Tomato UVI4 and CCS52B play important roles in hypocotyl elongation via modulation of cell cycle progression

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Abstract

Tomato hypocotyl elongation is a consequence of active cell division and expansion, both of which require precisely regulated cell cycle progression. Little is known about the function of APC/C regulators in cell cycle progression during hypocotyl growth. Here, we isolated and characterized the positive and negative APC/C regulators, SlCCS52 and SlUVI4 genes in tomato. We probed SlUVI4 and SlCCS52B roles in tomato hypocotyl elongation via modulating cell cycle progression. Light especially blue light represses the transcription of SlUVI4 and SlCCS52B to inhibit the hypocotyl elongation through the suppression of endoreduplication. MS basal salts and sugar both improve SlUVI4 and SlCCS52B transcription to enhance hypocotyl length via the promotion of endoreduplication. Hypocotyl elongation enhanced by heat might require auxin-induced repression of SlUVI4 transcription. Salt inhibits hypocotyl elongation possibly through ethylene-mediated modulation of SlUVI4 and SlCCS52B transcription. Genetic studies reveal that tomato deletion mutant of SlUVI4 and overexpression plants of SlCCS52B in Arabidopsis both exhibit shorter hypocotyl with enhanced endoreduplication. Thus, our results suggest that APC/C activities stimulated by SlCCS52 genes requires SlUVI4-meidated inhibitory machinery to reorchestrate cell cycle progression and facilitate hypocotyl elongation.

Tomato *UVI4* and *CCS52B* play important roles in hypocotyl elongation via modulation of cell cycle progression

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Footnotes:

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Abstract

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cycle progression during hypocotyl growth. Here, we isolated and characterized the positive and negative APC/C regulators, *SlCCS52*and *SlUVI4* genes in tomato. We probed *SlUVI4* and*SlCCS52B* roles in tomato hypocotyl elongation via modulating cell cycle progression. Light especially blue light represses the transcription of *SlUVI4* and *SlCCS52B* to inhibit the hypocotyl elongation through the suppression of endoreduplication. MS basal salts and sugar both improve *SlUVI4* and *SlCCS52B*transcription to enhance hypocotyl length via the promotion of endoreduplication. Hypocotyl elongation enhanced by heat might require auxin-induced repression of *SlUVI4* transcription. Salt inhibits hypocotyl elongation possibly through ethylene-mediated modulation of *SlUVI4* and *SlCCS52B* transcription. Genetic studies reveal that tomato deletion mutant of *SlUVI4* and overexpression plants of *SlCCS52B* in Arabidopsis both exhibit shorter hypocotyl with enhanced endoreduplication. Thus, our results suggest that APC/C activities stimulated by *SlCCS52* genes requires *SlUVI4* -meidated inhibitory machinery to reorchestrate cell cycle progression and facilitate hypocotyl elongation.

Keywords: Tomato, hypocotyl elongation, UVI4, CCS52, APC/C, cell cycle, light quality, heat, salt, plant hormones.

Introduction

Hypocotyl growth including elongation and thickening is important for the emergence of tomato seedlings from soil and resistance to lodging. In the dark, seedlings undergo skotomorphogenic development including closed cotyledons, formation of an apical hook and elongated hypocotyls. In the light, photomorphogenic development results in cotyledon expansion, leaf development, photosynthesis initiation and reduced hypocotyl elongation. Many mutants of light signaling components exhibit defective hypocotyl growth. Loss-of-function mutant of *phyB*(*Phytochrome B*), the red-light receptor, has elongated hypocotyls (de Lucas et al., 2008; Kim et al., 2016). *Phytochrome interacting factors* (*PIFs*) are transcription factors downstream of light signaling to positively regulate hypocotyl elongation, and monogenic*PIF* mutants have shorter hypocotyls than wild-type plants under light condition (Huq & Quail, 2002; Kim et al., 2003; Zhong et al., 2012). Ambient temperature also regulates hypocotyl elongation. High temperature promotes hypocotyl elongation through the increase of free IAA in Arabidopsis seedlings (Gray, Ostin, Sandberg, Romano, & Estelle, 1998). PIF4 promotes IAA biosynthesis, and is also required for high temperature induced hypocotyl elongation (Franklin et al., 2011; Koini et al., 2009; Stavang et al., 2009). PIF4 specifically binds to the biologically active Pfr form of phyB, which results in the degradation of PIF4 and prevents its transcription activity in the hypocotyl elongation (Huq & Quail, 2002; Park et al., 2012). PhyB-PIF signaling module also regulates red light-induced hypocotyl elongation under the warm temperature (Johansson et al., 2014). Blue light inhibits Arabidopsis hypocotyl elongation requiring the function of*cryptochrome 1* (*CRY1*) and *cryptochrome 2*(*CRY2*) (Ahmad & Cashmore, 1993). Under blue light, CRY1 directly interacts with PIF4 to inhibit its transcription activity and represses the hypocotyl elongation induced by high temperature (Ma et al., 2016).

Plant hormones have diverse and strong impacts on hypocotyl growth. Overproduction of auxin in plants promotes hypocotyl elongation (Boerjan et al., 1995; Romano, Robson, Smith, Estelle, & Klee, 1995; Zhao et al., 2001). Chemical screens identify a new auxin analog named pro-2,4-D which significantly promotes hypocotyl elongation (Savaldi-Goldstein et al., 2008). Exogenous application of gibberellins promotes Arabidopsis hypocotyl elongation under light via regulation of cellular elongation (Cowling & Harberd, 1999). Ethylene prevents the hypocotyl elongation in the dark, while promotes hypocotyl elongation in the light (Bleecker, Estelle, Somerville, & Kende, 1988; Smalle, Haegman, Kurepa, Van Montagu, & Straeten, 1997). Brassinosteroid (BR) stimulates hypocotyl elongation of pakchoi (*Brassica chinensis* cv Lei-Choi) (T. W. Wang, Cosgrove, & Arteca, 1993) and BR signaling is required for GA-induced hypocotyl elongation (Bai et al., 2012). Appropriate endogenous level of Abscisic acid (ABA) is essential for tomato hypocotyl elongation in the dark, which promotes the endoreduplication in hypocotyl cells and inhibits cytokinin biosynthesis (Humplik et al., 2015). Cytokinins inhibit hypocotyl elongation of Arabidopsis seedlings in the dark, while induce hypocotyl elongation in light-grown Arabidopsis plants via suppression of ethylene action or auxin transport (Smets, Le, Prinsen, Verbelen, & Van Onckelen, 2005).

Hypocotyl growth is species- and growth condition-dependent, resulting from cell division and expansion

(Gendreau et al., 1997; Raz & Koornneef, 2001; H. Wang & Shang, 2020). In Arabidopsis, hypocotyl growth is exclusively attributed to cell expansion (Boron & Vissenberg, 2014), while both cell division and expansion exist in hypocotyls of*Helianthus annuus* (Kutschera & Niklas, 2013). Accurate cell cycle progression including mitotic cycle and endoreduplication results in proper cell division and expansion during plant organ growth and development (De Veylder, Beeckman, & Inze, 2007; Sugimoto-Shirasu & Roberts, 2003). Cell cycle consists of four major phases, G1, S, G2 and M, and the progression is governed by the activity of cyclin-dependent kinases (CDKs)/cyclin protein complex, in which CDK is the catalytic subunit and cyclin is the regulatory subunit (Inze & De Veylder, 2006). CDK inhibitors (CKIs) or Kip-related proteins (KRPs) directly bind to the CDK/cyclin complexes to inhibit CDK activity (De Veylder et al., 2001; Lui et al., 2000; Sherr & Roberts, 1999; H. Wang, Fowke, & Crosby, 1997). SIAMESE(SIM)/SIAMESE RELATED (SMR) family proteins are another type of inhibitors of CDK activity controlling endocycle onset in Arabidopsis (Churchman et al., 2006; Kasili et al., 2010; Walker, Oppenheimer, Concienne, & Larkin, 2000). Cell cycle from G2 to M phase requires E3 ubiquitin ligase complexes Anaphase-promoting complex/cyclosome (APC/C) to degrade cyclins and inhibit CDK activity (Fulop et al., 2005; Marrocco, Bergdoll, Achard, Criqui, & Genschik, 2010; Peters, 2006; Sullivan & Morgan, 2007). APC/C is a large protein complex containing at least 11 different subunits referred as APC1 to APC11, in which APC2 and APC11 are core components with catalytic activities. APC/C has two types of activators, *cell division cycle 20* (*CDC20*) and *CELL CYCLE SWITCH52* (*CCS52*) (Fulop et al., 2005). Five *CDC20* homologs (*CDC20.1* - *CDC20.5*) and three *CCS52* homologs (*CCS52A1* , *CCS52A2* and *CCS52B*) were identified in Arabidopsis genome. *ULTRAVIOLET-B-INSENSITIVE4* (*UVI4*) and*OMISSION OF SECOND DIVISION1* (*OSD1*) are two negative regulators of APC/C, which have functional redundancy in the regulation of female gametophyte development (Bao & Hua, 2014; Heyman et al., 2011; Iwata et al., 2011). Either *UVI4* or *OSD1*overexpression leads to reduced plant size, lateral shoots and enhanced disease resistance (Bao, Yang, & Hua, 2013; Iwata et al., 2011).

Studies on APC/C activities in tomato hypocotyl growth were seldom reported. Here we accessed roles of the negative APC/C regulator*SlUVI4* and the positive regulator *SlCCS52B* in hypocotyl elongation via the modulation of cell cycle progression. High expression levels of both genes correlated with the robust hypocotyl elongation when seedlings grow under different lights or on the growth medium supplemented with MS salts and sugar. Salt, heat and plant hormones auxin and ethylene treatments affect their transcription distinctly to modulate hypocotyl elongation via the perturbation of cell cycle progression. Genetic studies revealed that *SlCCS52B*overexpression Arabidopsis plants and *SlUVI4* deletion mutants had defective hypocotyl elongation with enhanced endoreduplication. Together, our study establishes cellular and genetic connections between APC/C regulators, cell cycle progression and tomato hypocotyl elongation.

Materials and Methods

Plant materials and growth conditions

Tomato cultivar 'Heinz 1706', 'Moneymaker' and 'Ailsa Craig' seeds were obtained from Tomato Genetics Resource Center (*https://tgrc.ucdavis.edu/*) and US National Plant Germplasm System (*www.ars-grin.gov/*) respectively, and propagated in the greenhouse. Seeds were sterilized in 75% alcohol for 1 minute and 8% NaClO solution for 15 minutes, and washed with autoclaved water for five times, and planted on Murashige and Skoog (MS), non-MS (NMS) and MS with sugar (MSS) medium plates. Dry seeds were directedly sown at the different depth of soil mix containing 40% organic matters. All seedlings were grown in growth chamber at 25°C with 12-hour dark and 12-hour light condition.

Treatments with different lights, salt, heat and plant hormones

For light treatment, sterilized tomato seeds planted on MS growth medium were grown in red and blue light with the intensity of 100 μmol m^{-2} s^{-1} for 7 days, respectively. For salt stress and plant hormone treatment, sterilized tomato seeds were planted on MS growth medium supplemented with 150 mM NaCl or plant hormones of different concentrations and grown for 7 days. For heat treatment, sterilized tomato seeds were planted on MS growth medium and grown at 33°C for 7 days.

Hypocotyl growth measurement

Tomato seedlings grown under different conditions were photographed first, and their hypocotyl length and diameter in the middle of hypocotyl were measured by ImageJ software (*http://imagej.nih.gov/ij/*). Every experiment was independently repeated at least twice.

Cell Cycle analysis with flow cytometry

Hypocotyls of more than 3 seedlings were collected for cell cycle analysis. Samples were chopped in 'Aru' buffer and filtered by the metal mesh with 30μm pore size (Arumuganathan & Earle, 1991; Bao, Zhang, & Hua, 2014). More than 5,000 nuclei in each sample were stained with propidium iodide and measured in BD flow cytometer. Three biological replicates were analyzed for each tomato variety. Endocycle index or the cycle value were calculated to quantify the extent of endocycle using the following formula (Bainard, Bainard, Henry, Fazekas, & Newmaster, 2012; Barow & Meister, 2003): EI = (%4C nuclei x 1) + (%8C nuclei x 2) + (%16C nuclei x 3) + (%32C nuclei x 4).

Gene expression analysis

Total RNA from hypocotyls was extracted by RNAisoPlus (TaKaRa, Otsu, Japan, cat. #) following the manufacturer's instruction. The first strand complementary DNA (cDNA) synthesis using 2μg total RNA was performed following instructions provided with HiScript III RT SuperMix kit with gDNA wiper (Vazyme, cat. #R323-01) for qPCR analysis and HiScript III 1st Strand cDNA Synthesis Kit with gDNA wiper (Vazyme, cat. #R312-01) for full-length gene cloning. One microliter and 0.2μl of cDNA templates were used the amplification of *SlCBL1* gene by RT-PCR to determine the quality of cDNA and qPCR analysis, respectively.*SlCBL1* was used as a reference gene for qRT-PCR data normalization (Pombo et al., 2014). All primers were designed by on-line tools (*https://www.ncbi.nlm.nih.gov/tools/primer-blast/*) and listed in Supplementary Table 1.

Phylogenetic analysis

Amino acid sequences of *UVI4* and *CCS52* genes in Arabidopsis were obtained from the website (*www.arabidopsis.org*), and used for identification of their homologous genes in solanaceae species by BLAST in the website (*www.solgenomics.net*). Phylogenetic analysis using amino acid sequences were performed in the software MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

Plasmid construction and plant transformation

The coding sequence of each gene was first cloned into pDONR221 vector by BP reaction following the manufacture's instruction (Life Technologies, cat. #11789-020). These entry vectors were recombined into destination vectors by LR reactions (Life Technologies, cat. #11791-020). For Crispr-cas9 constructs, two guide RNAs, target 1 and 2, or Target 3 and 4 were designed following the instruction in the website *www.skl.scau.edu.cn*and included in primers for PCR amplification (Xie et al., 2017). DNA fragments amplified using the plasmid pCBC-DT1T2.2 as a template were ligated into pG3K-U6SC vector using Golden Gate Assembly method, and all constructs were transformed into *Agrobacterium* strains LBA4404 harboring the helper plasmids pVS1-VIR2 (Zhang et al., 2019). Transgenic Arabidopsis seedlings with overexpression constructs were generated through*Agrobaterium* -mediated floral dipping method (Clough & Bent, 1998). Transgenic tomato plants using cultivar 'Heinz 1706' were generated through*Agrobaterium* -mediated transformation as previously described (H. J. Sun, Uchii, Watanabe, & Ezura, 2006).

Yeast two hybrid and bimolecular fluorescence complementation assay

For Y2H analysis, all cDNAs of *SlUVI4* and *SlCCS52s* were cloned into pDEST-GADT7 and pDEST-GBKT7 (Rossignol, Collier, Bush, Shaw, & Doonan, 2007) and assays were performed following the user manual of Matchmaker Gold Yeast Two-Hybrid System (Clontech Laboratories, Inc.). For BiFC analysis, *SlUVI4* cDNA was cloned into the destination vector pSPYNE-35S GW and cDNAs of *SlCCS52* genes were cloned into the destination vector pSPYCE-35S GW (Walter et al., 2004). These vectors were transiently transformed into onion cells via Agrobacterium tumefacien strain GV3101 together with gene silencing

inhibitor P19 (Schutze, Harter, & Chaban, 2009; W. Sun, Cao, Li, Zhao, & Zhang, 2007). GFP signals were detected using Nikon fluorescence microscope ECLIPSE Ni-U.

Data analysis

Data were recorded and analyzed in Excel, and statistical analyses were performed in Data Processing System (DPS) software (Tang & Zhang, 2013). Means, standard errors and standard deviations of different replicates were calculated in Excel. Least significance difference test (LSD) was performed in DPS software to determine the statistical difference with p value less than 0.05.

Results

Proper sowing depth is required for successful emergence of tomato seedlings from soil.

We investigated effects of the sowing depth on tomato seed germination and hypocotyl elongation, both of which are important for tomato seedling production. We observed that the seedling emergence from soil was significantly reduced by 38% and 50% at the sowing depth of 4 and 5 cm compared to 2 and 3 cm, respectively (Figure 1A, B). We analyzed effects of the sowing depth on the seed germination. Germination rates of seeds sown at 4 and 5 cm deep soil reduced by 22% and 33% compared to that at 2 and 3 cm deep soil (Figure 1C), suggesting that reduction of seed germination in deep soil is one of factors influencing the seedling emergence from soil. We further analyzed hypocotyl elongation defects of tomato seedlings sown at 4 cm deep soil. Hypocotyls elongated to various directions due to large resistance, some of which may not be able to reach the surface of soil (Figure 1D). Taken together, these data indicated that both seed germination and hypocotyl elongation are significantly affected by the sowing depth, which results in dramatical reduction of seedling emergence from soil and the ultimate yield of plants.

Identification of APC/C regulators in tomato

We isolated tomato homologs of APC/C regulators through BLAST analysis in the website (www.solgenomics.net). Tomato has only one *UVI4* homologous gene *Solyc10g080400* in the genome referred as*SlUVI4* , which is different with other plant species such as Arabidopsis, rice, maize, strawberry, etc. We further identified*UVI4* homologous genes in other Solanaceae species, and revealed that pepper, potato and eggplant have one *UVI4* homolog, and tobacco and petunia have four and three homologs in the draft genome, respectively (Supplementary Figure S1A). Similar to Arabidopsis, tomato has three *CCS52* homologous genes referred as *SlCCS52s* including *Solyc08g080080*(*SlCCS52A1* , previously named as *SlCCS52A*),*Solyc12g056490* (*SlCCS52A2*) and *Solyc06g043150*(*SlCCS52B*) and four *CDC20* homologous genes referred as*SlCDC20s* including *Solyc03g096870* (*SlCDC20.1*),*Solyc08g005420* (*SlCDC20.2*), *Solyc06g072830*(*SlCDC20.3*) and *Solyc03g095210* (*SlCDC20.4*) (Supplementary Figure S1B). *SlCCS52A2* is newly isolated in tomato genome, and its amino acid sequence has 83.05% and 57.75% identity with SlCCS52A1 and SlCCS52B, respectively (Figure S2). We analyzed subcellular localizations of SlUVI4 and SlCCS52 proteins, and revealed that all of them have nuclear localization (Figure S3). SlCCS52A1 also has strong plasma membrane localization, and SlCCS52B has weak cytoplasmatic localization. We further tested the interactions between SlUVI4 and SlCCS52 proteins, and both yeast-two hybrid and bimolecular fluorescence complementation assay (BIFC) revealed that SlUVI4 could interact with SlCCS52A1 and SlCCS52A2, but not with SlCCS52B (Figure 2). All these data suggest that *SlUVI4* and *SlCCS52* genes might have similar functions as those in Arabidopsis.

Spatial and temporal expression of *SlUVI4* and*SlCCS52* genes in tomato hypocotyls

To understand the function of APC/C in tomato growth and development, we measured transcript abundance of *SlUVI4* and *SlCCS52s* using quantitative reverse transcription–polymerase chain reaction (qRT-PCR).*SlUVI4* had the highest expression level in stem and young fruit, and moderate expression in fully expanded leaf and ovary (Figure 3A).*SlCCS52A1* had high expression levels in most organs except root and stamen, and *SlCCS52A2* had relatively low expression levels in root, petal, sepal and young fruit (Figure 3B, C). *SlCCS52B* had similar tissue-specific expression patterns to *SlUVI4* (Figure 3D). These data suggested that *SlCCS52* genes are functionally redundant in the regulation of tomato organ growth and development.

We then analyzed the transcription of *SlUVI4* and *SlCCS52*genes in hypocotyls of day 1, 3, 5 and 7 after seed germination. All genes were highly expressed at day 1 followed by dramatically reduced transcripts afterwards (Figure 3E). The transcription of*SlCCS52A1* and *SlCCS52A2* did not change at day 3 and 5, and had slight reduction and increase at day 7 (Figure 3E). The transcription of *SlUVI4* and *SlCCS52B* had significant reductions at day 5 and 7 compared to day 3. Meanwhile, we measured the transcript abundances of APC/C core genes, *SlAPC2* and*SlAPC11* . Both *SlAPC2* and *SlAPC11* had dramatic reduction of transcript abundances at day 3, and restored the transcription afterwards (Figure S4). Taken together, these data indicate that APC/C activity is differentially regulated by*SlUVI4* and *SlCCS52* genes in various tissues, and*SlUVI4* and *SlCCS52B* may have more specific roles in the regulation of hypocotyl elongation.

Lights repress hypocotyl elongation through*SlUVI4* and*SlCCS52B* mediated cell cycle progression.

To investigate light effects on tomato hypocotyl growth, we measured hypocotyl length and diameter, and cell cycle progression in hypocotyl cells of cultivar 'Heinz 1706' under dark and different light conditions. The hypocotyl length was significantly longer under dark condition than white light condition, while the hypocotyl diameter was significantly thicker under white light condition (Figure S5A-C, E-G). Cell cycle progression in hypocotyl cells was consistent in the dark, resulting in relatively steady endoreduplication indices (EIs) (Figure S5D). Under white light condition, cell cycle progression in hypocotyl cells varied at different days, in which the proportion of 2C nuclei increased gradually after day 1 resulting in reduction of EI values (Figure S5H). Overall, EI values in the white light were smaller than that in the dark, which correlated with hypocotyl elongation. We then measured transcript abundances of *SlUVI4* and *SlCCS52* , and all of them had highest transcription at day 1 under both dark and light conditions (Figure S6). The transcription of *SlUVI4* and*SlCCS52B* gradually reduced during the hypocotyl elongation under light condition, which correlated with cell cycle progression. We further investigated effects of light qualities on hypocotyl growth, cell cycle progression and transcription of *SlUVI4* and*SlCCS52* genes. The hypocotyls under red light were significantly longer than that under blue light whereas diameters of hypocotyls gradually increased under both light conditions (Figure 4A-C, E-G). The proportion of 2C nuclei gradually increased under both light conditions resulting in reduction of EI values, which was more pronounced under red light condition (Figure 4D, H). qRT-PCR analyses revealed that the transcription of *SlUVI4* and *SlCCS52B* gradually reduced under both light conditions, which was parallel to changes in cell cycle progression (Figure 4I). Their transcript abundance accumulated more under red light than blue light condition, which correlated with longer hypocotyls under red light condition. We observed the reduction of*SlCCA52A1* not *SlCCS52A2* transcription during the hypocotyl growth, and *SlCCS52A1* expression was slightly higher under red light, suggesting weak roles of *SlCCS52A1* in hypocotyl elongation under different light conditions. All these data above suggest that*SlUVI4* and *SlCCS52B* play important roles in hypocotyl elongation through the regulation of cell cycle progression.

MS salts and sugar enhance hypocotyl elongation through*SlUVI4* and *SlCCS52B* mediated cell cycle progression.

To further understand roles of *SlUVI4* and *SlCCS52B* in tomato hypocotyl elongation, we checked whether their expressions were correlated with hypocotyl elongation under Murashige and Skoog (MS) salts and sugar treatments. Without MS salts in the growth medium (non-MS, NMS), the average hypocotyl lengths were 8.5, 18.6, 21.9 and 25.4 mm, and the average diameters of hypocotyls were 0.62, 0.76, 0.69 and 0.80 mm at day 1, 3, 5 and 7 (Figure 5A, B, C). With MS salts in the growth medium (MS), the average hypocotyl lengths were 9.5, 31.3, 35.9 and 39.2 mm, and the average diameters of hypocotyls were 0.76, 0.92, 0.81 and 0.89 mm at day 1, 3, 5 and 7 (Figure 5E, F, G). With MS salts and sugar both in the growth medium (MSS), the average hypocotyl lengths were 5.3, 28.1, 37.7 and 49.2 cm, and the average diameters of hypocotyls were 0.72, 0.89, 0.87 and 1.06 mm at day 1, 3, 5 and 7 (Figure 5I, J, K). These data above indicated that addition of MS salts with or without sugar significantly promoted hypocotyl elongation and thickening. Cell cycle analyses revealed that 16C nuclei only appeared in seedlings grown on MS and MS with sugar growth medium (Figure 5H, L), which resulted in the increase of EI values compared to that on non-MS medium (Figure

5D). In fact, addition of MS salts with sugar significantly increased abundances of 8C and 16C nuclei leading to higher EI values. qRT-PCR analyses of transcription of *SlUVI4* and *SlCCS52* genes in hypocotyls at day 7 showed that MS with or without sugar had highest and moderate transcriptions of*SlUVI4* and *SlCCS52B* , and only MS with sugar could enhance the transcription of *SlCCS52A1* and *SlCCS52A2* (Figure 5M). All these data suggested that MS salts with or without sugar promoted hypocotyl elongation possibly through *SlUVI4* and *SlCCS52B*coordinated cell cycle progression.

Heat promotes hypocotyl elongation through auxin-mediated transcriptional modulation of *SlUVI4* and *SlCCS52B*.

We increased incubation temperature during the emergence of tomato seedlings, and observed that 33°C treatment significantly promoted hypocotyl elongation of cultivar 'Heinz 1706' by 1.6- and 1.3-fold at NMS and MS growth medium compared to the normal growth temperature 25°C, but did not affect the hypocotyl thickness (Figure 6A, B, C). Cell cycle analysis revealed that 33°C treatment improved endoreduplication in hypocotyl cells compared to 25°C resulting in increases of EI values from 0.85 and 0.94 at 25°C to 0.99 and 1.00 at 33°C on NMS and MS growth medium, respectively (Figure 6D). Heat induced promotion of endoreduplication was more pronounced at NMS medium than MS medium, which was in accord with the rate of improvement on hypocotyl elongation. We also measured hypocotyl growth and cell cycle progression of tomato cultivars 'Moneymaker' and 'Alisa Craig', and observed the same phenomena on hypocotyl elongation and endoreduplication after high temperature treatment except a slight decrease of hypocotyl diameter of 'Alisa Craig' grown on MS medium at 33°C comparted to that at 25°C (Figure S7). qRT-PCR analysis revealed that 33°C treatment significantly inhibited the transcription of*SlUVI4* , but did not affect *SlCCS52B* transcription. The transcription of *SlCCS52A1* was not affected by 33°C treatment, while the transcript abundance of *SlCCS52A2* had a slight increase suggesting a weak role in the regulation of endoreduplication (Figure 6E).

To investigate functions of plant hormones in heat treatment, we firstly applied different concentrations of auxin (IAA), brassinosteroids (BRs), gibberellins (GAs) and ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in growth medium to analyze hypocotyl growth. We observed slight enhancement of hypocotyl elongation responding to low concentration of IAA (Figure 7A-C; Figure S8A, B). BR and GA did not have consistent effects on hypocotyl growth (Figure S8C-F). Higher concentration of ACC had more significant effects on hypocotyl elongation and thickening (Figure S8G, H). Cell cycle analysis in hypocotyls revealed no significant difference between the control and 0.1nM IAA treatment (Figure 7D). qRT-PCR analysis showed that*SlUVI4* transcriptions were significantly reduced, and*SlCCS52B* transcription had a slight reduction without statistical significance (Figure 7E), suggesting that reduced *SlUVI4*transcription and steady *SlCCS52B* transcription might be required to maintain endoreduplication in auxin induced hypocotyl elongation. Both *SlCCS52A1* and *SlCCS52A2* transcription were significantly reduced suggesting that they might not be as important as*SlCCS52B* in hypocotyl elongation. All the data above suggested that elevated temperature promoted hypocotyl elongation possibly due to auxin-mediated repression of *SlUVI4* transcription and maintenance of *SlCCS52B* transcription.

Salt stress represses hypocotyl elongation possibly through ethylene-mediated modulation of *SlUVI4* and *SlCCS52B*transcription

We investigated tomato hypocotyl growth under salt stress. NaCl with the concentration of 150 mM in the MS medium significantly inhibited the hypocotyl elongation by 39.3% compared to the control, but did not affect the hypocotyl thickening (Figure 8A-C). Cell cycle analysis revealed that the proportion of 2C and 4C nuclei reduced, and 8C and 16C dramatically increased which led to the increase of EI value from 1.01 to 1.19 (Figure 8D). Salt stress significantly promoted *SlUVI4* ,*SlCCS52A1* and *SlCCS52A2* transcription, and inhibited*SlCCS52B* transcription (Figure 8E). To determine the role of ethylene under salt stress condition, we applied 10μM ACC in the growth medium and measured hypocotyl growth, cell cycle progression, and the transcription of *SlUVI4* and *SlCCS52B* . The hypocotyl length was reduced by 38.2% and diameter was increased by 17.0% after 10μM ACC treatment (Figure 9A-C). The proportion of 2C and 4C nuclei decreased but 8C and 16C dramatically increased in ACC treatment, which resulted in a

higher EI value (Figure 9D). qRT-PCR analysis revealed that the *SlUVI4* transcript abundance increased by 32.3% and*SlCCS52B* transcript abundance decreased by 36.3% in ACC treatment, whereas *SlCCS52A1* and *SlCCS52A2* transcription were not altered (Figure 9E). Collectively, these data above suggested that salt stress might trigger ethylene production which disrupted the balance between *SlUVI4* and *SlCCS52B* to reprogram cell cycle progression and eventually lead to the alteration of hypocotyl elongation.

Mis-regulation of *SlUVI4* and *SlCCS52B* leads to repressed hypocotyl elongation.

To further determine the role of *SlUVI4* and *SlCCS52B*played in hypocotyl elongation, we generated *sluvi4* mutants using Crispr-Cas9 technology and *SlCCS52B* overexpression lines in Arabidopsis. We obtained several mutants with different mutation types. Tomato plants with mutations in the second exon of *SlUVI4* at T1 generation could not set up fruits, and we conducted reciprocal crosses with wild type plants 'Heinz 1706' and did not get success suggesting that the second exon is pivotal for *SlUVI4* function. Interestingly, all of mutants with the capability of fruiting only had mutations in the first exon, and the number of missing nucleotides is a multiple of 3, resulting in truncated proteins of SlUVI4 (Figure S9). Mutants of *sluvi4 cr-1* and *sluvi4 cr-2* lost 6 and 42 nucleotides encoding 2 and 14 amino acids, respectively. Both mutants exhibited significantly shorter hypocotyls than wild type, and*sluvi4 cr-1* phenotype was weaker than that of *sluvi4 cr-2*(Figure 10 A, B). Both mutants had thicker hypocotyls than wild type (Figure 10C). Cell cycle analysis revealed that *sluvi4* mutants had enhanced endoreduplication indicated by more 8C and 16C and a higher EI (Figure 10D). We further investigated three independent overexpression lines of *SlCCS52B* in Arabidopsis, which had significantly shorter hypocotyls compared to wild type (Figure 11). Cell cycle analysis revealed that overexpression of *SlCCS52B* also promoted endoreduplication in hypocotyl cells compared to wild type. Meanwhile, we obtained overexpression lines of *SlCCS52A2* in tomato, and three independent lines exhibited shorter hypocotyls without the alteration of cell cycle progression (Figure S10). All these data indicated that mis-regulation of *SlUVI4* and *SlCCS52B*affects tomato hypocotyl elongation through the modulation of cell cycle progression.

Discussion

Accurate cell cycle progression is important for hypocotyl elongation and thickening, which requires precisely mediated APC/C activities. Several positive regulators of APC/C, *SlCDC20* and *SlCCS52*genes were previously identified based on their homologous sequences to Arabidopsis, Medicago and human homologs (Mathieu-Rivet, Gevaudant, Sicard, et al., 2010). In this study, we isolated the negative regulator*SlUVI4* and new positive regulators, *SlCDC20.3* ,*SlCDC20.4* and *SlCCS52A2* using published tomato genome sequence in solgenomics website (Tomato Genome, 2012). Both*SlUVI4* and *SlCCS52B* had specifically high expressions in stems. Further analyses revealed that their transcript abundances correlated with cell cycle progression and hypocotyl elongation under different light conditions, and in MS salts and sugar treatments. They responded differently to exogenous hormones and environmental stresses to modulate cell cycle progression and hypocotyl elongation. Their overexpression lines in Arabidopsis and *sluvi4* mutants had altered cell cycle progression and hypocotyl elongation. Therefore, we draw the conclusion that *SlUVI4* and *SlCCS52B* coordinated cell cycle progression play important roles in the regulation of hypocotyl elongation.

UVI4 proteins are unique and conserved in the plant kingdom (d'Erfurth et al., 2009). Like most diploid Solanaceae species, tomato has only one*UVI4* gene in the genome indicating its central role in plant growth and development. In Brassicaceae species, a genome duplication brings about two homologous genes, *UVI4* and *OSD1* , which could be clearly distinguished via sequence similarity analysis (Mieulet et al., 2016). *UVI4* and *OSD1* mainly regulate mitosis and meiosis respectively, and knockout of both genes leads to the female gametophyte lethality (Bao & Hua, 2014; Hase, Trung, Matsunaga, & Tanaka, 2006; Heyman et al., 2011; Iwata et al., 2011). Here we presented results on tomato homologue *SlUVI4* in the regulation of cell cycle progression during hypocotyl elongation, while its roles in the gametophyte development require further studies. *CCS52* genes are activators of APC/C to promote the onset and progression of endoreduplication in plant organs (Cebolla et al., 1999; Vanstraelen et al., 2009). In tomato, only *SlCCS52A1* (previously as*CCS52A*) was reported to play important roles in fruit development through the regulation of endoreduplication (Mathieu-Rivet, Gevaudant, Sicard, et al., 2010). In Arabidopsis, UVI4 negatively

regulates APC/C activity through the interaction with CDC20 and CCS52 proteins. SlUVI4 can interact with SlCCS52A1 and SlCCS52A2, but not with SlCCS52B. Whether or not SlUVI4 can interact with the conserved domain of SlCCS52B remains to be further explored. And here we want to emphasize that the similar spatial and temporal expression patterns of *SlUVI4* and*SlCCS52B* imply important roles of their coordination in the regulation of tomato organ growth and development.

Hypocotyl elongation is based on cell division and expansion, which are maintained by APC/C activities. Based on our observations, the highest expression of *SlCCS52* genes right after the seed gemination results in the most robust APC/C activities to promote hypocotyl elongation, which consequently ensures the successful emergence from soil. Down-regulation of *SlCCS52A1* transcription led to the reduced plant heights and fruit size with decreased endoreduplication (Mathieu-Rivet, Gevaudant, Sicard, et al., 2010). Overexpression of*SlCCS52A1* in tomato resulted in the promotion of endoreduplication and abnormal growth phenotypes including impaired root and leaf growth (Mathieu-Rivet, Gevaudant, Cheniclet, Hernould, & Chevalier, 2010; Mathieu-Rivet, Gevaudant, Sicard, et al., 2010). Interestingly, overexpression lines of *SlCCS52B* in Arabidopsis generated in this study also had shorter hypocotyls with enhanced endoreduplication (Figure 11). Therefore, excessive APC/C activities triggered by overexpression of *SlCCS52* genes requires*SlUVI4* -mediated inhibition machinery to fine-tune the APC/C activities and facilitate plant growth and development. In this case, tomato hypocotyl might grow faster when both *SlUVI4* and*SlCCS52B* have higher transcript abundances, which proved to be true when tomato seedlings grew under different lights and in MS salts and sugar treatments. Light promotes cell division by activating photoreceptors to suppress cell division inhibitors (Okello, de Visser, Heuvelink, Marcelis, & Struik, 2016). Light inhibits the transcription of *KRP1* , a CDK inhibitor, and activates cell cycle genes such as*CDKB1;1* , *CDKB1;2* and *CYCA2;2* (Lopez-Juez et al., 2008). Under light conditions, the transcription of *SlUVI4* and*SlCCS52B* is growth-dependent, and higher in younger hypocotyls. Their reduced transcription during the hypocotyl growth correlated with enhanced cell division suggesting their coordination in endoreduplication.

Exogenous application of ethylene precursor ACC stimulates cambial cell division in *Populus* (Love et al., 2009), which may be the reason of increased tomato hypocotyl thickness after ACC treatment (Figure 9C). Application of ACC promotes *SlUVI4* transcription, whereas represses *SlCCS52B* transcription, which collectively results in the active cell division. However, we observed promotion of endoreduplication in hypocotyl cells, which is due to ethylene-induced DNA synthesis without cytokinesis through unknown mechanisms (Dan, Imaseki, Wasteneys, & Kazama, 2003). We observed the similar phenomena of hypocotyl elongation, cell cycle progression and transcriptions of*SlUVI4* and *SlCCS52B* under salt stress condition, suggesting that ethylene signaling is involved in salt responses. Ethylene drastically promotes hypocotyl elongation in nutrient-starved Arabidopsis seedlings under light condition, and might be a mediator of auxin-induced hypocotyl elongation (Smalle et al., 1997). High temperature promotes Arabidopsis hypocotyl elongation due to enhanced auxin synthesis or catabolism (Gray et al., 1998), which results in enhanced tomato hypocotyl elongation as well. High temperature induced promotion of endoreduplication in hypocotyl cells might be attributed to enhanced ethylene biosynthesis mediated by auxin (Kang, Newcomb, & Burg, 1971), which correlates with the repressed and promoted transcription of *SlUVI4* and *SlCCS52A2* in our study, respectively. However, the regulation machinery of *SlUVI4* and*SlCCS52A2* transcription by high temperature remains to be further studied.

The deletion mutant of *SlUVI4* and overexpression lines of*SlCCS52B* both have promoted endoreduplication in hypocotyl cells, indicating their antagonistic roles in cell cycle progression as the function of their homologues previously reported in Arabidopsis (Bao & Hua, 2014; Heyman et al., 2011; Iwata et al., 2011). *SlCCS52B*function remains to be further studied using tomato mutants. Hypocotyl elongation is dependent on the active cell division and consequent cell expansion, which are regulated by *SlUVI4* and *SlCCS52B* , respectively. Based on our study, we proposed a possible model of*SlUVI4* and *SlCCS52B* coordinated cell cycle progression (Figure S11). Under normal growth condition, light suppresses*SlUVI4* and *SlCCS52B* coordinated cell cycle progression to inhibit hypocotyl elongation. MS salts and sugar promote *SlUVI4*and *SlCCS52B* coordinated cell cycle progression to improve hypocotyl elongation. Under stress condition, salt stress triggers ethylene-mediated repression of *SlUVI4* and *SlCCS52B* to inhibit hypocotyl elongation. Heat

stimulates auxin and its mediated ethylene production to modulate *SlUVI4* and *SlCCS52B*function to promote hypocotyl elongation.

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Conflict of interest

The authors declare no potential conflict of interest.

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Figure legends

Figure 1. Sowing depth significantly affects both tomato seed germination and hypocotyl elongation. (A) Morphology of 10-day tomato cultivar 'Heinz 1706' seedlings after seeds were sown at different depth of soil. Dotted lines crossing the pots indicated the positions of seeds. Scale bar, 1cm. (B) Percentage of tomato seedlings emerged from soil. (C) Germination rate of tomato seeds at day 5 after sowing. (D) Morphology of 7-day tomato seedlings after sown at 4 cm depth of soil. Data are presented by means ± SE. Different letters indicate statistical significance ($p < 0.05$) determined by LSD Test in DPS Software.

Figure 2. Interactions between SlUVI4 and SlCCS52 proteins. (A) Yeast-two hybrid assay. Serial dilutions of yeast cells containing SlUVI4 fused with the activation domain (AD) and SlCCS52 fused with the DNA binding domain (BD) were plated on amino acid deficient yeast growth medium. (B) Confocal microscopy images from BiFC assay in onion epidermal cells. SlUVI4:YFP^N together with SlCCS52A1:YFP^C or SlCCS52A2:YFP^C were transiently expressed in onion epidermal cells. Images were taken at 48 h after incubation. Green signals are from YFP. Scale bar, 50 μm.

Figure 3. Gene expression of *SlUVI4* and *SlCCS52s* in different tissues and hypocotyls at different growth stages. The transcript abundance of *SlUVI4* (A), *SlCCS52A1* (B),*SlCCS52A2* (C) and *SlCCS52B* (D) in different tissues of tomato cultivar 'Heinz 1706' and hypocotyls at day 1, 3, 5 and 7 after seed germination (1d, 3d, 5d, 7d) were analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR). *SlCBL1*(Calcineurin B-like protein, Solyc12g015870) transcript abundance was used for data normalization. The transcript abundance of each gene in the root was used for all comparisons to calculate the relative expression. Data shown are means ± SE calculated from three independent experiments. Letters on the top of each column indicate statistically significant differences ($p < 0.05$) determined by LSD test in DPS software.

Figure 4. Different lights have distinct impacts on hypocotyl elongation and gene expression of *SlUVI4* and *SlCCS52s* . Tomato seedlings of cultivar 'Heinz 1706' were grown on MS medium under red light (A-D) and blue light (E-H) conditions. (A, E) Morphology of tomato seedlings. (B, F) Hypocotyl length. (C, G) Diameter in the middle of hypocotyl. Data represents means ± SE (n > 15). Different letters indicate statistical significance ($p < 0.05$) determined by LSD test in DPS Software. (D, H) Nuclear DNA distribution in hypocotyls. Data are presented by means ± SD. EI values are on the top of columns. (I) qRT-PCR analysis of *SlUVI4* , *SlCCS52B* ,*SlCCS52A1* and *SlCCS52A2* in hypocotyls using *SlCBL1*as a reference gene. Data shown are means ± SE calculated from three independent experiments.

Figure 5. MS salts and sugar enhance hypocotyl elongation through the promotion of *SlUVI4* and *SlCCS52B* transcription. Tomato seedlings were grown on non-MS agar (NMS, A-D), MS agar (MS, E-H) and MS agar with sugar (MSS, I-L) for 1, 3, 5, and 7 days after seeds were sown. (A, E, I) Morphology of tomato seedlings. (B, F, J) Hypocotyl length. (C, G, K) Diameter in the middle of hypocotyl. Data represents means ± SE. Different letters indicate statistical significance ($p < 0.05$) determined by LSD test in DPS Software. (D, H, L) Nuclear DNA ploidy distribution in hypocotyls. Data represents means ± SD. The number on the top of each column indicates EI. (M) qRT-PCR analysis of *SlUVI4* ,*SlCCS52B* , *SlCCS52A1* , and *SlCCS52A2* in hypocotyls using *SlCBL1* as a reference gene. Data shown are means ± SE calculated from three independent experiments. Letters on the top of each column indicate statistically significant differences ($p< 0.05$) determined by LSD test in DPS software.

Figure 6. Heat enhances hypocotyl elongation through the transcriptional modulation*SlUVI4* and *SlCCS52* genes. (A) Morphology of day seedlings grown on NMS and MS agar plates at 25°C and 33°C. (B) Hypocotyl length. (C) Diameter in the middle of hypocotyl. (D) Nuclear DNA ploidy distribution in hypocotyls. (E) qRT-PCR analysis of *SlUVI4* ,*SlCCS52B* , *SlCCS52A1* and *SlCCS52A2* in hypocotyls using *SlCBL1* as a reference gene. The transcript abundance of each gene at 25°C was used to calculate the relative expression. Data shown are means ± SE calculated from three independent experiments. The asterisk on the top of column indicates statistically significant differences ($p < 0.05$) determined by student$t$ -test.

Figure 7. Auxin promotes the hypocotyl elongation via the transcriptional modulation of *SlUVI4* and *SlCCS52* genes. (A) Morphology of tomato cultivar 'Heinz 1706' seedlings after 0.1 nM IAA treatment for 7 days. (B) Hypocotyl length. (C) Diameter in the middle of hypocotyl. (D) Nuclear DNA ploidy distribution in hypocotyls. (E) qRT-PCR analysis of *SlUVI4* and *SlCCS52s* in hypocotyls using *SlCBL1* as a reference gene. Data shown are means ± SE calculated from two independent experiments. The asterisk on the top of column indicates statistically significant differences ($p< 0.05$) determined by student t -test.

Figure 8. Salt stress inhibits hypocotyl elongation through the transcriptional modulation of *SlUVI4* and *SlCCS52* genes. (A) Morphology of tomato cultivar 'Heinz 1706' seedlings after 150 mM NaCl treatment for 7 days. (B) Hypocotyl length. (C) Diameter in the middle of hypocotyl. (D) Nuclear DNA ploidy distribution in hypocotyls. (E) qRT-PCR analysis of *SlUVI4* and *SlCCS52B* in hypocotyls using *SlCBL1* as a reference gene. Data shown are means ± SE calculated from three independent experiments. The asterisk on the top of column indicates statistically significant differences ($p< 0.05$) determined by student t -test.

Figure 9. Ethylene affects the hypocotyl elongation via the transcriptional modulation of *SlUVI4* and *SlCCS52* genes. (A) Morphology of tomato cultivar 'Heinz 1706' seedlings after 10μM ACC treatment for 7 days. (B) Hypocotyl length. (C) Diameter in the middle of hypocotyl. (D) Nuclear DNA ploidy distribution in hypocotyls. (E) qRT-PCR analysis of *SlUVI4*and *SlCCS52B* in hypocotyls using *SlCBL1* as a reference gene. Data shown are means ± SE calculated from three independent experiments. The asterisk on the top of column indicates statistically significant differences ($p < 0.05$) determined by student t -test.

Figure 10. Deletion mutants of *SlUVI4* exhibit defective hypocotyl elongation. (A) Morphology of 7-day seedlings grown on MS medium. (B) Hypocotyl length. (C) Diameter in the middle of hypocotyl. Data represents means ± SE (n > 19). Different letters indicate statistical significance ($p < 0.05$) determined by LSD test in DPS Software. (D) Nuclear DNA ploidy distribution in hypocotyls. Data are presented by means ± SD. EI values are on the top of columns.

Figure 11. Overexpression of *SlCCS52B* suppresses hypocotyl elongation. (A) Morphology of 6-day seedlings grown on 1/2 MS medium under 10μmol m^{-2} s^{-1} light. (B) Hypocotyl length. Data represents means ± SE (n > 50). Different letters indicate statistical significance ($p< 0.05$) determined by LSD test in DPS Software. (c) Nuclear DNA ploidy distribution in hypocotyls. Data are presented by means ± SD. EI values are on the top of columns.

Supporting Information

The following supplemental materials are available.

Table S1. Primer sequences used in this study.

Figure S1. Phylogenetic analysis of *UVI4* and *CCS52*homologous genes in *Solanaceae* species. The full-length amino acid sequences of UVI4 and CSS52 were isolated through BLAST analysis in the website (https://solgenomics.net/) and analyzed in the software MEGA X to determine their relationships. The bootstrap values are shown at the nodes. (A) Phylogenetic relationship among *UVI4* homologous genes. (B) Phylogenetic relationship among *CCS52* homologous genes.

Figure S2. Amino acid sequence alignment of tomato CCS52 homologs. Tomato *CCS52* homologous genes were isolated from the website*www.solgenomics.net*. Alignment of amino acid sequences was performed using online multiple sequence alignment program Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Figure S3. Subcellular protein localization. Fully expanded leaves of *N. benthamiana* were infiltrated with *A. tumefaciens* strain GV3101 containing different constructs with 35S promoter-driven GFP or *SlCCS52*genes fused with GFP.

Figure S4. Gene expression of APC/C core genes in hypocotyls at different growth stages after seed germinated. Hypocotyls of tomato cultivar 'Heinz1706' grown on agar with MS salts were harvested at day 1, 3, 5 and 7 (1d, 3d, 5d and 7d) after seeds germinated. qRT-PCR analysis of *SlAPC2* and *SlAPC11* in hypocotyls using *SlCBL1* as a reference gene. The transcript abundance of each gene at day 1 was used

for all comparisons to calculate the relative expression. Data shown are means ± SE calculated from three independent experiments. Letters on the top of each column indicate statistically significant differences ($p < 0.05$) determined by least significance difference test (LSD) in DPS software.

Figure S5. White light represses hypocotyl elongation through the suppression of endoreduplication. Tomato seedlings of cultivar 'Heinz 1706' were grown on MS medium in the dark (A-D) and white light (E-H) conditions. (A, E) Morphology of tomato seedlings. (B, F) Hypocotyl length. (C, G) Diameter in the middle of hypocotyl. Data represents means ± SE (n > 15). Different letters indicate statistical significance ($p < 0.05$) determined by LSD test in DPS Software. (D, H) Nuclear DNA distribution in hypocotyls. Data are presented by means ± SD. EI values are on the top of columns.

Figure S6. Gene expression of*SlUVI4* and *SlCCS52s* in hypocotyls at different growth stages in the dark and white light condition. Transcript abundance of*SlUVI4* (A), *SlCCS52B* (B), *SlCCS52A1* (C), and*SlCCS52A2* (D) in hypocotyls was analyzed by qRT-PCR. *SlCBL1* transcript abundance was used for data normalization. Data shown are means ± SE calculated from three independent experiments.

Figure S7. Heat promotes the endoreduplication to improve hypocotyl elongation of different tomato cultivars. Two tomato cultivars 'Alisa Craig' (A-C) and 'Moneymaker' (D-F) were grown on NMS and MS agar plates at 25°C and 33°C for days. (A, D) Hypocotyl length. (B, E) Diameter in the middle of hypocotyl. Data represents means ± SE (n > 15). Different letters indicate statistical significance ($p < 0.05$) determined by LSD test in Data Processing System (DPS) Software. (C, F) Nuclear DNA ploidy distribution in hypocotyls. Data represents means ± SD. EI values are on the top of columns.

Figure S8. Exogenous application of plant hormones affects hypocotyl elongation. Tomato seedlings of cultivar 'Heinz 1706' were grown on MS medium supplemented with different concentration of plant hormones IAA (A, B), BR (C, D), GA3 (E, F) and ACC (G, H). (A, C, E, G) Hypocotyl length. (B, D, F, H) Diameter in the middle of hypocotyls. Data represents means ± SE (n > 15). Different letters indicate statistical significance ($p < 0.05$) determined by LSD test in DPS Software.

Figure S9. Deletion mutations of*SlUVI4* generated using Crispr-Cas9 technology. (A) Target sites of the guide RNAs in Crispr-Cas9 gene editing system. (B) DNA sequence information of *SlUVI4 cr-1* and *cr-2* mutations. Nucleotides in blue are target sequences. The red hidden lines indicate missing nucleotides. Black lines indicate the protospacer-adjacent motif (PAM).

Figure S10. Overexpression of*SlCCS52A2* inhibits tomato hypocotyl elongation. (A) Morphology of 7-day seedlings grown on MS medium. (B) Hypocotyl length. (C) Diameter in the middle of hypocotyl. Data represents means ± SE (n > 15). Different letters indicate statistical significance ($p< 0.05$) determined by LSD test in DPS Software. (D) Nuclear DNA ploidy distribution in hypocotyls. Data are presented by means ± SD. EI values are on the top of columns.

Figure S11. A possible model for*SlUVI4* and *SlCCS52B* coordinated hypocotyl elongation under normal and stress conditions. Under normal growth condition, light represses *SlUVI4* and*SlCCS52B* coordinated endoreduplication to inhibit hypocotyl elongation. MS salts and sugar promote *SlUVI4* and *SlCCS52B*coordinated endoreduplication to enhance hypocotyl elongation. Under stress condition, salt triggers ethylene production which represses*SlUVI4* and *SlCCS52B* coordinated endoreduplication to inhibit hypocotyl elongation, while ethylene itself promotes endoreduplication via unknown cell cycle machinery. Heat-stimulated auxin production and auxin-mediated ethylene production reorchestrate*SlUVI4* and *SlCCS52B* coordinated endoreduplication to promote hypocotyl elongation.