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RESEARCH ARTICLE

Effects of Exogenous Trehalose on the Metabolism of Sugar and Abscisic Acid in Tomato Seedlings Under Salt Stress

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Abstract
Salt stress affects the growth and development of plants, which results in a decrease in crop quality and yield. In this study, we used tomato seedlings treated with salt and trehalose as experimental materials and analyzed them using the technique for order preference by similarity to ideal solution analysis to select the optimal trehalose concentration for treatment. We also determined the contents of sugar and abscisic acid (ABA) and detected the expression of genes involved in the metabolism of sugar and ABA by quantitative real-time PCR. Results showed that the optimal trehalose concentration was 2 mmol/L for tomato seedlings under salt stress. Exogenous trehalose decreased the starch content and increased the soluble sugar content by affecting the expression of genes related to the metabolism of starch and soluble sugar. Exogenous trehalose altered the accumulation and distribution of sugar by inducing the upregulation of sugar transporter genes. Furthermore, trehalose increased the ABA content to induce salt stress response by regulating the expression of genes related to the synthesis and metabolism of ABA. In conclusion, trehalose can effectively alleviate salt stress and enhance salt tolerance of tomato. These findings provide a novel perspective and a better resource to investigate the salt tolerance mechanism and a new method for alleviating salt stress in tomato.

Keywords Tomato · Trehalose · Salt stress · Sugar metabolism · Sugar transporter · Abscisic acid

Introduction

In recent years, increasing research attention has been paid to salt stress that can affect the growth and development of plants, especially in the stage of seed germination [1, 2]. The response of plants to salt stress is complex, which is achieved through a variety of physiological and biochemical reactions and molecular mechanisms, and also involves the differential expression of stress-related genes in the corresponding pathways [3–5].

Soluble sugars as osmoprotectants help in the alleviation of the negative effects of salt on plants. Rosa et al. [6] suggested that there was a significant increase in the levels of glucose, sucrose, and fructose in plants under salt stress, which played a vital role in carbon storage, osmotic protection, and free radical scavenging. Nemati et al. [7] demonstrated that exogenous glucose can limit the accumulation of Na^+ and stimulate the uptake of K^+, thereby maintaining ion homeostasis under salt stress.

Trehalose was first isolated from ryegrass by Wiggers in the late nineteenth century and was later found in bacteria, algae, yeast, lower plants, insects, and other invertebrates [8]. The amount of trehalose present in plants is extremely low, and excessive accumulation of trehalose can affect the metabolism of normal carbohydrates in plants and even inhibit their growth [9–11]. Trehalose produces a marked effect primarily in the form of signaling molecules under stress conditions [12]. The metabolic pathway of trehalose in plants is recognized as the Ots A–Ots B pathway, in which under the action of trehalose phosphate synthase, uridine diphosphate glucose and glucose-6-phosphate form trehalose-6-phosphate and uridine diphosphate and then generate trehalose and inorganic phosphates under the catalysis of trehalose phosphatase, as shown in Fig. 1 [13, 14]. Garcia et al. [15] reported in 1997 that when rice was exposed to

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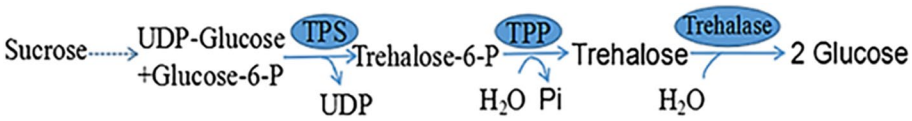

Fig. 1 Metabolic pathway of trehalose in plants

salt stress, the addition of low concentrations of trehalose (1–10 mmol/L) could protect its root integrity, protect root cells from abnormal cell division induced by severe salt stress, and excrete excessive Na^+ from cells by protecting ion pumps. Yang et al. [16] demonstrated that trehalose can increase the salt tolerance of plants, while exogenous trehalose may act as an inducer of biological processes involved in the salt stress response of plant.

Sugars not only participate in the metabolism of carbon and energy in cells but also play a key role in plant growth and stress resistance as a signal molecule [17]. The presence of an interaction has been suggested between sugars and plant hormones in metabolic pathways and stress response [18, 19]. Cheng et al. [20] found that exogenous glucose enhanced the expression of genes related to the biosynthesis of abscisic acid (ABA) and ultimately increased the ABA content in plants.

However, the effects of exogenous trehalose on sugar metabolism and the interaction between trehalose and ABA under salt stress are still unclear. In previous studies, we used transcriptome sequencing to screen the pathways and the related genes involved in protein metabolism and carbohydrate metabolism pathways in tomato [21]. In response to salt stress, the protein metabolism pathway was significantly enriched in seven pathways, with 17 key genes being differentially expressed, and the carbohydrate metabolism pathway was significantly enriched in six pathways, with 19 key genes being differentially expressed. Based on transcriptome sequencing results, this study analyzed the metabolic changes in sugar and ABA in tomato seedlings treated with trehalose and salt and determined the differential expression profiles of key genes involved in sugar metabolism. The purpose of this study was to investigate the effects of trehalose on salt stress and the interaction between sugar metabolism and trehalose on regulating the response to salt stress in tomato, which could provide a theoretical basis for improving crop yield, salt tolerance of plants, and the utilization rate of saline–alkali land in agricultural research.

Materials and Methods

Plant Material and Treatment

After soaking for 4–6 h at room temperature, tomato (cv. Ailsa Craig) seeds were sterilized with 75% ethanol and 10% NaClO, cultured at 25 °C for 8 d, and then cultured in Hoagland's nutrient solution for 4 weeks with illumination for 16 h/d at 25 °C. Trehalose and salt were applied to tomato seedlings with consistent growth, and each treatment was performed for 7 d as follows: (T1) control: 0 mmol/L Tre + 0 mmol/L NaCl; (T2) 0 mmol/L Tre + 200 mmol/L NaCl; (T3) 1 mmol/L Tre + 200 mmol/L NaCl; (T4) 2 mmol/L Tre + 200 mmol/L NaCl; (T5) 3 mmol/L Tre + 200 mmol/L NaCl; (T6) 5 mmol/L Tre + 200 mmol/L NaCl; and (T7) 10 mmol/L Tre + 200 mmol/L NaCl. Salt concentration was selected based on our previous research [21]. The formulation of Hoagland's nutrient solution was as follows: pH 6.0, 945 mg/L $Ca(NO_3)_2$, 607 mg/L KNO_3, 115 mg/L $(NH_4)_3PO_4$, 493 mg/L $MgSO_4$, 2.5 mL/L ferric salt solution, and 5 mL/L trace element.

Determination of Growth Indicators and Related Physiological Indicators

Plant height, fresh mass, and dry mass of tomato seedlings were measured, and the relative water content was calculated as follows:

$$\text{Relative water content} = \left[(\text{fresh mass} - \text{dry mass})/\text{fresh mass}\right] \times 100\% \quad (1)$$

The chlorophyll content of tomato leaves was measured by the acetone method as previously described in Ref. [22] with slight modification. The activity of peroxidase (POD) was determined by the guaiacol method, and the activity of superoxide dismutase (SOD) was determined by the photochemical reaction method of nitroblue tetrazolium according to previously described methods, with slight modification [23, 24]. Catalase kit was used to determine the activity of CAT enzyme according to the instructions (Solarbio, Beijing, China). The level of proline was determined as described in Ref. [25].

For the determination of malondialdehyde (MDA) levels, 1.0 g seedlings was added to 10 mL of 10% trichloroacetic acid solution, ground on ice, and then centrifuged at 5000 *g*. Then, 1 mL supernatant was aspirated and 3 mL of 0.6% thiobarbituric acid solution was added and heated in boiling water for 15 min. After cooling the liquid rapidly, absorbance values at 450, 532, and 600 nm were measured. The MDA level was determined according to the following formula:

$$c(\mu\text{mol/L}) = 6.45 \times \left(\text{OD}_{532} - \text{OD}_{600}\right) - 0.56 \times \text{OD}_{450} \quad (2)$$

Table 1 Primer sequences of genes related to sugar metabolism

Genes	GenBank number	Forward primer (F-primer 5′–3′)	Reverse primer (R-primer 5′–3′)
SS	NM_001247688.2	TTTTCTTGGGAAGCAACTCTG	CGACTCAAATGAGGAGGGACA
AGPase	NM_001247767.2	AAGAACTTCCACAAAGCCCTC	GGGACCCGACTTTATCCTCTA
BAM	NM_001247627.2	CCTTGTTCCATAGTGCCAGTG	AAGGTGGTGGATGGGATAGTC
AMY	XM_004235178.4	ACAGAGTGAGATGATGGTGGT	ATAAGCAAAGTGGTTGGTACA
SPS	NM_001246991.2	AGTAAGCAAGTCAACCCGATA	GGTGGTCAGGTGAAGTATGTT
SUS3	NM_001247875.2	CTCCCTTAGTCGTGGATTCTT	GATTGACAACTTTTCACCCTG
Wiv-1	NM_001246913.2	TCAAACCTCGTAACATCAAAG	CTAAACACCCACTCCACTCAG
TPP	XM_004237846.4	GGTTGGCAGAGTACAGTTTGA	AGATGAGGGAAGCAGTAAGAA
TPS1	NM_001246967.2	AAGGCACCAAATTCATTCCAT	TAACGGTCCAGTTTCTACGCA
Trehalase	XM_004245430.4	TCCATCTTGAACTGAAATCCC	ATAAGCCCCGTCCAGAATCGT
β-tublin	NM_001247878.2	CACGTGGGTCCAGCAATAC	GGTCAGCAGCACATCATGT

Table 2 Primer sequences of genes related to sugar transporter

Genes	GenBank number	Forward primer (F-primer 5′–3′)	Reverse primer (R-primer 5′–3′)
FRK2	NM_001246959.2	AGTTAGGATGCCACAAGGACA	CAATGGAAGTAGCAAAGGAGG
PFK	XM_004236594.4	CGACTTTGTTCTTTGCTTTGG	TCACGGAGTTATGGCAGGGTA
SUT1	NM_001302901.2	GCGGCGTACCCCAGAATGTTT	TTGCTGTATTCGTCGTCGGCTTTT
SUT4	NM_001247415.2	TTAGATTGGCACAGTTGATGG	TCACAGAAGAACTCGGGTAGC
MST3	NM_001329197.1	AGGAAATGATGTGAGTGAGCC	GATGCGATTGGTAGAAAGTGG
HXK2	NM_001247477.2	CAGAGTTAGGCAGAGGACCGT	ACATTGGCTGGTGGTAGATTC
HXK3	NM_001247781.2	TTTCCTCATTTTCAAGGTAGA	ATTATTCGGTGATTATGTCCC

Table 3 Primer sequences of genes related to ABA metabolism

Genes	GenBank number	Forward primer (F-primer 5′–3′)	Reverse primer (R-primer 5′–3′)
NCED1	NM_001247526.2	TTTCCATTCTTTCTCATCGTG	CAATGGAAGTAGCAAAGGAGG
NCED2	XM_004244759.4	AAAACTCCACGGGCATAGAAC	TTTACTGAAACGGAAAGATTAG
CYP707A1	NM_001247588.2	AATGGCATAAATGTATTGGGT	AATGGCATAAATGTATTGGGT
CYP707A2	XM_004244388.4	CTTGAAAGCCTGGAGGACTAA	AAAGAAAGAATGCTGGGAAAA

The optimum trehalose treatment concentration was selected using the technique for order preference by similarity to ideal solution (TOPSIS) analysis.

Determination of Starch and Soluble Sugar Contents Under Optimal Trehalose Concentration

Tomato seedlings (cultivated as mentioned earlier) with consistent growth were selected for four treatments (0 mmol/L Tre + 0 mmol/L NaCl; 0 mmol/L Tre + 200 mmol/L NaCl; 2 mmol/L Tre + 0 mmol/L NaCl; and 2 mmol/L Tre + 200 mmol/L NaCl), and each treatment was processed separately for 0, 1, 3, 5, and 7 d. Both roots and leaves of tomato were selected for determination.

Starch and soluble sugar contents were evaluated based on a previously reported protocol, with some modifications [25].

For the determination of trehalose content, 2 g tomato seedlings were ground into powder in a mortar with liquid nitrogen and homogenized by adding 2 mL of 80% ethanol and 1% polyvinylpolypyrrolidone (PVPP). The supernatant was concentrated in 200 mL distilled water and filtered by a microporous membrane (0.45 μm). Trehalose content was determined using the trehalose assay kit (Seebio, China).

Fig. 2 Tomato leaves grown under normal and salt-treated conditions

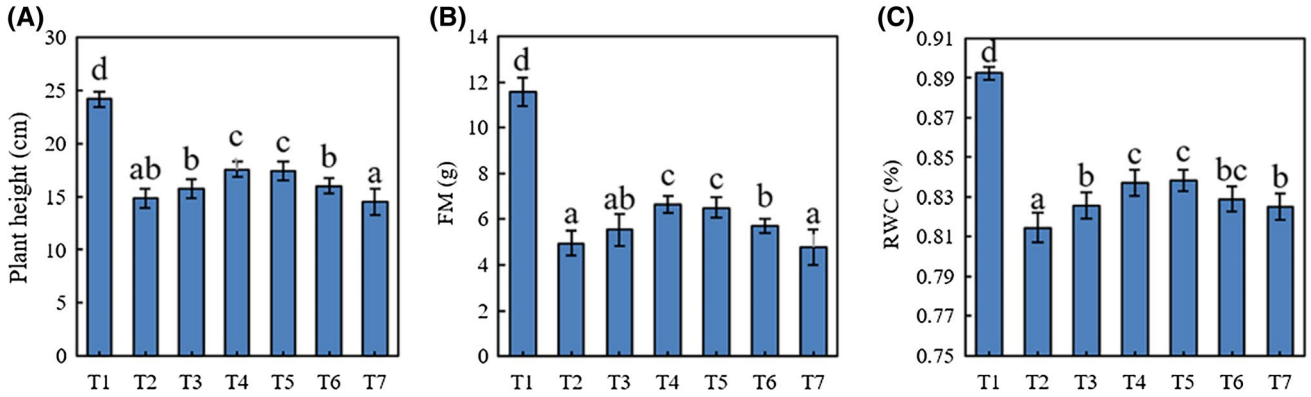

Fig. 3 Effect of trehalose concentration on tomato growth under salt stress. **A** Plant height, **B** fresh mass, and **C** relative water content

Determination of the Genes Expression Related to Sugar Metabolism Under Optimal Trehalose Concentration

Total RNA in tomato leaves and roots was extracted using the Column Plant RNAOUT kit (Tiandz, Beijing, China), and cDNA was synthesized following the instruction of the FastQuant RT kit (Tiangen Biotech, Beijing, China). CDS sequences of internal reference gene and target genes were searched in the NCBI database. The Primer 5.0 software was used to design specific primer sequences given in Table 1. PCR amplification and quantitative real-time PCR (qRT-PCR) were performed using S1000 thermal cycler (BIO-RAD, USA) and LightCycler96 (Roche, Basel, Switzerland), respectively. *β-Tublin* was selected as the internal reference gene [26].

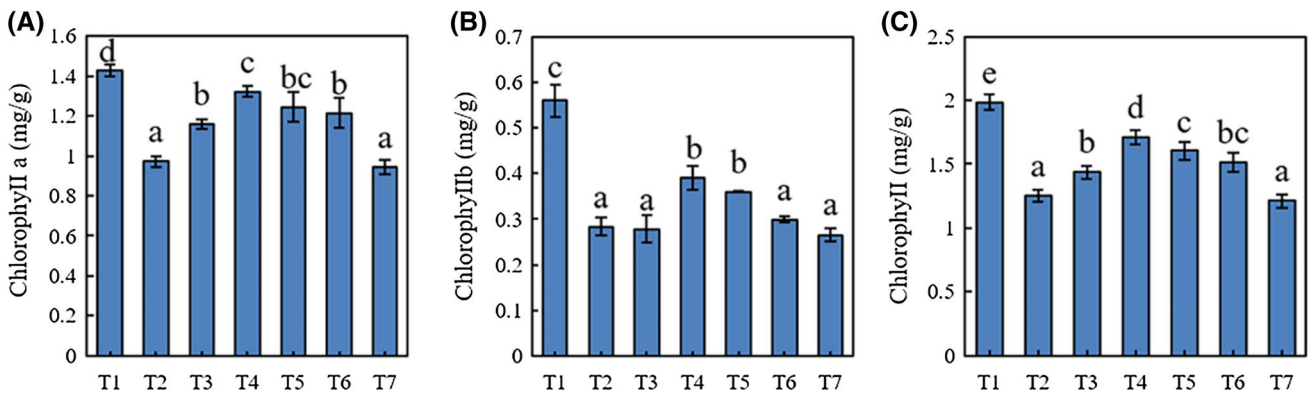

Fig. 4 Effect of trehalose concentration on the chlorophyll content of tomato leaves under salt stress. **A** Chlorophyll a, **B** chlorophyll b, and **C** total chlorophyll

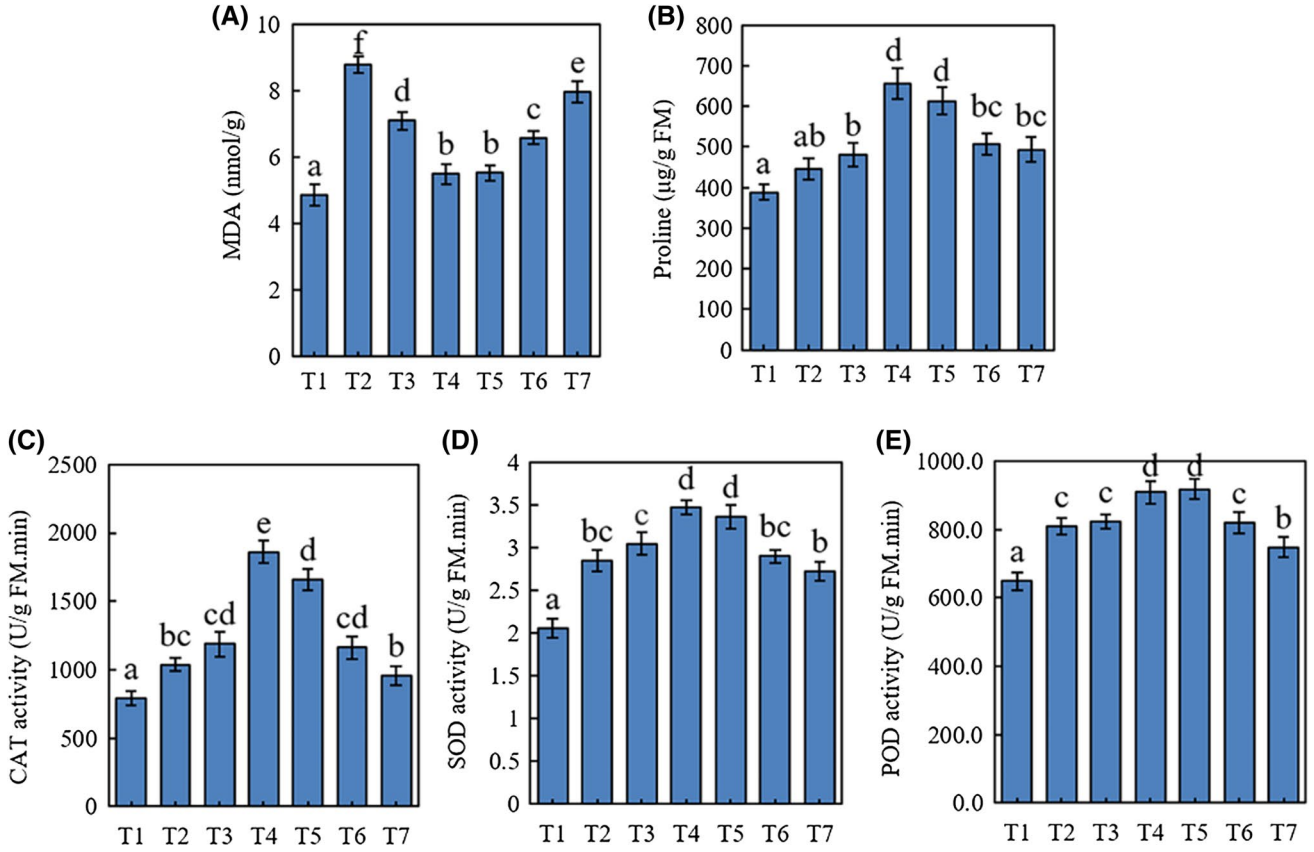

Fig. 5 Effects of different concentrations of trehalose on physiological indexes of tomato leaves under salt stress. **A** MDA, **B** proline, **C** CAT, **D** SOD, and **E** POD

Determination of the Genes Expression Related to Sugar Transporter Under Optimal Trehalose Concentration

The expression of genes related to sugar transporter was measured as mentioned earlier. The primer sequences are given in Table 2.

Determination of ABA Content and the Expression of Metabolism-Related Genes Under Optimal Trehalose Concentration

After being ground with liquid nitrogen, 0.1 g of tomato seedlings was added with 0.8 mL of 10% methanol and 1% acetic acid, and then extracted at 4 °C for 16 h. After centrifuged at 10000*g* for 15 min, the supernatant was aspirated and filtered through a 0.45-μm microporous membrane. The ABA content was determined using a Plant ABA ELISA test kit (Jianglai Biotechnology, Shanghai, China).

The expression of metabolism-related genes was determined as mentioned earlier. The primer sequences are given in Table 3.

Statistical Analysis

All experiments were repeated three times. Significance of differences ($p < 0.05$) between the mean values was determined by analysis of variance using the IBM SPSS Statistics 20 software (SPSS commercial software, USA).

Table 4 Appropriate treatment concentration evaluated by the TOPSIS method

Tre (mmol/L)	d_j^+	d_j^-	R_j	Rank
0	0.37966	0.04575	0.10755	5
1	0.26444	0.15939	0.37608	4
2	0.00487	0.41326	0.98834	1
3	0.07097	0.35692	0.83413	2
5	0.25677	0.18665	0.42094	3
10	0.39461	0.04742	0.10728	6

d_j^+, positive ideal solution of Euclidean distance; d_j^-, negative ideal solution of Euclidean distance; R_j, the closeness coefficient

Results

Screening for Optimal Trehalose Concentration

Under salt stress, the tomato leaves exhibited a withering state, and the plant height, fresh mass, and the relative water content (RWC) of the leaves were significantly reduced as shown in Figs. 2 and 3, respectively. However, exogenous trehalose improved the growth of tomato and increased the plant height, fresh mass, and the relative water content of tomato leaves, especially under treatment with 2 mmol/L trehalose, which was consistent with the results in Ref. [27].

Salt stress affects photosynthesis in plants. Salt stress can increase the number of free radicals in chloroplasts, thereby destroy the chlorophyll, and affect the photosynthesis of plants. After exogenous trehalose treatment, the reduction in chlorophyll content was improved and plant salt stress was alleviated [28]. The chlorophyll content in leaves decreased

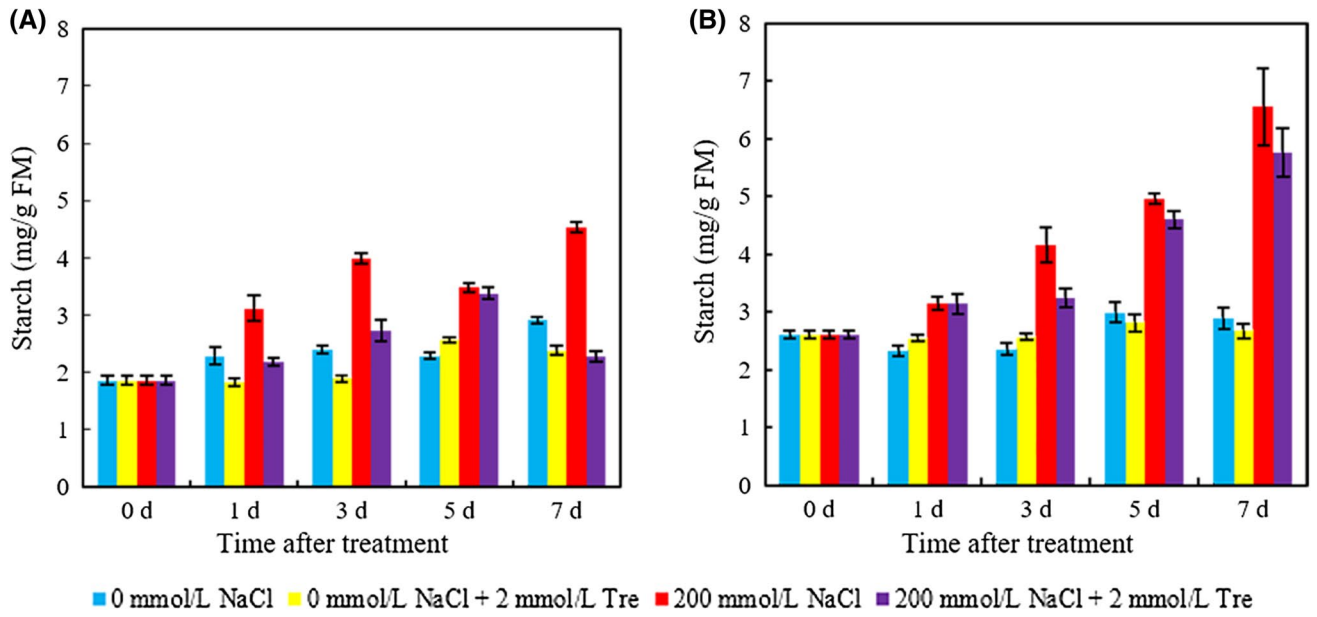

Fig. 6 Effect of exogenous trehalose on the starch content of tomato seedlings under salt stress. **A** Roots and **B** leaves

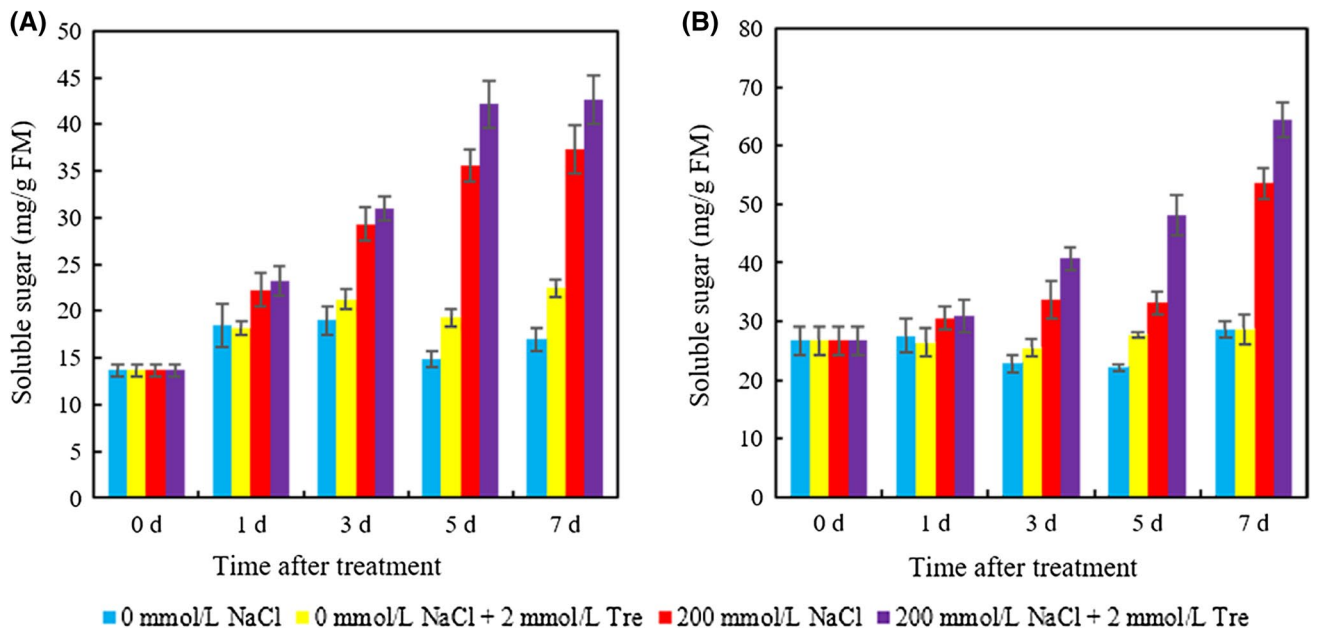

Fig. 7 Effect of exogenous trehalose on the soluble sugar content of tomato seedlings under salt stress. **A** Roots and **B** leaves

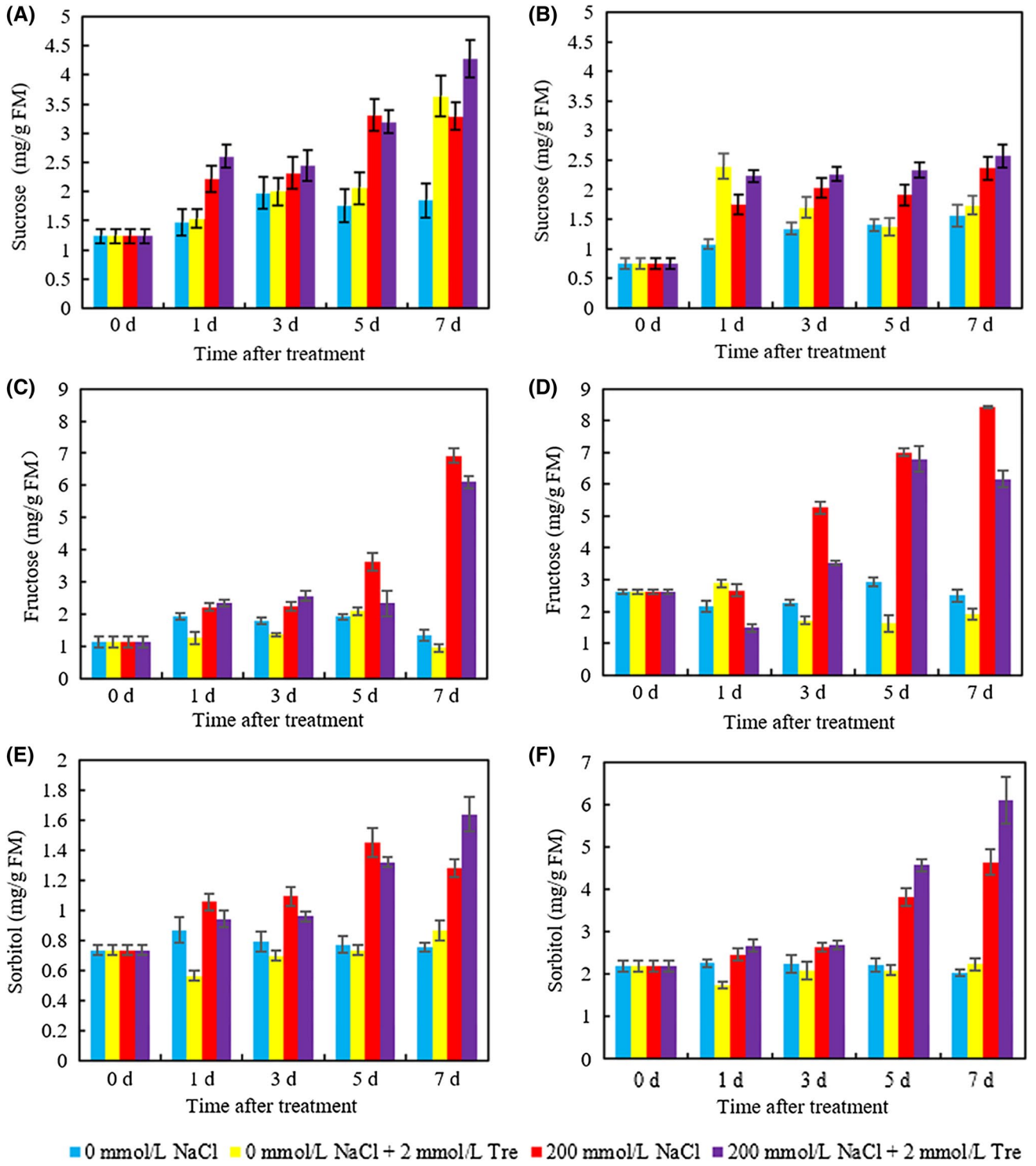

Fig. 8 Effects of exogenous trehalose on the contents of sucrose, fructose, and sorbitol in tomato seedlings under salt stress. **A** Sucrose in roots, **B** sucrose in leaves, **C** fructose in roots, **D** fructose in leaves, **E** sorbitol in roots, and **F** sorbitol in leaves

significantly after salt stress treatment (Fig. 4). However, after treatment with different concentrations of exogenous trehalose, the chlorophyll content increased initially and then decreased. The highest chlorophyll content was found to be with 2 mmol/L trehalose treatment.

The MDA content increased significantly after salt treatment as shown in Fig. 5A, indicating that salt stress

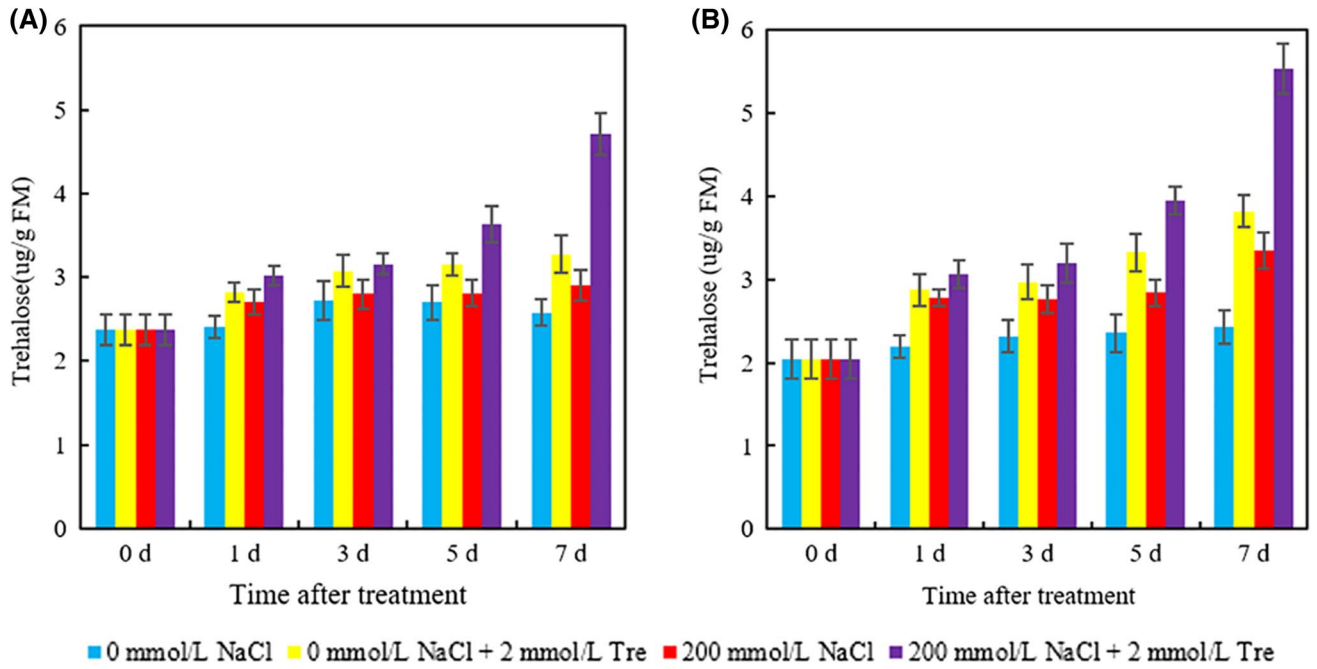

Fig. 9 Effect of exogenous trehalose on the trehalose content of tomato seedlings under salt stress. **A** Roots and **B** leaves

destroyed the cell membrane of plants severely, and a large number of free radicals and reactive oxygen species were produced that resulted in membrane damage. After treatment with different concentrations of trehalose, the MDA content decreased initially and then increased and was significantly reduced with 2 mmol/L trehalose treatment.

The accumulation of proline increases the osmotic potential of cells, regulates osmosis, and alleviates salt stress. Under salt treatment, proline showed a certain accumulation; after the application of trehalose, the proline content increased significantly, with the highest content being observed with 2 mmol/L trehalose treatment (Fig. 5B).

High salt content causes an imbalance of reactive oxygen species in plants, and plants remove the excess reactive oxygen species primarily through antioxidant enzymes such as SOD, POD, CAT, and other antioxidant enzymes and non-antioxidant enzymes such as ascorbic acid, glutathione (GSH), β-carotene, and mannitol [21]. It has been reported that the activities of SOD, CAT, and POD show a positive correlation with plant salt resistance [29]. In our study, the activities of these three antioxidant enzymes (POD, SOD, and CAT) increased after salt treatment, which indicated that salt stress could enhance the activities of these enzymes. After treatment with different concentrations of trehalose, the activities of the three antioxidant enzymes increased initially and then decreased, with the highest activity of CAT and SOD being detected with 2 mmol/L trehalose treatment (Fig. 5C, D). And the activity of POD reached the second highest, close to the highest, with 2 mmol/L trehalose treatment (Fig. 5E).

The TOPSIS mathematical model was used to analyze and screen the optimum exogenous trehalose concentration for alleviating salt stress in tomato seedlings. Results showed that the optimum concentration of trehalose was 2 mmol/L (Table 4). Therefore, this concentration was selected for subsequent experiments.

Effect of Exogenous Trehalose on Sugar Content of Tomato Seedlings Under Salt Stress

The accumulation of starch can lead to a negative feedback mechanism of photosynthesis, reduce the intensity of photosynthesis, and slow down plant growth. Under salt stress, starch accumulated in tomato leaves and reached the maximum content on the 7th day, whereas the addition of trehalose reduced starch accumulation, as shown in Fig. 6. The changes in the starch content of roots were consistent with those of leaves, but the starch content in roots was lower than that in leaves, which may be due to the fact that salt stress destroyed the transport mechanism of starch from leaves to roots and affected starch transport, thus primarily accumulating in leaves and causing a negative feedback mechanism of photosynthesis.

Plants regulate the cell osmotic potential by absorbing external inorganic salt ions and synthesizing small organic molecules such as proline and soluble sugar (sucrose, trehalose, etc.) to resist salt stress [30, 31]. Soluble sugar can be

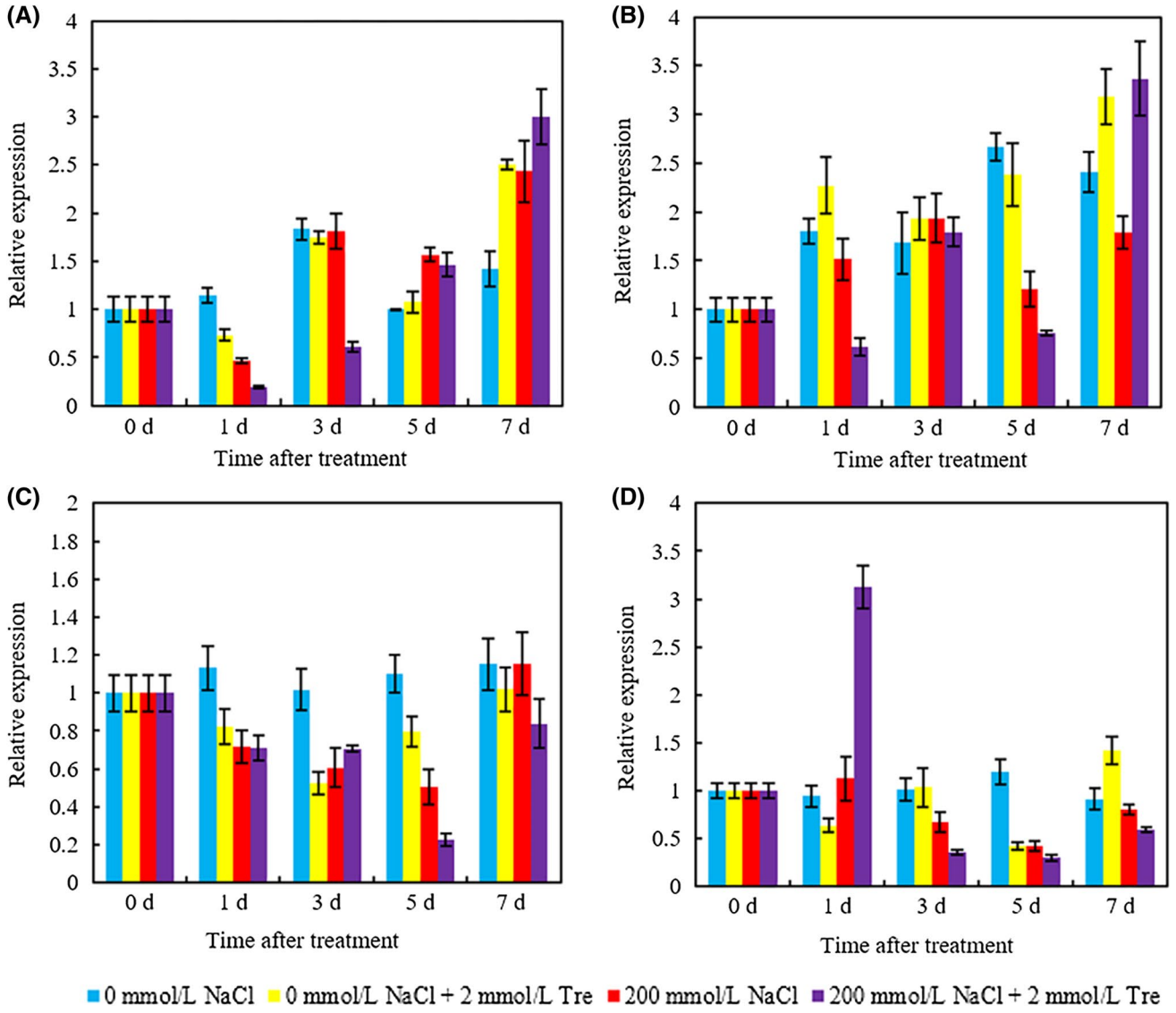

Fig. 10 Effect of exogenous trehalose on the expression of *SS* and *AGPase* in tomato seedlings under salt stress. **A** *SS* in roots, **B** *SS* in leaves, **C** *AGPase* in roots, and **D** *AGPase* in leaves

used as an osmotic substance to alleviate the ionic stress of plants and as a signal molecule to stimulate the response to salt stress. Our study showed that under salt stress, the soluble sugar contents increased in tomato roots and leaves, and exogenous trehalose significantly increased the soluble sugar contents, especially in the later stage (Fig. 7). It may be due to the fact that trehalose reduced the accumulation of starch and promoted the conversion of starch into soluble sugar.

As shown in Fig. 8, exogenous trehalose treatment under salt stress increased sucrose content, which is the primary product of photosynthesis. Exogenous trehalose contributed to the accumulation of sucrose, indicating that it may induce an increase in photosynthesis rate, which was consistent with the abovementioned results showing that exogenous trehalose increased the content of photosynthetic pigments. The change in fructose levels was inconsistent compared with sucrose levels. Under salt stress, the fructose content increased, whereas exogenous trehalose treatment significantly decreased the fructose content. It was probably because trehalose reduced the hydrolysis rate of sucrose, and the rate of sucrose synthesis was greater than the rate of degradation. With the progression of salt treatment, the sorbitol content increased gradually. Exogenous trehalose further induced an increase in sorbitol content in the later stage.

In general, the content of trehalose is extremely low in plants, and it is difficult to detect its presence directly. As shown in Fig. 9, the trehalose content under normal conditions was very low, and salt stress stimulated trehalose production, especially in leaves. The increase in trehalose

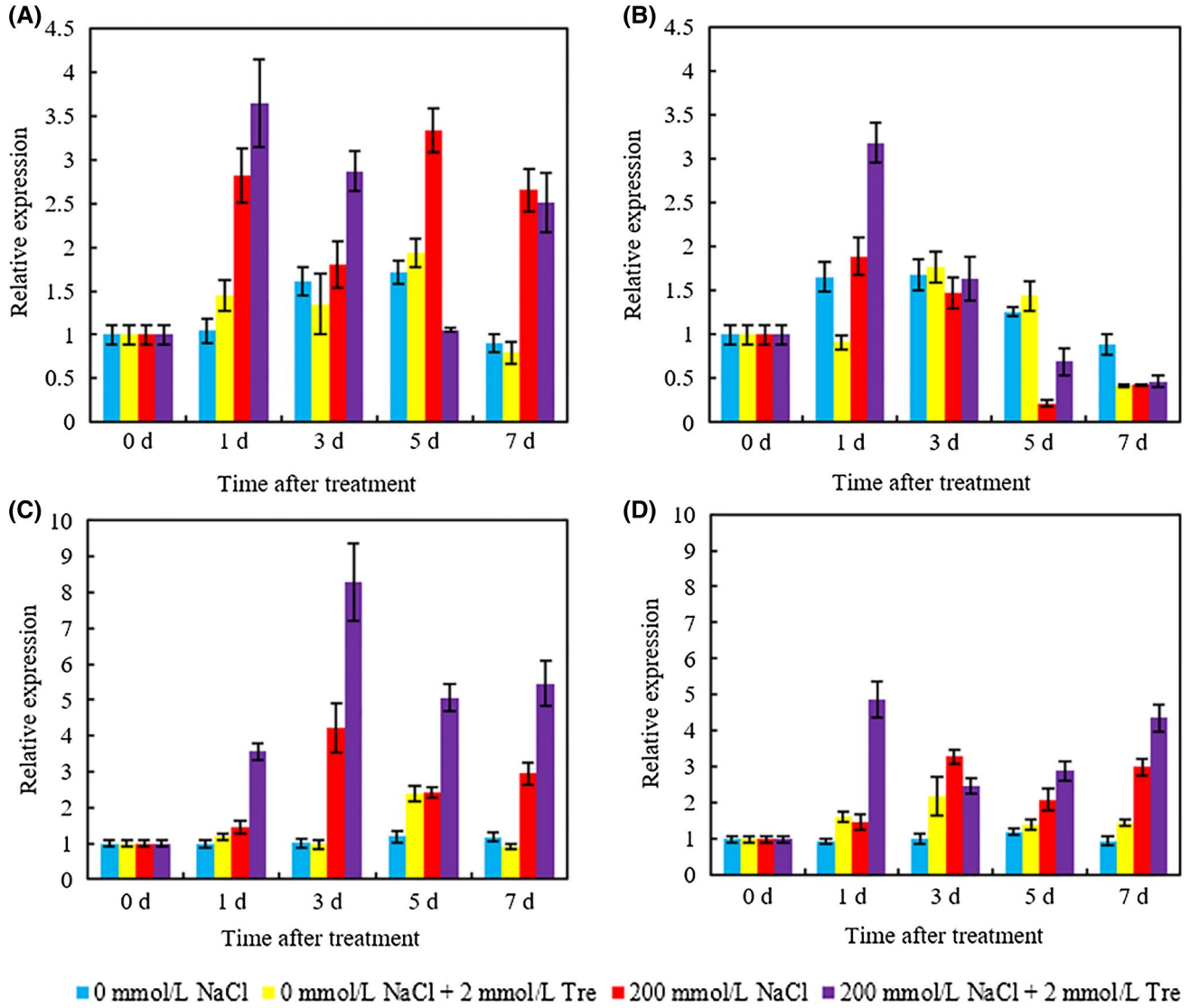

Fig. 11 Effect of exogenous trehalose on the expression of *BAM* and *AMY* in tomato seedlings under salt stress. **A** *BAM* in roots, **B** *BAM* in leaves, **C** *AMY* in roots, and **D** *AMY* in leaves

content confirmed that it enhanced the salt tolerance of tomato to some extent.

Effect of Exogenous Trehalose on Key Genes Involved in Sugar Metabolism in Tomato Seedlings Under Salt Stress

Starch is the primary product of plant photosynthesis and is generally degraded to resist stress. The starch synthase (*SS*) gene and the adenosine diphosphate glucose pyrophosphate (*AGPase*) gene play an important role in the starch synthesis pathway, while alpha amylase (*AMY*) and beta amylase (*BAM*) are the two key enzymes involved in plant starch degradation.

In our study, *SS* expression in tomato roots decreased initially and then increased under salt stress (Fig. 10). On the 1st day, exogenous trehalose significantly inhibited the expression of *SS*, which was consistent with the decrease in starch content. However, with the progression of salt treatment, *SS* expression gradually became stable. The change in the expression of *SS* in tomato leaves was approximately similar to that in roots. Under salt stress, *AGPase* expression in tomato roots was inhibited, especially in the case of exogenous trehalose treatment, which decreased its expression to 22.3% on the 5th day. However, in leaves, *AGPase* expression was increased significantly on the 1st day and then decreased with the progression of salt treatment.

As shown in Fig. 11, beta amylase played an important role in response to salt stress. The expression of the gene

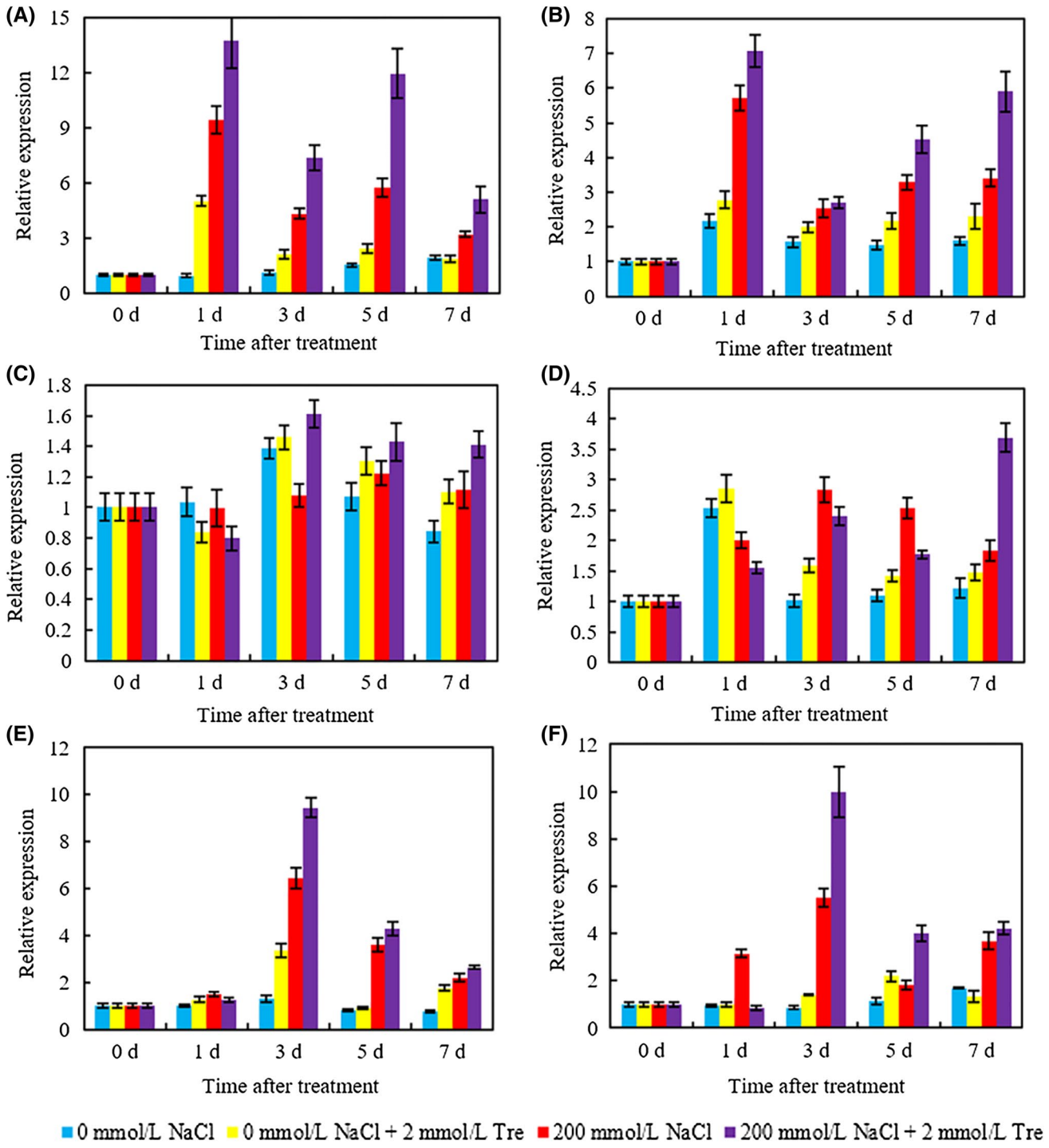

Fig. 12 Effect of exogenous trehalose on the expressions of *SPS*, *SUS3*, and *Wiv-1* in tomato seedlings under salt stress. **A** *SPS* in roots, **B** *SPS* in leaves, **C** *SUS3* in roots, **D** *SUS3* in leaves, **E** *Wiv-1* in roots, and **F** *Wiv-1* in leaves

BAM in tomato roots was significantly upregulated compared with the control group, especially under treatment with exogenous trehalose (except for the 5th day), which promoted the degradation of starch and reduced the feedback inhibition of photosynthesis. In leaves, *BAM* expression increased initially and then decreased, which was basically consistent with the previous changes in starch content. Salt + trehalose treatment significantly upregulated the expression of the α-amylase gene *AMY*, with the highest expression being observed on the 3rd day in roots, which

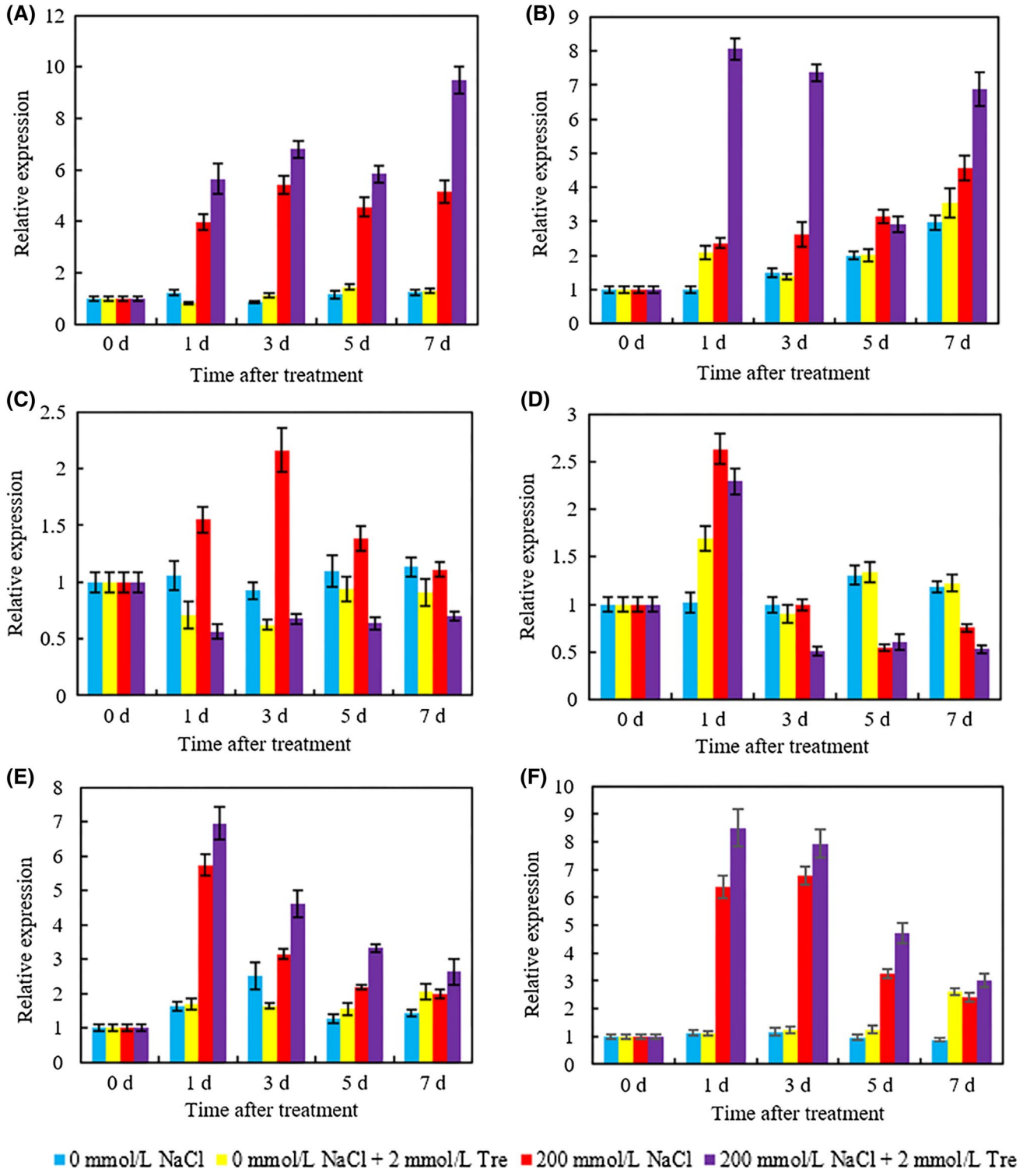

Fig. 13 Effect of exogenous trehalose on the expression of *TPS*, *TPP*, and *Tre* in tomato seedlings under salt stress. **A** *TPS* in roots, **B** *TPS* in leaves, **C** *TPP* in roots, **D** *TPP* in leaves, **E** *Tre* in roots, and **F** *Tre* in leaves

was approximately eight times of that in the control group and two times of that in the group of salt treatment alone, indicating that *AMY* was sensitive to trehalose. In leaves, the expression of *AMY* reached the peak on the 1st day and then decreased. The differential expression of *AMY* in different tissues indicated that its contribution to starch degradation was inconsistent and tissue specific.

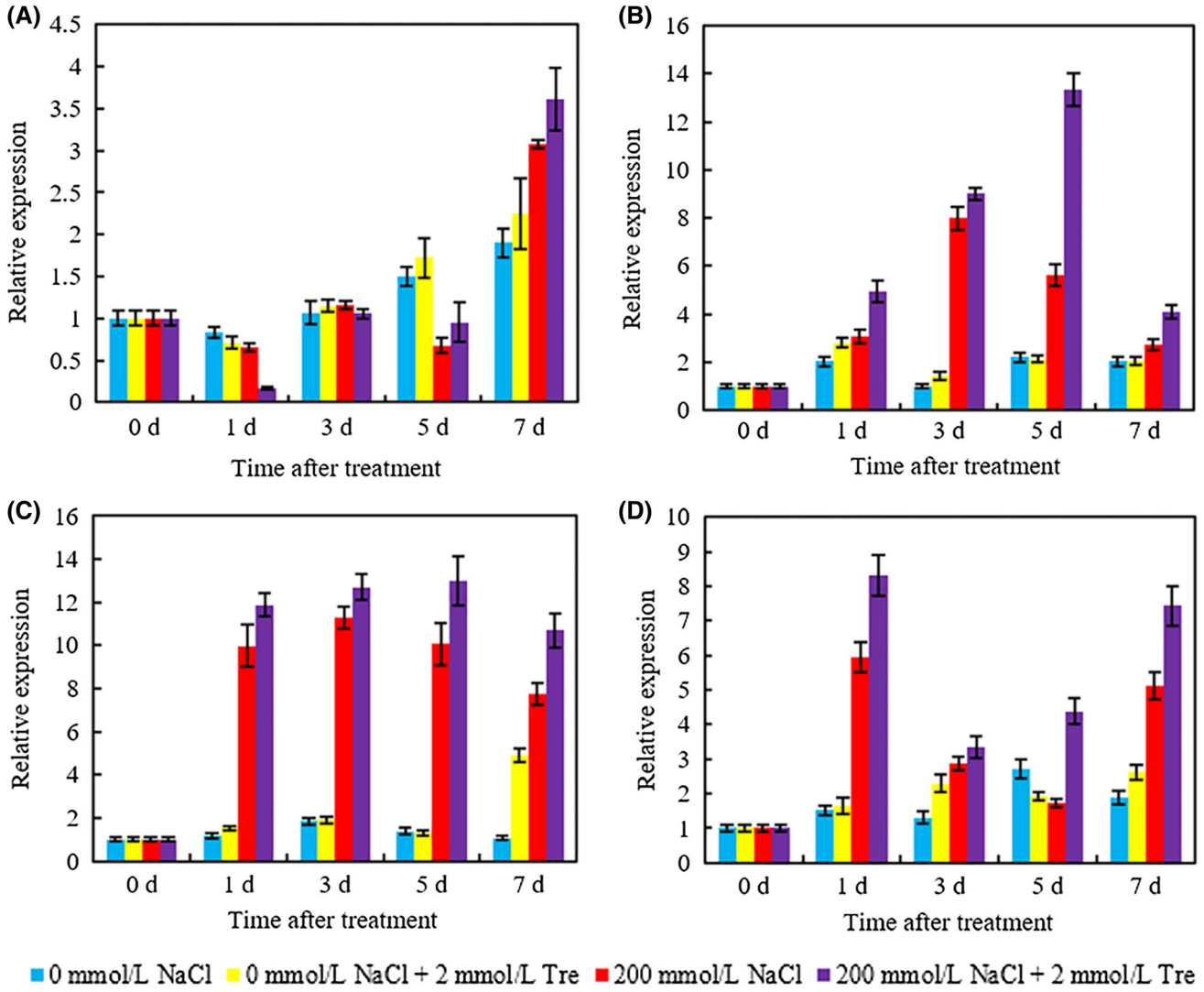

Fig. 14 Effect of exogenous trehalose on the expression of *FRK2* and *PFK* in tomato seedlings under salt stress. **A** *FRK2* in roots, **B** *FRK2* in leaves, **C** *PFK* in roots, and **D** *PFK* in leaves

Sucrose constitutes the highest proportion of sugars in plants and is directly related to plant stress resistance. The sucrose phosphate synthase (*SPS*) gene, the sucrose synthase (*SUS3*) gene, and the acid invertase (*Wiv-1*) gene are important in the sucrose metabolism pathway.

Figure 12 shows that *SPS* expression increased with the addition of exogenous trehalose under salt stress. Especially in roots, *SPS* expression was 14 times higher than that in the control group after 1 day of treatment, indicating that the effect of exogenous trehalose on roots was more obvious, which was consistent with the theory that roots are the most sensitive organs under salt stress. Sucrose synthase not only hydrolyzes sucrose but also synthesizes sucrose, regulating a reversible reaction. In roots, the expression of *SUS3* was not significantly different from that in the control group and tended to be stable. In leaves, *SUS3* expression decreased initially and then increased compared with that in the control group.

The enzyme acid invertase facilitates the hydrolysis of sucrose into glucose and fructose, which can significantly increase the cell osmotic potential. Salt treatment induced the expression of *Wiv-1* in roots and leaves. Especially on the 3rd day, *Wiv-1* expression reached the peak with salt + trehalose treatment, which was upregulated by 7.26 times and 10.68 times, respectively, indicating that *Wiv-1* may play an essential role in salt stress, while exogenous trehalose treatment significantly stimulated its expression.

The trehalose phosphate synthase (*TPS*) gene, the trehalose phosphatase (*TPP*) gene, and the trehalose (*Tre*) gene play an important role in the synthesis and metabolism of trehalose. As shown in Fig. 13, salt stress induced the expression of the *TPS* gene in roots and leaves, and the

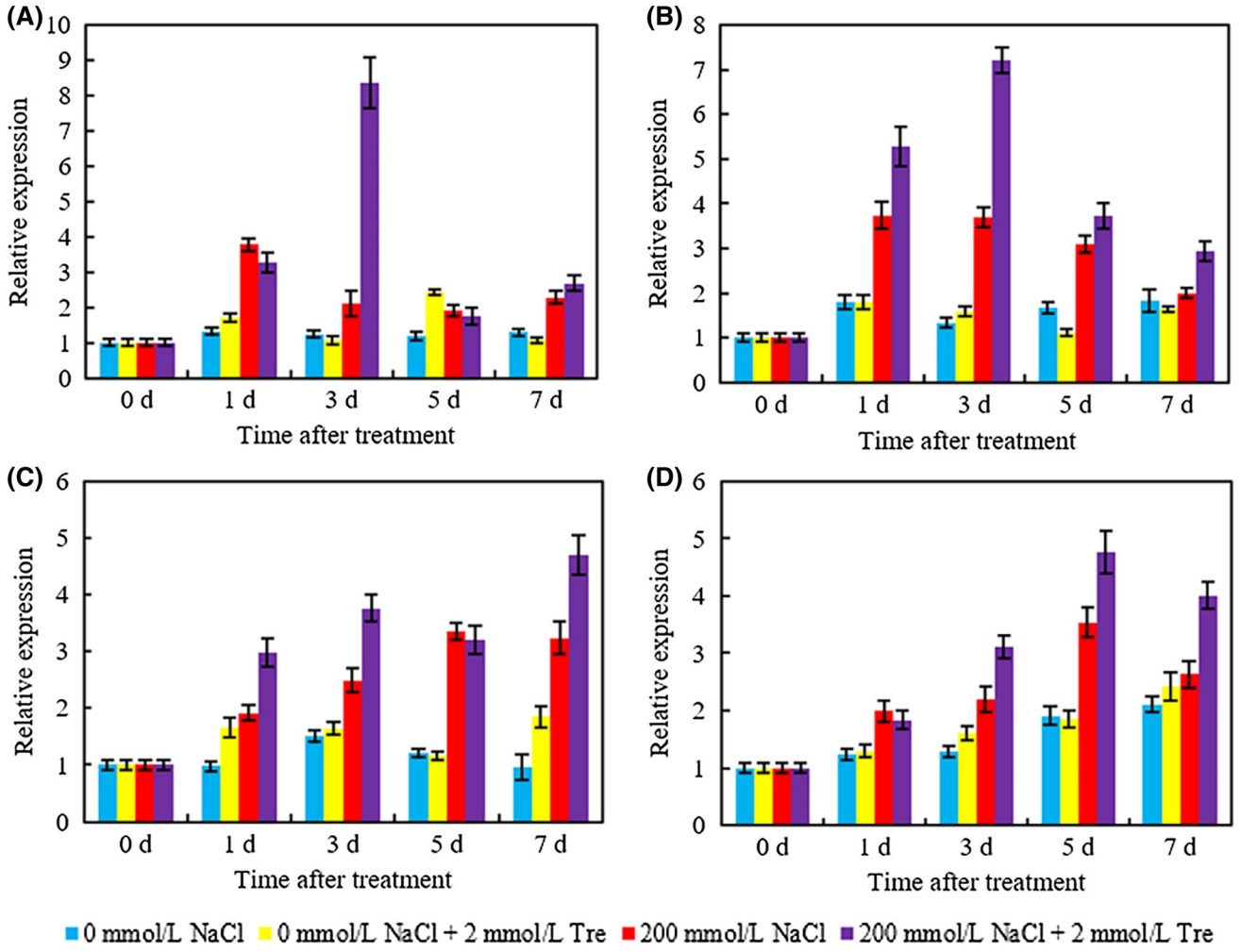

Fig. 15 Effect of exogenous trehalose on the expression of *HXK2* and *HXK3* in tomato seedlings under salt stress. **A** *HXK2* in roots, **B** *HXK2* in leaves, **C** *HXK3* in roots, and **D** *HXK3* in leaves

upregulation was higher with salt + trehalose treatment, which was consistent with the trend of trehalose content in Fig. 9. The expression of *TPP* was significantly upregulated under salt stress, reaching peaks on the 3rd and 1st day in roots and leaves, respectively. Exogenous trehalose treatment inhibited the expression of this gene, which may be caused due to the negative feedback regulation. The expression of *Tre* in roots and leaves increased initially and then decreased and was enhanced by the addition of trehalose and yielded a peak after 1 day.

Effects of Exogenous Trehalose on Sugar Signal Transduction Genes and Sugar Transporter Genes of Tomato Seedlings Under Salt Stress

Sucrose is converted into fructose under the action of sucrose synthase and hydrolase, and fructose enters the glycolysis cycle under the action of fructose phosphokinase and phosphokinase, participating in plant stress response. Under salt stress, the expression of fructokinase (*FRK2*) gene decreased initially and then increased in roots, whereas salt + trehalose treatment significantly inhibited its expression, which began to be upregulated on the 7th day (Fig. 14). In leaves, the expression of *FRK2* increased gradually under salt + trehalose treatment, reaching its peak on the 5th day.

As shown in Fig. 14C, D, salt stress significantly induced the expression of the phosphate fructokinase (*PFK*) gene, indicating that *PFK* may play an important role in response to salt stress. Moreover, salt + trehalose treatment further increased *PFK* expression in roots, which was upregulated by 9.3 times compared with that in the control group on the 1st day. *PFK* expression in tomato leaves was lower than that in roots but significantly upregulated.

Sucrose and hexose signal receptors are the major sugar signal receptors in plants. We selected two hexokinase genes, *HXK2* and *HXK3*, for this study (Fig. 15). Both genes

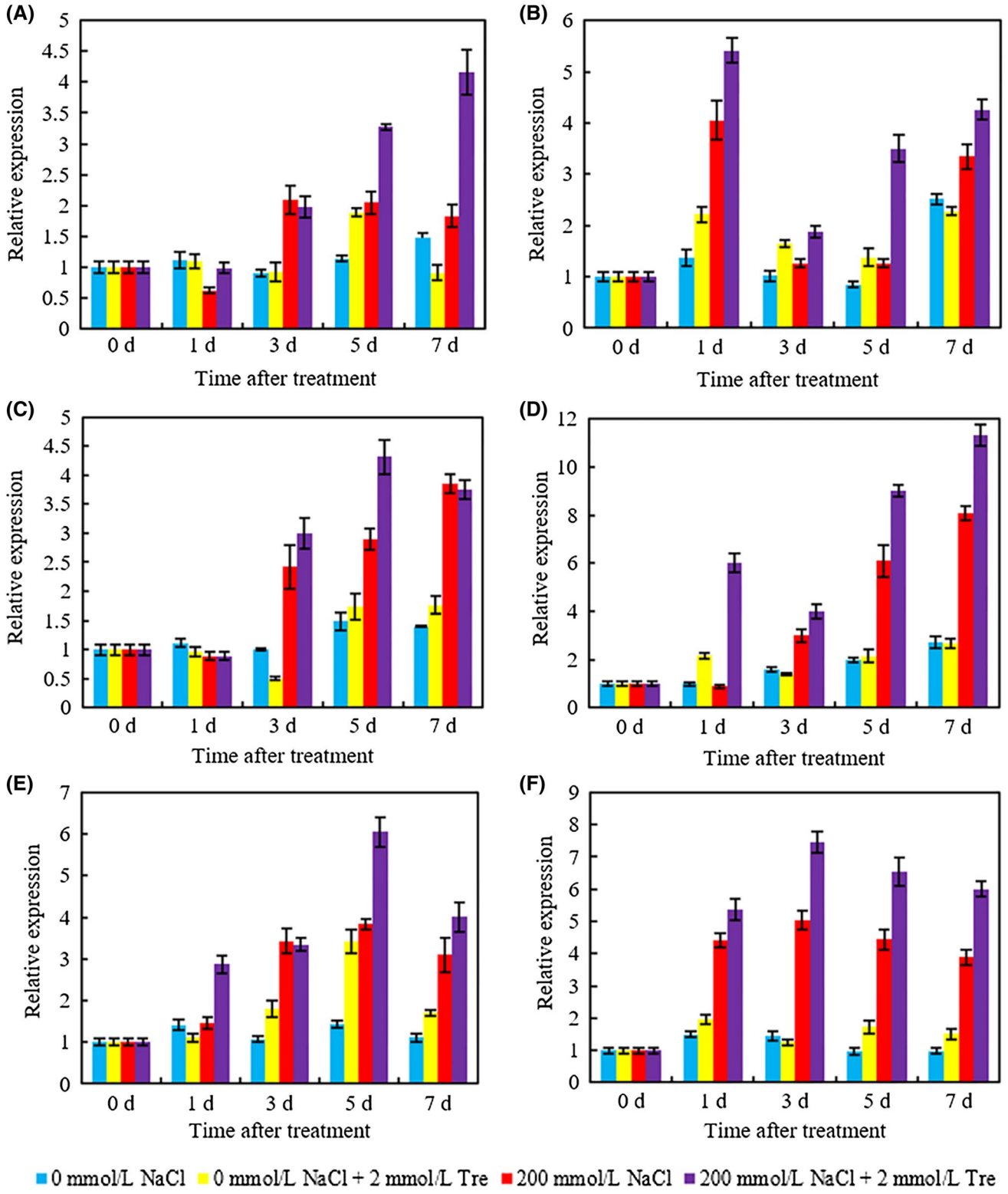

Fig. 16 Effect of exogenous trehalose on the expression of *MST3*, *SUT1*, and *SUT4* in tomato seedlings under salt stress. **A** *MST3* in roots, **B** *MST3* in leaves, **C** *SUT1* in roots, **D** *SUT1* in leaves, **E** *SUT4* in roots, and **F** *SUT4* in leaves

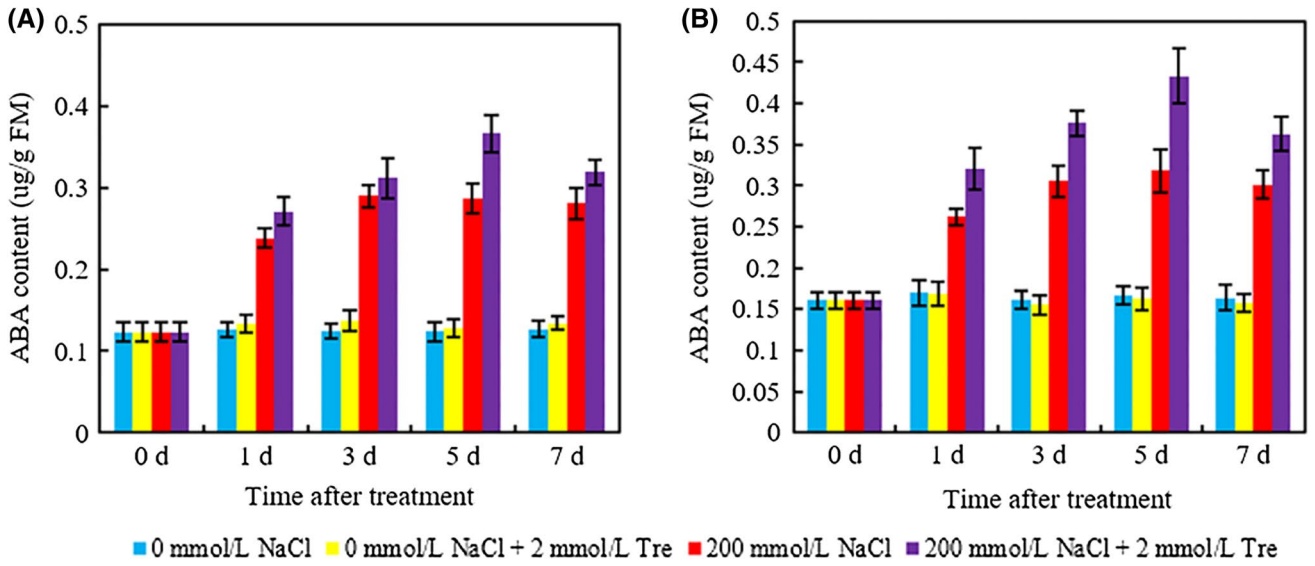

Fig. 17 Effect of exogenous trehalose on the ABA content in tomato seedlings under salt stress. **A** Roots and **B** leaves

were upregulated to varying degrees under salt stress, and exogenous trehalose was more conducive to the expression of hexokinase gene. Under salt + trehalose treatment, the expression of *HXK2* increased initially and then decreased, reaching its peak on the 3rd day. *HXK3* expression was upregulated with both salt treatment and salt + trehalose treatment.

The transport and distribution of sugar directly affect the normal growth and development of plants and also play an important role in plant stress. We analyzed the changes in the expression of the monosaccharide transporter (*MST3*) gene and the sucrose transporter genes (*SUT1* and *SUT4*) under salt stress. As shown in Fig. 16, the expression of *MST3* in roots was upregulated with salt treatment and significantly upregulated with salt + trehalose treatment. *MST3* expression in leaves was also induced by salt stress and reached the peak on the 1st day after salt + trehalose treatment. These results showed that both *SUT1* and *SUT4* were upregulated with salt + trehalose treatment, suggesting that these genes may be involved in the response to salt stress.

Effects of Exogenous Trehalose on ABA Synthesis and Metabolism of Tomato Seedlings Under Salt Stress

Under stress conditions, there is an increase in the ABA content in plants to resist external stress. As shown in Fig. 17, the ABA content increased significantly under salt stress, indicating that ABA was a stress-related hormone. After salt treatment, exogenous trehalose significantly increased the ABA content in tomato roots and leaves, reaching their peaks on the 5th day.

NCED1 and *NCED2* are key genes in the ABA synthesis pathway. As shown in Fig. 18, *NCED1* and *NCED2* in tomato roots and leaves were upregulated under salt treatment, especially when exogenous trehalose was added, indicating that exogenous trehalose had a significant positive effect on ABA synthesis.

CYP707A1 and *CYP707A2* are important genes in the ABA metabolic pathway. Figure 19 shows that the expression of *CYP707A1* was upregulated under salt stress, especially in roots. When treated for 3 days, *CYP707A1* expression was 1.62 times that of the control group. However, the addition of exogenous trehalose reduced the upregulated expression of *CYP707A1* under salt stress. *CYP707A2* expression was decreased, and exogenous trehalose further reduced its expression.

Discussion

Salt treatment inhibited the growth of tomato seedlings. After the application of trehalose, the plant damage was alleviated, whereas high concentrations of trehalose inhibited the growth of tomato seedlings. Although trehalose can provide a source of carbon for tomato, due to the inability of tomato to utilize carbon source under salt stress, ionic stress is aggravated, which reduces photosynthesis and affects tomato growth.

ROS accumulation increases significantly under salt stress, which may lead to cell membrane damage, oxidative stress, or cell death [32, 33]. In this study, we

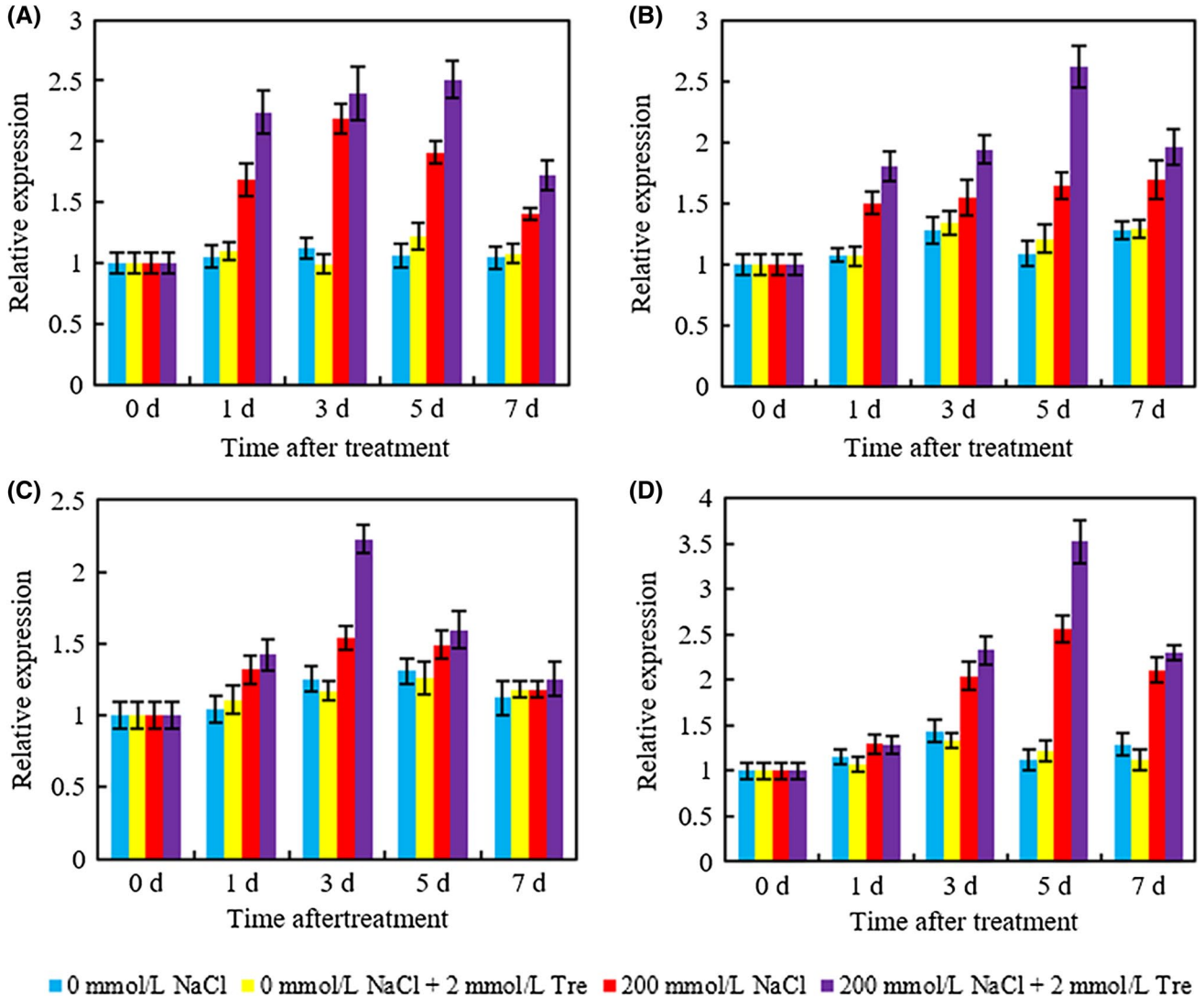

Fig. 18 Effect of exogenous trehalose on the expression of *NCED1* and *NCED2* in tomato seedlings under salt stress. **A** *NCED1* in roots, **B** *NCED1* in leaves, **C** *NCED2* in roots, and **D** *NCED2* in leaves

demonstrated that trehalose exerted the effects of enhancing the activities of antioxidant enzymes, increasing the proline content, and decreasing the MDA content, therefore alleviating the damage caused due to salt stress.

Under salt stress, starch accumulated in tomato roots and leaves, leading to a negative feedback mechanism of photosynthesis, which reduced the photosynthetic rate, decreased the chlorophyll content, and hindered starch transport. Exogenous trehalose treatment inhibited the expression of the starch synthesis genes *SS* and *AGPase* and promoted the expression of the starch metabolism genes *BAM* and *AMY*, thereby reducing the starch content, alleviating the negative feedback mechanism, and facilitating the accumulation of photoassimilation products.

Soluble sugars are primarily used as osmotic agents to prevent plant cells from dehydration and death and to maintain the internal stability of the cells under salt stress [34, 35]. When treated with salt, the content of various soluble sugars in different tissues of tomato seedlings increased significantly, while exogenous trehalose further increased their contents, especially that of sucrose. As an important osmotic regulator, sucrose can significantly increase the osmotic potential of plant cells and alleviate ionic stress. In addition, sucrose can eliminate ROS in plants. Exogenous trehalose administration significantly induced the expression of the *SPS* under salt stress and increased the sucrose content in tomato. The expression of the sucrose synthase gene *SUS3* increased initially and then decreased, whereas that of the acid invertase gene *Wiv-1* increased, which maintained a relative stability of sucrose content under salt stress.

The expression of the *TPS* in tomato was upregulated under salt stress, which was consistent with the findings of

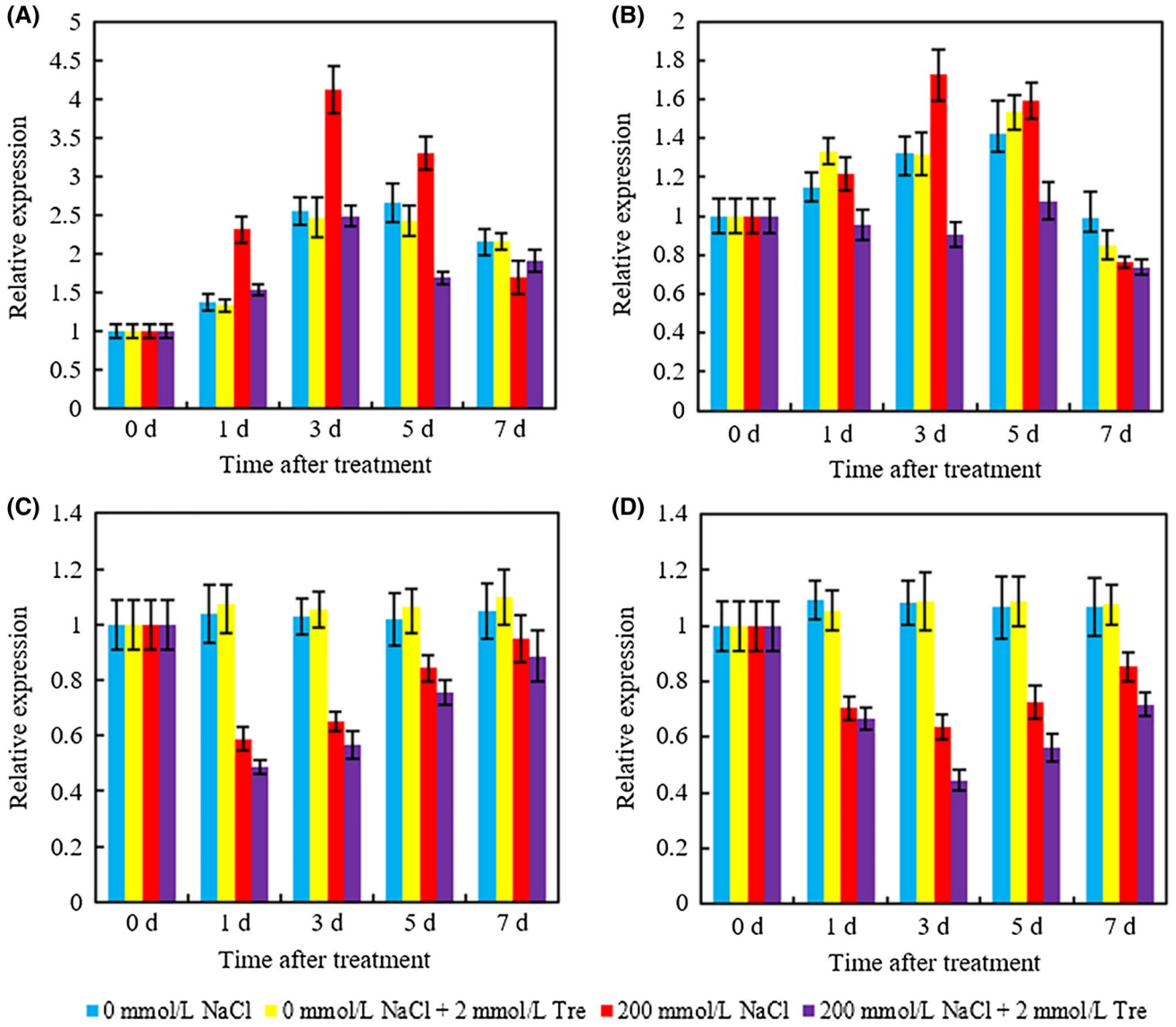

Fig. 19 Effect of exogenous trehalose on the expression of *CYP707A1* and *CYP707A2* in tomato seedlings under salt stress. **A** *CYP707A1* in roots, **B** *CYP707A1* in leaves, **C** *CYP707A2* in roots, and **D** *CYP707A2* in leaves

Li et al. [36] who showed that overexpression of *OsTPS1* enhanced the salt tolerance of rice and exogenous trehalose treatment further increased *TPS* expression. In our experiment, exogenous trehalose administration increased the content of endogenous trehalose in tomato, resulted in a negative feedback regulation, inhibited the expression of the *TPP*, enhanced the expression of trehalose gene, and promoted the conversion of excessive trehalose into glucose to maintain the stability of its content. *TPS*, *TPP*, and *Tre* exhibited differential expressions under salt stress, suggesting that the trehalose metabolic pathway could directly affect the salt tolerance of plants.

Fructose phosphokinase and fructokinase are two important enzymes in the plant glycolytic pathway, which can maintain the normal physiological function of plants and alleviate the physiological damage occurring in plants under stress [37]. The results of this study showed that the addition of exogenous trehalose could significantly upregulate the expressions of *FRK* and *PFK* under salt stress, suggesting that it could help plants accumulate sugars and maintain strong respiration. Sugar not only is the primary energy source for plant growth, but also can be used as a signal molecule to respond to plant stress. *HXK* is a sugar receptor that is affected by the external environment and forms a network signal system with plant hormones such as ethylene, ABA,

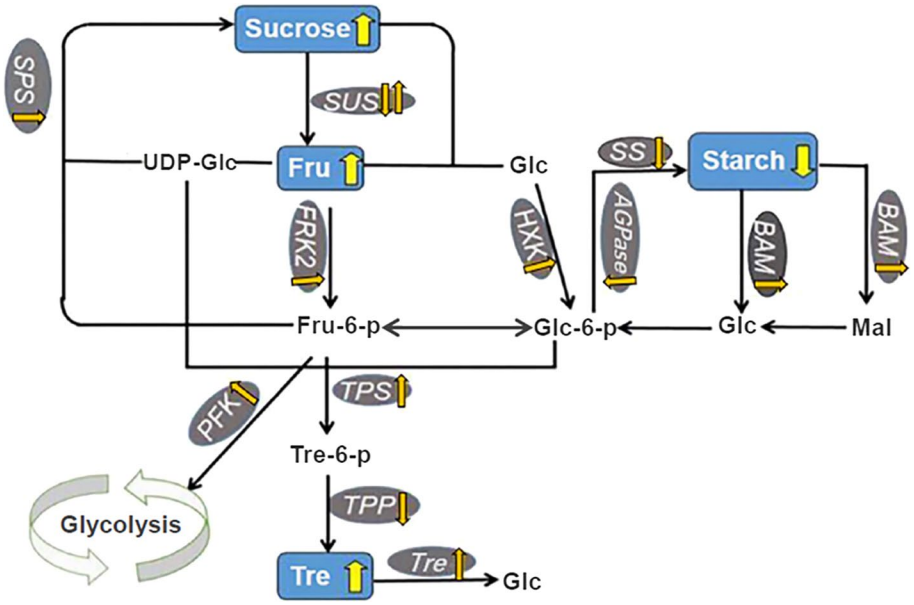

Fig. 20 Changes in glucose metabolism in tomato seedlings under salt stress by exogenous trehalose

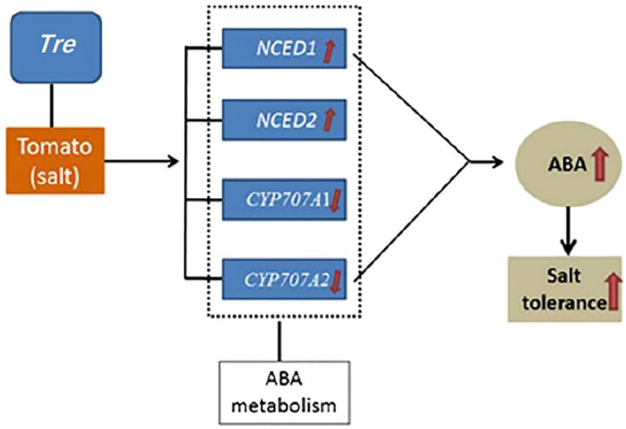

Fig. 21 Changes in ABA metabolism in tomato seedlings under salt stress by exogenous trehalose

and gibberellin. Exogenous trehalose treatment induced the upregulation of *HXK2* and *HXK3* to different degrees under salt stress. Sami et al. [38] reported that sugar and ABA interacted in the metabolic pathways of plants to influence drought and salt resistance.

Salt stress affects the activities of sugar transporters on plant membranes, which results in the abnormal transport of sugar and the inability of plants to obtain energy [39]. The sugar transporter genes exhibited different expression trends during osmotic stress, suggesting that the source–sink relationship of carbon may be related to the regulation of sugar transporter gene expression [40]. In addition, *AtSUC4* could regulate sucrose distribution and metabolism in response to drought stress [41]. The results of this study demonstrated that the expressions of the monosaccharide transporter (*MST3*) gene and the sucrose transporter genes *SUT1* and *SUT4* were significantly upregulated with salt + trehalose treatment. Trehalose may change the source–sink transport pathway of sugars, thereby changing the accumulation and distribution of sugars to respond to salt stress.

The entire process of plant growth and development is affected by the interaction between sugar and ABA. In this experiment, the ABA content of tomato seedlings accumulated significantly under salt stress, which was consistent with the results of Kong et al. [42] and Shu et al. [43], indicating that ABA directly responded to plant salt stress. Salt + trehalose treatment resulted in the upregulation of the ABA synthesis-related genes and the downregulation of metabolic genes, which indicated that the tomato plant increased its ABA content by accelerating ABA synthesis and reducing ABA decomposition. Saeedipour [44] found that differences in salt stress tolerance of rice plant were related to its ABA synthesis ability under stress conditions. Tomatoes overexpressing *NCED* accumulated more ABA and showed improved drought resistance. ABA primarily improves plant stress resistance by closing the stomata of plant leaves, reducing transpiration, and maintaining water in plants [45]. Therefore, trehalose acts as a signaling molecule by affecting the ABA anabolic pathway, leading to ABA accumulation and further improving the salt tolerance of tomato.

In summary, trehalose, a soluble, non-reducing disaccharide, is present in a low amount in plants; however, it regulates sugar accumulation and distribution by affecting the activities of sugar transporters and simultaneously regulates sugar metabolism and ABA metabolism, thereby significantly improving plant stress resistance (Figs. 20, 21). This study provides a greater understanding of the salt-tolerant mechanism of plants and has important value

for improving crop yield, salt tolerance of plants, and utilization of saline–alkali land in agricultural research.

Future research will continue to explore the effect of trehalose on tomato salt stress using transgenic technology to alter the expression of genes related to trehalose metabolism, which could help in further studying the salt tolerance mechanism of trehalose.

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