080) [18]. Tomato SlBES5 (also named SlBZR1) was reported to be an important transcriptional regulator in heat stress tolerance [19], autophagy and nitrogen starvation [20], and apical dominance [21]. While SlBES9 (also named SlBES1) was found involved in postharvest fruit softening [22]. Recently, another tomato BES1 family member SlBES8, was found to be critical for shoot apical meristem development, which controls the formation of flower and fruit. By contrary, distinct from overexpression of *SlBES8* in tomato, knockout of *SlBES8* displayed no obvious phenotypes [23]. It is probably due to the fact that the transcriptional abundance of *SlBES8* is extremely low in tomato. Of the nine BES1 family members, the expression of *SlBES8* is the lowest, while *SlBES2*, *SlBES5* and *SlBES9* have substantially higher expression levels than the other six members in various tomato organs (referring to data on TomExpress http://tomexpress.toulouse.inra.fr). Except for SlBES5, SlBES8 and SlBES9, little is known about the other six BES1 members in tomato. Whether tomato BES1 family is involved in fruit development and maturation is still unclear. However, previous reports found that overexpressing Arabidopsis *AtBZR1-1D* in tomato could affect fruit quality and pigment accumulation [24, 25], suggesting the potential functional significance of BES1 on fruit development and maturation in tomato.

In this study, tomato transcriptional factor SlBES2 was identified to be a transcriptional repressor and highly expressed at MG stage of fruit development. By genetically suppressing and overexpressing *SlBES2* in tomato, SlBES2 was found effective on the control of fruit photosynthetic apparatus through regulating genes related to light-harvesting proteins, chlorophyll metabolism and chloroplast development. Our study adds new actor to the complex mechanisms underlying photosynthetic apparatus regulation and the important role of SlBES2 in the regulation of fruit development and maturation.

Methods

Plant materials and growth conditions

Transgenic tomato lines were generated in this study using wild type Micro-Tom tomato (*Solanum lycopersicum* cv. Micro-Tom). The transgenic tomato lines and wild type control were transplanted in greenhouse after 12-day of germination on MS medium. The growth conditions were set to 16/8 hour light/dark cycle and 25/20 ℃ day/night temperature. Fruit samples of various developmental times or stages were collected according to the interval period between corresponding fruit developmental days and averaged breaker days, and samples of other organs were collected from one-month-old tomato plants. 2–4 days before breaker time was considered as mature green (MG) stage. Each sample was collected

from at least ten independent plants, and at least ten fruits were harvested for each fruit sample. At least three biological replicate samples were prepared for each material. To determine color of MG fruit, at least thirty fruits were harvested simultaneously, and color values of different parameters were measured with colorimeter on three points of the fruit equator section. All samples were frozen immediately and mixed thoroughly after grinded in liquid nitrogen. Wild type tobacco (*Nicotiana benthamiana* L.) leaves were used for all transient expression experiments in this study. Tobacco plants were planted on soil under the same growth conditions, and one-month-old plants were used for transformation and related analysis.

Gene cloning and generation of transgenic tomato lines

The open reading frame (ORF) sequence of SlBES2 was cloned from tomato cDNA prepared from mixed tomato organs. The sequence of *SlBES2* was confirmed by alignment in Sol Genomics Network (https://solgenomics.net/). For overexpressing *SlBES2* in tomato, the full-length open reading frame sequence of *SlBES2* was introduced to the pK303 plant expression vector downstream of two CaMV 35 S promoters [26]. For repressing *SlBES2* in tomato, a 339-bp gene-specific fragment ORF sequence of *SlBES2* was cloned and fused into the pCAMIBA1301 vector downstream of the CaMV 35 S promoter in both antisense and sense orientations. The specificity of selected fragment of *SlBES2* was tested by BLAST analysis in tomato genome (https://solgenomics.net/tools/blast/). These recombinant vectors were transformed into the *Agrobacterium tumefaciens* strain GV3101, and Agrobacterium mediated transformation of wild type tomato following standard methods. Positive transgenic individuals of T1 generation were screened in MS medium containing 125 mg L^{-1} kanamycin, and then confirmed by multiple PCR with plant DNA targeting at different vector genes. Homozygous individuals in each positive line of T2 generation were confirmed through checking its seeds in screening medium. Gene expression levels of SlBES2 of homozygous transgenic tomato lines were checked by qRT-PCR using specific primers, and transgenic lines with the best performance were selected for the following work. The T3 generation of homozygous transgenic lines were used for preliminary observation and RNA-seq analysis. The T4 generation lines were used for phenotyping, physiological determination, ultrastructural observation and qRT-PCR re-checking. All primer sequences used in this study are listed in Supplementary Table S1 of Supplementary files.

RNA isolation and quantitative real-time PCR

Total RNA was extracted with RNAprep Pure Plant Kit (Tiangen Biotech, China) according to the manufacturer's instructions. The first strand cDNA was synthesized from total RNA with PrimeScript™ RT reagent Kit (Takara, Japan) following the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was conducted on the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA) with TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Japan). At least two technical repetitions and three biological replicates were set for each sample in the qRT-PCR. The tomato housekeeping gene *Actin* (Solyc03g078400) was used as internal references to normalize gene expression levels. The relative gene expression was calculated according to the $2^{-\Delta\Delta CT}$ method.

Subcellular localization analysis

The full-length coding sequence of *SlBES2* without stop codon were amplified and fused into the pCXDG-GFP vector downstream of the CaMV 35 S promoter, and then the fused vector was transformed into *Agrobacterium tumefaciens* strain GV3101. Agrobacterium mediated transformation of tobacco leaf was used to transiently express the fusion protein in tobacco cell. The empty vector of pCXDG-GFP was set as control. After three-day infection, subcellular localization of green fluorescence was observed through the laser scanning confocal microscope Leica TCS SP8 (Germany).

Transactivation analysis in yeast

The full-length coding sequence of *SlBES2* cloned and ligated into pGBKT7-GAL4BD vector. Then the fused vector was transformed into Y2HGold yeast cells, and then cultivated on SD/−Trp medium plates with various concentration. The α-galactosidase activity of the transformants was detected by adding X-α-gal and the expression of AUR1-C was screened by adding Aureobasidin A (AbA) following the manufacturer's protocol (Clontech, USA). Transcriptional activator VP16 were used as positive control, and pGBKT7-GAL4BD vector alone were used as blank control.

Subcellular morphologic observation by TEM

The equator sections of fresh fruit at MG stage, containing epidermis and outer pericarp, were used for subcellular morphologic observation. Samples were cut into narrow strips and immediately prefixed with a 3% glutaraldehyde, then the tissue was postfixed in 1% osmium tetroxide, dehydrated in series acetone, infiltrated and embedded in epoxy resin (Epox 812). The semithin sections were stained with methylene blue and ultrathin sections were cut with diamond knife, stained with uranyl acetate and lead citrate. Sections were examined with JEM-1400-FLASH Transmission Electron Microscope (JEOL, Japan). Chloroplasts, grana and thylakoids were counted in at least six cells of three repeatable samples.

Pigment determination by HPLC

Extraction and determination of pigments in tomato MG fruit were performed under dark condition. 300 mg of sample was ground into powder and supersonically extracted with 3 mL of 80% cold methanol. After centrifugation, the supernatant was eluted through a 0.22 μm filter before injection. Fruit pigments were separated and determined on an Agilent 1260 Series liquid chromatograph system (Agilent Technologies). A YMC C30 column (4.6 × 250 mm, 5 μm) was employed for separating pigments. The mobile phase A consisted of 0.4% (w/v) ammonium acetate and 0.1% (w/v) BHT in methanol: methyltertbutylether (MTBE): water solution (90:7:3, v/v/v). The mobile phase B consisted of 0.4% (w/v) ammonium acetate and 0.1% (w/v) BHT methanol: MTBE: water (7:90:3, v/v/v). The program of gradient for phase A: phase B was 90:10 (%) at 0 min, 80:20 at 5 min, 70:30 (%) at 20 min, 10:90 (%) at 25 min, 10:90 (%) at 29 min, 90:10 (%) at 29.1 min, and 90:10 (%) at 37 min. The flow rate of mobile phases was maintained at 1 mL min^{-1}, the column temperature was 30 °C, the injection volume was 10 μL, and the spectrometry detector was set at 450 nm. The contents of each pigment were calculated from four biological replicates basing on corresponding standard curves.

Transcriptome profiling

Total RNA was extracted using RNAprep Pure Plant Kit (Tiangen Biotech, China) according to the manufacturer's instructions, and three biological replicates were prepared for each sample. RNA samples were sent to Shanghai Majorbio Bio-pharm Technology to conduct RNA-seq. The sequencing was performed on Illumina HiSeq2500. Raw reads were filtered and trimmed to remove adapters and low-quality reads. Clean reads were aligned to the latest tomato genome SL4.0 (https://www.solgenomics.net) to assemble putative transcripts. Gene expression levels were normalized and expressed as transcripts per million reads (TPM). Significant differentially expressed genes (DEGs) were identified by a threshold of $|\log_2$ fold change$| \geq 1$ and $p < 0.05$. Gene ontology enrichment analysis was performed with Goatools (version 0.6.5) software under a threshold of adjusted $p < 0.05$.

Dual-luciferase assay

For the determination of transcriptional activity of SlBES2, the full-length coding sequence of *SlBES2* was amplified and ligated into pEAQ-GAL4BD vector as an effector. The GAL4-binding element (5 × GAL4) was introduced to the double-luciferase reporter pGreenII 0800-LUC vector as the reporter. Transcriptional activator VP16 were used as positive control, and pEAQ-GAL4BD vector alone were used as blank control. For detecting the interaction between SlBES2 and promoter of candidate genes, the full-length coding sequence of *SlBES2* was cloned into the pGreenII 62-SK vector as an effector. A length of 2000-bp promoter sequence was cloned from tomato genome for each candidate genes, and then was fused into the original pGreenII 0800-LUC vector as the reporter. The empty pGreenII 62-SK vector was used as blank control. The effectors and reporters were transformed into *Agrobacterium tumefaciens* strain GV3101 respectively, and co-infected tobacco leaves for transient expression basing on the ratio of effector:reporter 9:1. The LUC and REN activities were measured using the Dual-Luciferase Reporter Assay System (Promega, USA) three days after infection. At least five biological replicates were performed for each co-infection. The ratio of LUC/REN was calculated to determine the transcriptional activation activity of SlBES2 on the promoter of candidate genes.

Yeast two-hybrid assay

The full-length coding sequence of *SlBES2* was cloned and inserted pGBKT7 vector as bait, and full coding sequences of candidate protein were cloned and constructed into pGADT7 as prey respectively. The empty vector pGADT7 and pGBKT7, as well as pGADT7-T, pGBKT7-53 and pGBKT7-Lam that supplied by the Matchmaker Gold Yeast Two-Hybrid System (Takara, Japan) were used as negative or positive controls. The recombined plasmids were co-transformed into Y2H Gold yeast strain cells according to the protocol. Yeast culture mediums SD/−Leu/−Trp and SD/−Ade/−His/−Leu/−Trp (Clontech, USA) with or without X-α-gal were used to culture and screen positive transformants.

Firefly luciferase complementation imaging (LCI) assay

Firefly luciferase complementation imaging assay was performed according to the protocol as previously reported [27]. The full-length coding sequence of *SlBES2* was cloned and inserted into the pCAMBIA-cLUC vector to generate a C-terminal luciferase protein, and the full-length coding sequence for candidate interacting proteins were cloned and inserted into the pCAMBIA-nLUC vector to generate an N-terminal luciferase protein. Empty vectors were used as control. The recombinant plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101, and co-infected tobacco leaves for transient expression. Four different co-infection were performed on the one leaf by quarter method. After three days infection, infected leaves were collected form plants and sprayed evenly with 1 millimolar of luciferin (Promega, USA), and then immediately incubated in dark for 10 min to quench the fluorescence before taking the LUC photo under a cooled CCD imaging apparatus (Alliance, UK). For each pair of experiments, at least six independent leaves were collected for LUC imaging. The electronic

image files were scanned by software Image J to obtain gray value of each quartered area basing on appropriate circular spot of same size.

Results

Localization, expression and activity of transcriptional factor SlBES2

SlBES2 (Solyc02g063010), a core member of tomato BES1 transcriptional factor family, encode a protein of 320 amino acid, which has 58.2% and 56.9% identity with its close homologs in tomato, respectively SlBES5 (Solyc04g079980, also named SlBZR1) and SlBES9 (Solyc12g089040, also named SlBES1). SlBES2 protein also share 53.4%, 51.5%, and 48.2% identify with its close homologs in Arabidopsis (Supplementary Figure S1 of Supplementary files), respectively AtBES1 (At1g19350), AtBZR1 (At1g75080) and AtBEH1 (At3g50750). Gene expression analysis of *SlBES2* in different tomato organs and fruit developmental stages showed that the expression of *SlBES2* gradually increased during fruit development, reached its highest level at the stage of mature green (MG), and then decreased towards fruit ripening (Fig. 1A). Transient expression analysis of SlBES2 fused with green fluorescent protein (GFP) in tobacco showed that SlBES2 was localized in both the nucleus and cytoplasm (Fig. 1B). In addition, transactivation analysis

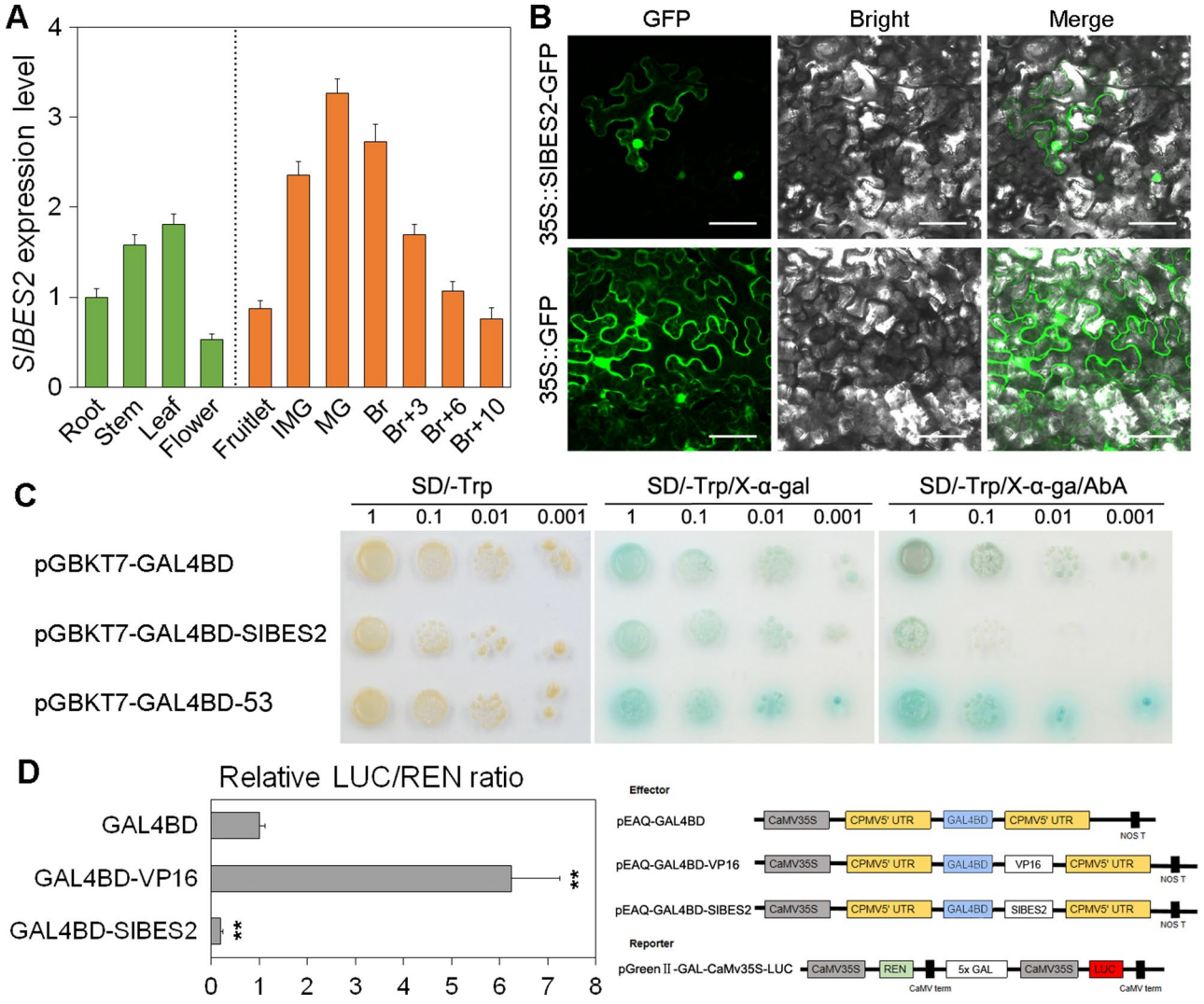

Fig. 1 Molecular characterization of transcriptional factor SlBES2. **(A)** Expression pattern of *SlBES2* in different tomato organs (green columns) and different fruit developmental stages (orange columns). IMG, immature green stage; MG, mature green stage; Br, breaker stage; Br + n, days after breaker. Gene expression levels were obtained by qRT-PCR and all expressed relatively to root. **(B)** Subcellular localization of SlBES2 fused with green fluorescent protein (GFP) in tobacco leaf. **(C)** Autoactivation analysis of SlBES2 protein in yeast by Y2H system. pGBKT7 alone was used as negative control, while pGBKT7-53 was used as positive control. **(D)** Analysis of SlBES2 transcriptional activity in tobacco leaf by dual-luciferase assay. GAL4BD alone was used as blank control, while transcriptional activator VP16 was used as positive control. Value of each column and bar above represents mean ± SE (A, $n = 3$; D, $n = 5$). Asterisk indicates statistical significance compared with control (*$p < 0.05$ and **$p < 0.005$)

of SlBES2 in yeast indicated that SlBES2 has no visible transactivation activity (Fig. 1C). Transcriptional activity of SlBES2 was further determined by dual-luciferase assay in tobacco. Contrary to the performance of transcriptional activator and blank control, SlBES2 exhibited significant transcriptional inhibition activity (Fig. 1D).

SlBES2 regulates fruit chlorophyll accumulation

To investigate the functional significance of SlBES2 in tomato, SlBES2-repressing (RNA interference, SlBES2-Ri) and SlBES2-overexpressing (SlBES2-OE) transgenic tomato lines were generated, of which SlBES2-Ri-5, SlBES2-Ri-6, SlBES2-OE-1 and SlBES2-OE-3, transgenic lines were selected in this study (Supplementary Figure S2 of Supplementary files). Compared to wild type tomato, the expression levels of *SlBES2* were up to 92% decreased in MG fruit and 81% decreased in leaf of SlBES2-Ri lines, while up to 22-fold increased in MG fruit and 26-fold increased in leaf of SlBES2-OE lines (Fig. 2A). As shown in Fig. 2B and C, the color of MG fruits on SlBES2-overexpressing tomato lines display markedly dark green phenotype compared to wild-type at mature green stage, in contrast, SlBES2-repressing tomato lines bore paler MG fruits. The differences of fruit color between SlBES2-overexpressing / SlBES2-repressing lines and wild type was proved to be statistically significant through quantitative determination with colorimeter (Fig. 2D). To find out the specific pigments that dominate the fruit color among the transgenic lines, the contents of various fruit pigments in MG fruit were measured by HPLC. As shown in Fig. 2E, the contents of xanthophyll, zeaxanthin and β-carotenoid were significantly increased in SlBES2-OE lines, but not obviously changed in SlBES2-Ri lines. However, the contents of chlorophyll a and chlorophyll b were significantly enhanced in SlBES2-OE lines, while reduced in SlBES2-Ri lines. The distinct phenotypes between SlBES2-OE and SlBES2-Ri lines suggest that SlBES2 may involve in the regulation of fruit chlorophyll accumulation.

SlBES2 regulates fruit chloroplast development

As chlorophyll mostly resides in the grana thylakoid membrane, and changes in the abundance of chloroplast and grana stacks have strong effects on chlorophyll levels, it is necessary to observe the status of chloroplast development in fruit. The number of chloroplast and granum in epidermis cells of MG fruit was counted under transmission electron microscope (Fig. 3A). Indeed, when compared with wild type control, neither the number of chloroplasts per cell nor the number thylakoids per granum was significantly altered in the MG fruits of transgenic lines (Fig. 3B and C). However, in line with the dark green phenotype, the number of thylakoids per chloroplast in MG fruit was significantly increased in SlBES2-OE lines, while reduced in SlBES2-Ri lines (Fig. 3D). The finding supports that the active role of SlBES2 plays an important role in the regulation of chloroplast development.

Comparative transcriptome analysis of SlBES2 on fruit development

A genome-wide transcriptomic profiling was performed on tomato fruits of SlBES2-overexpressing, SlBES2-repressing tomato lines, and wild type at MG stage. The principal component analysis (PCA) revealed that the three biological replicates of each material were closely clustered together, while different materials were clearly separated far away (Fig. 4A). Differentially expressed genes (DEGs) were identified in SlBES2-overexpressing and SlBES2-repressing tomato through comparative transcriptome analysis against wild type (Fig. 4B). In total, 5862 DEGs were obtained in SlBES2-OE lines, while 2827 DEGs were obtained in SlBES2-Ri lines, of which 2092 DEGs are common between SlBES2-OE and SlBES2-Ri lines (Fig. 4C). The complete lists of the DEGs are available in Supplementary Table S2 of Supplementary files. In order to identify specific functions impacted by SlBES2, the 2092 common DEGs were identified by gene ontology (GO) annotation analysis and KEGG pathway enrichment analysis. As the result shown, most of the DEGs are annotated as metabolic or cellular process basing on biological process classification, and catalytic activity or binding protein basing on molecular function (Fig. 4D). The KEGG pathway enrichment analysis showed that the genes that classified as photosynthesis antenna (light-harvesting) proteins take up the top one position among all enriched KEGG pathway terms (Fig. 4E). Besides, the result also indicate that SlBES2 is involved in multiple metabolic pathways, such as plant hormone signal transduction, porphyrin and chlorophyll metabolism and carbon fixation in photosynthetic organisms. These results support the putative regulatory role of SlBES2 in tomato fruit photosynthetic apparatus.

SlBES2 regulates fruit photosynthetic apparatus-related genes at both transcriptional and posttranscriptional levels

To mine candidate genes regulated by SlBES2 during fruit development, the transcriptional abundance of DEGs related to light-harvesting proteins, chlorophyll metabolism, and chloroplast development were screened out and expressed as heatmaps (Fig. 5). For the DEGs encoding light-harvesting proteins (Fig. 5A), they are all up-regulated in SlBES2-OE, but rarely varied in SlBES2-Ri. For the DEGs involved in chlorophyll metabolism, a total of five DEGs were down-regulated in SlBES2-OE (Fig. 5B), including the genes referring to chlorophyll breakdown, e.g. *Green-Flesh* (GF, Solyc08g080090) and

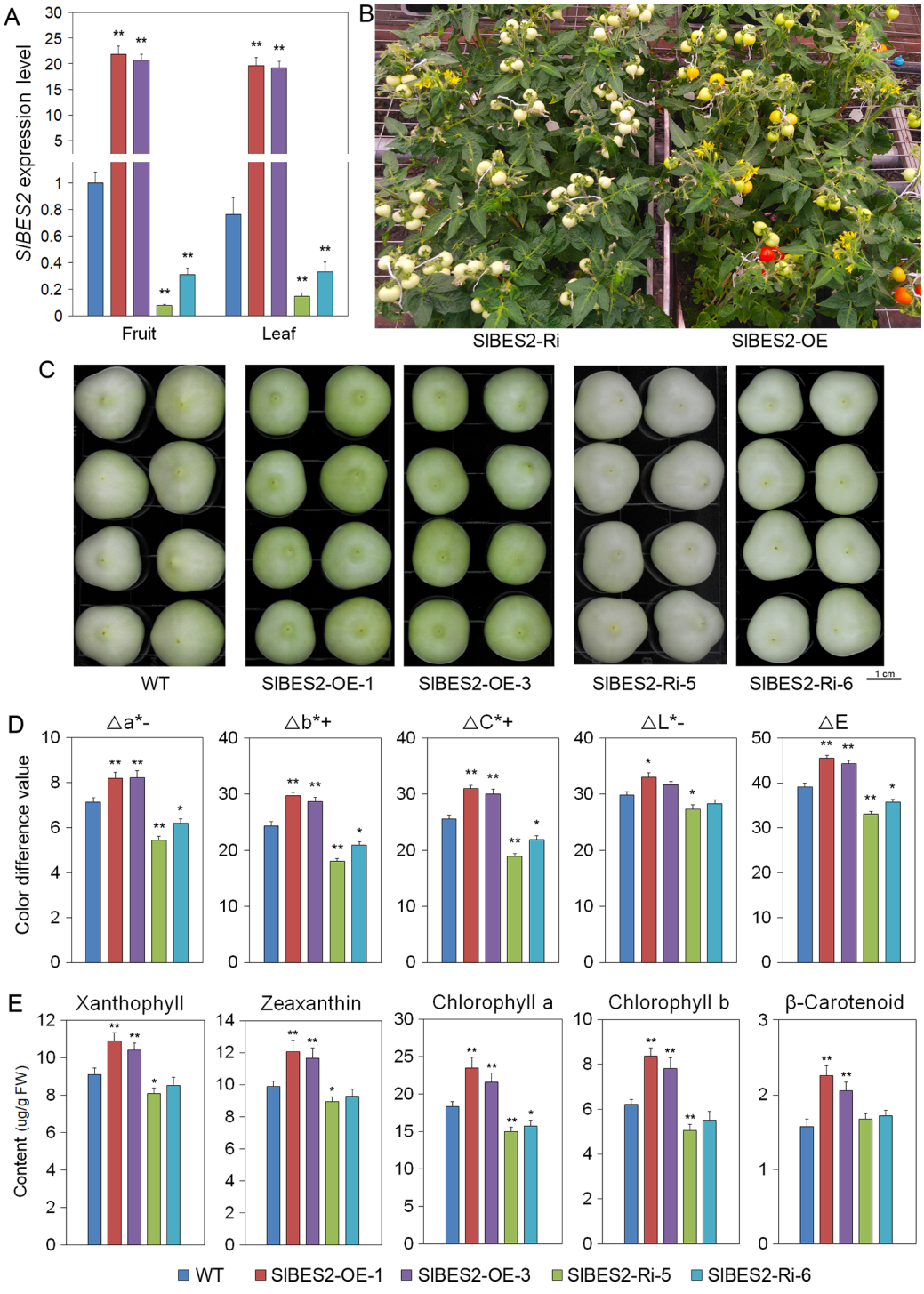

Fig. 2 Phenotypes of SlBES2-overexpressing and SlBES2-repressing tomato lines. **(A)** Relative gene expression of *SlBES2* in MG fruit and leaf of different transgenic tomato lines and wild type control. **(B)** Phenotype of transgenic plants at MG stage. **(C)** Phenotype of fruit at MG stage. **(D)** Quantitative determination of MG fruit color with colorimeter. (**E**) Contents of various fruit pigments in MG fruit. Value of each column and bar above represents mean ± SE (A, $n = 3$; D, $n \geq 30$; E, $n = 4$). Asterisk indicates statistical significance compared with wild type (*$p < 0.05$ and **$p < 0.005$)

Non-Yellow Coloring 1 (NYC1, Solyc07g024000), of which only *SlNYC1* was significantly up-regulated in SlBES2-Ri. Among the DEGs associated with chloroplast development (Fig. 5C), the genes encoding GOLDEN2-like 1 (GLK1, Solyc07g053630), GOLDEN2-like 2 (GLK2, Solyc10g008160) and ELONGATED HYPOCOTYL 5 (HY5, Solyc08g061130) were significantly up-regulated in SlBES2-OE and down-regulated in SlBES2-Ri. The

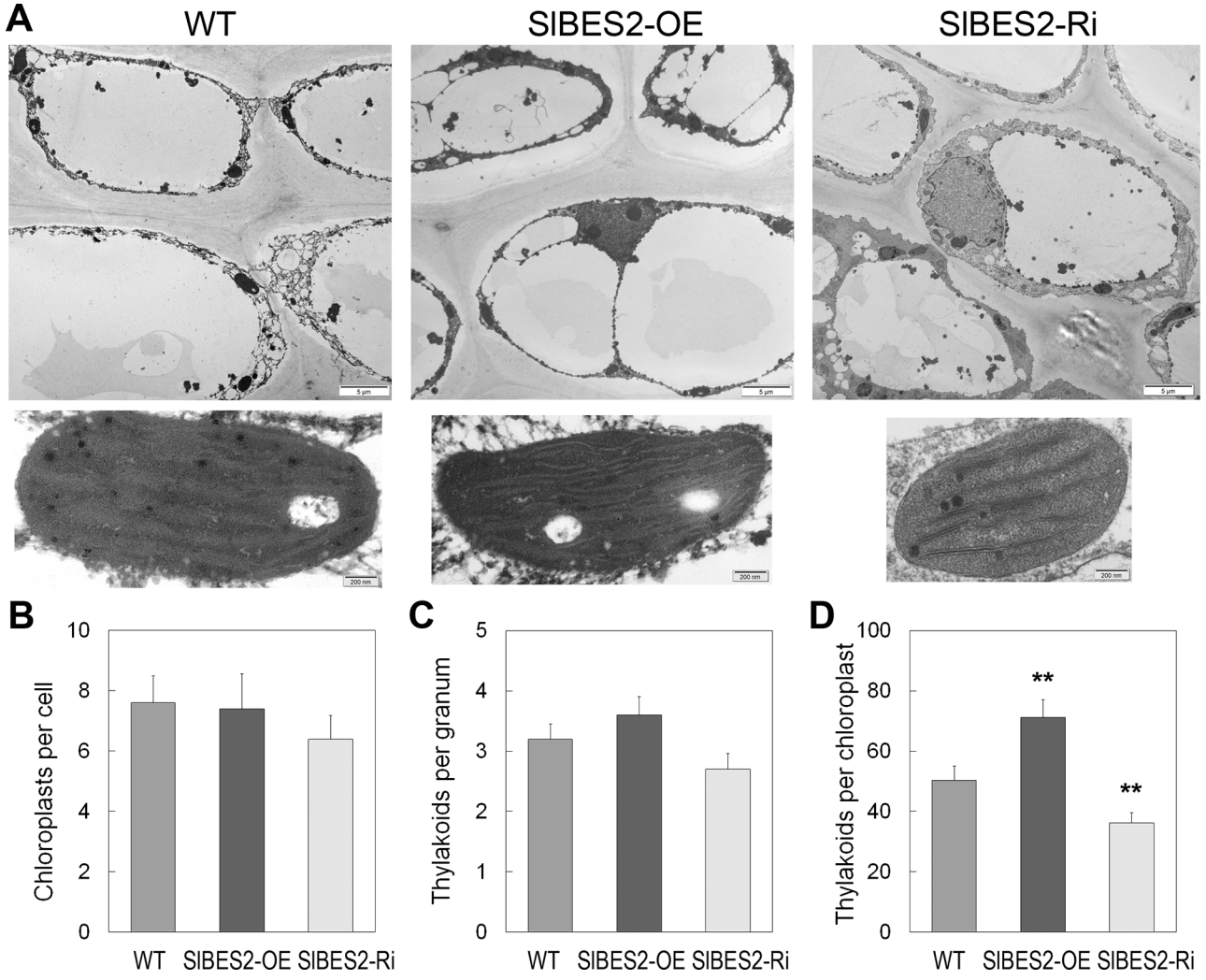

Fig. 3 Observation of fruit chloroplast development in SlBES2-overexpressing and SlBES2-repressing tomato lines. **(A)** Ultrastructure of fruit epidermis cell and chloroplast under transmission electron microscope. **(B)** Counting the number of chloroplasts per cell. **(C)** Counting the number thylakoids per granum. **(D)** Counting the number of thylakoids per chloroplast. Value of each column and bar above represents mean ± SE (for B, $n = 6$; C and D, $n \geq 10$). Asterisk indicates statistical significance compared with wild type (*$p < 0.05$ and **$p < 0.005$)

expression levels of *SlBES2* and several candidate genes were rechecked in the next generation of the transgenic lines by qRT-PCR (Fig. 5D). As the result shown, *SlLHCA4*, *SlLHCB1*, *SlLHCB3*, *SlGLK1*, *SlGLK2* and *SlHY5* were significantly up-regulated in SlBES2-OE fruit, but only *SlGLK2* and *SlHY5* were down-regulated in SlBES2-Ri. Notably, *SlNYC1* and *Green-Flesh* were down-regulated in SlBES2-OE and up-regulated in SlBES2-Ri. Overall, these results suggest that SlBES2 not only act as a repressor, but may also work as an activator.

To identify target genes regulated by SlBES2, the transcriptional activation ability of SlBES2 on the promoters of some candidate genes in chlorophyll metabolism and chloroplast development were analyzed in vivo by dual-luciferase reporter assay system (Fig. 6A). As the result shown (Fig. 6B), the transcription of *SlNYC1* (Solyc07g024000) and *Green-Flesh* (Solyc08g080090) were significantly repressed by SlBES2. Given SlBES2 was found having transcriptional inhibition activity, *SlNYC1* and *Green-Flesh* are supposed to be the target genes of SlBES2, mediating the regulation of fruit chlorophyll accumulation.

To explore interacting proteins of SlBES2, protein-protein interaction analysis between SlBES2 and SlGLK1, SlGLK2, SlTAGL1, SlAPRR2 or SlHY5 respectively, were performed by both yeast two-hybrid (Y2H) system and LUC-firefly luciferase complementation imaging (LCI) assay system. Among them, only SlHY5 showed clear protein interaction with SlBES2 in both Y2H test (Fig. 6C) and LCI assay (Fig. 6D), and the interaction was proved to be statistically significant basing on quantitative analysis (Fig. 6E). Considering SlHY5 has been reported to be an important photosynthetic regulator for fruit maturation and exhibited similar function as SlBES2

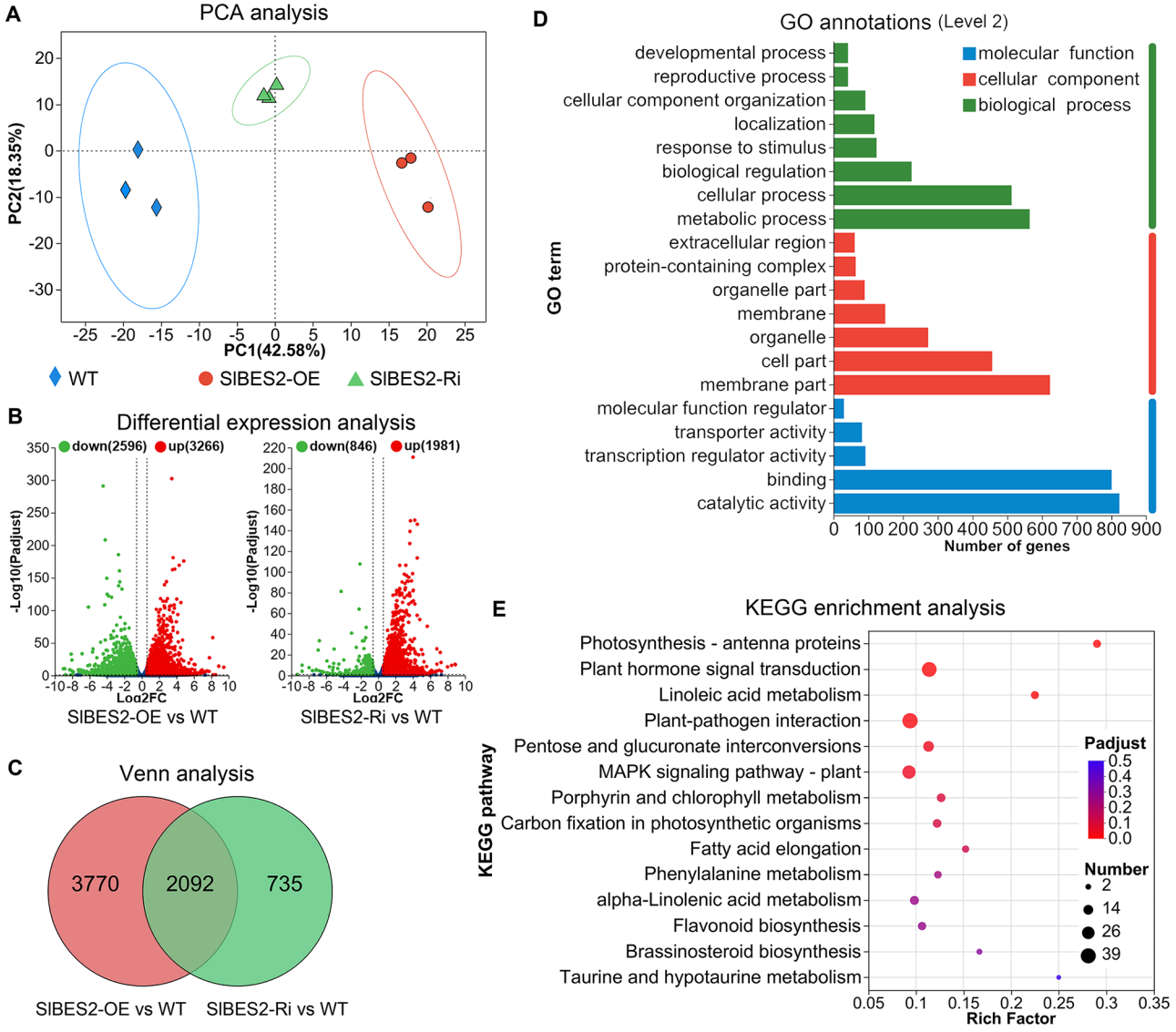

Fig. 4 Comparative transcriptome analysis of fruit between SlBES2-overexpressing and SlBES2-repressing tomato. **(A)** Principal component analysis (PCA) of transcriptome data among different materials. **(B)** Differential expression analysis of transcriptome data of SlBES2-overexpressing or SlBES2-repressing tomato lines versus wild type. **(C)** Venn analysis of differentially expressed genes (DEGs) between SlBES2-overexpressing and SlBES2-repressing tomato lines. **(D)** Gene ontology (GO) annotation analysis of common DEGs between SlBES2-overexpressing and SlBES2-repressing tomato lines. **(E)** KEGG pathway enrichment analysis of common DEGs between SlBES2-overexpressing and SlBES2-repressing tomato lines

on MG tomato fruit, the regulation of fruit photosynthetic apparatus by SlBES2 probably involves the coordination of SlHY5.

To sum up, SlBES2 can not only act as a transcriptional repressor, but also work as a posttranscriptional regulator, which coordinatively regulates fruit photosynthetic apparatus development in tomato.

Discussion

The regulatory role of BES1 on fruit development have been found in other studies. As reported, ectopic overexpressing Arabidopsis *AtBZR1-1D* (an increased dephosphorylated AtBZR1 mutant) in tomato not only enhanced fruit carotenoid accumulation and fruit quality, but also enhanced fruit chlorophyll accumulation at mature green (MG) stage [24], which is quite similar with our findings from the SlBES2-OE lines. The slight difference on phenotype is probably due to different tomato materials used in our study. In addition, comparative proteomics analysis of the AtBZR1-1D-overexpressing tomato fruit revealed that photosynthetic apparatus is the top one markedly enhanced pathway by AtBZR1-1D, and plant hormone signaling is also highly in accordance with our results of comparative transcriptome analysis between SlBES2-OE/SlBES2-Ri tomato and wild type. These

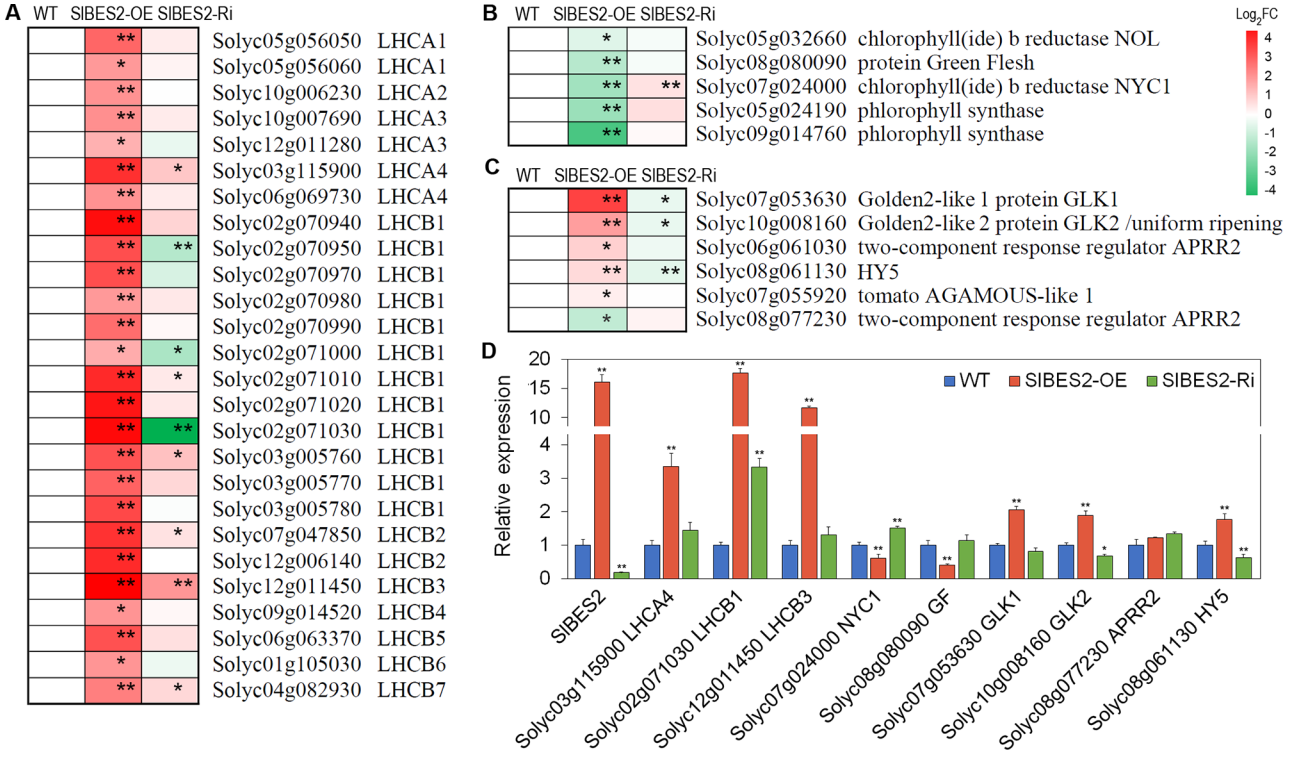

Fig. 5 Expression levels of genes related to fruit photosynthetic apparatus in SlBES2-overexpressing and SlBES2-repressing tomato. **(A)** Heatmap showing the expression levels of DEGs encoding light-harvesting proteins. **(B)** Heatmap showing the expression levels of DEGs involved in chlorophyll metabolism. **(C)** Heatmap showing the expression levels of DEGs associated with chloroplast development. **(D)** Validation of the expression levels of DEGs related to fruit photosynthetic apparatus by qRT-PCR. Gene expression levels were all expressed relatively to wild type. Value of each column and bar above represents mean ± SE ($n = 3$). Asterisk indicates statistical significance compared with wild type (*$p < 0.05$ and **$p < 0.005$)

findings all support the important role of BES1 in regulation of fruit photosynthetic apparatus.

Chlorophyll is not only indispensable for photosynthesis, but also crucial for fruit maturation. Chlorophyll breakdown in plant mainly starts from the chlorophyll cycle which interconverts chlorophyll a and chlorophyll b. Chlorophyll b must be converted into chlorophyll a before degradation. Non-Yellow Coloring 1 (NYC1) and NYC1-Like (NOL) are thylakoid membrane-localized chlorophyll(ide) b reductases, catalyzing the conversion of chlorophyll b to 7-hydroxymethyl chlorophyll a. The product can be further catalyzed by 7-hydroxymethyl chlorophyll a reductase (HCAR) and converted to chlorophyll (a). Chlorophyll a can be catalyzed by chlorophyllide a oxygenase (CAO) and regenerate chlorophyll (b). A group of chloroplast Stay-Green proteins (SGR/NYE) are responsible for the conversion of chlorophyll a to pheophytin a, which is the initial step of chlorophyll breakdown [28]. The *nyc1* mutant of rice showed a stay-green phenotype [29, 30]. Green-Flesh is a major Stay-Green protein in tomato. The *green-flesh* tomato mutant, caused by an amino acid substitution in Green-Flesh protein, bear mature fruit with muddy brown color due to obstruction of chlorophyll degradation plus accumulation of lycopene [31]. As revealed by these reports, SlNYC1 and Green-Flesh either work as a critical rate limiting enzyme in the pathway of chlorophyll breakdown, and down-regulation of their transcription can effectively suppress the breakdown of chlorophyll and cause dark green phenotype. SlNYC1 is not only required for degradation of chlorophyll, but also required for the degradation of light-harvesting complex and grana [28]. The functions of SlNYC1 and Green-Flesh are highly consistent with our finding that overexpressing SlBES2 in tomato suppressed the transcription levels of *SlNYC1* (Solyc07g024000) and *Green-Flesh* (Solyc08g080090), and enhanced chlorophyll accumulation in MG fruit. Given that SlBES2 alone worked as a transcriptional repressor, and promoter of *SlNYC1* and *Green-Flesh* were negatively regulated by SlBES2, together, SlBES2 was believed to be a critical transcriptional regulator on fruit chlorophyll metabolism through regulating the transcription of *SlNYC1* and *Green-Flesh*, the key rate limiting enzymes for chlorophyll breakdown.

Chlorophyll breakdown occurs on of thylakoids inside of chloroplast and is tightly associated to chloroplast development [32]. In this study, GOLDEN2-like transcription factors SlGLK1 and SlGLK2 was found up-regulated in MG fruit of SlBES2-OE tomato. GLKs play essential roles in plant chloroplast development and

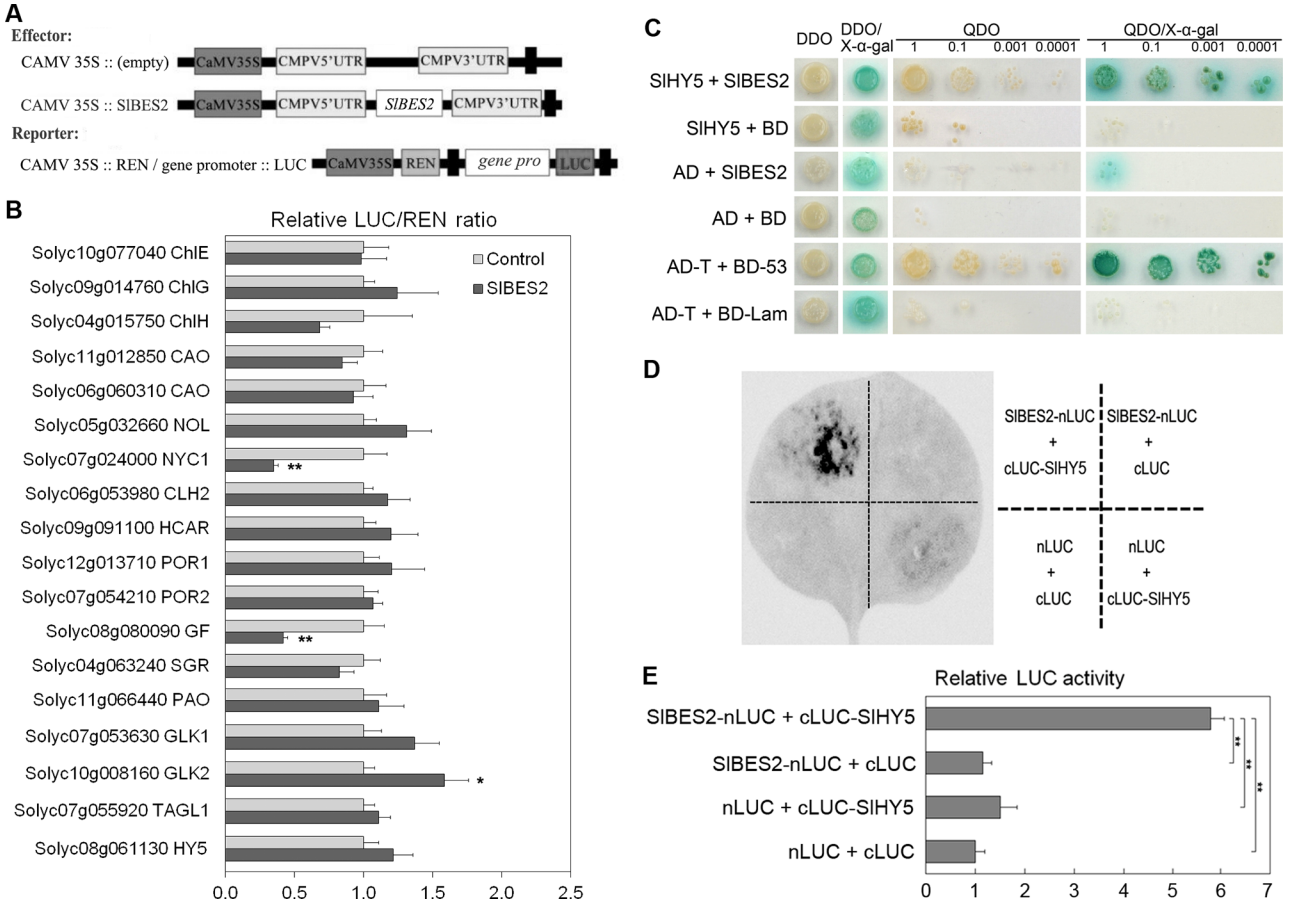

Fig. 6 Verification of the regulatory role of SlBES2 on genes related to fruit photosynthetic apparatus. **(A)** Diagram showing vector constructs of reporter and effector used for dual-luciferase reporter assay system. The empty pGreenII 62-SK vector was used as blank control. **(B)** Transcriptional activation activity of SlBES2 on the promoters of candidate genes in tobacco leaves basing on dual-luciferase reporter assay. **(C)** Protein-protein interaction between SlBES2 and SlHY5 tested by yeast two-hybrid system. AD-T plus BD-53 was used as the positive control while AD-T plus BD-Lam as negative control, DDO represents SD/-Leu/-Trp medium; QDO represents SD/-Ade/-His/-Leu/-Trp medium. **(D)** Protein-protein interaction between SlBES2 and SlHY5 determined in tobacco leaves by LUC-firefly luciferase complementation imaging assay system. **(E)** Quantitative analysis of protein-protein interaction of SlBES2 and SlHY5 basing on LUC-firefly luciferase complementation imaging assay. Value of each column and bar above represents mean ± SE (for B, $n = 5$; E, $n = 6$). Asterisk indicates statistical significance compared with corresponding control (*$p < 0.05$ and **$p < 0.005$)

photosynthetic apparatus [33]. In tomato, SlGLK1 and SlGLK2 positively regulate chlorophyll level in both leaf and fruit, and the loss function of SlGLK2 results in the *uniform ripening* (*u*) tomato mutants [1, 2]. As similar as the phenotype of overexpression and repression of GLKs in tomato, the SlBES2-OE tomato fruit exhibited dark green phenotype while SlBES2-Ri tomato was pale, suggesting the involvement of GLKs in SlBES2-regulated fruit chloroplast development. However, tomato full-length SlGLK2 encodes a 310-amino acid protein, but in the *u* mutant only 80-amino acid fragment can be synthesized due to a frameshift mutation and premature stop codon. Micro-Tom tomato is a classical *u* mutants, as a result, SlGLK2 does not work in all the transgenic lines and wild type no matter how it is regulated. The dual-luciferase assay revealed that the promoter of *SlGLK1*, but not *SlGLK2*, can be significantly activated by SlBES2, indicating that SlGLK1 is probably responsible for the control of chloroplast development in tomato. Recent finding revealed that SlGLK1 involves in the regulation of plant photomorphogenesis and chloroplast development through BIN2 phosphorylating [34]. As a major target protein of BIN2, BES1 is phosphorylated by BIN2 and BIN2-BES1 interacting module is critical for BES1's function [14]. Given that direct protein-protein interaction between SlGLK1 and SlBES2 was not detected in tomato, it is possible that the competition between module BIN2-GLK1 and BIN2-SlBES2 control the regulation of photomorphogenesis and chloroplast development in tomato.

LONG HYPOCOTYL5 (HY5) is a basic Leu zipper transcription factor that works downstream of multiple photoreceptors. As a critical regulator in the light signaling pathway, HY5 is involved in a subset of physiological processes in plant growth and development, such as photomorphogenesis, pigment biosynthesis, chloroplast development [35, 36]. As recently reported, loss of

function of SlHY5 in tomato impairs fruit pigment accumulation, and SlHY5 regulates fruit maturation at both the transcriptional and translational levels [4]. Notably, MG fruit of SlHY5 mutants exhibits much paler skin than wild type, which is quite similar as the phenotype of SlBES2-Ri tomato in our study. *SlHY5* was up-regulated in SlBES2-OE tomato and down-regulated in SlBES2-Ri tomato, which are also consistent with the function of SlHY5 on fruit development. However, as reveled by the dual-luciferase assay, the positive regulation of *SlHY5* by SlBES2 is indirect at transcriptional level. But direct protein-protein interaction between SlBES1 and SlHY5 was proved to be real by both in vitro and in vivo verification. BES1-HY5 protein interaction has been previously identified in Arabidopsis and it is important for the regulation of plant photomorphogenesis [37]. Therefore, the regulator role of SlBES2 on fruit photosynthetic apparatus should also rely on the posttranscriptional regulation of SlHY5. Besides, considering HY5 also plays a crucial role for GLKs on chloroplast development [38, 39], and both SlHY5 and SlGLKs were up-regulated by SlBES2 in SlBES2-OE, it can be concluded that module SlHY5-SlGLKs also involves in the regulation of fruit photosynthetic apparatus by SlBES2 in tomato.

Conclusions

Overall, our study demonstrate that SlBES2 plays an important role in the regulation of fruit photosynthetic apparatus through either transcriptional repression of genes involved in chlorophyll breakdown, e.g. *NYC1* and *Green Fresh*, or posttranscriptional regulation of proteins associated with plant photomorphogenesis and chloroplast development, e.g. SlHY5 and SlGLKs. The photosynthetic apparatus is the basis of the higher plants, which determines fruit development and maturation. In this regard, our findings may provide insights into allowing a tight control of the target genes for fruit photosynthetic apparatus development, defining potential breeding strategies aiming at improving fruit postharvest and quality in horticulture crops.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06085-w.

Supplementary Material 1: Table S1 The primers used in this study.

Supplementary Material 2: Table S2 The list of common DEGs with gene annotation between SlBES2-OE and SlBES2-Ri tomato lines by comparative transcriptome analysis versus wild type tomato.

Supplementary Material 3: Figure S1 Alignment and cluster analysis of tomato and Arabidopsis BES1 family.

Supplementary Material 4: Figure S2 PCR identification of resistant SlBES2-overexpressing and SlBES2-repressing tomato plants.

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Author contributions

MH: Conceptualization, Investigation, Validation, Formal analysis, Data curation, Funding acquisition, Writing – original draft, Writing – review & editing. WL, DS and GA: Investigation, Validation, Validation Data curation. BH and ZL: Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

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Data availability

The RNA sequencing data are available at NCBI database with accession numbers from SRR31559214 to SRR31559218 and from SRR31559637 to SRR31559640. All the other data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Powell AL, Nguyen CV, Hill T, Cheng KL, Figueroa-Balderas R, Aktas H, et al. Uniform ripening encodes a golden 2-like transcription factor regulating tomato fruit chloroplast development. Science. 2012;336(6089):1711–5.
2. Nguyen CV, Vrebalov JT, Gapper NE, Zheng Y, Zhong S, Fei Z, et al. Tomato GOLDEN2-LIKE transcription factors reveal molecular gradients that function during fruit development and ripening. Plant Cell. 2014;26(2):585–601.
3. Pan Y, Bradley G, Pyke K, Ball G, Lu C, Fray R, et al. Network inference analysis identifies an APRR2-like gene linked to pigment accumulation in tomato and pepper fruits. Plant Physiol. 2013;161(3):1476–85.
4. Wang W, Wang P, Li X, Wang Y, Tian S, Qin G. The transcription factor SlHY5 regulates the ripening of tomato fruit at both the transcriptional and translational levels. Hortic Res. 2021;8(1):83.
5. Yin YH, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J. A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. Cell. 2005;120(2):249–59.
6. Cui XY, Gao Y, Guo J, Yu TF, Zheng WJ, Liu YW, et al. BES/BZR transcription factor TaBZR2 positively regulates Drought responses by activation of TaGST1. Plant Physiol. 2019;180(1):605–20.
7. Li H, Ye K, Shi Y, Cheng J, Zhang X, Yang S. BZR1 positively regulates freezing Tolerance via CBF-Dependent and CBF-Independent pathways in Arabidopsis. Mol Plant. 2017;10(4):545–59.
8. Min HJ, Cui LH, Oh TR, Kim JH, Kim TW, Kim WT. OsBZR1 turnover mediated by OsSK22-regulated U-box E3 ligase OsPUB24 in rice BR response. Plant J. 2019.
9. Zhao X, Dou L, Gong Z, Wang X, Mao T. BES1 hinders ABSCISIC ACID INSENSITIVE5 and promotes seed germination in Arabidopsis. New Phytol. 2019;221(2):908–18.

10. Liu X, Zhao C, Gao Y, Xu Y, Wang S, Li C et al. A multifaceted module of BRI1 ETHYLMETHANE SULFONATE SUPRESSOR1 (BES1)-MYB88 in growth and stress tolerance of apple. Plant Physiol. 2021.
11. Hu J, Sun S, Wang X. Regulation of shoot branching by Strigolactones and brassinosteroids: conserved and specific functions of Arabidopsis BES1 and Rice BZR1. Mol Plant. 2020;13(6):808–10.
12. Wang WX, Lu XD, Li L, Lian HL, Mao ZL, Xu PB, et al. Photoexcited CRYPTOCHROME1 interacts with dephosphorylated BES1 to regulate Brassinosteroid Signaling and Photomorphogenesis in Arabidopsis. Plant Cell. 2018;30(9):1989–2005.
13. Wang F, Gao Y, Liu Y, Zhang X, Gu X, Ma D, et al. BES1-regulated BEE1 controls photoperiodic flowering downstream of blue light signaling pathway in Arabidopsis. New Phytol. 2019;223(3):1407–19.
14. He G, Liu J, Dong H, Sun J. The Blue-Light receptor CRY1 interacts with BZR1 and BIN2 to modulate the phosphorylation and nuclear function of BZR1 in repressing BR Signaling in Arabidopsis. Mol Plant. 2019;12(5):689–703.
15. Cheng PL, Liu YN, Yang YM, Chen H, Cheng H, Hu Q et al. CmBES1 is a regulator of boundary formation in chrysanthemum ray florets. Hortic Res. 2020;7(1).
16. Chen LG, Gao ZH, Zhao ZY, Liu XY, Li YP, Zhang YX, et al. BZR1 family transcription factors function redundantly and indispensably in BR signaling but exhibit BRI1-Independent function in regulating Anther Development in Arabidopsis. Mol Plant. 2019;12(10):1408–15.
17. Chen WY, Lv MH, Wang YZ, Wang PA, Cui YW, Li MZ et al. BES1 is activated by EMS1-TPD1-SERK1/2-mediated signaling to control tapetum development in *Arabidopsis thaliana*. Nat Commun. 2019;10.
18. Su DD, Xiang W, Wen L, Lu W, Shi Y, Liu YD et al. Genome-wide identification, characterization and expression analysis of BES1 gene family in tomato. BMC Plant Biol. 2021;21(1).
19. Yin YL, Qin KZ, Song XW, Zhang QH, Zhou YH, Xia XJ, et al. BZR1 transcription factor regulates heat stress tolerance through FERONIA receptor-like kinase-mediated reactive oxygen Species Signaling in Tomato. Plant Cell Physiol. 2018;59(11):2239–54.
20. Wang Y, Cao JJ, Wang KX, Xia XJ, Shi K, Zhou YH, et al. BZR1 mediates Brassinosteroid-Induced Autophagy and Nitrogen Starvation in Tomato. Plant Physiol. 2019;179(2):671–85.
21. Xia X, Dong H, Yin Y, Song X, Gu X, Sang K, et al. Brassinosteroid signaling integrates multiple pathways to release apical dominance in tomato. Proc Natl Acad Sci. 2021;118(11):e2004384118.
22. Liu HR, Liu LH, Liang DY, Zhang M, Jia CG, Qi MF et al. SlBES1 promotes tomato fruit softening through transcriptional inhibition of PMEU1. Iscience. 2021;24(8).
23. Su D, Wen L, Xiang W, Shi Y, Lu W, Liu Y, et al. Tomato transcriptional repressor SlBES1.8 influences shoot apical meristem development by inhibiting the DNA binding ability of SlWUS. Plant J. 2022;110(2):482–98.
24. Liu LH, Jia CG, Zhang M, Chen DL, Chen SX, Guo RF, et al. Ectopic expression of a BZR1-1D transcription factor in brassinosteroid signalling enhances carotenoid accumulation and fruit quality attributes in tomato. Plant Biotechnol J. 2014;12(1):105–15.
25. Liu LH, Liu HR, Li S, Zhang X, Zhang M, Zhu N et al. Regulation of BZR1 in fruit ripening revealed by iTRAQ proteomics analysis. Sci Rep-Uk. 2016;6.
26. Ren MZ, Qiu SQ, Venglat P, Xiang DQ, Feng L, Selvaraj G, et al. Target of Rapamycin regulates Development and ribosomal RNA expression through kinase domain in Arabidopsis. Plant Physiol. 2011;155(3):1367–82.
27. Chen H, Zou Y, Shang Y, Lin H, Wang Y, Cai R, et al. Firefly luciferase complementation imaging assay for protein-protein interactions in plants. Plant Physiol. 2008;146(2):368–76.
28. Kuai B, Chen JY, Hortensteiner S. The biochemistry and molecular biology of chlorophyll breakdown. J Exp Bot. 2018;69(4):751–67.
29. Kusaba M, Ito H, Morita R, Iida S, Sato Y, Fujimoto M, et al. Rice NON-YELLOW COLORING1 is involved in light-harvesting complex II and grana degradation during leaf senescence. Plant Cell. 2007;19(4):1362–75.
30. Sato Y, Morita R, Katsuma S, Nishimura M, Tanaka A, Kusaba M. Two short-chain dehydrogenase/reductases, NON-YELLOW COLORING 1 and NYC1-LIKE, are required for chlorophyll b and light-harvesting complex II degradation during senescence in rice. Plant J. 2009;57(1):120–31.
31. Barry CS, McQuinn RP, Chung MY, Besuden A, Giovannoni JJ. Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the green-flesh and chlorophyll retainer mutations of tomato and pepper. Plant Physiol. 2008;147(1):179–87.
32. Wang P, Grimm B. Connecting Chlorophyll Metabolism with Accumulation of the photosynthetic apparatus. Trends Plant Sci. 2021;26(5):484–95.
33. Waters MT, Wang P, Korkaric M, Capper RG, Saunders NJ, Langdale JA. GLK transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis. Plant Cell. 2009;21(4):1109–28.
34. Zhang D, Tan W, Yang F, Han Q, Deng X, Guo H, et al. A BIN2-GLK1 signaling Module integrates Brassinosteroid and Light Signaling to repress Chloroplast Development in the Dark. Dev Cell. 2021;56(3):310–24. e7.
35. Gangappa Sreeramaiah N, Botto Javier F. The multifaceted roles of HY5 in Plant Growth and Development. Mol Plant. 2016;9(10):1353–65.
36. Wang F, Zhang L, Chen X, Wu X, Xiang X, Zhou J, et al. SlHY5 integrates temperature, light, and hormone signaling to Balance Plant Growth and Cold Tolerance. Plant Physiol. 2019;179(2):749–60.
37. Li QF, He JX. BZR1 interacts with HY5 to Mediate Brassinosteroid- and light-regulated Cotyledon opening in Arabidopsis in darkness. Mol Plant. 2016;9(1):113–25.
38. Kobayashi K, Baba S, Obayashi T, Sato M, Toyooka K, Keränen M, et al. Regulation of root greening by light and auxin/cytokinin signaling in Arabidopsis. Plant Cell. 2012;24(3):1081–95.
39. Cackett L, Luginbuehl LH, Schreier TB, Lopez-Juez E, Hibberd JM. Chloroplast development in green plant tissues: the interplay between light, hormone, and transcriptional regulation. New Phytol. 2022;233(5):2000–16.

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