**Abstract:** Heat stress (HS) has been considered as a severe threat to crop yields in recent years. Sucrose, as a major product of photosynthesis, plays an important role in plant growth and stress response. Sucrose phosphate synthase (SPS) is a key rate-limiting enzyme in the sucrose synthesis pathway in plants. However, its molecular mechanism and signaling pathway remain unclear. In this study, we identified a novel SPS gene (*SlSPS*) in tomato and generated over-expression and knock-out of *SlSPS* gene transgenic tomato plants to investigate its biological functions related to the growth and thermotolerance of tomato. Over-expression of *SlSPS* gene increased the growth and biomass of transgenic tomato plants, such as fresh weight, dry weight, plant height, fruit weight and root length. In contrast, knock-out of *SlSPS* gene decreased the growth and biomass of transgenic tomato plants. Under heat stress, the survival rates were positively correlated with the expression level of *SlSPS* gene in different tomato varieties. Furthermore, *SlSPS*-overexpressing tomato plants showed higher SPS activity and sucrose content and heat stress resistant phenotypes. By comparison, knock-out tomato plants showed lower SPS activity and sucrose content and susceptible to heat stress. The determination of several reference values of oxidative stress parameters were also consistent with their heat resistance of these transgenic plants. In summary, *SlSPS* gene could positively mediate the growth and thermotolerance in tomato plants.

**Keywords:** sucrose phosphate synthase; heat stress; tomato; plant growth

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**1. Introduction**

Temperature is an important factor that can dramatically affect plant growth and geographical distribution. The extremely high temperature is a part of climate change caused by global warming and has caused huge damage to crop production worldwide [1,2]. Each Celsius degree increase in global mean temperature would reduce the global yields of wheat (*Triticum aestivum*) by 6.0%, rice (*Oryza sativa*) by 3.2%, maize (*Zea mays*) by 7.4%, and soybean (*Glycine max*) by 3.1%, on average [3]. Heat stress has negative effects on photosynthetic activity in plants, and water loss caused by heat impairs cell division and plant growth [4]. It is necessary to elucidate molecular mechanisms underlying heat stress response and thermotolerance in plants.

As sessile organisms, plants must adapt to various environmental stresses, such as heat, drought, cold, and poor lighting. Heat shock proteins and reactive oxygen species (ROS)-scavenging enzymes are major proteins that are induced by HS and are well-known target genes of HS-responsive transcription factors [5,6]. The Ca^{2+}-dependent signaling plays a possible role in HS response [7]. In addition, some phytohormones, such as auxin, abscisic acid (ABA), brassinosteroids (BRs), salicylic acid (SA) and ethylene (ET), are also involved in HS signaling in different plant species [8]. The production of glycinebetaine in tobacco reduces HS-induced photo-oxidation by enhancing the protection of photosystem II, and is associated with elevated levels of antioxidants and increased thermotolerance [9].
Two ethylene response factors, ERF95 and ERF97, regulate heat stress response through the formation of the EIN3-ERF95/ERF97-HSFA2 transcriptional cascade [10].

Several heat stress-related genes have been cloned and their roles have been validated in tomato. The mitogen-activated protein kinase 1 (SlMPK1) gene has been identified and cloned in tomato, and it can negatively regulate the antioxidant defense signaling; and the Serine-Proline-Rich protein Homolog (SISPRIH), a substrate of SlMPK1, is also involved in multiple signaling pathways associated with heat stress response [11,12]. SIMAPK3 is a negative regulator of thermotolerance in tomato by regulating expressions of antioxidant enzymes and HSPs/HSFs [13]. Over-expression of tomato DnaJ protein20 (SIDnaJ20) enhances the thermotolerance in transgenic tomato plants, whereas its suppression increases the sensitivity of tomato to heat stress [14]. Over-expression of Arabidopsis thaliana receptor-like kinase ERECTA (ER) improves the thermotolerance in tomato and reduced expression of a tomato ER allele decreases thermotolerance [15]. Betaine aldehyde dehydrogenase (BADH) and choline oxidase (COD) are key enzymes in the synthesis of glycinebetaine (GB), and two kinds of transgenic tomato plants which were transformed with BADH gene and codA gene, respectively, showed enhanced thermotolerance due to the positive role of GB in response to heat stress [16].

Sucrose, as the major transport form of photo-assimilated carbon, plays a crucial role in plant development and growth by serving as a source of carbon skeletons and energy for sink organs in plants [17]. Sucrose also acts as a key player in stress perception and signaling [18]. Sucrose phosphate synthase (SPS) is a key rate-limiting enzyme in the biosynthesis of sucrose and is one of the key enzymes necessary for sucrose to enter various metabolic pathways [19]. In addition, SPS also plays a vital role in plant growth, yield and quality, and response to abiotic stresses [20–23]. Many studies on its function in response to environmental stimuli at different species have been reported. The changes in the expression of SPS and sucrose synthase (SUS), sucrose content and arrangement of chloroplast starch may play a significant role in the cold response in M. giganteus and maize plants [24]. In waxy maize, heat stress decreases the activity of SPS and SUS, leading to the reduction in grain weight and starch deposition [25]. Heat stress induces reproductive failures in chickpea (Cicer arrietinum) due to impaired sucrose metabolism in leaves and anthers, which is caused by the decreased activity of SPS and SUS [26]. However, it is still unclear whether SPS plays a role in the growth and stress resistance in tomato.

In this study, we generated knock-out and over-expressing transgenic lines in tomato cv Micro-Tom, identified their phenotypes and physiological indexes, and investigated the effects of heat stress on the plant growth. Our results highlight the role of SlSPS gene in positively mediating the growth and thermotolerance in tomato.

2. Materials and Methods
2.1. Plant Materials and Growth Condition

The cherry tomato (Solanum lycopersicum L. cv. Micro-Tom), as an effective model system [27], was used in this study, which was further used to generate transgenic lines. Both Micro-Tom and transgenic seeds were sterilized, sown and grown in pots inside a growth chamber at 28 °C/15 °C under a 14-h light/10-h dark photoperiod with 60–70% humidity. Plant height, fresh weight and dry weight were determined at 4 weeks. Fruits were collected and weighed at 12 weeks. For the test of root length, seeds of Micro-Tom, over-expression lines (OE) and knock-out lines (CR) were sterilized and sown on 1/2 MS medium under 18-h light (25 °C)/6-h dark (18 °C) cycle conditions. The lengths of primary roots were measured at 2 weeks. At least 20 seeds of each line were used for these experiments.

2.2. Heat Stress Treatment

At four-week seedling stage, each line of Micro-Tom, OE and CR plants was divided into two groups. The control group (CK) were grown under previous condition and the heat stress group (HS) were treated at 42 °C/28 °C (light/dark) with consistent photoperiod
and humidity about two weeks. Leaves were collected and immediately frozen in liquid nitrogen at 0 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h and two weeks after heat stress treatment, and then stored at −80 °C for further experiments. Experiments on survival rates under heat stress were conducted on tomato varieties preserved in our lab, in which at least 20 seedlings were used.

2.3. Vector Construction and Genetic Transformation

Knock-out plants were generated using CRISPR/Cas9 technology. Briefly, single-guide RNAs targeting the first exon of SISPS gene (Sl07g007790) were cloned downstream of AtU3b promoter, in the CRISPR/Cas9 binary vector pCAMBIA1300-pYAO-cas9 (From Yao-Guang Liu Lab). For SISPS over-expression, the full length SISPS protein-coding sequence (CDS) were amplified from Micro-Tom and cloned into plant binary vector pCAMBIA1300 to generate the expression vector pCaMV35S:: SISPS. Genomic sequence and coding sequence information was downloaded from Sol Genomics Network (https://solgenomics.net/, accessed on 29 May 2022). The plasmids were then introduced into the Agrobacterium tumefaciens strain GV3101, and then introduced into Micro-Tom according to standard protocols [28]. The transgenic tomato plants were confirmed by quantitative real-time PCR (qPCR) or PCR detection and direct sequencing. Transgenic plants of T3 generation were used for further experiment.

2.4. Expression Analysis

Total RNA was extracted using MiniBEST Universal RNA Extraction Kit (TaKaRa, Dalian, China). First-strand cDNA was synthesized using a PrimeScript RT reagent kit (TaKaRa) and qPCR was conducted on an ABI Prism 7500 Fast Real-time PCR System (Applied Biosystem, Waltham, MA, USA) using SYBR Premix Ex Taq II kit (Tli RNaseH plus) according to the manufacturer’s instructions (TaKaRa, Dalian, China). The expression level of tomato eukaryotic initiation factor gene (eiF, Solyc12g096000) was used as an internal control. The relative expression levels of the genes of interest were calculated using the $2^{-\Delta\Delta CT}$ method. All primers used are listed in Supplementary Table S1.

2.5. DAB Staining

Hydrogen peroxide (H$_2$O$_2$) is one of the most important reactive oxygen species. The accumulation of H$_2$O$_2$ in leaves was detected using 3,3′-diaminobenzidine (DAB). After heat stress treatment, the leaves were harvested and soaked into DAB solution (pH 3.8, 1 mg/mL). After vacuum infiltration, the leaves were incubated at 28 °C for 8–12 h and boiled in 95% ethanol for 10 min twice. Then, the leaves were rehydrated in different concentrations of ethanol (85%, 70%, 50%) and preserved in 50% glycerol for microscopic observation.

2.6. Determination of Physiological Indexes

Physiological indexes (content of proline, malondialdehyde, O$_2^-$ and H$_2$O$_2$ and enzyme activity of superoxide dismutase, peroxidase and catalase) were determined according to manufacturer’s instruction (Suzhou Comin Biotechnology Co., Ltd., product No. PRO-2-Y, MDA-2-Y, SA-2-Y, H2O2-2-Y, SOD-2-Y, POD-2-Y and CAT-2-Y, http://cominbio.com/, accessed on 29 May 2022). Each group have three biological replicates. Briefly, frozen samples were ground under cryogenic conditions, divided into several vials and immediately weighed (around 100 mg per sample). These physiological indexes were determined using a UV-visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan, UV-2600i).

3. Results

3.1. Generation of Over-Expression and Knock-Out of SISPS Gene Transgenic Plants

To generate over-expression SISPS gene construct (Sl07g007790), we amplified its coding sequence and cloned it into plant binary vector to generate the expression vector pCaMV35S:: SISPS. As for knock-out SISPS gene by CRISPR/Cas9 technology, we
cloned targeted sequence to generate knock-out construct pCambia1300-pYAO-cas9-SlSPS. Homozygous knock-out plants were obtained until T3 generation. After Agrobacterium-mediated transformation, transgenic seedlings were identified by qPCR or PCR detection and direct sequencing. Expression analysis showed that the expression of SlSPS gene was up-regulated more than 10-fold in over-expression plants (OE: SlSPS-OE1, SlSPS-OE2 and SlSPS-OE3) compared to that in Micro-Tom, and was down-regulated at least 3-fold in knock-out plants (CR: SlSPS-CR1, SlSPS-CR2 and SlSPS-CR3) (Figure 1A,B). Sequencing analysis showed that the knock-out lines have nucleotides insertion (+1 bp) or deletion (−2 bp/−4 bp) (Figure 1C). These results indicated that transgenic plants were successfully generated for further analyses.

Figure 1. Expression level of SlSPS gene in over-expression lines (SlSPS-OE1, SlSPS-OE2 and SlSPS-OE3) (A) and knock-out lines (SlSPS-CR1, SlSPS-CR2 and SlSPS-CR3) (B). **p < 0.01, comparisons with Micro-Tom plants using Student’s t-tests. Target sites of SlSPS coding sequence in the knock-out lines (C). Nucleotide or dash in red denotes insertion or deletion in the corresponding site.

3.2. Effects of Over-Expression and Knock-Out of SlSPS Gene on Plant Phenotypes

To evaluate the effects of the expression of SlSPS gene on plant phenotypes, several phenotypic traits of transgenic plants were analyzed at different stages. At the seedling stage (2-week, 3-week, 4-week) and flowering stage, the over-expression plants were larger than plants, while the knock-out plants were smaller (Figure 2A–D). At the two-week seedling stage, growing under light condition, the root length of over-expression plants was longer than that of Micro-Tom plants, and the root length of knock-out plants was shorter (Figure 3A,B). At the four-week seedling stage, the height, fresh weight and dry weight of knock-out plants were significantly lower than those of wide-type plants (Figure 2E–G).
Figure 2. Phenotypes and biomass determination of transgenic lines (OE and CR) and Micro-Tom. Phenotypes of seedlings were observed at 2-week (A), 3-week (B), 4-week (C) and flowering stage (D). Determination of plant height (E), fresh weight (F) and dry weight (G) were performed at four-week seedling stage (n = 20 for each line). Values in (E–G) are means ± SD. * p < 0.05, ** p < 0.01, comparisons with Micro-Tom plants using Student’s t-tests.

Figure 3. Root length (A,B) and fruit weight (C) in transgenic lines and Micro-Tom plants. Root length and fruit weight were analyzed at 2-week and 12-week seedling stage (n = 20 for each line). Values in (B,C) are means ± SD. ** p < 0.01, comparisons with the Micro-Tom plants using Student’s t-tests.
At the ripening stage of fruits (about 12-week stage), the individual fruit weight (Figure 3C) was significantly increased in over-expression lines and decreased in knock-out lines than in Micro-Tom plants. Therefore, the expression of SlSPS gene could significantly affect multiple phenotypic traits (size, height and biomass) of transgenic tomato plants, and the plant size and biomass in transgenic lines and Micro-Tom plants were positively correlated with their expression level.

3.3. Effects of Heat Stress on Expression of SlSPS Gene, SPS Enzyme Activity, and Sucrose and Soluble Sugar Contents

Our previous work showed that SlSPS expression and SPS activity at the seedling stage, mature green stage and red ripe stage were up-regulated in SlSPS-overexpressing tomato plants and down-regulated in knock-out plants [29]. After 2-week heat stress treatment, the survival rate was positively correlated with the expression level of SlSPS gene in plants, with a correlation coefficient of over 0.8 (Figure 4A,B), suggesting that SlSPS gene might positively respond to heat stress. Under heat stress, the expression of SlSPS gene increased immediately at 1 h and reached the peak level at 2 h, and then gradually decreased at 4 h. At 12 h and 24 h, the expression of SlSPS gene returned to the initial level (Figure 4C). Since the expression of SlSPS gene was up-regulated during 1–12 h, SPS protein was accordingly accumulated and its activity significantly increased (Figure 4D).

![Figure 4. Relationship between survival rates and expression level of SlSPS gene under 2-week heat stress (A,B). (n = 20 for each varieties). The horizontal axis denotes tomato varieties used in this experiment (A). The scatters and regression curve represent the survival rates and expression level and their correlation (B). Expression level (C) and SPS enzyme activity (D) of Micro-Tom were analyzed at 0 h, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h after high temperature treatment. Values in (C,D) are means ± SD. (n = 3 for each time point).](image-url)
Since the susceptibility or resistance phenotypes to heat stress appeared at 96 h (Figure 5A), we measured SPS activity (Figure 5C), sucrose content (Figure 5D) and soluble sugar content (Figure 5E). Under heat stress, compared to Micro-Tom plants, the SISPS-overexpressing plants exhibited higher level of SPS activity and the contents of sucrose and soluble sugar, but knock-out plants showed decreased SPS enzyme activity and lower contents of sucrose and soluble sugar. These results suggested that heat stress could alter SISPS expression, SPS activity and the contents of sucrose and soluble sugar content in plants.

Figure 5. Seedlings of transgenic plants and Micro-Tom (A) and DAB staining assay results (B) at 96 h high temperature treatment. SPS enzyme activity (C), sucrose content (D) and soluble sugar content (E) at 96 h high temperature treatment. Values in (C–E) are means ± SD. **p < 0.01, comparisons with the Micro-Tom plants using Student’s t-tests. (n = 3 for each line) Seedlings of transgenic plants and Micro-Tom plants under 2-week heat stress (F).
3.4. Effects of the Expression of SlSPS Gene on Thermotolerance

To evaluate the effect of SlSPS on the susceptibility of plants to heat stress, we treated four-week seedlings with heat stress. After 2-week heat stress treatment, the knock-out plants exhibited high susceptibility to heat stress, including withered and dying condition and reduced survival rate, while SlSPS-overexpressing plants grew normally, with few leaves turning yellow, indicating that they had high tolerance to heat stress (Figure 5F). At 96 h after the onset of heat stress treatment, DAB staining showed that the levels of H$_2$O$_2$ in leaves were ranked as follows: knock-out plants > Micro-Tom plants > SlSPS-overexpressing plants (Figure 5B). These results suggested that over-expression of SlSPS gene enhanced the thermotolerance in tomato plants.

3.5. Effects of the Expression SlSPS Gene on Oxidative Stress Parameters and Antioxidant Enzymes

To observe the effects of SlSPS gene on oxidative stress and antioxidant enzymes in response to heat stress, we measured several key physiological indexes (Figure 6). Proline (Pro) is a multifunctional amino acid that plays an important role in plants under various abiotic stresses. In this study, we found that, at normal temperature, the background level of Pro in SlSPS-overexpressing plants was significantly higher than those in Micro-Tom plants and knock-out plants, but there was no significant difference between Micro-Tom plants and knock-out plants. After heat stress, the contents of Pro significantly increased in all three types of plants, and the content of Pro in SlSPS-overexpressing plants was significantly higher than those in Micro-Tom plants and knock-out plants, and there was no significant difference between Micro-Tom plants and knock-out plants (Figure 6A). Malondialdehyde (MDA) is an important biomarker of membrane lipid peroxidation caused by oxidative stress in plants. Heat stress significantly increased MDA levels in Micro-Tom plants and knockout plants but did not affect the level of MDA in SlSPS-overexpressing plants (Figure 6B), suggesting that the over-expression of SlSPS could alleviate lipid peroxidation caused by heat stress. Various abiotic stresses can induce the production of reactive oxygen species (ROS) in plants, which are highly toxic and cause damage to proteins, lipids, carbohydrates and DNA. ROS include free radicals (such as O$_2^-$, OH, etc.) and non-radical (molecular) forms (H$_2$O$_2$). Heat stress could significantly increase the levels of O$_2^-$ and H$_2$O$_2$ in knock-out plants and Micro-Tom plants, but not SlSPS-overexpressing plants (Figure 6C,D), suggesting that the over-expression of SlSPS could reduce the production of ROS caused by heat stress in plants.

Antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), are crucial to scavenge ROS and enhance heat tolerance in plants. The over-expression plants had significantly higher enzyme activities than Micro-Tom plants treated by high temperature, and meanwhile the activities of these enzymes of knock-out lines declined sharply than over-expression plants and Micro-Tom plants, although heat stress could significantly decrease the activities of these enzymes in three types of plants (Figure 6E–G). These results indicated that knock-out of SlSPS decreased the activities of ROS-scavenging enzymes, while over-expression of SlSPS also had elevated effects on these enzymes.

Taken together, compared to Micro-Tom plants, SlSPS-overexpressing plants had higher levels of Pro, higher activities of ROS-scavenging enzymes, lower level of ROS and MDA under heat stress. In contrast, knock-out plants accumulated more ROS and MDA but had lower activities of ROS-scavenging enzymes. These results could explain the heat resistance phenotypes of SlSPS-overexpressing plants and heat susceptibility of knock-out plants.
4. Discussion

Global warming has posed a severe threat to ecosystem and caused a decrease in agricultural yield [2,3]. High temperature also influences the production and quality of tomato fruits [30]. In this study, we found that a sucrose phosphate synthase gene, SlSPS, could not only regulate plant growth but also enhance thermotolerance in tomato plants.
SPS plays a crucial role in sucrose biosynthesis by directly regulating the synthesis and distribution of sucrose and starch in plants [19]. Previously, it has been reported that over-expression of maize SPS in tomato plants grown with CO₂ enrichment leads to decreased foliar carbohydrate accumulation [31]. Furthermore, it is involved in the growth, yield, fruit quality, and abiotic stress response in plants [20–23]. For example, SPS is regulated by protein phosphorylation and shows a circadian pattern of activity in tomato [32]. In this study, we found that manipulation of SlSPS gene caused various phenotypic changes in tomato plants (Figures 2 and 3). Over-expression of SlSPS gene increased root length, fresh weight and dry weight at seedling stage, and also increased the fruit weight, whereas knock-out of SlSPS gene exerted opposite effects, indicating that SlSPS could positively regulate the growth and development of tomato plants. These results were consistent with previous studies on Arabidopsis, muskmelon and potato [20–22,33]. In Arabidopsis, the growth of rosettes, flowers and siliques of double mutant sps1/spsc and triple mutant sps1/spsa2/spsc was reduced compared with Micro-Tom plants [33]. Previously, we found that over-expression of SlSPS gene in Arabidopsis increased SPS activity and the levels of the sucrose and soluble sugar [34]. Antisense repression of SPS in transgenic muskmelon affected plant growth and fruit development, such as smaller leaves, shorter plant height and smaller stem diameter [20]. Over-expression of a maize SPS gene improved yield characters of potato under field conditions [22].

In addition to the regulation of plant growth, SPS plays a vital role in abiotic stress response, such as osmotic pressure, extreme temperatures, salinity and drought [24,35–38]. When plants are exposed to abiotic stress, SPS activity tends to increase, thereby increasing the contents of soluble sugars (such as sucrose) and altering the osmotic pressure of the cells, which allow them to resist the stressful environment. It was reported that low temperature caused a significant increase in SPS transcript level in kiwifruit [39]. In wheat, the activity of SPS in seedlings of drought-tolerant cultivar under normal growth condition is significantly higher than that of non-drought-tolerant one; and drought stress significantly increases the activity of SPS in drought-tolerant cultivar, suggesting that SPS is more involved in regulating sucrose accumulation in the drought-tolerant cultivar than in the non-drought-tolerant cultivar; the change of carbon partitioning in favor of sucrose synthesis, leading to the accumulation of sucrose as a defense mechanism against drought stress [38]. Osmotic stress can induce the transcription of two SPS genes encoding AtSPS2F and AtSPS4F in Arabidopsis thaliana, but not AtSPS1F and AtSPS3F [35]. Low temperature stress can increase the activity of SPS in winter wheat spikes, and there is a negative correlation between sucrose content, SPS activity and ABA content and grain number per ear and the 1000-grain weight [40]. Here, we found that the thermotolerance was enhanced by increased expression of SlSPS gene, and vice versa (Figures 4 and 5). The over-expression of SlSPS gene increased SPS activity and sucrose content, leading to enhanced growth of seedlings. In contrast, the expression of SPS gene was downregulated in knock-out plants; leading to impaired growth and higher susceptibility to high temperature.

Oxidative damage to cell membranes has been considered as a common event under abiotic stress that can be assessed by measuring MDA content and ion leakage [41]. Oxidative damage can be caused by the overproduction of ROS, and maintaining a low steady-state levels of ROS is important for the growth and development of plants [42]. Various antioxidant enzymes, such as SOD, POD and CAT, play a crucial role in regulating the production and scavenging of ROS and enhancing the thermotolerance in plants [43,44]. For example, SlDnaJ20 over-expression enhances the thermotolerance of transgenic tomatoes by reducing the accumulation of ROS and alleviating the photoinhibition of photosystem II (PSII) [14]. In addition, knockout of SIMAPK3 enhances tolerance to heat stress in tomato plants by decreasing ROS contents and increasing the activities and transcript levels of antioxidant enzymes [13]. In this study, we found that heat stress increased the levels of MDA, H₂O₂ and O₂⁻ and decreased activities of antioxidant enzymes in knock-out plants; in contrast, the over-expression of SlSPS significantly increased Pro level and activities of antioxidant enzymes but decreased the contents of MDA, H₂O₂ and O₂⁻ (Figure 6).
These results suggested that over-expression of SlSPS could enhance the thermotolerance of tomato plants by increasing the contents of Pro and the activities of antioxidant enzymes under heat stress.

Although SPS is known to play many biological functions in plant development and stress adaption, its molecular mechanism is not clear. How does the signal pathway of SlSPS protein regulate high temperature response in tomato? Could SlSPS play potential roles on reproductive and floral development? Furthermore, whether the higher BRIX, which is a determining parameter for industrial processing, would come with the manipulation of SlSPS gene expression? How does SlSPS affect tomato fruit quality during fruit development? How do upstream regulatory factors regulate SlSPS expression? The work will continue to study the mechanism of SlSPS in heat stress and the affection of the plant growth, including flowering development and fertilization process. Our research will provide theoretical foundation and new genetic germplasm resources.

5. Conclusions

In our study, we found SlSPS gene plays an important role in regulating the growth of tomato plants at both seedling and ripening stages. Many phenotypic traits were affected, including height, fresh weight, dry weight, root length and fruit weight and SlSPS gene usually positively modulated these traits. The over-expression plants were usually larger than Micro-Tom plants, while the knock-out plants were smaller. Under heat stress, tomato cultivars with higher survival rates always had higher SlSPS gene expression level, and the correlation rates were 0.8. Elevated expression of SlSPS gene could increase sucrose content, soluble sugar content and SPS enzyme activity, and reduce ROS damage, thereby enhancing thermotolerance in tomato plants, and vice versa. The findings highlight the importance of SlSPS gene in improving the growth and thermotolerance of tomato plants and its potential application in germplasm improvement.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8060491/s1, Table S1: primer sequence.

Author Contributions: Y.Z. and W.Z. designed the experiments. Y.Z., Y.L. and D.Z. perform the experiments and analyzed the data. Y.L. drew the figure and wrote the manuscript. Y.L. and W.Z. modified the manuscript. All authors have read and agreed to the published version of the manuscript.

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