Strigolactone-Mediated Trehalose Enhances Salt Resistance in Tomato Seedlings

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Abstract: Strigolactones (SLs) are newly discovered plant hormones that modulate a variety of physiological and biochemical processes and plant stress responses. In this study, SLs’ synthetic analog, GR24, significantly improved the growth of tomato seedlings under salt stress, while SLs’ synthesis inhibitor, TIS108, inversed the positive role of SLs, indicating that SLs could effectively enhance salt-stress resistance in tomato. To further explore the mechanism of SL-modulated trehalose (Tre) in response to salt stress, Tre metabolism was analyzed during this process. GR24 increased the endogenous Tre and starch contents and decreased the glucose (Glu) level under salt-stress conditions. Additionally, the TPS and TPP activities were enhanced by GR24 and the activity of THL was inhibited by GR24 under salt stress; thereafter, Tre biosynthesis-related genes, including TPS1, TPS2, TPP1, and TPP2, were also upregulated by GR24 under salt stress. However, the function of GR24 in Tre metabolism was inhibited by TIS108. Thus, the results indicated that GR24 improved the expression levels or activities of Tre biosynthesis-related genes or enzymes and inhibited the transcript level or activity of genes or enzymes related to Tre degradation, respectively, resulting in an increase in the endogenous Tre level and, therefore, weakening the salt toxicity of tomato seedlings.

Keywords: strigolactone; trehalose; salt; regulator pathway; genes expression

1. Introduction

Plant growth usually faces many kinds of environmental stresses. By 2050, it is expected that 50% of cultivable land will be lost [1]. Plants are sensitive to environmental stresses, such as salt stress. There are many reasons for the increase in soil salinity, including natural occurrences, climate change, the rise in the sea level, drought evaporation, and different agricultural irrigation methods [2]. An increasing number of studies have reported that salt stress influences plant growth and development. For instance, Hasanuzzaman et al. [3] and Ahmad et al. [4] concluded that salt stress could reduce root/shoot weight, branch length, plant height, branching, leaf number, and early vegetative development. Salt stress also interferes with photosynthetic activity and respiration, reduced enzyme activity, sugar metabolism, protein and nucleic acid synthesis, impaired ion homeostasis, and osmotic and hormonal balance [5,6].

As a general rule, different crops have different ranges of adaptation to the salt content of soil. Previous research showed that NaCl was harmful to seed germination and seedling growth in the range of 0–150 mM [7]. For instance, Hu et al. [8] determined the effect of the methylation of N6-methyladenosine mRNA in Arabidopsis on its salt tolerance under 100 and 150 mM of NaCl. Therefore, the enhancement of salt tolerance in plants is a top priority in agricultural research.

Strigolactones (SLs) were first found in the root exudates of cotton [9]. Subsequently, SLs, as an important signaling molecule, were described as germination stimulants of witchweed seeds [9,10]. SLs could stimulate the spore germination, hyphal branching,
and metabolic activity of beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF) [11], in establishing a symbiotic relationship between plants and AMF, which plays an important role in adapting to stress [12]. Marro et al. [13] reported that SLs protect plants from damage to plant parasitic nematodes by enhancing mycorrhizal colonization.

Recently, SLs have been recognized as new types of plant hormones that play a regulatory role in various plants growth and development, including seedling growth [14], leaf senescence [15], photo morphogenesis [16], and the shaping of root system architecture [17]. SLs play pivotal roles in response to diverse abiotic and biotic stresses [18]. For instance, exogenous GR24, as a positive regulator, could enhance the resistance to low-light, drought, and salt in Arabidopsis [19–21]. In addition, the exogenous application of SLs was effective in reducing the negative influence of salt stress in sunflowers [18].

Sugars are a source of carbon units and metabolic energy, and act as signal molecules to report the carbon status in cells [22,23]. Trehalose (Tre) is a nonreducing disaccharide; its synthesis pathway requires two stages and it is highly conserved in some plants, including tomato [24,25]. Trehalose-6-phosphate synthetase (TPS) concentrates uridine diphosphate glucose (UDPG) and glucose 6-phosphate (G6P) to produce trehalose-6-phosphate (T6P). Trehalose-6-phosphate phosphatase (TPP) dephosphorylates T6P to form Tre. The upregulation of TPS/TPP genes increase Tre levels, thereby promoting growth and development in plant, including seed germination, root growth, and flowering [26,27].

In contrast to Tre synthesis, the trehalase THL directly decomposes Tre to produce two molecules of glucose (Glu). Furthermore, T6P hydrolase (TPHase) hydrolyzes T6P to produce G6P and Glu. The THL hydrolases include neutral trehalase (NTH) and acidic trehalase (ATH) [28,29]. The Tre pathway has close contact with abiotic stress resistance [30–32]. In plants, Tre could be produced under extreme environments, including salt-stress, drought-stress, and cold-stress environments [33]. The pre-sowing Tre treatment of seeds could promote the normal growth of radish plants under drought stress, which is associated with significant increases in chlorophyll A content, total soluble sugars, water-use efficiency, photosynthesis, free proline content and the activity of the SOD enzyme [34]. Additionally, the overexpression of the OsTRE1 gene helps rice to adapt to salt stress [35].

The planting area of tomato (Solanum lycopersicum L.) is huge, and tomato is a very popular cash crop throughout the world. However, abiotic stresses seriously reduce the productivity and quality of tomato. Previous research has shown that SLs and Tre could promote the growth and development of plants under salt stress. However, we have no clear understanding of the interplay between SLs and Tre under the influence of abiotic stresses.

As a result, we carried out the current experiment and hypothesized that crosstalk between SLs and Tre is likely to play an important role in improving the growth of tomato seedlings under salt stress. This research may provide an important foundation for future studies of the interaction between SLs and Tre in plants under stress.

2. Materials and Methods

2.1. Plant Materials

In this study, the tomato “Micro-Tom” (Nanjing Ebiosci Biological Technology Co., Ltd., Nanjing, China) was selected as the plant material. The seeds were placed in a conical flask containing water, and then put in a Shaking Incubator to germinate for 3 days. The conditions for seed germination were 180 rpm at 28 °C, in darkness. The germinated seeds were planted. The four-leaf seedlings were transferred to conical flasks containing 1/2 Hogland solution to grown for 21 days. The growth conditions of the tomato seedlings were as follows: a cycle of 14 h in light at 250 μmol m⁻² s⁻¹, photon irradiance at 25 °C and 10 h in darkness at 20 °C, with 70% relative humidity.

2.2. Treatments

Previous studies showed that salt stress stimulated by NaCl significantly inhibits the growth of tomato seedlings, especially with increased concentrations of NaCl. To study the effect of NaCl on the seedlings of tomato, NaCl concentrations were set to 150 mM
NaCl [36,37]. Tomato seedlings with similar growth were selected and treated with NaCl (150 mM), NaCl + SLs synthetic analog GR24 (15 μM), and NaCl + SLs synthesis inhibitor TIS108 (3 μM) in Hogland solution under salt stress. The tomato seedlings were immersed in different treatments for 7 days, and the Hogland solution served as the control. The concentrations of GR24 and TIS108 were selected by referring to [37] and [38]. After 7 days of treatment, the samples were frozen in liquid nitrogen, then stored quickly at −80 °C for future use.

2.3. Measurement of Morphological Indexes

The distance from the stem base to the growth point of a plant is defined as the plant height. Plant heights and root lengths were measured by a vernier caliper. The phenotype was captured by a Canon camera (EOS 700D).

2.4. Determination of Tre, Starch and Glu Content

According to the manufacturer’s instructions, as provided in the Tre Content Detection Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China, BC0330), the content of Tre was determined. The specific steps were as follows:

1. The foil bag was equilibrated at room temperature for 20 min; the required strip was sealed immediately and the remaining strips were placed in self-sealing bags at 4 °C.
2. The standard wells and the sample well were set up. Fifty μL of the standard liquid of each concentration was added into the standard wells, and one standard well corresponded to one concentration.
3. Ten μL of sample and 40 μL of sample solvent were added into the sample well, successively. No additions were made to the blank well.
4. Apart from the blank well, 100 μL horseradish peroxidase (HRP)-labeled detection antibody was added to all standard wells and to the sample well, respectively, and the reaction well was sealed with a sealing film. The reaction well was incubated in a 37 °C water bath or incubator for 60 min.
5. The liquid was discarded, and the board was pat dried on absorbent paper, and each microplate well was filled with washing reagent and allowed to stand for 1 min. The washing reagent was discarded, and the board was pat dried on absorbent paper, the board was washed 5 times.
6. In each microplate well, 50 μL of substrate A and substrate B were added and incubated at 37 °C in the dark for 15 min.
7. The 50 μL termination solution was added into each microplate well, and the OD value of each microplate well was measured at 450 nm within 15 min. The Tre content was calculated in the sample, based on the standard curve.

The content of starch was determined using iodine colorimetry [39]. An 0.2 g sample was taken and ground in 2 mL of distilled water, and the seed powder was mixed with 3.2 mL of 60% perchloric acid for approximately 10 min. After centrifugation at room temperature, 5000 × g for 5 min, the supernatant was extracted into a 10 mL tube, and the volume was fixed with distilled water. The absorbance value was determined at 660 nm. The assay method of Glu was modified slightly, according to study of Saqib et al. [40].

The 3,5-dinitrosalicylic acid (DNS) reagent was prepared as follows: first, 3,5-dinitrosalicylic acid (6.3 g) was added in 2 M of sodium hydroxide solution (262 mL), and the mixture was stirred until completely dissolved (liquid A). Next, potassium sodium tartrate (185 g) was dissolved into 500 mL hot water (liquid B). Liquid A and liquid B were mixed, phenol and sodium sulfite (5 g each) were added and refrigerated to room temperature, and the volume was adjusted to exactly 1000 mL with distilled water. Then, 0.5 g samples were accurately weighed, ground into homogenate with 10 mL distilled water, and diluted in a 50 mL volumetric flask and mixed. After shaking well, the samples were put in a 50 °C water bath for about 10 min and refrigerated to room temperature. Then, they were centrifuged at 4 °C, 4000 × g, for about 15 min. Two mL of supernatant was mixed with 1.5 mL DNS. Subsequently, the samples were placed into a boiling water bath for about 5 min and refrigerated to room temperature.
Then, miscible liquids were filtered into a 25 mL volumetric bottle. The absorbance value was measured at 540 nm.

2.5. Determination of TPP and TPS Activities

According to the manufacturer’s instructions, as set out in the ELISA kit (Yuanmu Biotechnology, Shanghai, China), the activities of TPP and TPS were determined [37]. The extraction steps of the enzyme were performed as follows: samples (0.5 g) were ground quickly with liquid nitrogen and transferred to a test tube. PBS (9 mL, 0.01 M, pH 7.4) was added to prepare the homogenate and collected into test tubes. The homogenate was centrifuged at 4 °C, 4000× g, for 15 min, and the supernatant was collected for detection. Then, the enzyme activity was determined according to the following steps.

In the plate well, the 10 µL enzyme solution and 50 µL of HRP-conjugate reagent were added, and antibodies were incubated at 37 °C for about 30 min. The board was washed in the dark, then 100 µL of chromogen solution was added and incubated into the board at 37 °C for 15 min. The absorbance was monitored at 450 nm. According to the manufacturer’s instructions for THL, as set out in Activities Detection Kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China, BC2510), the activity of THL was determined.

2.6. Real-Time RT-PCR Analysis

The tomato leaves (0.5 g) were ground in liquid nitrogen in pre-cooled mortar and collected in a centrifuge tube, according to the study of Huang et al. [41]. TRIzol (1 mL) was added to the centrifuge tube and incubated at 4 °C for about 10 min. The 200 µL chloroform was added, incubated for 5 min, and centrifuged at 4 °C, 12,000× g, for about 15 min. The supernatant was mixed with an equal volume of isopropanol, which was incubated at −20 °C for about 1 h. Then, the supernatant was washed twice with 75% ethanol and collected into the adsorption column. Finally, RNA was dissolved with ddH2O, which did not contain RNase. The collected total RNA was transformed into single-stranded cDNA, as recommended by the manufacturer. RNA (500 ng) that originated from various treatments was applied in the synthesis of first-strand cDNA, which happened in 10 µL reactions having 2 µL of AMV reverse transcriptase XL (AG, China) and 2.5 µM of random primer. The reaction system was as follows: 10 mL 2 × SuperReal PreMix Plus, 0.6 mL of 10 mM forward primers, 0.6 mL of 10 mM reverse primers, 2 mL of cDNA, and 6.8 mL of RNase-free ddH2O.

According to the manufacturer’s instructions, as set out in the ABI Step One Plus system (Applied Biosystems, Carlsbad, CA, USA) and SYBR® Premix Ex Taq™ II (AG, Shanghai, China) were used to reverse transcribe the RNA. Then, gene-specific primers were applied, as shown in Table 1. The PCR cycling conditions were as follows: 95 °C for 15 min and 40 cycles of 95 °C for 10 s and 60 °C for 20 s. The actin transcript level was normalized and the relative expression level was expressed as the value of the relative expression level relative to the line in level with the control sample at a specified time. The expression level of the gene was calculated by $2^{-\Delta\Delta CT}$. Briefly, $\Delta CT = CT$ (target gene) – $CT$ (internal reference gene). $\Delta\Delta CT = \Delta CT$ (test group) – $\Delta CT$ (control group). Each sample was set to three biological replicates.

2.7. Statistical Analysis

All data were determined in three independent biological replicates for each experiment. Data statistical analysis was carried out via SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). We adopted Duncan’s ($p < 0.05$) method for significance analysis. The boxplots were established by Origin 2021 software.
Table 1. Primer sequence for qRT-PCR.

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene ID</th>
<th>Primer Sequence (5’–3’)</th>
</tr>
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<tbody>
<tr>
<td>TPS1</td>
<td>LOC100135703</td>
<td>GGGCAGAAACGAGTGATGCTGTAAG CGACAGAGCGAGATGGAGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCAAGGAAACGAGAGACTTGAGTGC CCAAAGAGGTGATGCTGTC</td>
</tr>
<tr>
<td>TPS2</td>
<td>LOC101250326</td>
<td>ACTCGAAACCACCAAACATGCTCTC</td>
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<tr>
<td></td>
<td></td>
<td>ATCTCAGACCAGGTTACTCAG AATGAATTCGTCGTCAGAG</td>
</tr>
<tr>
<td>TPP1</td>
<td>LOC101259279</td>
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<tr>
<td></td>
<td></td>
<td>TGTTTGTACGGTGCTGTCGTC AATGAATTCGTCGTCAGAG</td>
</tr>
<tr>
<td>TPP2</td>
<td>LOC101245612</td>
<td>ACTCTCAGCACCAGTACTCAG AATGAATTCGTCGTCAGAG</td>
</tr>
<tr>
<td>Actin</td>
<td>NC_015447</td>
<td>AATGAACTTCGTTTCAGGTTTC</td>
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3. Results

3.1. Effect of GR24 and TIS108 on Tomato Seedling Growth under Salt Stress

Compared with the control, the NaCl treatment significantly reduced root length and plant height by 23.96% and 11.36%, respectively. However, NaCl + GR24 treatment significantly increased root length and plant height. The TIS108 treatment reversed the positive effect of GR24 (Figure 1a,b). Figure 1c shows a perfect match with the changes in tomato phenotypes revealed by the above results. The results suggest that the application of SLs could effectively promote the growth of tomato seedlings under salt stress.

![Figure 1](image-url)

Figure 1. Effects of GR24 and TIS108 on plant height, root length, and phenotype in tomato seedlings under salt stress. (a) Plant height; (b) maximum root length; (c) phenotype. Every value stands for the average ± standard error (SE). At the same time period, different letters express notable differences among different treatments ($p < 0.05$). GR24: SLs’ synthetic analog; TIS108: SLs’ synthesis inhibitor.
3.2. SLs Were Involved in the Conversion among Tre, Starch, and Glu

As shown in Figure 2a, in comparison to the control, the NaCl treatment significantly reduced the Tre content, by 6.80%. However, the Tre content was raised under NaCl + GR24 treatment, even exceeding 9.55% of the control. The Tre content in the TIS108 treatment was 17.26% lower than the content in the NaCl treatment (Figure 2a). Similarly, the changes in the starch were in full agreement with the Tre levels mentioned above, under different treatments (Figure 2b). The Glu content was increased under salt stress. However, the increase was inhibited in the application of the GR24. NaCl + TIS108 treatment led to a marked increase in Glu content, by 1.75%, compared to the NaCl treatment alone (Figure 2c). These results imply that the addition of exogenous SLs promotes starch condensation to synthesize Tre and inhibits Tre being degraded to Glu, thereby protecting tomato plants from salt stress.

Figure 2. The GR24 and TIS108 affect the content of Tre (a) starch (b) and Glu (c) in tomato seedlings under salt stress. Every value stands for the average ± standard error (SE). At the same time period, different letters express notable differences among different treatments (p < 0.05). GR24: SLs’ synthetic analog; TIS108: SLs’ synthesis inhibitor.

3.3. Effect of SLs on TPS, TPP, and THL Activities under Salt Stress

As can be seen in Figure 3, the activities of TPS and TPP in the NaCl treatment were 38.17% and 42.70% lower than those in the control, but the activities of TPS and TPP increased significantly after the application of GR24. The application of TIS108 reduced the activities of TPS and TPP in NaCl-treated tomato leaves, which were about 55.64% and 87.61% of the activities with the NaCl treatment (Figure 3a,b). Salt stress resulted in a significant increase, of 83.64%, in THL activity, compared to that in the control (Figure 3c).
THL activity was reduced significantly in tomato seedlings treated with GR24 under salt stress. However, TIS108 enhanced the THL activity. This indicated that SLs play a role in controlling Tre content by upregulating its biosynthesis enzymes TPS and TPP and downregulating its degradation enzyme THL.

![Graphs showing TPS, TPP, and THL activities under different treatments](image)

**Figure 3.** GR24 and TIS108 affect TPS activity (a), TPP activity (b), and THL activity (c) in tomato seedlings under salt stress. Every value stands for the average ± standard error (SE). At the same time period, different letters express notable differences among different treatments (p < 0.05). GR24: SLs’ synthetic analog; TIS108: SLs’ synthesis inhibitor.

3.4. SLs Regulated the Transcriptional Level of TPS1, TPS2, TPP1, and TPP2 Genes under Salt Stress

After NaCl treatment, the expression level of TPS1 had no significant difference, compared to that of the control (Figure 4a). NaCl + GR24 treatment led to a significant upregulation in TPS1 expression, by 604.12%, as compared to that of the NaCl treatment. Treatment with NaCl + TIS108 markedly reduced the expression level of TPS1. The TPS2, TPP1, and TPP2 expressions showed very similar changes under different treatments. The expression levels of TPS2, TPP1, and TPP2 in the NaCl treatment were 80.36%, 76.77%, and 91.39% lower than those in the control, respectively. The expression levels of TPS2, TPP1, and TPP2 were significantly upregulated by the NaCl + GR24 treatment, compared to the NaCl treatment. However, the NaCl + TIS108 treatment significantly reduced the positive effects of TPS2, TPP1, and TPP2 under salt stress (Figure 4b–d). The expression of these genes contributes to Tre accumulation. Thus, we concluded that SLs enhanced salt-stress resistance in tomato seedlings by upregulating TPS1, TPS2, TPP1, and TPP2, leading to Tre accumulation.
Figure 4. GR24 and TIS108 affect the expression of TPS1 (a), TPS2 (b), TPP1 (c), and TPP2 (d) genes in tomato seedlings under salt stress. Every value stands for the average ± standard error (SE). At the same time period, different letters express notable differences among different treatments (p < 0.05). GR24: SLs’ synthetic analog; TIS108: SLs’ synthesis inhibitor.

4. Discussion

Salt stress is a prevalent phenomenon that restricts plant growth. Salt stress prevents the growth of the roots and leaves of tomato seedlings, restraining photosynthesis and transpiration, resulting in a reduction in crop yield and quality [42]. Previous research indicated that SLs could efficiently relieve the oxidative damage induced by salt stress in plants [43,44]. The role of SLs is very wide, including promoting stem elongation and internode length, increasing root hairs and nodules numbers, and inhibiting adventitious roots and bud outgrowth [45]. SLs also inhibit the polar transport of auxin in stems by downregulating the PIN1 gene, thus inhibiting branching [46]. The purpose of this study was to determine the relationship between SLs and Tre and the regulatory mechanisms of salt tolerance in tomato seedlings.

The height and root length of tomato seedlings treated with NaCl (150 mM) were significantly inhibited, compared to the control, while GR24 could relieve this inhibition (Figure 1). When the SLs’ synthesis inhibitor TIS108 was added, the root length and plant height of tomato seedlings were lower than they were with NaCl treatment. It was proved that SLs could improve the salt tolerance of tomato seedlings, restoring normal growth (Figure 1). The results of [47] were consistent with our study; they reported that GR24 relieved the inhibition of growth in ornamental sunflower, led by 150 mM NaCl via raising the transpiration ratio, the stomatal opening, and the photosynthetic rate. Ma et al. [48] found that GR24 improved the photosynthesis in Brassica napus L., promoting the growth
of shoots and roots under salt stress. Cheng et al. [46] reported that SLs stimulated the expression of ethylene (ET) biosynthetic genes, and that ET directly affected the root hair (RH) elongation by signal transduction. Furthermore, auxin efflux and polar auxin transport (PAT) affected auxin levels in RH cells, which mediated SLs’ promotion of root hair elongation [49] and helped plants to absorb nutrients and water from the soil [50], thereby promoting the development of roots. Moreover, brassinosteroids (BRs), ET, and auxin, as plant growth regulators (PGR), could modulate plant development and enhance stress resistance. BRs contribute to the development of lateral roots (LRs) and adventitious roots (ARs). Moreover, BRs crosstalk with SLs through NO signaling, thereby enhancing or reducing the formation of LRs and ARs [51]. In addition, salt stress inhibits the plant height, the stem diameter, the leaf area, and the root length of tomato, while the interaction of SLs and NO reverse this inhibition by enhancing photosynthesis and antioxidant capacity [37,52]. Hira et al. [53] observed that SLs can improve the morphological, physiological, and biochemical properties of sunflower under salt stress and enhance salt tolerance. The seed dormancy of Arabidopsis was broken, because SLs raised the GA level [46]. SLs increased the content of abscisic acid (ABA) in the bud to achieve the effect of inhibiting branch development [54]. Therefore, there is sufficient evidence to prove that SLs play a key role in regulating tomato adaptation to salt stress.

It is well-known that starch, Tre, and Glu are types of polysaccharide, disaccharide, and monosaccharide, respectively [55,56]. Under salt stress conditions, starch was hydrolyzed to soluble sugars, such as Glu and sucrose, which could ensure the survival of wheat in a short time [5]. Furthermore, starch could be degraded into maltose, and maltose could synthesize Tre [57]. Tre in turn degrades to Glu, which synthesizes Tre [58,59].

Many studies have proven that the accumulation of Tre is beneficial for plants in adapting to salt stress [60–62]. For example, Tre improved the salt tolerance of watermelon by maintaining the balance of cellular metabolism [60]. Yang et al. [61] also found that Tre could alleviate the damage to tomato plants caused by salt stress. At present, it has been reported that SLs interact with sugar to regulate plant growth and development. For instance, SLs play a key role in the transduction of sugar signaling molecules that participate in the development of Arabidopsis thaliana seedlings [62]. Takahashi et al. [63] also confirmed that SLs may be involved in Tre metabolism, promoting Tre biosynthesis, which regulates tomato adaptation to salt stress. The above reports imply that SLs may promote the biosynthesis of Tre by mediating the transformation among starch, Glu, and Tre, and then regulate Tre in response to salt stress. However, further research is needed in support of this hypothesis.

Tre metabolism in plants is related closely with activities of TPS, TPP, and THL. TPS and TPP play positive roles in the synthesis of Tre, while THL promotes the degradation of Tre. Under certain conditions, TPS condensed Glu to T6P, which was in turn dephosphorylated by TPP to form Tre [59]; the increase in T6P inhibited starch degradation [64]. Additionally, Kolbe et al. [65] found that the expression of TPS leads to a large increase in the AGPase of transgenic Arabidopsis. AGPase is a rate-limiting enzyme that plays a key role in starch biosynthesis, and an increase in AGPase activity can be used as a signal of starch synthesis [66]. Here, NaCl treatment significantly decreased the activities of TPS and TPP. This inhibition was reversed with the addition of GR24 (Figure 3). TIS108 treatment led to reductions in TPS and TPP activities (Figure 3). It was further confirmed that SLs increased Tre content in the tomato seedlings by increasing the activities of TPS and TPP, thereby reducing the damage to tomato seedlings caused by salt stress (Figure 3). This is consistent with other reports. The TPS/TPP pathway contributed to Tre biosynthesis, which responds
Moreover, THL was completely opposite—the activity of THL was raised under NaCl stress and NaCl + TIS108 treatment, while its activity decreased under NaCl + GR24 treatment. This indicated that SLs maintained Tre content by weakening the activity of THL. Analysis showed that increases in TPS and TPP activities and a decrease in THL activity can be used as a signal of Tre effectiveness. Therefore, we concluded that SLs regulate the response of Tre to salt stress by increasing the activities of TPS and TPP and decreasing the activities of THL.

Zhang et al. [43] found that SLs could induce the transcription of CsMAX2 genes that help for Arabidopsis in adaptation to salt stress. Zhang et al. [48] also revealed that SLs could induce the transcription of genes related to tryptophan metabolism, plant hormone signaling, and photosynthesis in response to salt stress. Previous studies demonstrated that both ABA and SLs were derived from carotenoids [68,69]. Under heat stress, the application of SLs downregulated LjNCED2, resulting in a decrease in the ABA level [70] and promoting Arabidopsis seed germination [71]. Furthermore, the expression of ABA synthesis-related genes (TaNCED1/2, TaSDR, TaZEP, TaABA3, and TaAAO) led to an increase in ABA content.

The ABA signal transduction could improve salt resistance in wheat by regulating stomatal closure [72]. Exogenous ABA enhanced the freezing tolerance of grapevines by inducing an accumulation of sugars in buds and decreasing stomatal conductance [73]. For years, many transcriptomic studies have provided evidence that the expression of genes involved in Tre biosynthesis regulates plant adaptation to abiotic stress. As reported by Lei et al. [74], the PoTPS1 gene changed Glu from UDPG to G6P, laying the foundation for the synthesis of Tre, and the content of Tre also increased with the expression of PoTPS1. For example, the allelic variations of TPS genes help with the adaptation of Cicer arietinum L. to salt stress [75]. In addition, the TPP genes are induced in maize seedlings under drought stress [76]. Similarly, under salt stress, OsTPP1 is transiently expressed and accompanied by an elevation of Tre content [77]. It has been demonstrated that the expression levels of Tre biosynthesis-related genes TPS1, TPS2, TPP1, and TPP2 in tomato seedlings were decreased under NaCl treatment. Surprisingly, the genes were induced by application of GR24. One key finding was that the expression levels of TPS1, TPS2, TPP1, and TPP2 genes were significantly reduced in the absence of SLs (Figure 4). The molecular evidence supports the conclusion that SLs participate in Tre synthesis through the upregulation of TPS1, TPS2, TPP1, and TPP2 genes, and then promote tomato-seedling growth.

This study is the first to propose and verify that SLs respond to salt stress by promoting the biosynthesis of Tre, laying the foundation for the application of SLs in agricultural practices. Future work should further explore the deep mechanisms of SLs under salt stress and excavate key genes and receptors to improve salt tolerance in tomato. SLs are very expensive, and it is difficult to apply them directly to crop production. Our results could provide a theoretical basis for the breeding work of tomato salt-tolerant cultivars.

5. Conclusions

In this article, we found that SLs plays an important role in alleviating the inhibition of tomato seedlings caused by salt stress. Additionally, we reported, for the first time, the action mechanism of SLs in enhancing salt resistance by regulating Tre metabolism. Our results indicate that GR24 enhances the accumulation of endogenous Tre and starch, promotes Glu decomposition, increases TPS and TPP activities, and decreases THL activity, and that the expression levels of TPS1, TPS2, TPP1, and TPP2 genes related to Tre biosynthesis were upregulated (Figure 5).
Figure 5. Role model of SLs in salt stress. Under salt stress, SLs promoted tomato seedling growth by regulating the metabolism of Tre. SLs accelerated the conversion of Glu to Tre by enhancing TPS and TPP activities. In addition, SLs inhibited the degradation of Tre by weakening THL activity. Moreover, SLs upregulated the Tre biosynthesis genes TPS1, TPS2, TPP1, and TPP2. (Green “→” indicates promotion; blue “→” indicates some active participation in the corresponding process; red “→” indicates increasing or decreasing; black “→” indicates treatment; the blocking line “⊣” indicates inhibition).

Author Contributions: X.L. (Xuefang Lu) was responsible for drawing and conceived the project and wrote the initial manuscript. X.L. (Xiaojun Liu) was also responsible for writing. J.X. and Y.L. were responsible for formatting. Y.C. was responsible for collecting data. C.L. and W.Y. were responsible for funding acquisition, obtaining resources, and writing and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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