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Identification and expression of the trehalose-6-phosphate synthase gene family members in tomato exposed to different light spectra

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Abstract: Light is the source of energy for plants. Light wavelengths, densities and irradiation periods act as signals directing morphological and physiological characteristics during plant growth and development. To evaluate the effects of light wavelengths on tomato growth and development, *Solanum lycopersicum* (cv. micro-Tom) seedlings were exposed to different light-quality environments, including white light and red light supplemented with blue light (at ratios of 3:1 and 8;1, respectively). Tomatoes grown under red light supplemented with blue light displayed significantly shorter stem length, a higher number of flower buds and rate of fruit set, but an extremely late flowering compared to white-light-grown plants. To illustrate the mechanism underlying the inhibition of stem growth and floral transition mediated by red/blue light, 10 trehalose-6-phosphate synthase (TPS) genes were identified in tomato, and bioinformatics analysis was performed. qRT-PCR analysis showed that *SlTPS*s were expressed widely throughout plant development and *SlTPS1* was expressed at extremely high levels in stems and buds. Further analysis of several flowering-associated genes and microRNAs showed that the expressions of *SlTPS1*, *SlFT* and *miR172* were significantly downregulated in tomato grown under red and blue light compared with those grown under white light, whereas miR156 transcript levels were increased. A regulatory model underlying vegetative growth and floral transition regulated by light qualities is presented. Our data provide evidence that light quality strongly affects plant growth and phase transition, most likely via the TPS1-T6P signaling pathway.

Key words: TPS; light wavelength treatments; vegetative growth; floral transition; tomato

INTRODUCTION

Plants are exposed to various environmental conditions constantly. The most dramatic variation is in the daily rhythm under a light-dark cycle. Irradiative changes could be perceived by red- and blue-light photoreceptors and gene expression was regulated in plants in response to various light environments [1,2]. Phytochromes are biliprotein photoreceptors that respond to different light wavelengths; they are responsive to changes in light quality and quantity, light direction and period length, enabling plants to respond optimally to changed light conditions [3]. Light acts as signals regulating seed germination, seedling establishment, the proper development of photosynthetic machinery, the architecture of the vegetative plant, the timing of flowering, tuberization, bud dormancy, and potentially the allocation of resources to root, stem, leaf, and storage organs. The phytochromes also display regulatory functions mediating light responses, such as growth inhibition, leaflet or organelle movement and phototropism. The recent discovery of phytochrome-related proteins in photosynthetic cyanobacteria and nonphotosynthetic eubacteria has opened new avenues for investigating biliprotein photosensory function [3].

The transition of plants from vegetative to reproductive development is crucial for their successful reproduction. Several external and endogenous factors were found to participate in phase transition, such as day length, temperature, hormonal status, and carbohydrate availability [4]. Energy status, which was indicated by light density and periods, was found to affect plant growth and development [5]. Trehalose-6-phoshate (T6P) is the metabolic precursor of the non-reducing disaccharide trehalose. It is the product of the condensation reaction of uridine diphosphate (UDP)-glucose and glucose-6-phosphate (G6P), which is catalyzed by trehalos-6-phoshate synthase (TPS) [6].

T6P, found only in trace amounts in most plants, has been suggested as the signaling molecule that transduces messages of carbohydrate availability to other signaling pathways [7]. *TPS1* loss-of-function mutants are embryonic-lethal [8]. Embryo defects in *tps1* mutants can be rescued by *TPS1* overexpression in seeds, but the seedlings develop slowly and senesce before entering into the reproductive phase [9]. Elevating TPS1 in *tps1-2* mutants results in a delayed floral transition [10]. These results indicated that *TPS1* is required for the initiation of flowering. Further evidence showed that the T6P pathway regulated floral transition at two sites in the plant: the leaves and the shoot apical meristem (SAM) [11]. In the leaves, TPS1 induces the FLOWERING LOCUS T (FT) gene, which is then transported to the SAM and promotes floral formation; T6P acts as an indicator of a plant's carbon status in the SAM [11].

To study the effects of different combinations of light wavelengths on tomato growth and development, micro-Tom (*Solanum lycopersicum*) seedlings were exposed to different light-quality environments, including white light and red LEDs supplemented with blue light. Interestingly, tomato grown under red LEDs supplemented with 1:3 or 1:8 blue light displayed shorter stem length and an extremely late flowering compared to those of white-light-grown plants. To further explore the regulatory mechanism, 10 *SlTPS* that may participate in energy fluency were identified and investigated at the transcription level during the processes of plant vegetative and reproductive development and in response to changes in light quality. Our data provided the evidence that different light qualities have strongly significant effects on plant growth and phase transition.

MATERIALS AND METHODS

Plant materials and light treatments

Tomato (*Solanum lycopersicum* cv. Micro-Tom) plants were grown in a standard culture chamber under the following conditions: 16/8 h day/night cycle, 23°C, 80% relative humidity. The light density was adjusted to 2000 μmol $s^{-1}m^{-2}$ via the methods for determining light density according to Thimijan [12]. Seeds were sterilized, germinated in 1/2 MS medium for 7 days, and then seedlings of similar sizes were transplanted to pots containing nutrient soil.

To evaluate the effects of light quality (spectral distribution of light) on tomato growth, the seedlings were exposed to different light conditions: white light and red light supplemented with blue light (red/blue-light photosynthetic photon flux density (PPFD) ratios were 3:1 and 8:1, respectively). Three types of LEDs were used: InGaN/YAG white LEDs (color temperature of 6500K), red gallium-aluminum-arsenide (GaAlAs) LEDs and InGaN blue LEDs. Red and blue LEDs have a peak emission at 660 nm and 450-470nm, respectively. Seedlings under white light were designated as the control.

Identification of tomato TPS genes

The TPS full-length cDNA sequences of *Arabidopsis* were obtained from the nucleotide database of NCBI (http://www.ncbi.nlm.nih.gov/nucleotide/) and the corresponding amino acid sequences were also obtained. To identify new homologues in tomato, the complete cDNA and protein sequences of TPS gene families in *Arabidopsis* were subjected to BLASTN and TBLASTN searches against SGN tomato WGS chromosomes (version SL2.40) (http://solgenomics.net/tools/blast) [13]. Taken together, 10 potential TPS genes in tomato were identified from the currently available genomic databases.

Bioinformatic analyses of tomato TPS genes

After searching for *SlTPS*s, the bioinformatics tool FGENESH (http://linux1.softberry.com/berry) was used to analyze and predict those unknown *SlTPS*s. A conserved domain database (http://www.ncbi.nlm.nih.gov/Structure/cdd/) was used for functional annotation of the proteins. Deduced amino acid sequences of *SlTPS*s were aligned with the homologous proteins in *Arabidopsis* using ClustalX 2.0 software in the default setting. A phylogenetic tree was constructed using the MEGA (version 5.0) software by the neighbor-joining (N-J) method.

RNA isolation and quantitative real-time PCR

Different tissues and fruits from different developmental stages were harvested, frozen in liquid nitrogen and stored at -80°C. Total RNA was isolated using Trizol Reagent (Invitrogen, USA) according to the manufacturer's instructions. Total RNA used for cDNA synthesis

was treated with *DNase* I (Fermentas, Thermo, USA) to remove contaminating genomic DNA. First-strand cDNA was reverse transcribed from 2 μg of total RNA using the RevertAid™ First Strand cDNA synthesis kit (Fermentas, Thermo, USA) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was conducted on a CFX Connect Detection system (Bio-Rad, USA). PCR amplification reactions were performed in a 20-μL mixture containing 10 μL of FastSYBR Mixture (CWBIO, China), 2.5 ng of total RNA and 1 μL each of the forward and reverse primers (5 μM). PCR detection were performed by the three-step method: incubation at 95°C for 5 min, followed by 40 cycles at 95°C for 5 s, 60°C for 5 s and 72°C for 5 s. Relative expression levels were calculated based on the $2^{-\Delta\Delta Ct}$ method. Actin (Slactin-51, accession number Q96483) was used as the reference gene for expression analysis of 10 *TPS* and *FT* genes.

To investigate the expression of miRNAs, poly(A) was added to the total RNA using *Escherichia coli* poly(A) polymerase (NEB, UK) at 37°C for 30min and then reverse transcription was performed and adaptor was added with an oligo(dT) adapter primer (AAGCAGTGGTATCAACGCAGAGTACTTTTTTTTTTTTTTTTTTTTTTTVN) [14]. *U6* (accession number X51447.1) was used as reference gene for expression analysis of miRNA. Gene accession numbers and primers for qRT-PCR are listed in Table 1. For all qRT-PCR experiments, at least three biological replicates were performed and each reaction was run in triplicate.

Table 1. Primers for qPCR.

Gene name	Primer name	Sequence(5' to 3')
Solyc07g062140.2.1	TPS2140qForward	GCCTATTCATCATCTGGACCGTTCT
	TPS2140qreverse	AAGTTATGCCTGTGGCGTTTTTCTC
Solyc02g071590.1.1	TPS1590qForward	TCTTTTCCATTATCTCCCACTTCCG
	TPS1590qreverse	GCTGTCCTTGGTCCTCTACTCCTTC
Solyc08g076650.2.1	TPS6650qForward	CAAGATGGTGAGGAAGGATGGAACT
	TPS6650qreverse	ATGGAAATGGGCTATGGAGGAAGAA
Solyc01g005210.2.1	TPS5210qForward	TAACGAAATGCAAGAGAAAGGAATG
	TPS5210qreverse	AGGCAAATGTAATAGATGTGGTGGG
Solyc10g007950.2.1	TPS7950qForward	TGCCTTGGTTTGTTTGGTTTCCTTC
	TPS7950qreverse	CGGTTTCTTCCTTCAATCCGTTTCT
Solyc07g055300.2.1	TPS5300qForward	TATCAAACCCTAAAATCCACCCCCT
	TPS5300qreverse	CTCATTCCAACTAAAACTCCAGCCC
Solyc02g072150.2.1	TPS2150qForward	CTATCCTGTTGTAGTCGCATGTTGG
	TPS2150qreverse	CTCTTGACGCTTGTTTGAATTTCTT
Solyc05g005750.2.1	TPS5750qForward	TGATGTGGAGCAGAGTGAACAGGAG
	TPS5750qreverse	GACGAAGGAAATGGGCTATGAAGAA
Solyc04g025940.2.1	TPS5940qForward	GGGATACAACAGCTGAAAAGTGGTG
	TPS5940qreverse	GATGGGAATGGGCTATGAAGAAAGA
Solyc07g006500.2.1	TPS6500qForward	CCTTCATAGTCCTTTCCCGTCGTCT
	TPS6500qreverse	TCTTTCAATTCCTTCGCCTTTTTCG
Solyc05g053850.2.1	SlFT-F	TGTGGTTTACAACAATAGGGTGG
	SlFT-R	TGTCTAGAATCGATTATGTCCGG
miR156(M00016)	qmiR156-F	TTGACAGAAGATAGAGAGCAC
miR172(M00046)	qmiR172-F	AGAATCTTGATGATGCTGCA

RESULTS

Identification and multiple sequence analysis of SlTPS genes

In total, 10 SlTPS genes were identified via the BLAST search against the tomato genome sequence. Based on multiple alignments of TPS protein sequences in *Arabidopsis* and tomato, the SlTPS protein families clustered into three subfamilies/subclasses. three TPS proteins (Solyc02g072150.2.1, Solyc10g007950.2.1 and Solyc07g055300.2.1) showed close homology to AtTPS7, while Solyc01g005210.2.1 and Solyc08g076650.2.1 displayed high similarity to AtTPS5 and AtTPS6, respectively. The five TPS gene homologs mentioned above belonged to subclass I. It was found that Solyc07g006500.2.1 was most closely homologous to AtTPS11, while Solyc05g005750.2.1 and Solyc04g025940.2.1 were more closely homologous to AtTPS8, AtTPS9 and AtTPS10 genes, which belonged to subclass II. Two TPS proteins (Solyc07g062140.2.1 and Solyc02g071590.1.1), belonged to subclass III, and have high sequence similarity with AtTPS1 (Fig. 1).

Expression patterns of SlTPS genes in different tissues and organs

To investigate the potential functions of SlTPS genes, their expression profiles were determined by qRT-PCR in different tissues including root, stem (St), leaf (L), bud, flower (F) and four stages of fruit development. It is apparent that *SlTPS*s were widely expressed throughout the plant development (Fig. 2). Compared with other tissues, the 10 SlTPS genes in fruits were expressed at relatively high levels, and their expression patterns during fruit development and ripening could be divided into three types. The transcripts of four genes including Solyc07g062140.2.1, Solyc02g071590.1.1, Solyc08g076650.2.1 and Soly-

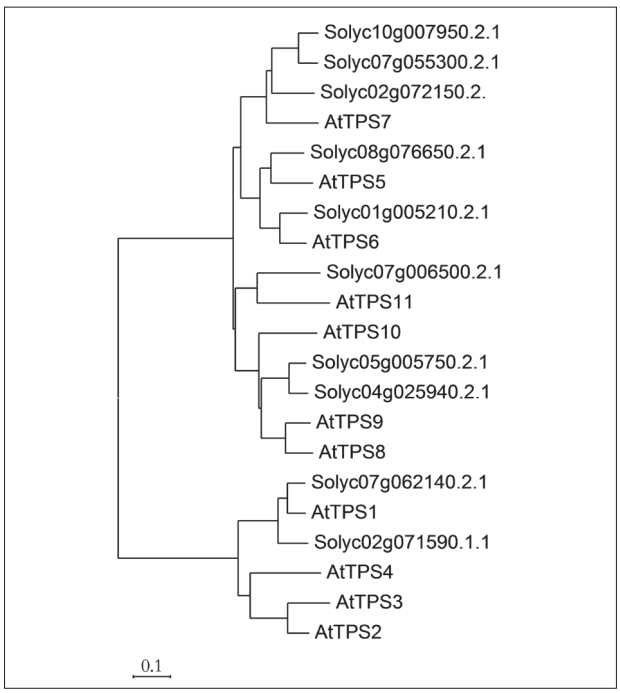

Fig. 1. Phylogenetic analysis of TPS proteins. The phylogenetical tree was generated using MEGA5.0 program by the neighbor-joining method. The accession numbers of TPS genes in *Arabidopsis* were as follows. AtTPS1 (AT1G78580.1), AtTPS2 (AT1G16980.1), AtTPS3 (AT1G17000.1), AtTPS4 (AT4G27550.1), AtTPS5 (AT4G17770.1), AtTPS6 (AT1G68020.2), TPS7 (AT1G06410.1), AtTPS8 (AT1G70290.1), AtTPS9 (AT1G23870.1), AtTPS10 (AT1G60140.1), AtTPS11 (AT2G18700.1).

c10g007950.2.1 were decreased following fruit ripening, while Solyc07g055300.2.1 showed the reverse pattern, indicating their potential roles in fruit ripening. In addition, other TPS genes were consistently expressed at relatively high levels throughout fruit developmental stages. With respect to floral organs, all TPS genes were expressed at extremely low levels in floral buds with the exception of Solyc02g071590.1.1, which was strongly expressed in leaf and bud. Four genes (Solyc07g055300.2.1, Solyc07g006500.2.1, Solyc04g025940.2.1 and Solyc07g062140.2.1) upregulated remarkably during the flowering process. With respect to the vegetative organs, there were 7, 2 and 1 TPS genes expressed at extremely high levels in stems, roots and leaves, respectively (Fig. 2).

Effects of light quality on floral transition in tomato

The vegetative and reproductive growth of tomato under different irradiation sources after a 50-day cul-

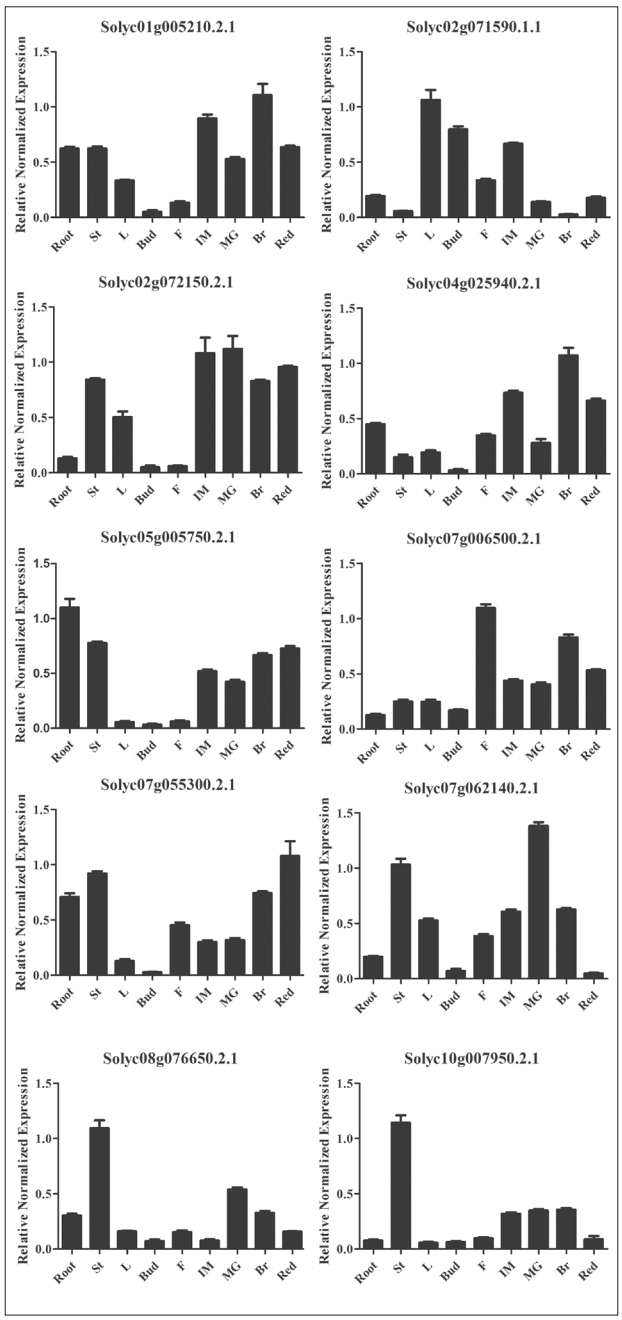

Fig. 2. Expression patterns of *SlTPS* in different organs. Root, stem (St), leaves (L), floral buds (Bud), flowers (F), immature green fruit (IM), mature green fruit (MG), breaker (Br) and red fruit (Red) were collected for investigation.

ture after germination was investigated. The morphological and physiological characteristics of at least 30 plants, including flowering time, length of internodes, number of flower buds and the rate of fruit set, were observed and collected. The results showed that the flowering time was significantly delayed in tomato

Table 2. Floral timing in tomatoes grown under environments with different light-quality. At least 30 plants were investigated for each treatment.

Light Treatments	Flowering time (Mean dpg)	SE	P value
White light	40.25	0.3541	N
Red LEDs and 1:3 blue light	46.34	0.4267	<0.0001

dpg – days post germination. SE – standard error.

Table 3. Flowering ratio in seedlings grown under environments with different light-quality 50 days post germination.

Light Treatment	Total Buds	Flowered Number
white light	15	13
Red LEDs and 1:3 blue light	15	2
Red LEDs and 1:8 blue light	0	0

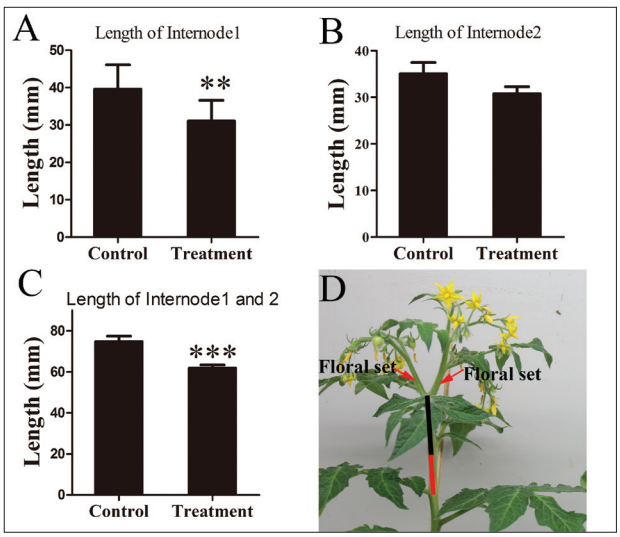

Fig. 3. Internode lengths in tomato seedlings grown under different light-quality environments. Control – white-light-grown plants, Treatment – seedlings grown under red LEDs and 1:3 blue light environment, internode 1 – the first internode below flowering branch (black bar), and internode 2 – the second internode below flowering branch (red bar). Student's t-test was used to analyze the significance. One asterisk (*) – significant ($0.01<p<0.05$) differences between samples, two asterisks (**) – significant ($0.001<p<0.01$) differences between samples, and three asterisks (***) – significant ($p<0.001$) differences between samples.

seedlings grown under red light supplemented with blue light compared with those grown under white light. The tomato grown under red and blue light (at a ratio of 3:1) flowered at approximately 46 days post germination (dpg), and 40 dpg in plants under white light (Table 2). Meanwhile, the flowering ratio significantly differed under different irradiation sources and various blue to red LED ratios. Statistical analysis showed that 13/15 buds flowered under

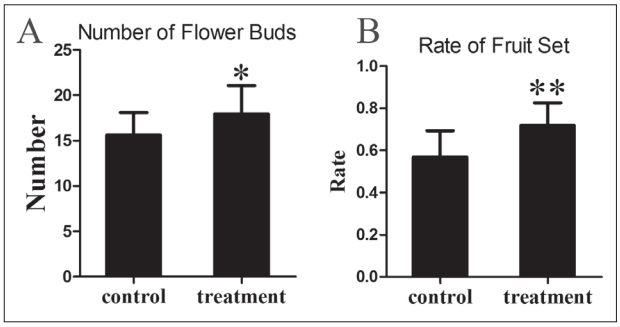

Fig. 4. Number of flower buds and rates of fruit set under different light-quality environments. Control – white-light-grown plants, Treatment – seedlings grown under red LEDs and 1:3 blue light environment. Significance was analyzed using the same methods as mentioned above.

white light at 50 dpg, but only 2/15 buds flowered under 75% red and 25% blue LEDs (3:1). There were no flowers observed in seedlings under red and blue LEDs (8:1 ratio) (Table 3). However, these seedlings restated their floral transition later when transferred to white light conditions at 50 dpg. In terms of vegetative growth, the lengths of two internodes below the first flowering branch were distinctly shortened in seedlings under 75% red and 25% blue LEDs compared to controls (Fig. 3). Interestingly, we observed that the plants grown under red and blue LEDs (3:1 ratio) produced more flower buds and the rate of fruit set was significantly increased compared to those in seedlings under white light treatment (Fig. 4).

Expression of flowering-associated genes and microRNAs

TPS gene families play important roles in plant growth regulation, especially *TPS1*. According to the abovementioned results, Solyc02g071590.1.1 showed homology to *AtTPS1*, and was named *SlTPS1-1*. *SlTPS1-1* was strongly expressed in flower buds, suggesting a potential important role in the initiation of flowering. To reveal the molecular mechanism underlying whether floral transition was regulated by light quality, the transcripts of *SlTPS1-1* and floral transition-related genes or microRNA, such as *FT*, *miR172* and *miR156*, were determined by qRT-PCR in tomato plants under different light treatments. The expressions of *SlTPS1-1*, *SlFT* and miR172 were significantly downregulated in tomato grown under red and blue LEDs (at 3:1 and 8:1 ratios) compared with

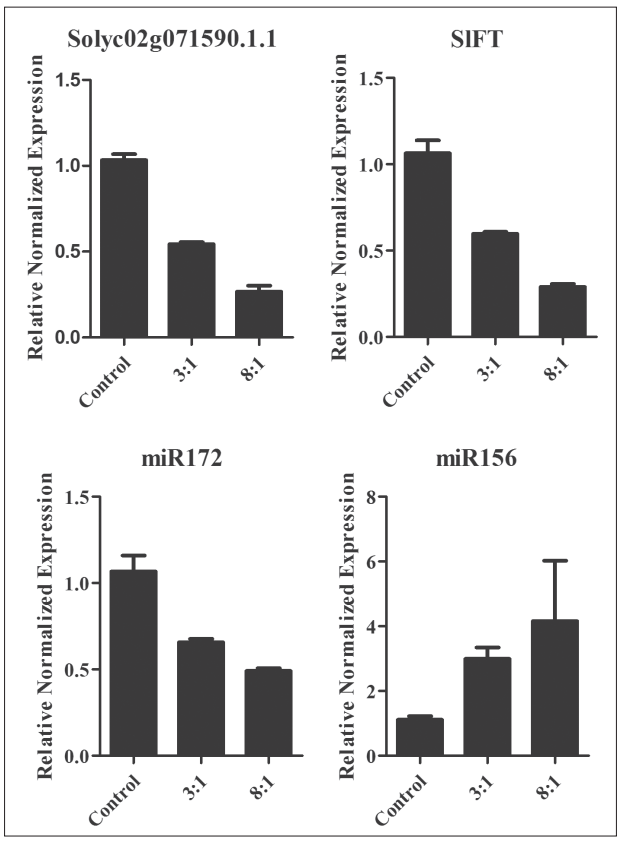

Fig. 5. Expression of flowering-associated genes and microRNAs. The expression patterns of Solyc02g071590.1.1 (SlTPS1), SlFT, miR172 and miR156 were determined by qRT-PCR. Control – white-light-grown plants, 3:1 – seedlings grown under red LEDs and 1;3 blue light environment, 8:1 – seedlings grown under red LEDs and 1:8 blue light environment.

those grown under white light, whereas miR156 transcript levels were obviously increased (Fig. 5).

DISCUSSION

Light provides an energy source for the plants via photosynthesis, and reception of the light signal is crucial for optimizing plant growth and reproduction. About 150 years ago, scientists noticed that different light wavelengths could result in different energy conversion efficiency [15]. In many species, red light has the highest quantum yield for CO_2 fixation among various light wavelengths in the photosynthetically active region (PAR) of the spectrum (400-700 nm, which includes green and blue light) [16,17]. In view of the high photosynthetic quantum yield derived from red light, high-efficiency red LEDs have been used to produce optimal light environments for photosynthesis and the growth of plants. Combinations of different light wavelengths were also proved to enhance quantum yields [18]. Interestingly, consistent treatment with red light alone resulted in poor photosynthetic performance, which is converse to the opinion that red light is the most efficient energy source [19-22]. Actually, carbon utilization during plant development does not merely depend on carbon assimilation. For example, carbon starvation treatment for even short periods leads to growth inhibition, which cannot be recovered immediately after carbon supplementation [23], indicating that there might be a time difference between energy status energy sensing. The plants may evolve a regulation mechanism to perceive sugar signals and cope with the various environments.

There is mounting evidence that trehalose-6-P (T6P) is an important sugar signal in plants [7,24,25]. T6P is synthesized by TPS which belongs to a small gene family [25]. There are 11 TPS genes in *Arabidopsis*, which were clustered into two subfamilies: Class I TPSs (*TPS1*, *TPS2*, *TPS3* and *TPS4*) and Class II TPSs (*TPS5*, *TPS6*, *TPS7*, *TPS8*, *TPS9*, *TPS10* and *TPS11*) [26]. Among the TPS genes in *Arabidopsis*, only TPS1 shows demonstrable TPS activity [27], and mutation of tps1 in *Arabidopsis* resulted in embryo maturation defects along with abnormalities throughout vegetative growth and floral transition [10]. In this study, two SlTPS1-like genes (Solyc02g071590.1.1 and Solyc07g062140.2.1) were identified in tomato, similar to those reported in rice [28]. Interestingly, Solyc02g071590.1.1 (SlTPS1-1) was shown to be highly expressed in leaves, while other SlTPSs were not (Fig. 2), indicating that SlTPS1-1may act as a key player in energy sensing in tomato.

Previous studies showed that different light quality has a distinct effect of on morphologic characteristics during plant growth and reproduction. In strawberry, stem elongation was promoted and inhibited under red and blue light, respectively. Plantlets cultured under 70% red+30% blue showed higher leaf and root number, higher plant height and root length, and greater fresh and dry weight compared to those cultured under 90% red+10% blue and 80% red+20% blue. Goins et al [20] reported that wheat grown under red LEDs alone displayed greater main culm length, fewer subtillers and a lower seed yield on harvest day

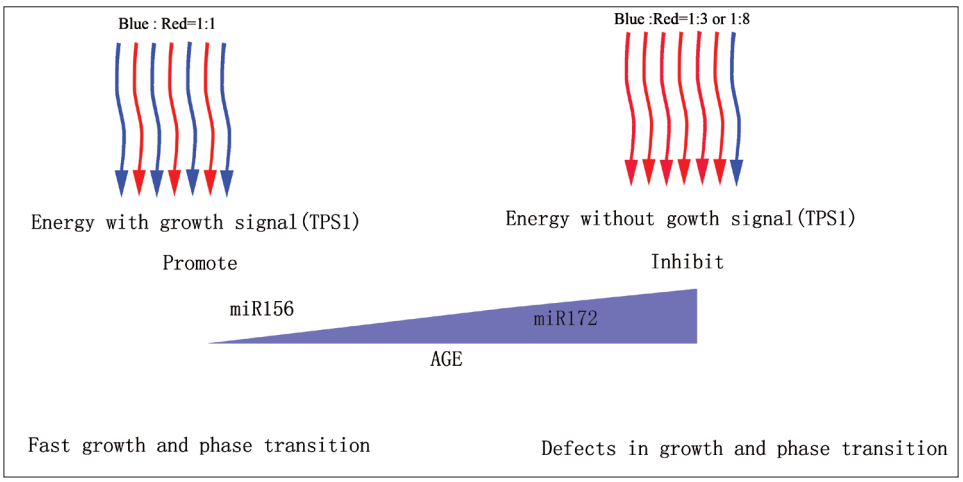

Fig. 6. A hypothesized model for the regulation of vegetative growth and floral transition by light quality. When tomato plants were grown under white light, TPS1 were expressed at higher levels in order to produce abundant sugars as carbon source. Higher levels of TPS1 suppressed the expression of miR156, which could promote plant aging and lead to fast growth and floral transition. On the contrary, when tomato plants were exposed to a high ratio of red to blue light, TPS1 expression were downregulated to accommodate CO_2 assimilation because of increased net leaf photosynthesis rate, and therefore miR156 expression was upregulated. Subsequently, higher levels of miR156 suppressed the transcripts of the SPL gene and resulted in delayed flowering. miR172 as miR156, antagonizes phase transition.

compared to white-light-grown plants, while wheat grown under red LEDs supplemented with 10% blue light produced a seed yield close to that of white light. However, we observed that tomato grown under red LEDs supplemented with 1/3 blue light demonstrated shorter internodes (Fig. 3), but higher flower numbers and rates of fruit set (Fig. 4) compared to those of white-light-grown plants. These results suggest that there is an optimal threshold level for blue light for optimal growth and reproduction under a red-based light source, which might depend on plant species. It has been demonstrated that flowering time was delayed significantly under red LEDs and blue light treatment (Table 2), especially in the tomato under higher ratios of red to blue light (Table 3).

To illustrate the mechanism underlying the regulation of vegetative growth and floral transition via light wavelength, the energy signaling pathway-associated gene *SlTPS1-1* was investigated at the transcription level. The results showed that *SlTPS1-1* was downregulated significantly (Fig. 5), indicating that TPS-related sugar sensing and signaling pathways were significantly suppressed in tomato plants grown under red LEDs. The *FT* gene integrates several external and endogenous cues controlling flowering, with FT protein moving directly from the leaves to the shoot apex, behaving as a long-distance signal [31]. Previous studies suggested that the SPL gene, as downstream targets of FT, function in the control of flowering time and phase change. Here, *FT* was downregulated in tomato grown under red and blue light (Fig. 5), which can delay flowering via affecting *SPL* expression [29]. miR156 was considered as an age marker in plants, downregulated with increasing plant age [29]. miR172 was proved to be as a miR156 antagonist on regulating phase transition in plants [30]. Compared to white light grown plants, the expressions of miR156 and miR172 were upregulated and downregulated, respectively (Fig. 5), in tomato plants grown under red LEDs supplemented with blue light. This might be the reasons that the vegetative phases were maintained and flowering was inhibited in tomato plants grown under higher ratios of red/blue light. The upregulation of miR156 in tomato grown under red/blue light would suppress the expression of SPL, the target of miR156 [32], and subsequently inhibit flowering in an FT-independent manner [29]. Recent research demonstrated the correlation of sugar signaling and miR156 expression and their roles in regulating the floral pathway. It showed that T6P was able to modu-

late the expression of the targets of miR156, partially via the miR156-dependent age-related pathway [11].

Finally, a regulatory mechanism underlying TPS1 functions on plant vegetative and reproductive growth via energy signaling pathway mediated by red/blue light, and eventually influencing the growing of the plant, was hypothesized (Fig. 6). When tomato plants were grown under white light, TPS1 were expressed at higher levels in order to produce abundant sugars as a carbon source. Higher levels of TPS1 suppressed the expression of miR156, which could promote plant aging and lead to fast growth and floral transition. On the contrary, when tomato plants were exposed to high ratios of red to blue light, TPS1 expression were downregulated to accommodate CO2 assimilation because of an increased net leaf photosynthesis rate, and therefore miR156 expression was upregulated. Subsequently, higher levels of miR156 suppressed the transcripts of SPL gene and resulted in delayed flowering.

CONCLUSION

In this work, defects in phase transition including juvenile-to-adult transition and vegetative-to-reproductive phase transition were observed in tomatoes grown in environments under a high ratio of red to blue light. The key regulator in energy status sensing, *SlTPS1*, was downregulated in plants grown in the presence of red and blue light as compared to those grown under white light. This indicates that an imbalance in light source would affect the energy sensing pathway, thereby modulating miR156, miR172 and *SlFT*, and eventually inhibiting vegetative growth and floral transition.

Authors' contribution: Juan Lou designed the experiment and wrote the manuscript, Zexiong Chen and Juan Lou performed all the experiments.

Conflict of interest disclosure: We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work.

REFERENCES

1. Schaffer R, Landgraf J, Accerbi M, Simon V, Larson M, Wisman E. Microarray analysis of diurnal and circadian-regulated genes in Arabidopsis. Plant Cell. 2001;13(1):113-23.
2. Tepperman JM, Hudson ME, Khanna R, Zhu T, Chang SH, Wang X, Quail PH. Expression profiling of phyB mutant demonstrates substantial contribution of other phytochromes to red-light-regulated gene expression during seedling de-etiolation. Plant J. 2004;38(5):725-39.
3. Smith H. Phytochromes and light signal perception by plants – an emerging synthesis. Nature. 2000;407(6804):585-91.
4. Srikanth A, Schmid M. Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci. 2011;68(12):2013-37.
5. Koch KE. Carbohydrate-Modulated Gene Expression in Plants. Annu Rev Plant Physiol Plant Mol Biol. 1996;47:509-40.
6. Cabib E, Leloir LF. The biosynthesis of trehalose phosphate. J Biol Chem. 1958;231(1):259-75.
7. Lunn JE, Feil R, Hendriks JH, Gibon Y, Morcuende R, Osuna D, Scheible WR, Carillo P, Hajirezaei MR, Stitt M. Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADP glucose pyrophosphorylase and higher rates of starch synthesis in Arabidopsis thaliana. Biochem J. 2006;397(1):139-48.
8. Eastmond PJ, van Dijken AJ, Spielman M, Kerr A, Tissier AF, Dickinson HG, Jones JD, Smeekens SC, Graham IA. Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. Plant J. 2002;29(2):225-35.
9. Gomez LD, Gilday A, Feil R, Lunn JE, Graham IA. AtTPS1-mediated trehalose 6-phosphate synthesis is essential for embryogenic and vegetative growth and responsiveness to ABA in germinating seeds and stomatal guard cells. Plant J. 2010;64(1):1-13.
10. van Dijken AJ, Schluepmann H, Smeekens SC. Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. Plant Physiol. 2004;135(2):969-77.
11. Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M. Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science. 2013;339(6120):704-7.
12. Thimijan ,R. W., and R.D. Heins. Photometrics, radiometric and quantum light units of measure: A review of procedures for interconversion. HortScience. 1983;18(6):818-822
13. The tomato genome sequence provides insights into fleshy fruit evolution. Nature. 2012;485(7400):635-41.
14. Xian Z, Huang W, Yang Y, Tang N, Zhang C, Ren M, Li Z. miR168 influences phase transition, leaf epinasty, and fruit development via SlAGO1s in tomato. Journal of experimental botany. 2014;65(22):6655-66.
15. Hoover WH. The dependence of carbon dioxide assimilation in a higher plant on wavelength of radiation. Smithson Inst Misc. 1933;95:1-13.
16. McCree KJ. Significance of enhancement for calculations based on the action spectrum for photosynthesis. Plant Physiol. 1972;49(5):704-6.
17. Efimtsev EI, Boichenko VA, Litvin FF. Action spectra of photosynthesis and liberation of hydrogen in bacteria, algae and higher plants. Dokl Akad Nauk SSSR. 1975;220(5):1238-40.
18. Hogewoning SW, Wientjes E, Douwstra P, Trouwborst G, van Ieperen W, Croce R, Harbinson J. Photosynthetic quantum yield dynamics: from photosystems to leaves. Plant Cell. 2012;24(5):1921-35.

19. Brown CS, Schuerger AC, Sager JC. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. J Am Soc Hortic Sci. 1995;120(5):808-13.
20. Goins GD, Yorio NC, Sanwo MM, Brown CS. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J Exp Bot. 1997;48(312):1407-13.
21. Yorio NC, Goins GD, Kagie HR, Wheeler RM, Sager JC. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. HortScience. 2001;36(2):380-3.
22. Matsuda R, Ohashi-Kaneko K, Fujiwara K, Kurata K. Effects of blue light deficiency on acclimation of light energy partitioning in PSII and CO2 assimilation capacity to high irradiance in spinach leaves. Plant Cell Physiol. 2008;49(4):664-70.
23. Smith AM, Stitt M. Coordination of carbon supply and plant growth. Plant Cell Environ. 2007;30(9):1126-49.
24. Schluepmann H, Pellny T, van Dijken A, Smeekens S, Paul M. Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2003;100(11):6849-54.
25. Kolbe A, Oliver SN, Fernie AR, Stitt M, van Dongen JT, Geigenberger P. Combined transcript and metabolite profiling of Arabidopsis leaves reveals fundamental effects of the thiol-disulfide status on plant metabolism. Plant Physiol. 2006;141(2):412-22.
26. Leyman B, Van Dijck P, Thevelein JM. An unexpected plethora of trehalose biosynthesis genes in Arabidopsis thaliana. Trends Plant Sci. 2001;6(11):510-3.
27. Blazquez MA, Santos E, Flores CL, Martinez-Zapater JM, Salinas J, Gancedo C. Isolation and molecular characterization of the Arabidopsis TPS1 gene, encoding trehalose-6-phosphate synthase. Plant J. 1998;13(5):685-9.
28. Zang B, Li H, Li W, Deng XW, Wang X. Analysis of trehalose-6-phosphate synthase (TPS) gene family suggests the formation of TPS complexes in rice. Plant Mol Biol. 2011;76(6):507-22.
29. Wang JW, Czech B, Weigel D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. Cell. 2009;138(4):738-49.
30. Wu G, Park MY, Conway SR, Wang JW, Weigel D, Poethig RS. The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. Cell. 2009;138(4):750-9.
31. Baurle I, Dean C. The timing of developmental transitions in plants. Cell. 2006;125(4):655-64.
32. Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU. Dissection of floral induction pathways using global expression analysis. Development. 2003;130(24):6001-12.