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ORIGINAL ARTICLE

ABA signaling rather than ABA metabolism is involved in trehalose-induced drought tolerance in tomato plants

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Abstract

Main conclusion **Trehalose increased drought tolerance of tomato plants, accompanied by reduced water loss and closed stomata, which was associated with the upregulated ABA signaling-related genes expression, but not in ABA accumulation.**

Abstract Drought is one of the principal abiotic stresses that negatively influence the growth of plant and yield. Trehalose has great agronomic potential to improve the stress tolerance of plants. However, little information is available on the role of ABA and its signaling components in trehalose-induced drought tolerance. The aim of this study is to elucidate the potential mechanism by which trehalose regulates ABA in response to drought stress. In this study, 6-week-old tomato (*Solanum lycopersicum* cv. Ailsa Craig) plants were treated with 0 or 15.0 mM trehalose solution. Results showed that trehalose treatment significantly enhanced drought tolerance of tomato plants, accompanied by encouraged stomatal closure and protected chloroplast ultrastructure. Compared with controls, trehalose-treated plants showed lower hydrogen peroxide content and higher antioxidant enzymes activities, which contributed to alleviate oxidative damage caused by drought. Moreover, trehalose treatment decreased ABA content, which was followed by the downregulation of ABA biosynthesis genes expression and the upregulation of ABA catabolism genes expression. In contrast, exogenous trehalose upregulated transcript levels of ABA signaling-related genes, including *SlPYL1/3/4/5/6/7/9*, *SlSnRK2.3/4*, *SlAREB1/2*, and *SlDREB1*. These results suggested that trehalose treatment enhanced drought tolerance of tomato plants, and it's ABA signaling rather than ABA metabolism that was involved in trehalose-induced drought tolerance in tomato plants. These findings provide evidence for the physiological role of trehalose and bring about a new understanding of the possible relationship between trehalose and ABA.

Keywords Abscisic acid · Chloroplast ultrastructure · Drought stress · Stomatal closure · Tomato · Trehalose

Abbreviations

AREB	ABA-responsive element-binding factor
NCED	9-*cis*-Epoxycarotenoid dioxygenase
PYR/PYL/RCAR	The pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptor
ROS	Reactive oxygen species
SnRK2s	Sucrose non-fermenting 1-related protein kinase 2s
RWC	Relative water content

Introduction

Drought is a significant limiting factor for agricultural productivity and generally inhibits plant growth (Terry et al. 2007), the improvement of plant drought tolerance is important in modern agriculture. Tomato (*Solanum lycopersicum*) is a horticultural commodity of great economic importance,

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which constitutes an important part of agriculture industry (Wang et al. 2017a, b).

Trehalose is a nonreducing disaccharide composed of an α,α-(1,1)-glycosidic bond linking two glucose units, which is an important stress-induced metabolite in plants under environmental resistance (Fernandez et al. 2010). Previous studies demonstrated that trehalose acted as an elicitor of plant defense mechanisms (Fernandez et al. 2010), which played important roles in response to diverse abiotic stresses, such as salinity (Chang et al. 2014), heat (Luo et al. 2010), deficient nitrogen (Lin et al. 2017), and drought (Ali and Ashraf 2011; Ma et al. 2013). Exogenous trehalose treatment significantly improved drought tolerance of maize and wheat plants, by (1) protecting the photosynthetic performance and enhancing photosynthetic activity (Ali and Ashraf 2011), (2) adjusting plant water status (Ali and Ashraf 2011), and (3) increasing the activities of some key antioxidant enzymes coupled with enhanced production of non-enzymatic antioxidants (Ali and Ashraf 2011; Ma et al. 2013). Although it has been demonstrated that antioxidant capacity played a crucial role in trehalose-mediated drought response, signaling pathway that is responsible for trehalose-induced drought tolerance in tomato plants needs to be investigated.

ABA is a vital phytohormone involved in response to various abiotic stresses, and its level rapidly increases under drought conditions (Raghavendra et al. 2010). The accumulation of endogenous ABA is regulated by a balance between biosynthesis and catabolism (Kushiro et al. 2004). The 9-*cis*-epoxycarotenoid dioxygenase (NCED) is a key enzyme in ABA biosynthesis (Qin and Zeevaart 1999). Overexpression of *SlNCED1* has been shown to result in ABA accumulation and increase drought resistance in tomato (Thompson et al. 2000). ABA is primarily catabolized to form 8′-hydroxy ABA through hydroxylation with ABA 8′-hydroxylase, which is mediated by proteins encoded by the *CYP707A* gene family (Nambara and Marion-Poll 2005). In tomato, silencing *SlNCED1* or *SlCYP707A2* differently decreased or increased the ABA contents (Ji et al. 2014). Moreover, a previous study has shown that, exogenous trehalose treatment increased ABA content under normal conditions. As for drought stress, trehalose-treated soybean plants exhibited enhanced plant growth and reduced ABA content (Asaf et al. 2017). However, the role of ABA in trehalose-induced drought tolerance in tomato plants is not clearly understood.

ABA plays a pivotal role in response to drought stress by upregulating ABA-responsive signaling components that control water status and stomatal closure, thereby enhancing plant adaptation to drought stress (Vishwakarma et al. 2017). Under stress conditions, ABA signaling pathway which incorporates the pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptor (PYR/PYL/RCAR), type 2C protein phosphatase (PP2Cs), and sucrose non-fermenting 1-related protein kinase 2s (SnRK2s) (Ma et al. 2009; Park et al. 2009), is activated and phosphorylates downstream substrates, such as ABA-responsive element-binding factors (AREBs)/ABA-responsive binding factors (ABFs), which further modulate ABA-responsive genes expression during ABA response (Yoshida et al. 2014). In tomato, *SlAREB1/ABF2*, *SlAREB2/ABF4*, and dehydration-responsive element-binding protein 1 (*DREB1*) have been identified and characterized as ABA-responsive signaling components (Hsieh et al. 2010; Orellana et al. 2010; Jiang et al. 2017). Furthermore, a previous study indicated that ectopic expression of a gene related to trehalose biosynthesis resulted in an ABA-insensitive phenotype (Nelson Avonce and Iturriaga 2004). Endogenous manipulation of trehalose level in *Arabidopsis thaliana* could regulate the vegetative growth in a mechanism involving ABA signaling transduction (Gómez et al. 2010). These results suggested that there might be a correlation between trehalose and ABA signaling. However, it is still unclear if ABA signaling could be regulated by exogenous trehalose under drought conditions.

Since increasing research efforts aimed at studying the induced tolerance by trehalose treatment both in plants and in animals, the present study investigated the role of ABA and its signaling components in trehalose-induced drought tolerance of tomato plants. Our results showed that trehalose treatment increased drought tolerance of tomato plants, accompanied by protected chloroplast ultrastructure and increased stomatal closure. The increase in drought tolerance was associated with the elevated antioxidant enzymes activities and the upregulated ABA signaling-related genes expression, but not in ABA accumulation. This study provided further insights into the role of trehalose in abiotic stress, and showed that under drought stress, trehalose could exert a positive influence on ABA signaling, while exerting a negative influence on ABA metabolism.

Materials and methods

Plant material and treatments

All tomato (*Solanum lycopersicum* cv. Ailsa Craig) seeds were sown in plastic pots (diameter in 7 cm, height in 10 cm) filled with a mixture of seedling substrate, soil, and vermiculite (2/1/1, by vol.). The seedlings were cultured in a greenhouse at 25 ± 2 °C, 60–65% relative humidity and 18/6-h dark/light cycle (Wang et al. 2017a, b). Six weeks later, tomato plants were divided into four groups of 60 plants each for different treatment. All plants were sprayed with different concentrations of trehalose solution (1.5, 15.0, and 45.0 mM) or water (as control) in both sides of leaves (Mostofa et al. 2015; Lin et al. 2017). Water was chosen as the control treatment for trehalose because it was considered not to introduce potential complications. Afterward, all plants

were placed in a dark chamber for 4 h for full absorption of trehalose solution, and then these plants were subjected to drought stress by stopping irrigation. Three biological replicates were carried out in this experiment.

The third to fifth leaves from the top of a stem (fully mature leaves) were sampled from four plants per treatment after 4 h absorption of trehalose solution (time 0), on day 0, 0.5, 1, 2, 3, 5, and 7 under drought stress, and then rapidly frozen in liquid nitrogen and stored at − 80 °C before analysis. Control: water-treated plants under drought stress. Trehalose: 15.0 mM trehalose-treated plants under drought stress. All experiments were conducted in triplicate.

Measurement of survival rate

Plants in each group were withheld water for 11 consecutive days, and then rehydrated with adequate water, representative images were taken after re-watering for 3 days, and the survival rate was calculated.

Measurement of relative water content (RWC)

RWC in tomato plants was calculated using the equation: RWC (%) = (fresh weight–dry weight)/(rehydrated weight–dry weight). Leaves were excised from each group and their fresh weights were scored immediately. After submerging under deionized water at 4 °C for 24 h, the rehydrated weights were determined. Finally, the dry weights were measured when leaves were dried at 70 °C for 48 h (Gaxiola et al. 2001).

Scanning electron microscopy (SEM)

On the fifth day after drought treatment, four leaf tissues were collected from each treatment group. Samples were processed according to Li et al. (2015) with some modifications. Leaf samples were fixed with 4% (v/v) glutaraldehyde solution in 0.1 M phosphate-buffered saline (PBS; pH 6.8), and then rinsed five times with PBS. Afterward, samples were dehydrated in a graded ethanol series, vacuum dried, and gold-coated. Finally, SEM was performed on a JSM-6360LV microscope (Jeol Ltd., Tokyo, Japan) and photos were taken simultaneously. The stomatal aperture was calculated by examining 11 stomata per group using Image J software (Martin and Stimart 2005).

Transmission electron microscopy (TEM)

On the fifth day after drought treatment, leaf segments (1–2 mm^2) having no veins were taken using a scalpel. The first-phase preparations were processed using the method of Wang et al. (2017a, b). Leaf segments were fixed with 2.5% (v/v) glutaraldehyde solution in 0.1 M PBS (pH 7.2) for 2 h at room temperature. After washing twice with 0.1 M PBS (pH 7.2), samples were postfixed with 1% osmic acid in 0.1 M PBS (pH 7.2) for 3 h, and then dehydrated with a gradient ethanol solution (30, 50, 70, 90, and 100%). Finally, samples were washed with propylene oxide, and later embedded in Spurr´s resin. With a thickness of 80 nm, the ultrathin sections were stained with uranyl acetate and lead citrate. Leaf cells were observed with a transmission electron microscope (HITACHI 7500, Tokyo, Japan) at 80 kV, and photos were taken simultaneously. The numerical data was calculated using the software Image J, by analyzing on average 20 micrographs per group (Vassileva et al. 2012).

Measurement of MDA content, ion leakage and H_2O_2 content

Malondialdehyde (MDA) content was assayed using the 2-thiobarbituric acid reaction method of Ding et al. (2007). MDA content was expressed as mmol g^{-1} FW. Ion leakage level of leaf discs was measured immediately according to the method of Wang et al. (2017a, b). Hydrogen peroxide (H_2O_2) content was measured with a H_2O_2 Detection Kit (A064, Jiancheng, Nanjing, China) according to the manufacturer's instruction, and it was expressed as mmol g^{-1} FW (Wang et al. 2017a, b).

Measurement of antioxidant enzymes activities

For measurement of antioxidant enzymes activities, frozen leaf sample (0.4 g in powder form) was homogenized with 5 mL of cold 0.1 M PBS (pH 7.0). The homogenate was centrifuged at 12,000*g* for 10 min at 4 °C, and the supernatant was collected and used for enzymes activities determination (Wang et al. 2017a, b). Catalase (CAT, EC 1.11.1.6) activity was measured following the consumption of H_2O_2 at 240 nm (Larrigaudière et al. 2004). Peroxidase (POD, EC 1.11.1.7) activity was measured by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation (Doerge et al. 1997). The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured by recording the decrease in absorbance of ascorbic acid at 290 nm (Nakano and Asada 1981). All enzyme activities were calculated based on fresh weight, and were expressed as U g^{-1} FW.

Measurement of ABA content

The indirect competitive enzyme-linked immunosorbent assay was used to determine ABA content (Wang et al. 2017a, b). 0.2 g of frozen sample in powder form was extracted and homogenized with 2 mL of precooled 80% (v/v) methanol with 1 mM butylated hydroxytoluene, and then the extract was incubated for 48 h at 4 °C. After centrifugation at 12,000*g* for 15 min at 4 °C, the supernatant

was collected and passed through a C18 Sep-Pak classic cartridge (Waters Corp., Millford, MA, USA). The residue was dissolved with 2 mL of phosphate buffer sodium (0.1 mM, pH 7.5, contained 1% (v/v) Tween 20 and 1% (w/v) gelatin) for further determination of ABA content, and it was expressed as ng g^{-1} FW.

Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from 150 mg of frozen leaf sample using an *EasyPure* Plant RNA Kit (Beijing Transgen Biotech Co. Ltd., Beijing, China). The total RNA was quantified by a NanoDrop 2000 Photometer spectrophotometer (Thermo Scientific, Waltham, MA, USA), and stored at − 80 °C. The cDNA was synthesized with 2 μg of RNA using a *TransScript* One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (Beijing Transgen Biotech). All qRT-PCR were performed with TransStart Top Green qPCR SuperMix (Beijing Transgen Biotech) (Wang et al. 2017a, b). The qRT-PCR thermal cycle condition was as follows: pre-denaturation at 94 °C for 30 s, 40 cycles at 94 °C for 5 s, 60 °C for 15 s, 72 °C for 15 s, with a melt cycle from 65 to 95 °C (Wang et al. 2017a, b). The relative expression for each gene was normalized to *β-Actin* Ct value and calculated using the $2^{-\Delta\Delta Ct}$ method. Primers used for qRT-PCR analysis were listed in Supplementary Table S1. All experiments were run in triplicate with different cDNAs synthesized from three biological replicates.

Statistical analysis

All data were obtained from three independent replicates and were subjected to two-way analysis of variance (ANOVA). The mean differences were compared with Duncan's multiple range test using the statistical analysis software SPSS 20.0 (IBM Corp., Armonk, NY, USA). Differences with $P < 0.05$ were considered significant.

Results

Effects of trehalose treatment on the phenotype of tomato plants

To determine the effects of trehalose treatment on drought tolerance of tomato plants, three different concentrations of trehalose (1.5, 15.0 or 45.0 mM) were used. After 5 days' exposure to drought, control plants displayed obvious symptoms of seriously wilted leaves and bent stems, whereas trehalose-treated plants showed significantly less severe symptom with only a few wilted leaves (Fig. 1a). The group with 15.0 mM trehalose treatment performed better than 1.5 and 45.0 mM treatment groups (Fig. 1a). Thus, 15.0 mM trehalose treatment was selected for the further study. RWC decreased significantly as days go on after stopping watering in all plants, and 15.0 mM trehalose treatment retarded the decrease in RWC compared with control. RWC in trehalose-treated plants was significantly higher than that in control (Fig. 1b). Moreover, after 3 days of re-watering (all plants were withheld water for 11 consecutive days), 15.0 mM trehalose-treated plants recovered better than control plants, with a majority of leaves remained green (Fig. 1c). The survival rate of 15.0 mM trehalose-treated plants was 70.0%, whereas that of control plants was only 20.0% (Fig. 1d). These results suggested that 15.0 mM trehalose-treated plants suffered less damage than control plants under drought stress, implying that trehalose treatment enhanced drought tolerance of tomato plants.

Effects of trehalose treatment on the chloroplast ultrastructure of tomato plants

The electron microscopic images of chloroplasts were observed on the fifth day under drought stress. In control plants, drought stress changed chloroplast shape from spindle-shape into round, accompanied by a significant increase in the width-to-length ratio of chloroplast (Fig. 2a, c). In contrast, most chloroplasts in trehalose-treated plants remained spindle-shaped, and the width-to-length ratio of chloroplast was lower than that in control (Fig. 2b, c). Moreover, in control plants, starch granules were completely depleted with a large number of osmiophilic plastoglobules (Fig. 2a, d). But in trehalose-treated plants, starch granules were well preserved, occupying 23.0% of the chloroplast area, and few osmiophilic plastoglobules were presented (Fig. 2b, d).

Effects of trehalose treatment on the stomatal closure of tomato plants

The lower surfaces leaf samples were scanned at 500× magnification on the fifth day under drought stress. Drought stress caused the stomata to be closed in both groups, whereas trehalose treatment reinforced stomatal closure compared with control. Most stomata completely closed in trehalose-treated plants, while remained partially opened in control (Fig. 3a, b). The stomatal aperture in control plants was 314.3% larger than that in trehalose-treated plants (Fig. 3c), suggesting that trehalose treatment encouraged stomatal closure under drought stress.

Effects of trehalose treatment on MDA content, ion leakage, H_2O_2 content and antioxidant enzymes activities

Malondialdehyde (MDA) content increased in both groups under drought stress, while trehalose treatment retarded the

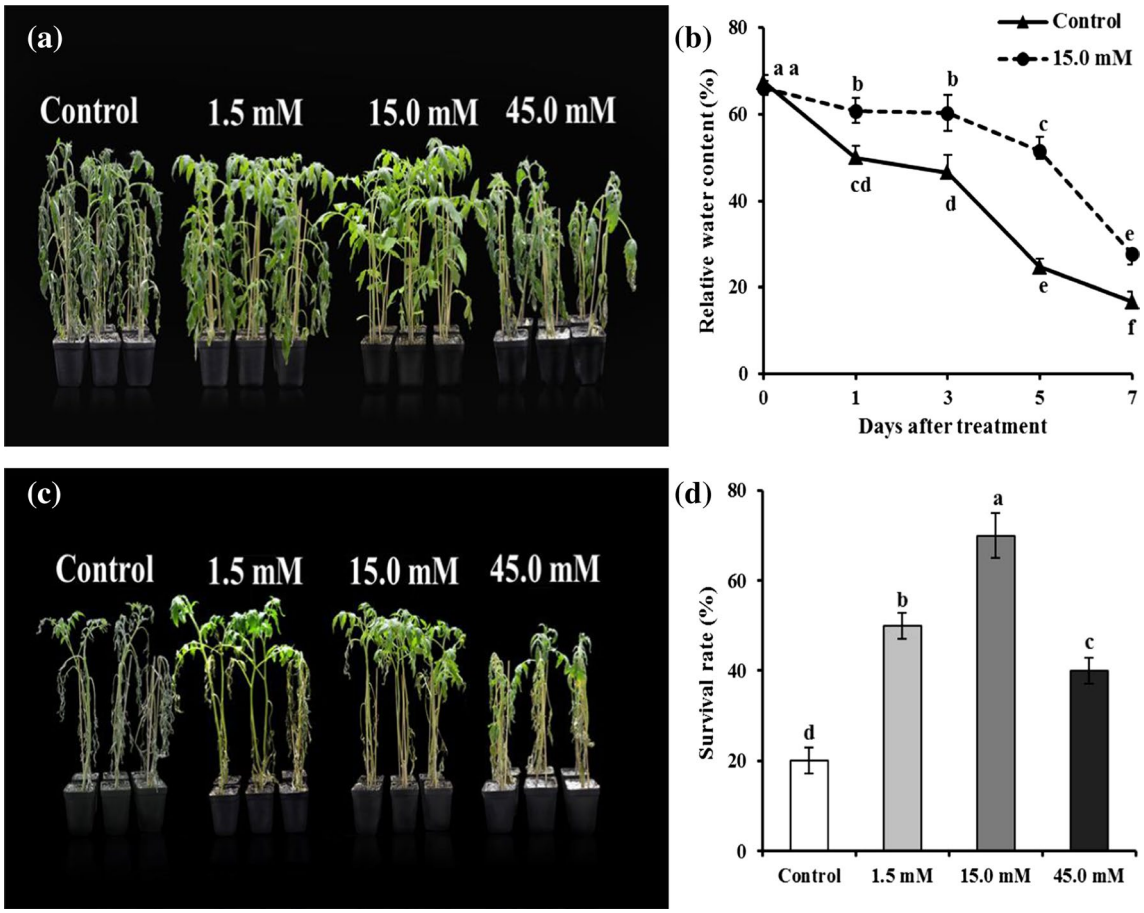


Fig. 1 Phenotype of trehalose-treated tomato plants under drought stress. **a** Effects of different concentrations of trehalose (0, 1.5, 15.0, or 45.0 mM) on the phenotype of tomato plants on the fifth day under drought stress. **b** Effects of trehalose treatment on RWC under drought stress. **c** Rehydrated phenotype of control and trehalose-treated tomato plants. All tomato plants were withheld water for 11 consecutive days, and then re-watered for 3 days. **d** Survival rate of tomato plants described in **c**. Data are the mean values of three biological replicates ± SD. Values followed by different letters were significantly different according to Duncan's multiple range test at $P < 0.05$

increase in MDA. MDA contents in trehalose-treated plants were significantly lower than those in control (Fig. 4a). Ion leakage in trehalose-treated plants was 22.4, 84.8, 84.6, and 49.8% lower than those in control on days 1, 3, 5, and 7 (Fig. 4b). On the first day, there was no significant difference in H_2O_2 content, but the contents of H_2O_2 in control increased rapidly and became significantly higher than those in trehalose-treated plants through the remaining drought period (Fig. 4c). Results from DAB staining (on the fifth day) further showed that trehalose treatment reduced H_2O_2 accumulation under drought stress (Fig. S1).

Catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities were higher in trehalose-treated plants than those in control during the whole drought period (Fig. 4d–f). CAT activities displayed a mark increase and peaked on the third day at 99.23 U g^{-1} FW in trehalose-treated plants, which was 31.5% higher than that in control (Fig. 4d). POD activities in trehalose-treated plants showed a fluctuating increase and were significantly higher than those in control (Fig. 4e). The activities of APX were 22.5, 27.2, 41.8, and 34.6% higher after trehalose treatment than those in controls on days 1, 3, 5, and 7 (Fig. 4f).

Effects of trehalose treatment on ABA content and the expression of genes involved in ABA metabolism

The concentration of ABA in trehalose-treated plants decreased on the first day, and then increased slowly through the remaining drought period. Treatment with trehalose notably suppressed the elevation of ABA contents, which were 26.5, 19.1, 37.1, and 28.9% lower than those in control on days 1, 3, 5, and 7 (Fig. 5a). Transcript levels of genes involved in ABA metabolism were evaluated at the transcriptional level using qRT-PCR (Fig. 5b–e). On the whole, transcript levels of the ABA biosynthesis genes *SlNCED1*

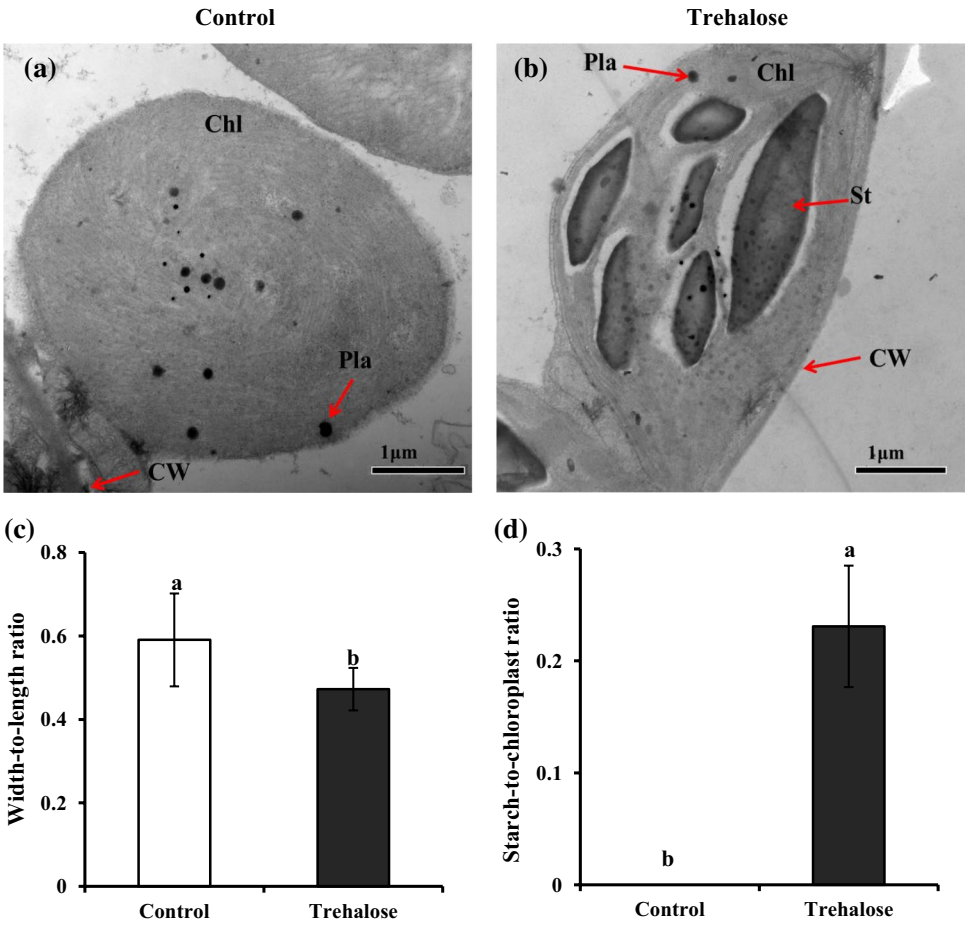


Fig. 2 Effects of 15.0 mM trehalose treatment on chloroplast ultrastructure on the fifth day under drought stress detected by TEM. Scale bars = 1 μm. **a** Tissues taken from control plants. **b** Tissues taken from trehalose-treated plants. **c** Width-to-length ratio of chloroplast. **d** Chloroplast area occupied by starch granule. *CW* cell wall, *Chl* chloroplast, *Pla* osmiophilic plastoglobule, *St* starch granule. Data are the means of at least 17 chloroplasts ± SD. Values followed by different letters were significantly different according to Duncan's multiple range test at $P < 0.05$

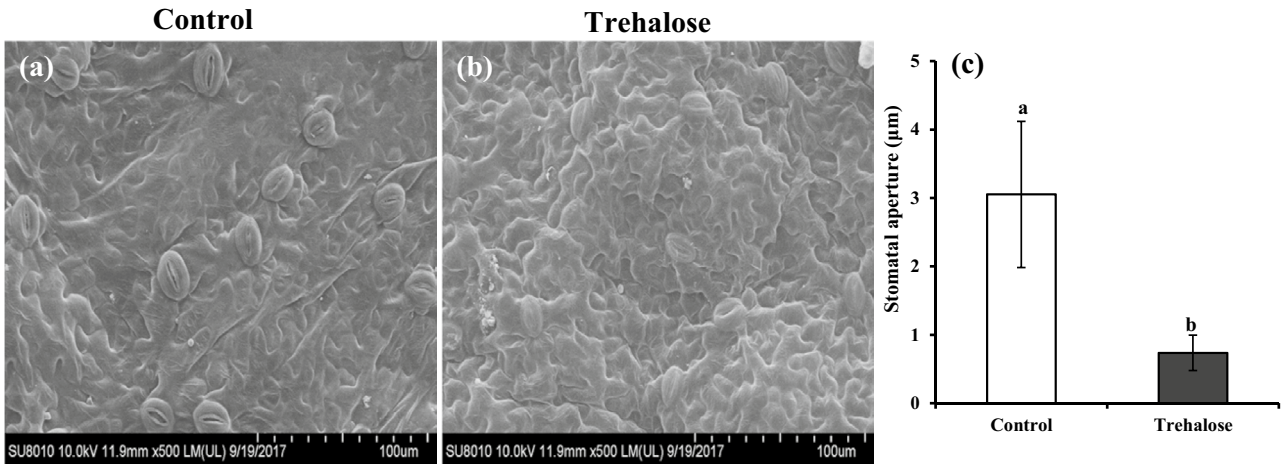


Fig. 3 Effects of 15.0 mM trehalose treatment on stomatal closure on the fifth day under drought stress detected by SEM. Scale bars = 100 μm. **a** Tissues taken from control plants. **b** Tissues taken from trehalose-treated plants. **c** Stomatal aperture. Data are the means of 11 stomata ± SD. Values followed by different letters were significantly different according to Duncan's multiple range test at $P < 0.05$

and *SlNCED2* were lower in trehalose-treated plants than those in control (Fig. 5b, c). Under drought stress, two ABA catabolism genes, *SlCYP707A1* and *SlCYP707A2*, were upregulated, and their transcript levels were significantly higher in trehalose-treated plants than in control (Fig. 5d, e). The present results revealed that trehalose treatment downregulated ABA biosynthesis and upregulated ABA catabolism genes expression in response to drought stress.

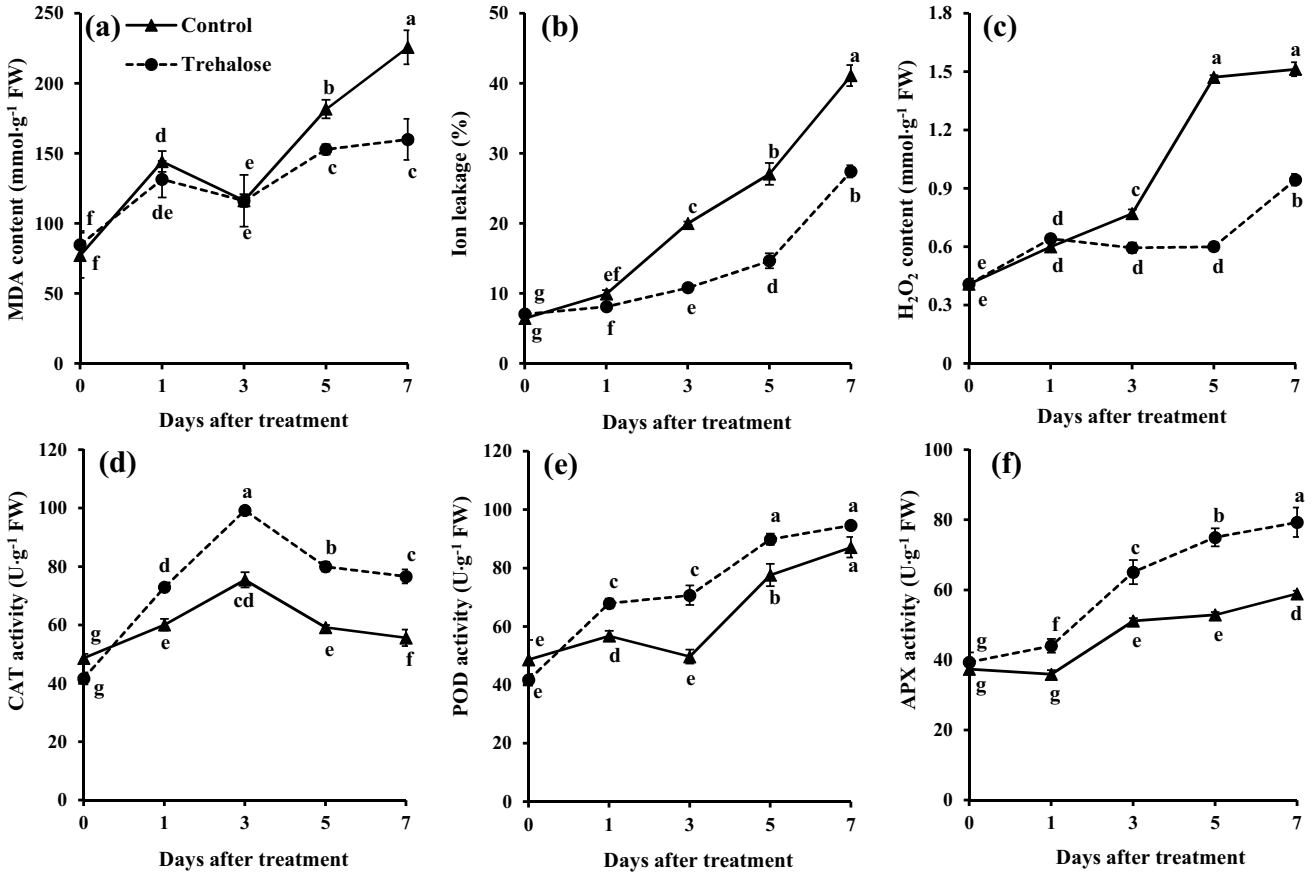


Fig. 4 Effects of 15.0 mM trehalose treatment on **a** MDA content, **b** ion leakage level, **c** H_2O_2 content, and the activities of antioxidant enzymes **d** CAT, **e** POD, and **f** APX under drought stress. Data are the mean values of three biological replicates ± SD. Values followed by different letters were significantly different according to Duncan's multiple range test at $P < 0.05$

Effects of trehalose treatment on the expression of genes involved in ABA signaling pathway

Trehalose treatment upregulated the expression levels of genes involved in ABA signaling under drought stress (Fig. 6). Transcript levels of *SlPYL1* in trehalose-treated plants were 21.6, 58.6, and 22.8% higher than those in control on days 0.5, 1 and 2 (Fig. 6a). Transcript levels of *SlPYL3* were enhanced by trehalose treatment, with values nearly 35.4% and 84.6% higher than those in control plants on days 0.5 and 1 (Fig. 6b). The expression patterns of *SlPYL4* were similar between control and trehalose-treated plants, and *SlPYL4* expression levels were 1.39 and 1.53 times higher after trehalose treatment than those in control on days 0.5 and 1 (Fig. 6c). Drought treatment markedly decreased *SlPYL5/6/7* genes relative expression, which maintained a lower level of transcription in control plants. Treatment with trehalose strongly triggered the transcription expression of these three genes, which were significantly higher than those in control on days 0.5 and 2 (Fig. 6d–f). The *SlPYL9* expression levels in trehalose-treated plants were 1.52, 1.57, and 2.05 times higher than those in control on days 0.5, 1 and 2 (Fig. 6g). The initial expression of *SlSnRK2.3* was lower (0.5-fold) in trehalose-treated plants, while expression levels of *SlSnRK2.3* in trehalose-treated plants were 98.4, 63.4, and 30.9% higher than those in control through the remaining drought period (Fig. 6h). The expression patterns of *SlSnRK2.4* were similar between control and trehalose-treated plants, and treatment with trehalose showed higher *SlSnRK2.4* expression levels on days 1 and 2 (Fig. 6i). In trehalose-treated plants, transcript levels of *SlAREB1* were upregulated, which were 1.47-, 2.17-, 2.44-, and 1.05-fold higher than those in control (Fig. 6j). The relative expression of *SlAREB2* in trehalose-treated plants was 102.5%, 84.8%, 67.4% higher than those in control (Fig. 6k). The *SlDREB1* expression levels were also significantly enhanced by trehalose treatment except on day 2 (Fig. 6l).

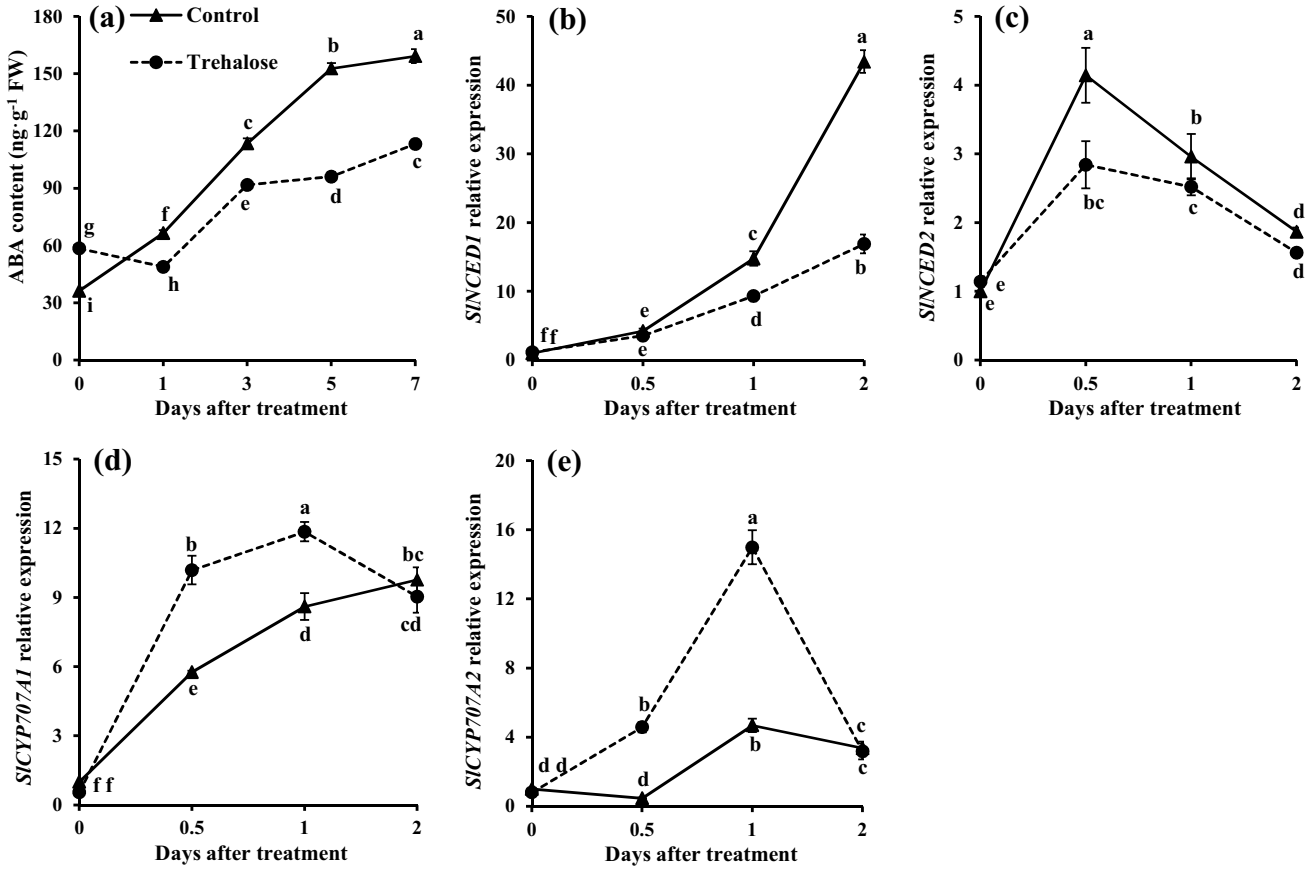


Fig. 5 Effects of 15.0 mM trehalose treatment on **a** ABA content, and the relative expression of **b** *SlNCED1*, **c** *SlNCED2*, **d** *SlCYP707A1*, and **e** *SlCYP707A2* under drought stress. Data are the mean values of three biological replicates ± SD. Values followed by different letters were significantly different according to Duncan's multiple range test at $P < 0.05$

Discussion

In recent years, the induced resistance by trehalose treatment has been increasingly studied in plants. Although it has been previously reported that trehalose treatment could enhance drought tolerance of maize and wheat, little information is available on the role of ABA and its signaling components in trehalose-induced drought tolerance. Moreover, there have not been any studies about the effects of exogenous trehalose on the responses of both, stomata and chloroplast ultrastructure. Therefore, the aim of this study is to elucidate the potential mechanism by which trehalose regulates ABA in response to drought stress.

In tomato plants, drought stress significantly upregulated expression levels of genes related to endogenous trehalose biosynthesis (Fig. S2), suggesting that trehalose might play a pivotal role in the modulation of metabolism to drought stress in tomato plants. It has been reported that excessive trehalose could inhibit seedling growth (Schluepmann et al. 2004). Thus, we started an experiment with different concentrations of trehalose solutions to find out the appropriate one. The group with 15.0 mM trehalose treatment performed better under drought stress than other treatment groups, exhibiting better performance and less wilting symptoms than controls (Fig. 1a). After re-watering, growth was resumed more remarkable in 15.0 mM trehalose-treated plants (Fig. 1c, d). Based on these, 15.0 mM trehalose was selected for the research. The results showed that exogenous trehalose treatment enhanced drought tolerance of tomato plants.

Previous studies showed that plant drought resistance had certain correlation with changes of chloroplast ultrastructure (Guofu et al. 2006). This change was suggested an important trait related to plant drought susceptibility (Vassileva et al. 2012). In our study, the typical chloroplast fine ultrastructure was observed in trehalose-treated plants, with normally preserved starch granules and less accumulated osmiophilic plastoglobules (Fig. 2). It has been reported that starch granules can be preferentially associated with enhanced effectiveness of photosynthesis, and the missing of starch granules seems to be a sign for lower photosynthetic activity in drought-stressed plants (Zellnig et al. 2010). Additionally, damage from drought stress always coincides with

accumulation of large osmiophilic plastoglobules (Bhuiyan and van Wijk 2017), which reveals the lipid peroxidation of thylakoid membrane system by ROS accumulation (Munne-Bosch and Alegre 2004). A previous study demonstrated that exogenous spermidine enhanced drought tolerance of maize, the plastids of which exhibited a better integrated thylakoid structure, due to the preservation of starch granules and less accumulation of large osmiophilic plastoglobules (Li et al. 2018), thus supporting our present data. These results implied that trehalose treatment could protect the chloroplast ultrastructure from oxidative damage under drought conditions.

Membrane damage and cell membrane instability induced by drought stress have been used as criteria to assess the degree of drought tolerance (Sairam et al. 2005). Drought-stressed enhanced the membrane lipid peroxidation in plants, expressed as MDA content, which could adversely affect the membrane integrity under oxidative stress (Heath and Packer 1968). Besides MDA, ion leakage is also a widely used index to evaluate the membrane integrity and instability under abiotic stress (Bernardo et al. 2017). In our study, both MDA content and ion leakage level increased in both groups under drought stress, while the levels in trehalose-treated plants were significant lower than those in controls (Fig. 4a, b). Thus, trehalose treatment mitigated cell membrane damage caused by drought, and this was in agreement with the alleviated damage of chloroplast ultrastructure (Fig. 2).

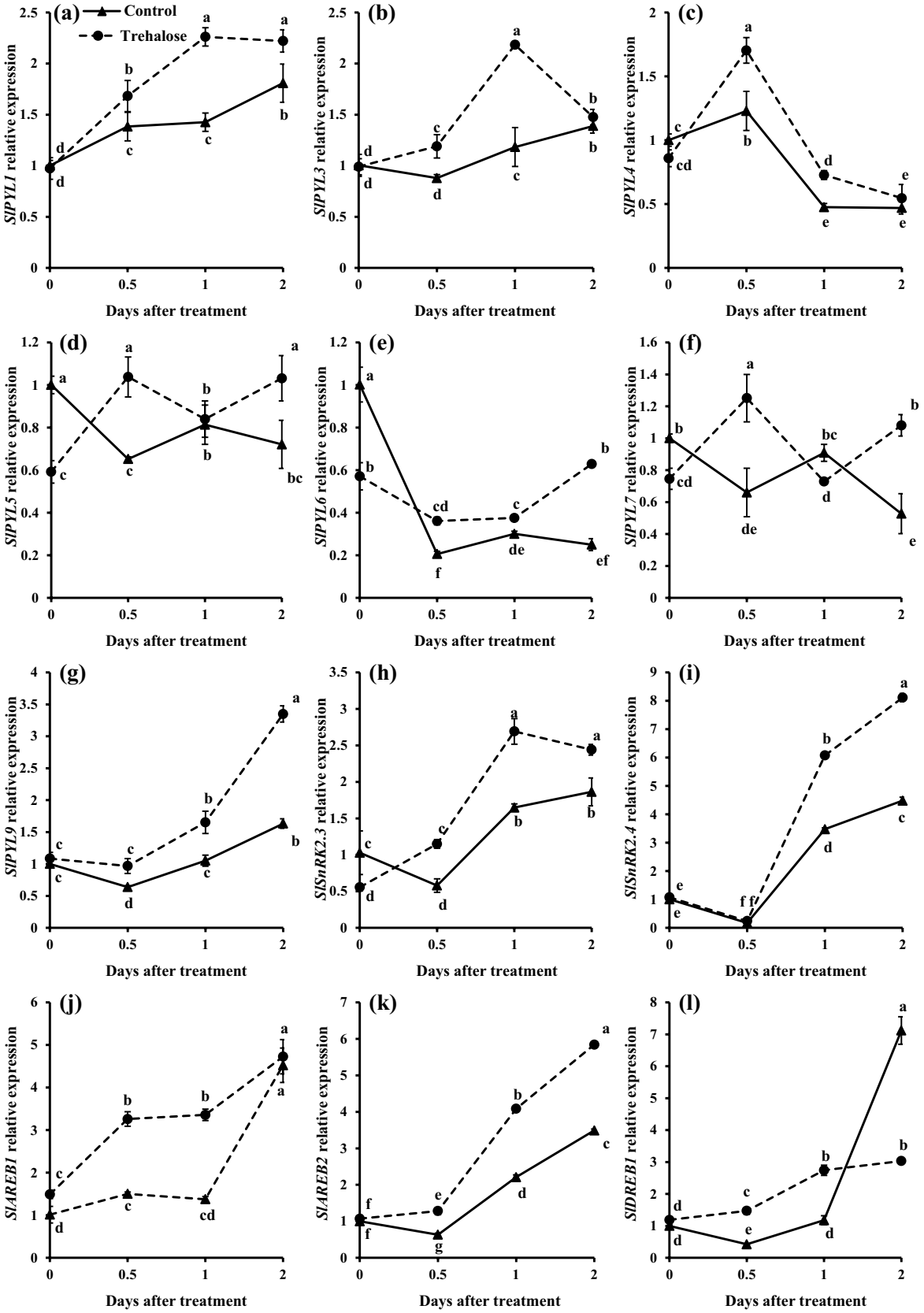
Exposure of plants to drought stress caused rapid ROS accumulation, which resulted in irreparable metabolic dysfunction and even cell death (Vassileva et al. 2012). H_2O_2 is a prominent ROS species and considered as an indicator of oxidative stress, which increases significantly under drought conditions (Żamojć et al. 2015). A previous study revealed that melatonin-treated wheat plants showed enhanced drought tolerance with lower H_2O_2 content (Cui et al. 2017). This was consistent with our results that trehalose-treated plants had lower H_2O_2 content than control (Fig. 4c), indicating that trehalose treatment decreased ROS accumulation. Ultimately, it alleviated oxidative damage to both chloroplast ultrastructure and cell membrane caused by drought (Figs. 2, 4a, b).

As a general adaptation strategy, antioxidant enzymes are deployed in plants to protect them from oxidative damages caused by ROS overproduction, such as APX, CAT and POD, which effectively scavenge ROS in a harmonized manner (Kadkhodaie et al. 2013). Present data showed that activities of antioxidant enzymes were significantly higher in trehalose-treated plants than those in control under drought stress (Fig. 4d–f). A previous study in peach plants also demonstrated that the enhancement of drought tolerance was related to the increase in antioxidant enzymes activities of APX, CAT, and POD (Wang et al. 2019). Trehalose-induced increase in antioxidant enzymes activities suppressed H_2O_2 accumulation (Fig. 4c), which decreased the susceptibility to oxidative stress, protecting both chloroplast ultrastructure and cell membrane from oxidative damage. Taken together, these results demonstrated that antioxidant enzymes were involved in the drought response induced by trehalose in tomato plants.

Maintenance of high RWC in drought-resistant cultivars has been reported to be an adaptation to drought stress in several crop species (Meher et al. 2018). In our study, drought stress reduced RWC, while trehalose-treated plants showed higher RWC than control plants (Fig. 1b). Difference in RWC was consistent with the phenotype between trehalose-treated and control plants, which suggested that trehalose treatment could retain water more effectively than control under drought stress.

Plants lose water primarily by transpiration through the stomata on their leaves. The regulation of stomatal closure is important for water conservation under drought stress (Lim et al. 2015). In our study, compared with control, trehalose treatment reinforced stomatal closure and prevented stomata opening (Fig. 3), which led to reduced water loss through stomata (Fig. 1b). Similar result was gained from plants exhibited drought-tolerant phenotype, which was characterized by lower levels of water loss with increased stomata closure (Wang et al. 2018a, b). These results revealed that exogenous trehalose-induced stomatal closure, which was associated with the improved drought tolerance.

ABA is a pivotal player by reducing water loss by inducing stomatal closure through changing the expression of many stress-responsive genes, which further leads to enhanced tolerance to drought stress (Holbrook et al. 2002; Lim et al. 2015). PYLs are confirmed ABA receptors, and SlPYL1, SlPYL4, SlPYL5, SlPYL7, and SlPYL9, are identified as ABA-dependent receptors (Chen et al. 2016). Previous studies demonstrated that *SlPYL1/3* could be prominently induced or increased by drought treatment, and *SlPYL3/4/5/6/7/9* genes expression could be upregulated by exogenous ABA in tomato plants, which responded to drought or ABA treatment at the transcriptional levels (Sun et al. 2011; Zhu et al. 2018). SnRK2s are key components of ABA signaling transduction, which are important for the ABA-induced activation of ABA-responsive genes through phosphorylation of transcription factors, such as AREBs/ABFs (Furihata et al. 2006; Sun et al. 2011). A previous study reported that *SlSnRK2.3/4* expression could be positively induced by drought in tomato plants (Sun et al. 2011). Moreover, transcript levels of *SlAREB1* and *SlAREB2* were significantly upregulated by ABA and drought, and *SlAREB1*-overexpressing transgenic tomato plants showed increased tolerance to drought stress (Hsieh et al. 2010; Orellana et al. 2010). DREB transcription factors positively regulated the expressions of stress-responsive genes, thereby increasing the tolerance to


(a)
Control
Trehalose
SlPYL1 relative expression
(b)
SlPYL3 relative expression
(c)
SlPYL4 relative expression
(d)
SlPYL5 relative expression
(e)
SlPYL6 relative expression
(f)
SlPYL7 relative expression
(g)
SlPYL9 relative expression
(h)
SlSnRK2.3 relative expression
(i)
SlSnRK2.4 relative expression
(j)
SlAREB1 relative expression
(k)
SlAREB2 relative expression
(l)
SlDREB1 relative expression
Days after treatment

◂**Fig. 6** Effects of 15.0 mM trehalose treatment on the relative expression of **a** *SlPYL1*, **b** *SlPYL3*, **c** *SlPYL4*, **d** *SlPYL5*, **e** *SlPYL6*, **f** *SlPYL7*, **g** *SlPYL9*, **h** *SlSnRK2.3*, **i** *SlSnRK2.4*, **j** *SlAREB1*, **k** *SlAREB2*, and **l** *SlDREB1* under drought stress. Data are the mean values of three biological replicates ± SD. Values followed by different letters were significantly different according to Duncan's multiple range test at $P < 0.05$

drought via ABA-dependent pathway (Wang et al. 2018a, b). *R*elative expression of *SlDREB1* was strongly induced by drought and exogenous ABA, and overexpression of the *SlDREB1* gene in transgenic *A. thaliana* showed enhanced tolerance to drought stress (Jiang et al. 2017). These results supported our present data that trehalose treatment markedly upregulated transcript levels of *SlPYL1/3/4/5/6/7/9*, *SlSnRK2.3/4*, *SlAREB1/2*, and *SlDREB1* compared with controls. Our present result was consistent with the lower level of RWC and encouraged stomatal closure shown in the trehalose-treated plants (Figs. 1b, 3), which implied that the improved drought tolerance in trehalose-treated plants could be partly attributed to the activation of ABA signaling-related genes expression.

However, it's noteworthy that ABA content was lower in trehalose-treated plants than that in control under drought stress, and trehalose treatment selectively suppressed the upregulation of *SlNCED1/2* (both for ABA biosynthesis) and promoted the upregulation of *SlCYP707A1/2* (both for ABA catabolism), which indicated that trehalose might participate in the negative regulation of ABA metabolism under drought stress (Fig. 5). This was consistent with a previous study that exogenous trehalose treatment reduced endogenous ABA content under drought conditions in soybean plants (Asaf et al. 2017). Besides, the present results implied that trehalose treatment reduced endogenous ABA level by enhancing ABA degradation and suppressing ABA synthesis, which was supported by a previous study in apple plants (Li et al. 2015). However, a previous report showed that increasing endogenous ABA content by exogenous application or overexpressing genes, could upregulate ABA signaling-related genes expression, thereby positively affect stress tolerance of plants (Vishwakarma et al. 2017), which is inconsistent with our present data. A possible explanation is that trehalose played an essential role in regulating ABA signaling-related genes expression under drought stress, which might be independent on ABA as supported by a previous study in *A. thaliana* stating that the effects of trehalose on starch metabolism and plants growth were mediated by an ABI4-dependent, but also an ABA synthesis-independent mechanism (Ramon et al. 2007). In brief, trehalose treatment might positively activate the ABA signaling pathway, which trigged stomatal closure and reduced water loss in response to drought stress.

Conclusion

In summary, this study demonstrated that appropriate trehalose treatment enhanced drought tolerance, and induced stomatal closure as well as protected chloroplast ultrastructure of tomato plants. The increase in drought tolerance was partially associated with the enhancement of antioxidant enzymes activities, which reduced ROS accumulation and protected both, cell membrane and chloroplast ultrastructure, from oxidative damage caused by drought. Moreover, trehalose treatment upregulated ABA signaling-related genes expression levels, and activated the ABA signaling pathway, which played an important role in regulating stomatal closure and water loss. By enhancing ABA degradation and suppressing its synthesis, less ABA was accumulated in trehalose-treated plants. Taken together, these results revealed that trehalose treatment enhanced drought tolerance of tomato plants, and it's ABA signaling rather than ABA metabolism that was involved in trehalose-induced drought tolerance. This study provided insights into the regulatory mechanism of trehalose-induced drought tolerance, providing data for further understanding of the role that trehalose played against abiotic stress in tomato plants.

Author contribution statement YWQ, and SL conceived and designed the experiments; YWQ, LR and ZSJ performed the experiments; YWQ, WL and SL analyzed the data. YWQ and ZRR wrote the manuscript. ZRR, SJP and SL made manuscript revisions. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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