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SlSPS, a Sucrose Phosphate Synthase Gene, Mediates Plant Growth and Thermotolerance in Tomato

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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 (SlSPRH1), a substrate of SlMPK1, is also involved in multiple signaling pathways associated with heat stress response [11,12]. SlMAPK3 is a negative regulator of thermotolerance in tomato by regulating expressions of antioxidant enzymes and HSPs/HSFs [13]. Over-expression of tomato DnaJ protein20 (SlDnaJ20) 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 arietinum*) 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 *SlSPS* gene (Sl07g007790) were cloned downstream of AtU3b promoter, in the CRISPR/Cas9 binary vector pCAMBIA1300-pYAO-cas9 (From Yao-Guang Liu Lab). For *SlSPS* over-expression, the full length *SlSPS* protein-coding sequence (CDS) were amplified from Micro-Tom and cloned into plant binary vector pCambia1300 to generate the expression vector pCaMV35S:: *SlSPS*. 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_2O_2) is one of the most important reactive oxygen species. The accumulation of H_2O_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

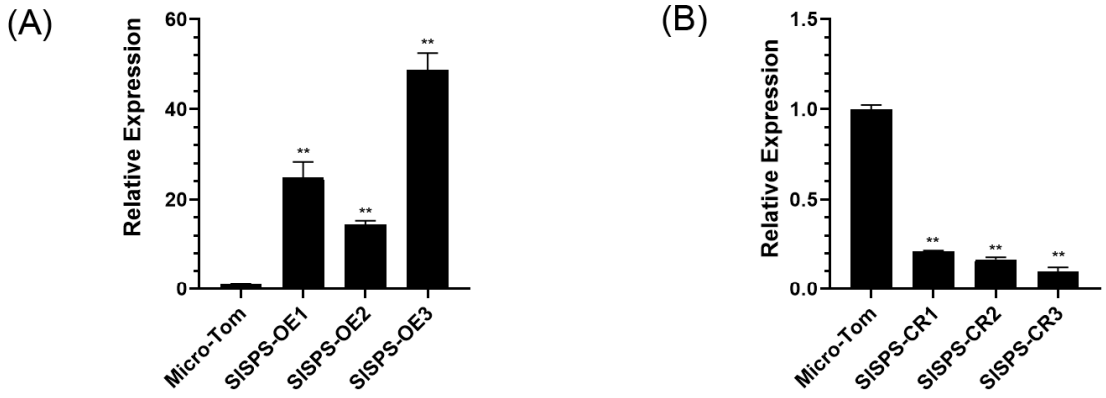
Physiological indexes (content of proline, malondialdehyde, O_2^- and H_2O_2 and enzyme activity of superoxide dismutase, peroxidase and catalase) were determined according to manufacturer's instruction (Suzhou Comin Biotechnology Co., Ltd., product No. PRO-2-Y, MDA-2-Y, SA-2-Y, H2O2-2-Y, SOD-2-Y, POD-2-Y 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 Cororation, Kyoto, Japan, UV-2600i).

3. Results

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

To generate over-expression SlSPS gene construct (Sl07g007790), we amplified its coding sequence and cloned it into plant binary vector to generate the expression vector pCaMV35S:: SlSPS. As for knock-out SlSPS 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.



(C)

Micro-Tom	ATGGCGGGAAACGATTGGATTAACAGTTACTTAGAGGCGATACTGGATGTAGGACCAGGGCTAGATGATAAGA	
SlSPS-CR1	ATGGCGGGAAACGATTGGATTAACAGTTACTTAGAGGCGATACTGGATGTAGGACCAGGGACTAGATGATAAGA	(+1 bp)
SlSPS-CR2	ATGGCGGGAAACGATTGGATTAACAGTTACTTAGAGGCGATACTGGATGTAGGACCAGGG--AGATGATAAGA	(−2 bp)
SlSPS-CR3	ATGGCGGGAAACGATTGGATTAACAGTTACTTAGAGGCGATACTGGATGTAGGACCAGGG----ATGATAAGA	(−4 bp)

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).

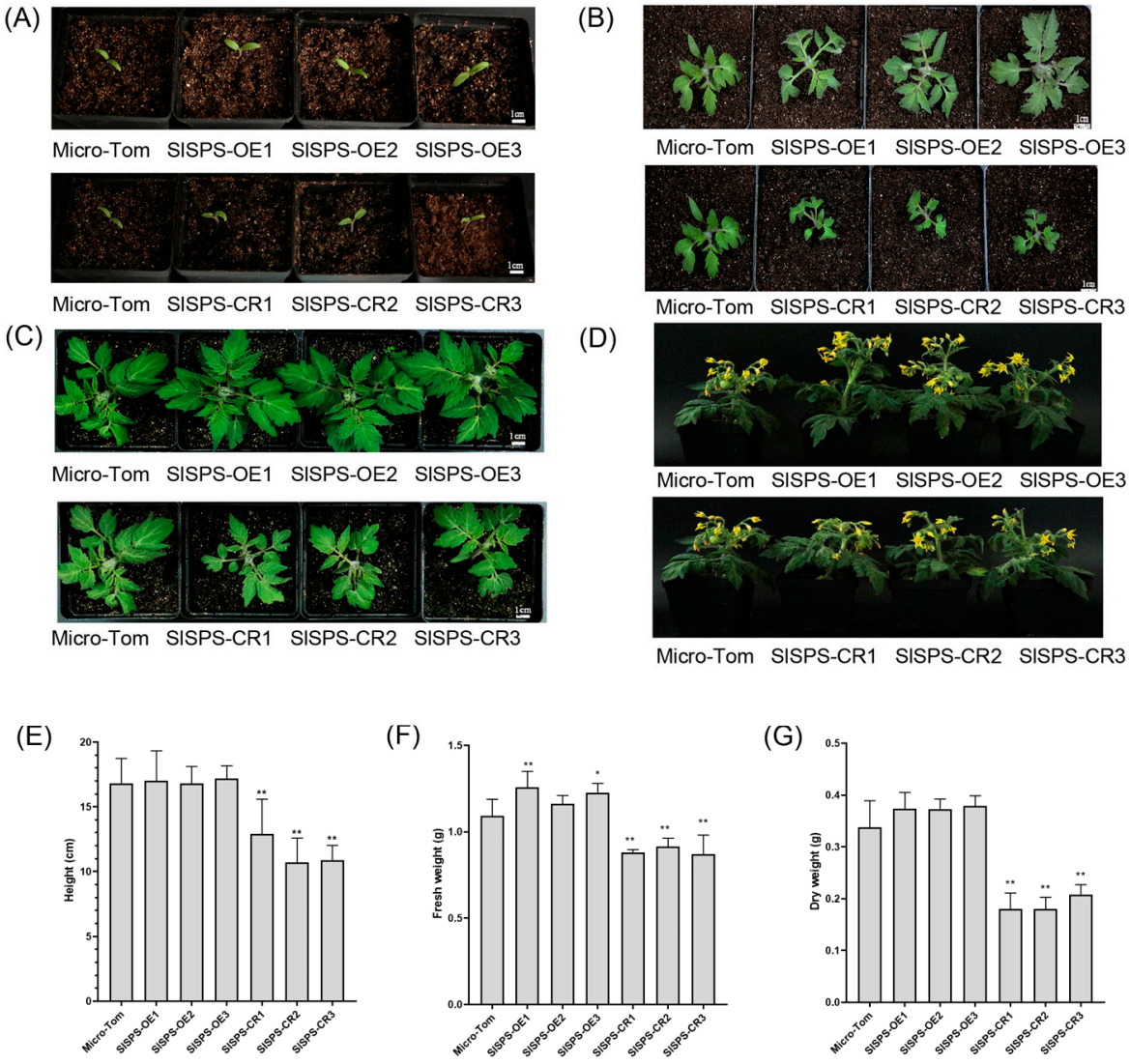


Figure 2. Phenotypes and biomass determination of transgenic lines (OE and CR) and Micro-Tom. Phenotypes of seedlings were observed at 2-week (**A**), 3-week (**B**), 4-week (**C**) and flowering stage (**D**). Determination of plant height (**E**), fresh weight (**F**) and dry weight (**G**) were performed 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.

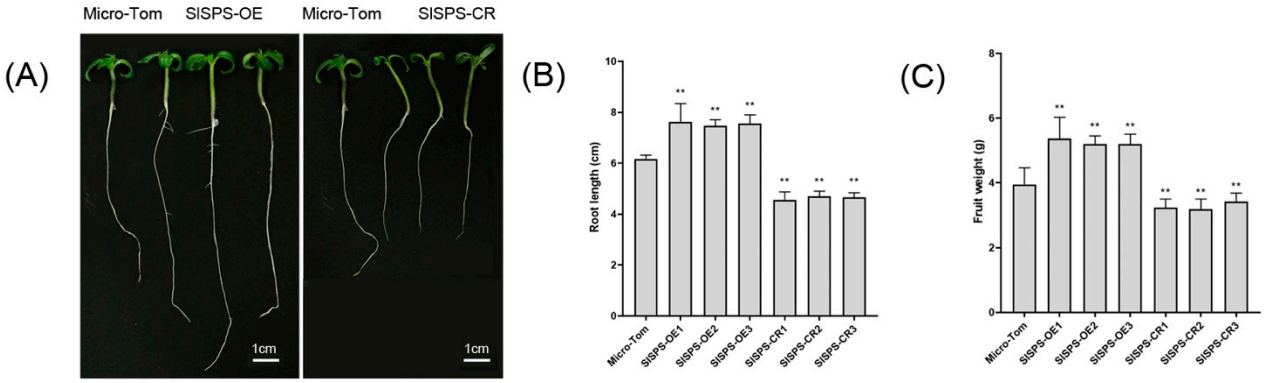


Figure 3. Root length (**A**,**B**) and fruit weight (**C**) in transgenic lines and Micro-Tom plants. Root length and fruit weight were analyzed at 2-week and 12-week seedling stage (n = 20 for each line). Values in (**B**,**C**) are means ± SD. ** $p < 0.01$, comparisons with the Micro-Tom plants using 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).

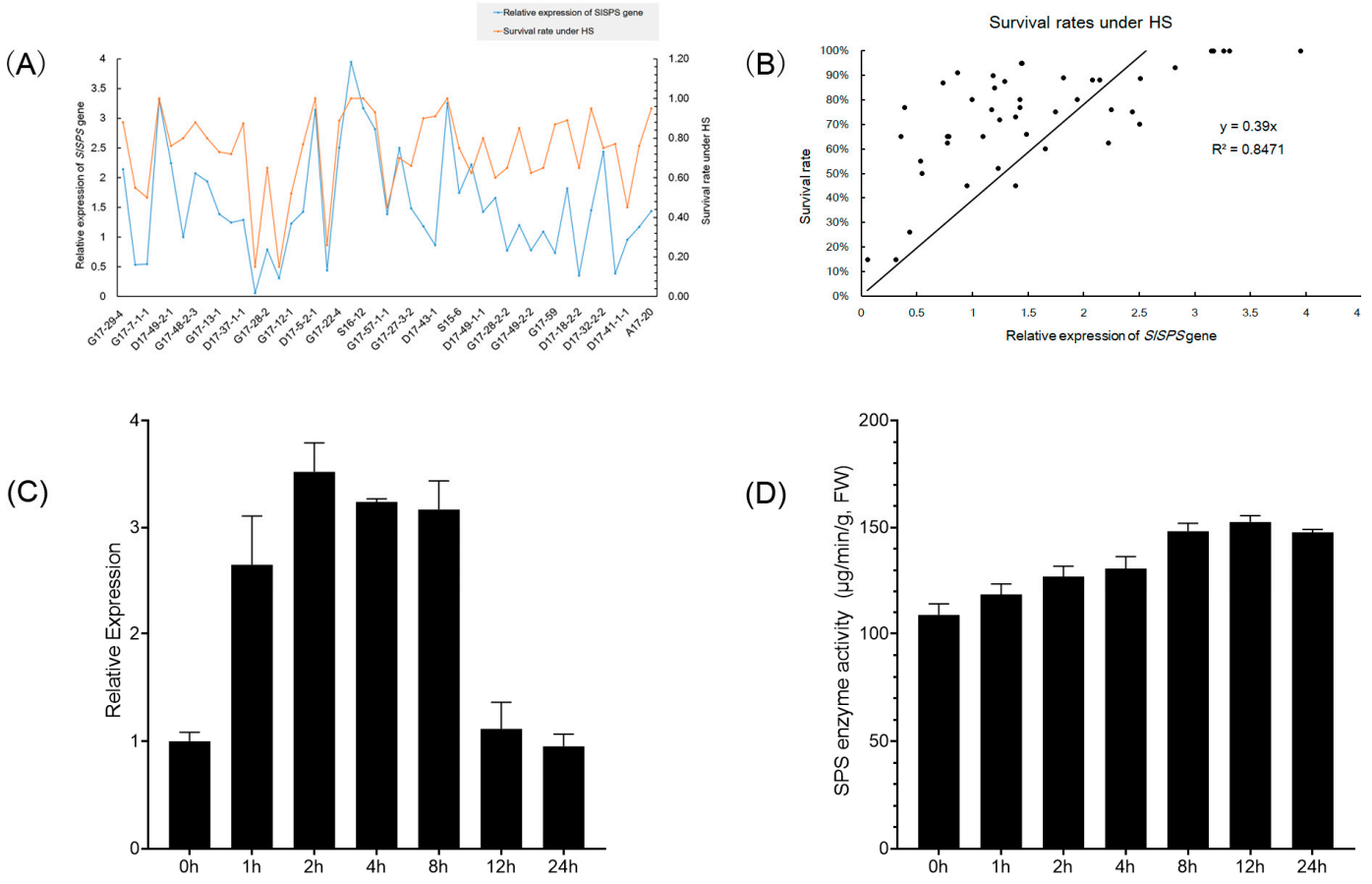


Figure 4. Relationship between survival rates and expression level of *SlSPS* gene under 2-week heat stress (**A**,**B**). (n = 20 for each varieties). The horizontal axis denotes tomato varieties used in this experiment (**A**). The scatters and regression curve represent the survival rates and expression 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).

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 *SlSPS*-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 *SlSPS* expression, SPS activity and the contents of sucrose and soluble sugar content in plants.

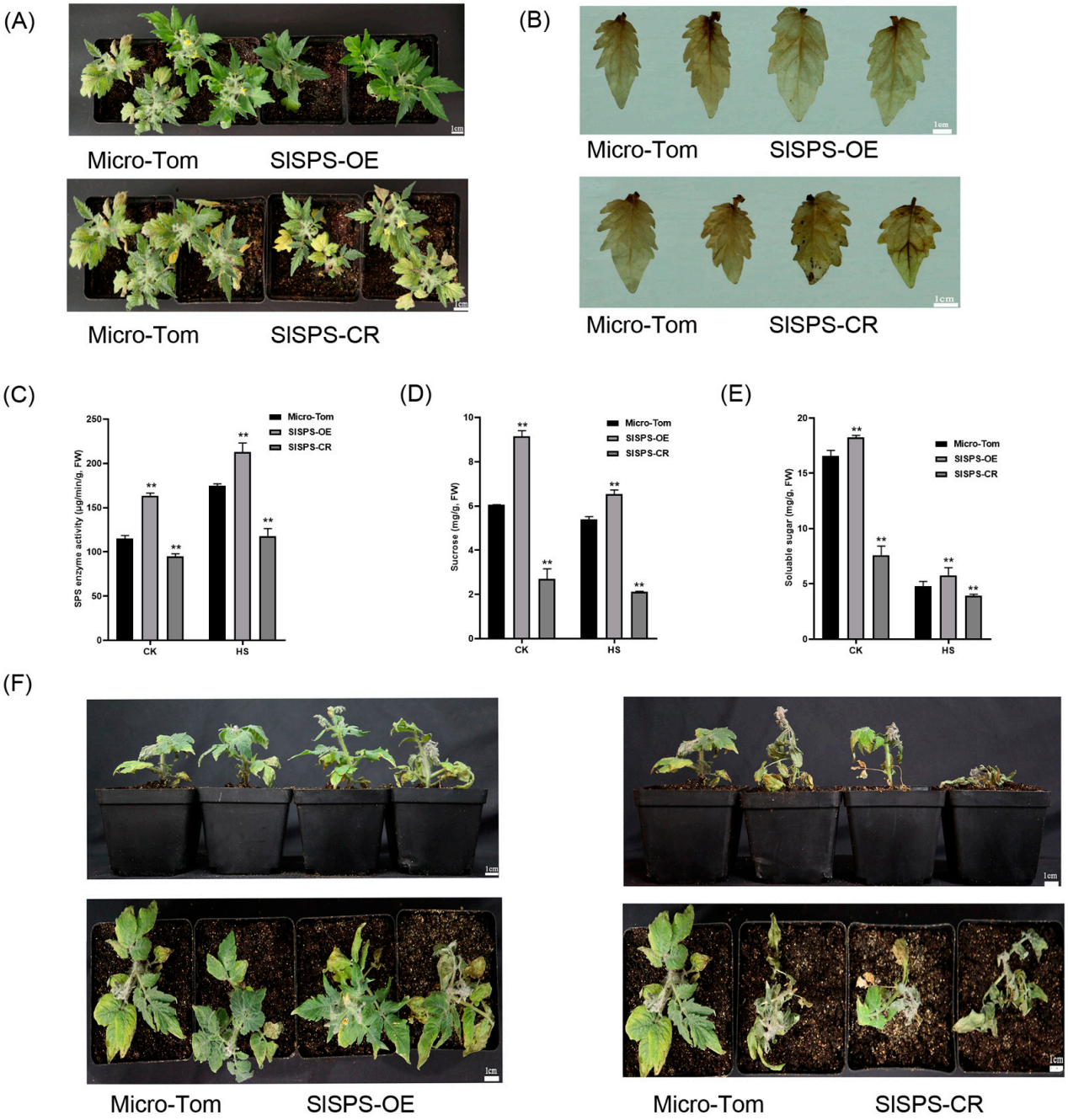


Figure 5. Seedlings of transgenic plants and Micro-Tom (**A**) and DAB staining assay results (**B**) at 96 h high temperature treatment. SPS enzyme activity (**C**), sucrose content (**D**) and soluble sugar content (**E**) at 96 h high temperature treatment. Values in (**C**–**E**) are means ± SD. ** $p < 0.01$, 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_2O_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 Antioxidtant 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_2O_2). Heat stress could significantly increase the levels of O_2^- and H_2O_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.

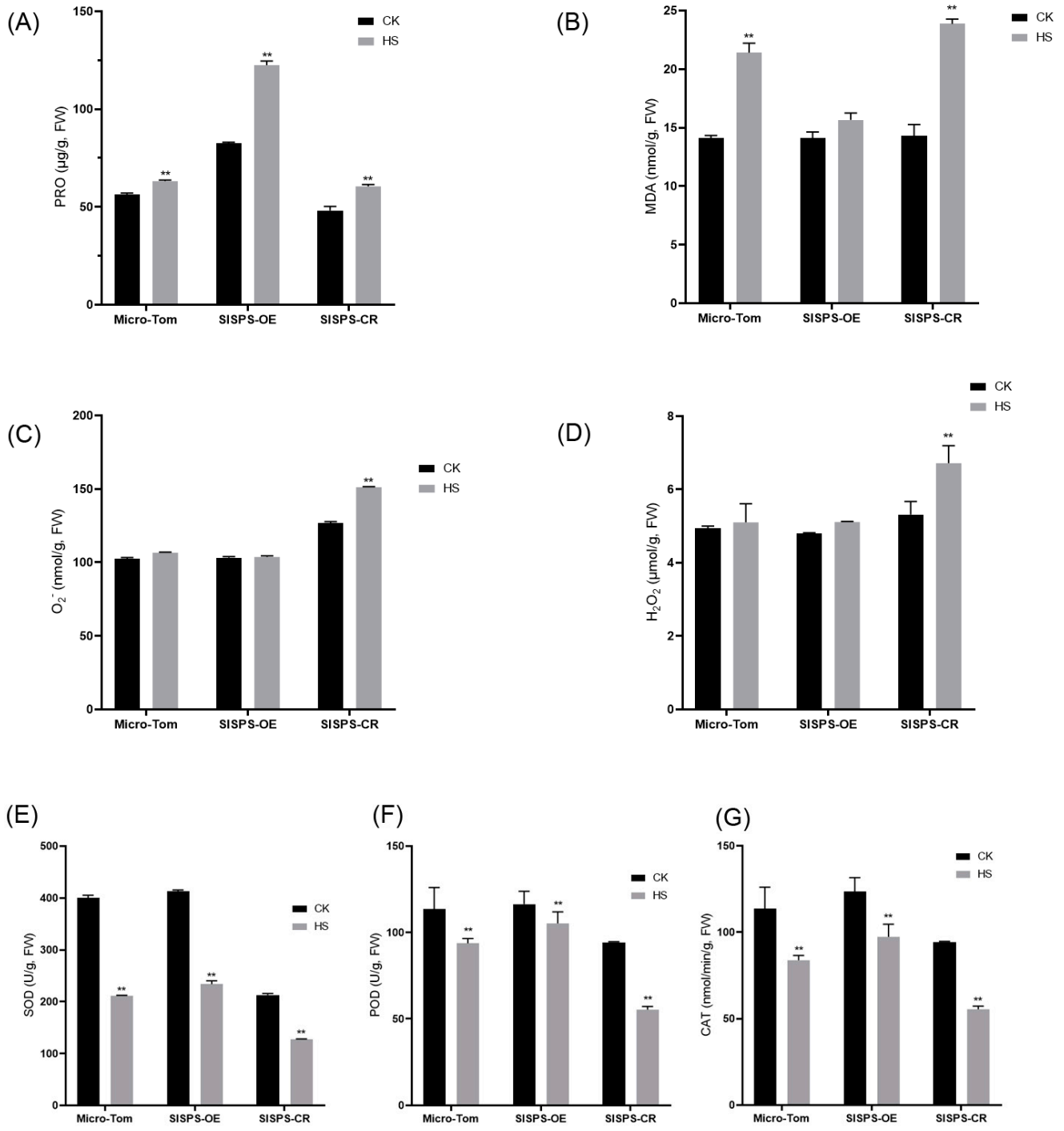


Figure 6. Reference values of oxidative stress parameters of leaves under HS treatment for 2 weeks. Contents of Pro (**A**), MDA (**B**), O_2^- (**C**) and H_2O_2 (**D**) and enzyme activities of SOD (**E**), POD (**F**), and CAT (**G**). CK and HS represent the control group and heat stress group, respectively. Values are means ± SD. ** $p < 0.01$, comparisons with the control group using Student's *t*-tests. (n = 3 for each group).

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 CO_2 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 circadiam 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 *spsa1/spsc* and triple mutant *spsa1/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 *SlSPS* 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 SlMAPK3 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, H_2O_2 and O_2^- 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, H_2O_2 and O_2^- (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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References

1. Lesk, C.; Rowhani, P.; Ramankutty, N. Influence of extreme weather disasters on global crop production. *Nature* **2016**, *529*, 84–87. [CrossRef] [PubMed]
2. Lobell, D.B.; Schlenker, W.; Costa-Roberts, J. Climate trends and global crop production since 1980. *Science* **2011**, *333*, 616–620. [CrossRef] [PubMed]
3. Zhao, C.; Liu, B.; Piao, S.; Wang, X.; Lobell, D.B.; Huang, Y.; Huang, M.; Yao, Y.; Bassu, S.; Ciais, P.; et al. Temperature increase reduces global yields of major crops in four independent estimates. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 9326–9331. [CrossRef] [PubMed]
4. Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. *Int. J. Mol. Sci.* **2013**, *14*, 9643–9684. [CrossRef]

5. Khan, Z.; Shahwar, D. Role of Heat Shock Proteins (HSPs) and Heat Stress Tolerance in Crop Plants. In *Sustainable Agriculture in the Era of Climate Change*; Roychowdhury, R., Choudhury, S., Hasanuzzaman, M., Srivastava, S., Eds.; Springer International Publishing: Cham, Switzerland, 2020; pp. 211–234.
6. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]
7. Zeng, H.; Xu, L.; Singh, A.; Wang, H.; Du, L.; Poovaiah, B.W. Involvement of calmodulin and calmodulin-like proteins in plant responses to abiotic stresses. *Front. Plant Sci.* **2015**, *6*, 600. [CrossRef]
8. Li, N.; Euring, D.; Cha, J.Y.; Lin, Z.; Lu, M.; Huang, L.-J.; Kim, W.Y. Plant Hormone-Mediated Regulation of Heat Tolerance in Response to Global Climate Change. *Front. Plant Sci.* **2021**, *11*, 2318. [CrossRef]
9. Yang, X.; Liang, Z.; Lu, C. Genetic engineering of the biosynthesis of glycinebetaine enhances photosynthesis against high temperature stress in transgenic tobacco plants. *Plant Physiol.* **2005**, *138*, 2299–2309. [CrossRef]
10. Huang, J.; Zhao, X.; Bürger, M.; Wang, Y.; Chory, J. Two interacting ethylene response factors regulate heat stress response. *Plant Cell* **2021**, *33*, 338–357. [CrossRef]
11. Ding, H.; Wu, Y.; Yuan, G.; Mo, S.; Chen, Q.; Xu, X.; Wu, X.; Ge, C. In-depth proteome analysis reveals multiple pathways involved in tomato SlMPK1-mediated high-temperature responses. *Protoplasma* **2019**, *257*, 43–59. [CrossRef]
12. Ding, H.; He, J.; Wu, Y.; Wu, X.; Ge, C.; Wang, Y.; Zhong, S.; Peiter, E.; Liang, J.; Xu, W. The Tomato Mitogen-Activated Protein Kinase SlMPK1 Is as a Negative Regulator of the High-Temperature Stress Response. *Plant Physiol.* **2018**, *177*, 633–651. [CrossRef]
13. Yu, W.; Wang, L.; Zhao, R.; Sheng, J.; Zhang, S.; Li, R.; Shen, L. Knockout of SlMAPK3 enhances tolerance to heat stress involving ROS homeostasis in tomato plants. *BMC Plant Biol.* **2019**, *19*, 354. [CrossRef]
14. Wang, G.; Cai, G.; Xu, N.; Zhang, L.; Sun, X.; Guan, J.; Meng, Q. Novel DnaJ Protein Facilitates Thermotolerance of Transgenic Tomatoes. *Int. J. Mol. Sci.* **2019**, *20*, 367. [CrossRef]
15. Shen, H.; Zhong, X.; Zhao, F.; Wang, Y.; Yan, B.; Li, Q.; Chen, G.; Mao, B.; Wang, J.; Li, Y.; et al. Overexpression of receptor-like kinase ERECTA improves thermotolerance in rice and tomato. *Nat. Biotechnol.* **2015**, *33*, 996–1003. [CrossRef]
16. Zhang, T.; Li, Z.; Li, D.; Li, C.; Wei, D.; Li, S.; Liu, Y.; Chen, T.H.H.; Yang, X. Comparative effects of glycinebetaine on the thermotolerance in codA- and BADH-transgenic tomato plants under high temperature stress. *Plant Cell Rep.* **2020**, *39*, 1525–1538. [CrossRef]
17. Lemoine, R. Sucrose transporters in plants: Update on function and structure. *Biochim. Biophys. Acta* **2000**, *1465*, 246–262. [CrossRef]
18. Keunen, E.; Peshev, D.; Vangronsveld, J.; Van Den Ende, W.I.M.; Cuypers, A.N.N. Plant sugars are crucial players in the oxidative challenge during abiotic stress: Extending the traditional concept. *Plant Cell Environ.* **2013**, *36*, 1242–1255. [CrossRef]
19. Huber, S.C.; Huber, J.L. Role and regulation of sucrose-phosphate synthase in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1996**, *47*, 431–444. [CrossRef]
20. Tian, H.; Ma, L.; Zhao, C.; Hao, H.; Gong, B.; Yu, X.; Wang, X. Antisense repression of sucrose phosphate synthase in transgenic muskmelon alters plant growth and fruit development. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 365–370. [CrossRef]
21. Haigler, C.H.; Singh, B.; Zhang, D.; Hwang, S.; Wu, C.; Cai, W.X.; Hozain, M.; Kang, W.; Kiedaisch, B.; Strauss, R.E.; et al. Transgenic cotton over-producing spinach sucrose phosphate synthase showed enhanced leaf sucrose synthesis and improved fiber quality under controlled environmental conditions. *Plant Mol. Biol.* **2007**, *63*, 815–832. [CrossRef]
22. Ishimaru, K.; Hirotsu, N.; Kashiwagi, T.; Madoka, Y.; Nagasuga, K.; Ono, K.; Ohsugi, R. Overexpression of a Maize SPS Gene Improves Yield Characters of Potato under Field Conditions. *Plant Prod. Sci.* **2008**, *11*, 104–107. [CrossRef]
23. Whittaker, A.; Martinelli, T.; Farrant, J.M.; Bochicchio, A.; Vazzana, C. Sucrose phosphate synthase activity and the co-ordination of carbon partitioning during sucrose and amino acid accumulation in desiccation-tolerant leaf material of the C4 resurrection plant Sporobolus stapfianus during dehydration. *J. Exp. Bot.* **2007**, *58*, 3775–3787. [CrossRef]
24. Bilska-Kos, A.; Mytych, J.; Suski, S.; Magoń, J.; Ochodzki, P.; Zebrowski, J. Sucrose phosphate synthase (SPS), sucrose synthase (SUS) and their products in the leaves of *Miscanthus* × *giganteus* and *Zea mays* at low temperature. *Planta* **2020**, *252*, 23. [CrossRef]
25. Yang, H.; Gu, X.; Ding, M.; Lu, W.; Lu, D. Heat stress during grain filling affects activities of enzymes involved in grain protein and starch synthesis in waxy maize. *Sci. Rep.* **2018**, *8*, 15665. [CrossRef]
26. Kaushal, N.; Awasthi, R.; Gupta, K.; Gaur, P.; Siddique, K.H.M.; Nayyar, H. Heat-stress-induced reproductive failures in chickpea (*Cicer arietinum*) are associated with impaired sucrose metabolism in leaves and anthers. *Funct. Plant Biol. FPB* **2013**, *40*, 1334–1349. [CrossRef]
27. Parrotta, L.; Aloisi, I.; Faleri, C.; Romi, M.; Del Duca, S.; Cai, G. Chronic heat stress affects the photosynthetic apparatus of *Solanum lycopersicum* L. cv Micro-Tom. *Plant Physiol. Biochem.* **2020**, *154*, 463–475. [CrossRef]
28. McCormick, S. Transformation of tomato with *Agrobacterium tumefacines*. In *Plant Tissue Culture Manual: Supplement 7*; Lindsey, K., Ed.; Springer: Dordrecht, The Netherlands, 1997; pp. 311–319.
29. Zeng, D.; Liu, Y.; Zhang, H.; Yang, X.; Zhu, L.; Zhu, W.; Zhang, Y. Cloning and expression analysis of tomato *SlSPS1* gene. *Mol. Plant Breed.* **2021**, *19*, 3896–3904. [CrossRef]
30. Lokesha, A.N.; Shivashankara, K.S.; Laxman, R.H.; Geetha, G.A.; Shankar, A.G. Effect of High Temperature on Fruit Quality Parameters of Contrasting Tomato Genotypes. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 1019–1029. [CrossRef]

31. Murchie, E.H.; Sarrobert, C.; Contard, P.; Betsche, T.; Foyer, C.H.; Galtier, N. Overexpression of sucrose-phosphate synthase in tomato plants grown with CO_2 enrichment leads to decreased foliar carbohydrate accumulation relative to untransformed controls. *Plant Physiol. Biochem.* **1999**, *37*, 251–260. [CrossRef]
32. Jones, T.L.; Ort, D.R. Circadian Regulation of Sucrose Phosphate Synthase Activity in Tomato by Protein Phosphatase Activity. *Plant Physiol.* **1997**, *113*, 1167–1175. [CrossRef]
33. Bahaji, A.; Baroja-Fernández, E.; Ricarte-Bermejo, A.; Sánchez-López, Á.M.; Muñoz, F.J.; Romero, J.M.; Ruiz, M.T.; Baslam, M.; Almagro, G.; Sesma, M.T.; et al. Characterization of multiple SPS knockout mutants reveals redundant functions of the four Arabidopsis sucrose phosphate synthase isoforms in plant viability, and strongly indicates that enhanced respiration and accelerated starch turnover can alleviate the blockage of sucrose biosynthesis. *Plant Sci. Int. J. Exp. Plant Biol.* **2015**, *238*, 135–147.
34. Liu, Y.; Zhu, L.; Yang, X.; Zhu, W.; Zhang, H.; Zhang, Y. Ectopic expression of *SlSPS* gene (Sucrose Phosphate Synthase) promotes plant type in Arabidopsis thaliana. *J. Hebei Agric. Univ.* **2021**, *44*, 34–40.
35. Solís-Guzmán, M.G.; Argüello-Astorga, G.; López-Bucio, J.; Ruiz-Herrera, L.F.; López-Meza, J.E.; Sánchez-Calderón, L.; Carreón-Abud, Y.; Martínez-Trujillo, M. *Arabidopsis thaliana* sucrose phosphate synthase (sps) genes are expressed differentially in organs and tissues, and their transcription is regulated by osmotic stress. *Gene Expr. Patterns GEP* **2017**, *25–26*, 92–101. [CrossRef] [PubMed]
36. Almadanim, M.C.; Alexandre, B.M.; Rosa, M.T.G.; Sapeta, H.; Leitão, A.E.; Ramalho, J.C.; Lam, T.T.; Negrão, S.; Abreu, I.A.; Oliveira, M.M. Rice calcium-dependent protein kinase OsCPK17 targets plasma membrane intrinsic protein and sucrose-phosphate synthase and is required for a proper cold stress response. *Plant Cell Environ.* **2017**, *40*, 1197–1213. [CrossRef]
37. Hagemann, M.; Marin, K. Salt-induced Sucrose Accumulation is Mediated by Sucrose-phosphate-synthase in Cyanobacteria. *J. Plant Physiol.* **1999**, *155*, 424–430. [CrossRef]
38. Nemati, F.; Ghanati, F.; Ahmadi Gavlighi, H.; Sharifi, M. Comparison of sucrose metabolism in wheat seedlings during drought stress and subsequent recovery. *Biol. Plant.* **2018**, *62*, 595–599. [CrossRef]
39. Langenkämper, G.; McHale, R.; Gardner, R.C.; MacRae, E. Sucrose-phosphate synthase steady-state mRNA increases in ripening kiwifruit. *Plant Mol. Biol.* **1998**, *36*, 857–869. [CrossRef]
40. Zhang, W.; Wang, J.; Huang, Z.; Mi, L.; Xu, K.; Wu, J.; Fan, Y.; Ma, S.; Jiang, D. Effects of Low Temperature at Booting Stage on Sucrose Metabolism and Endogenous Hormone Contents in Winter Wheat Spikelet. *Front. Plant Sci.* **2019**, *10*, 498. [CrossRef]
41. Zang, X.; Geng, X.; Wang, F.; Liu, Z.; Zhang, L.; Zhao, Y.; Tian, X.; Ni, Z.; Yao, Y.; Xin, M.; et al. Overexpression of wheat ferritin gene TaFER-5B enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging. *BMC Plant Biol.* **2017**, *17*, 14. [CrossRef]
42. Haider, M.S.; Jaskani, M.J.; Fang, J. 14-Overproduction of ROS: Underlying molecular mechanism of scavenging and redox signaling. In *Biocontrol Agents and Secondary Metabolites*; Jogaiah, S., Ed.; Woodhead Publishing: New Delhi, India, 2021; pp. 347–382.
43. Esposito, M.P.; Nakazato, R.K.; Pedroso, A.N.V.; Lima, M.E.L.; Figueiredo, M.A.; Diniz, A.P.; Kozovits, A.R.; Domingos, M. Oxidant-antioxidant balance and tolerance against oxidative stress in pioneer and non-pioneer tree species from the remaining Atlantic Forest. *Sci. Total Environ.* **2018**, *625*, 382–393. [CrossRef]
44. Tutar, O.; Marin-Guirao, L.; Ruiz, J.M.; Procaccini, G. Antioxidant response to heat stress in seagrasses. A gene expression study. *Mar. Environ. Res.* **2017**, *132*, 94–102. [CrossRef]