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Regulating Function of Exogenous Salicylic Acid on Sugar Metabolism in Tomato Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NC designed the study. Authors BZZ, ZFW performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BZZ, WTP and CXZ managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: SA (Salicylic acid) is a key regulator for sugar metabolic pathway, but the regulating function on sugar metabolism in tomato seedlings is imperfect. In this study, tomato seedlings were sprayed with SA, and then soluble sugar contents, enzyme related to sugar metabolism levels, gene expressions were determined.
Study Design: In order to analyze the mechanism of SA in sugar metabolic pathway, tomato seedlings were sprayed with SA, and then soluble sugar contents, enzyme related to sugar metabolism levels, gene expressions were determined.
Place and Duration of Study: College of Biological Science and Technology, between December 2021 and May 2022.
Methodology: The expressions of genes related to sugar metabolism in tomato were measured by qRT-PCR. The contents of soluble sugar in tomato seedings were measured with HPLC (High performance liquid chromatography).
Results: The sugar metabolism in tomato seedlings was affected by SA treatment for 6 h. The contents of fructose, glucose and sucrose were improved in tomato seedlings by SA treatment. Between 6 to 24 h, enzymatic activities of SlAI, SlNI and SlSS were higher than those in controls, while there was little change in enzyme activity of SlSPS.

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Conclusion: The contents of soluble sugar, enzyme activities related to sugar metabolism had changed considerably by SA treatment. Especially from 6 h to 24 h after SA treatment, the contents of fructose and glucose were significantly higher than those of the control group, and the changes of sugar contents were mainly related to the increases of enzyme activities of AI, NI and SS, suggesting that SA treatment mainly affected the activities of SlAI, SlNI and SlSS to promote the accumulations of soluble sugars.

Keywords: SA; sugar metabolism; tomato seedlings.

1. INTRODUCTION

Sugar metabolism plays an important role in plant life activities. Carbohydrate is not only the product of photosynthesis, but also the substrates for respiration, and it is important for plant growth and development as well as energy metabolism [1-2]. Therefore, sugar metabolism is closely related to plant growth and development. Related enzymes that catalyze glucose metabolism include acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) [3]. Previous studies in our lab have shown that plant hormones, such as IAA, ABA and GA, impact carbohydrate partitioning by controlling enzyme activities about sugar metabolism in tomato [4]. While other study shows, SA can influence enzyme activities to control the process of sugar metabolism [5].

Salicylic acid, also known as o-hydroxybenzoic acid, is a small molecule phenolics and widely distributes in plants [6]. At the same time, SA participates in regulation of physiological processes such as growth, development, maturity and aging. And it also involves in inducible process of anti-stress responses like salt resistance, drought resistance and low temperature resistance [7]. Low concentration of SA promotes tomato seedlings growth, increase in root-shoot ratio. Physiological effects of SA on plants are multifaceted, and different concentrations of SA have different effects on different plant species, different varieties of the same species or different organs of the same species [8]. During the after-ripening and fruit softening in kiwifruit (*Actinidia deliciosa*), ASA inhibits amylase activity, hexose accumulation, increase in ASA activity and decline in acid invertase activity, while delays sugar accumulation, fruit ripening and fruit softening. In cucumber seedling roots, SA promotes amylase activity and it causes the fall of sucrose phosphate synthase activity [9]. The right amount of SA significantly increases the volume and sugar degree of tomato fruits by controlling the activities of invertase, sucrose synthase and sucrose phosphate synthase, and then improves tomato fruit quality [10]. Under salt stresses, SA promotes enzymatic activities of AI and NI to get more fructose and glucose which participate in osmoregulation and surviving salt adversity [11].

But during the growth and development of tomato seedlings, how SA controls sugar content in leaves, enzyme related to sugar metabolism levels and the rules of gene expression are unclear and lack systematic exposition. Thus, plants of *Solanum lycopersicum* were used as test material which were sprayed with SA in this study, and systematically researched the changing rule of sugar metabolism in tomato seedlings. It would provide a basis for studying the regulation mechanism of sugar metabolism and a theoretical basis for spraying SA in production practices to control growth and development of tomato seedlings.

2. MATERIALS AND METHODS

2.1 Test Material

Solanum lycopersicum cv. “Zhongshu No. 6” was used as test material in this study.

Seeds were disinfected by temperature difference method in 58℃ and gave rise to sprout without lighting at 25℃, and then they were seeded into the soil.

Tomato seedlings were sprayed with 0.5 mmol·L^{-1} of SA when tomato seedlings grew to the 4-leaf stage. Samples were taken at 0 h, 6 h, 12 h, 24 h, 48 h and 72 h after treatment, respectively. The samples were frozen with liquid nitrogen and stored in -80 ℃ refrigerator.

2.2 Test methods

2.2.1 qRT-PCR

Gene sequences were derived from NCBI database, and designed real-time PCR primers by Primer 5.0 (Table 1).

Table 1. Real-time PCR primer sequences

Gene	Accession number	Sequence
Actin	Q96483	F 5'-TGTCCCTATTTACGAGGGTTATGC-3'
		R 5'-AGTTAAATCACGACCAGCAAGAT-3'
SlSPS	AB051216.1	F 5'-CGGTGGATGGCAAAACG-3'
		R 5'-GGCAATCGGCCTCTGGT-3'
SlSS	AJ011319	F 5'-GGACAGGAATAAGCCCATCA-3'
		R 5'-TCGGTCTCCACCAACCAC-3'
SlTIV1	M81081.1	F 5'-ACGGGTATGTGGGAGTGT-3'
		R 5'-TCGGGTGTCCATTTGTTC-3'

2.2.2 Expression analysis of genes related to sugar metabolism in tomato

The extraction of total RNA in tomato leaves and qRT-PCR processing were carried out according to the method of Zhao [12]. The reverse transcription of total RNA in tomato leaves was performed by the FastKing RT Kit. Three replicates were performed for each sample.

2.2.3 Measuring enzyme activity related to sugar metabolism in tomato

The extraction of SlSPS, SlSS, SlAI, SlNI and estimation of enzymic activities were used by the method of Yu [13].

2.2.4 Determination of soluble sugar contents in tomato leaves

The contents of various soluble sugars in tomato leaves were determined by high performance liquid chromatography (HPLC). The specific extraction and content determination were carried out according to the method of Yu [13].

3. RESULTS AND ANALYSIS

3.1 Effects of SA Treatment on Sugar Contents in Tomato Seedling Leaves

By SA treatment, the changes of sugar contents in tomato leaves were seen in the Fig.1. The fructose content between 6-24 h in SA treatment group was higher than that in distilled water control group. And the glucose content of SA treatment group was higher than that of control group except 24 h. The sucrose contents in 6 h and 72 h were higher than those in the control group. In brief, the soluble sugar contents in tomato seeding leaves were improved at different periods by SA treatment.

3.2 Effects of SA Treatment on Enzymes Related to Sugar Metabolism in Tomato Seedlings

By SA treatment, enzymatic activities of SlAI, SlNI, SlSS and SlSPS were shown a trend of increasing first and then decreasing, and they were higher than in controls between 6 and 24 h. While SlSPS activity had no significant change, it was only slightly higher than the control group at 6 h and 24-48 h, respectively (Fig.2). SA had no significant effect on sucrose phosphate synthase activity, but promoted the activities of SlAI, SlNI and SlSS. Comd with the control group, the activities of these three enzymes were increased significantly.

3.3 Effects of SA Treatment on Gene Expressions Related to Sugar Metabolism in Tomato Seedlings

Effects of SA treatment on tomato seedlings were seen in the Fig.3. SA inhibited the gene expressions related to sugar metabolism in tomato seedlings. By SA treatment, gene expression of *SlSPS* showed a trend of decreasing first and then increasing, and its gene expression level was lower than that of the control group except for 24 h. Gene expression of *SlSS* showed a trend of increasing first and then decreasing, and the gene expression levels were lower than those of the control group. Gene expression of *SlTIV1* was lower than control group after 24 h. The levels of these gene expressions were lower than the control group within 72 h by SA treatment.

4. DISCUSSION

SA is a novel plant growth regulator. At present, there are many studies on its role in the regulation of physiological processes, such as

plant growth, development, maturity and aging, and the induction of stress responses, such as salt resistance, drought resistance, low temperature resistance, ultraviolet resistance and heavy metal resistance [14].

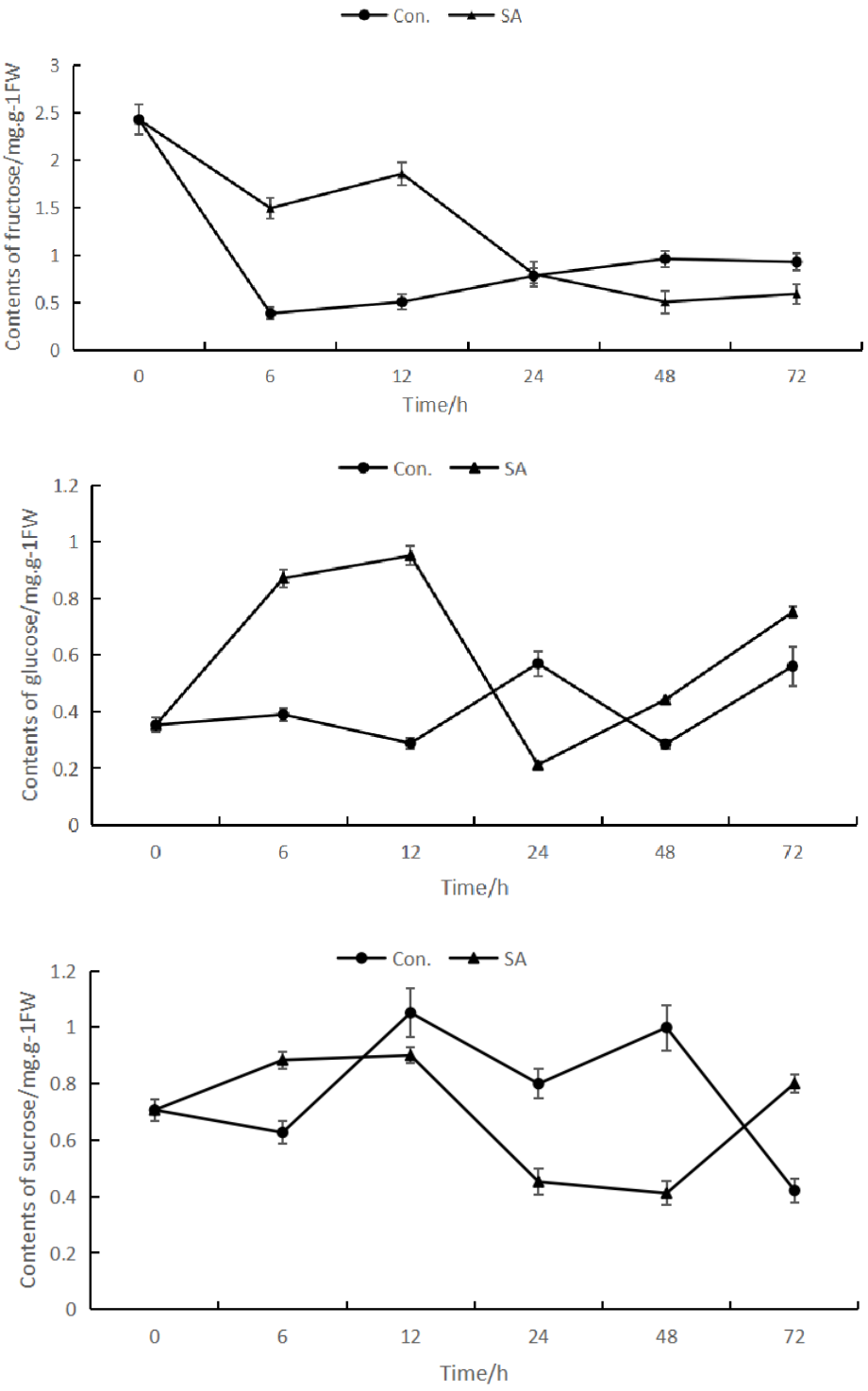


Fig. 1. Changes of sugar contents in tomato leaves treated with SA

Note: Con., control; SA, salicylic acid treatment

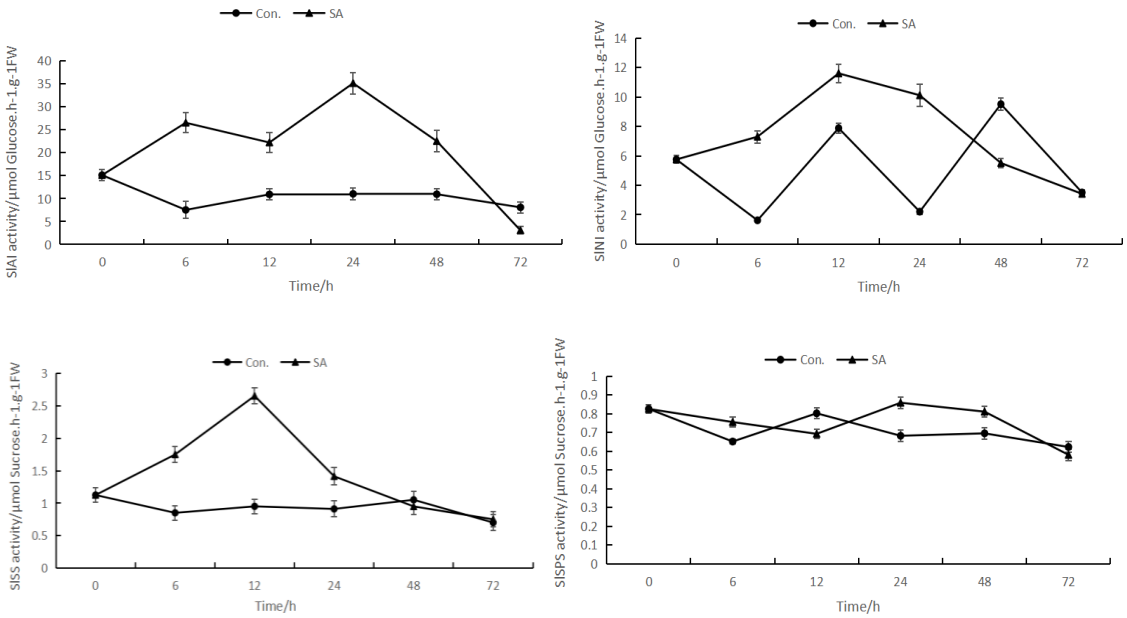
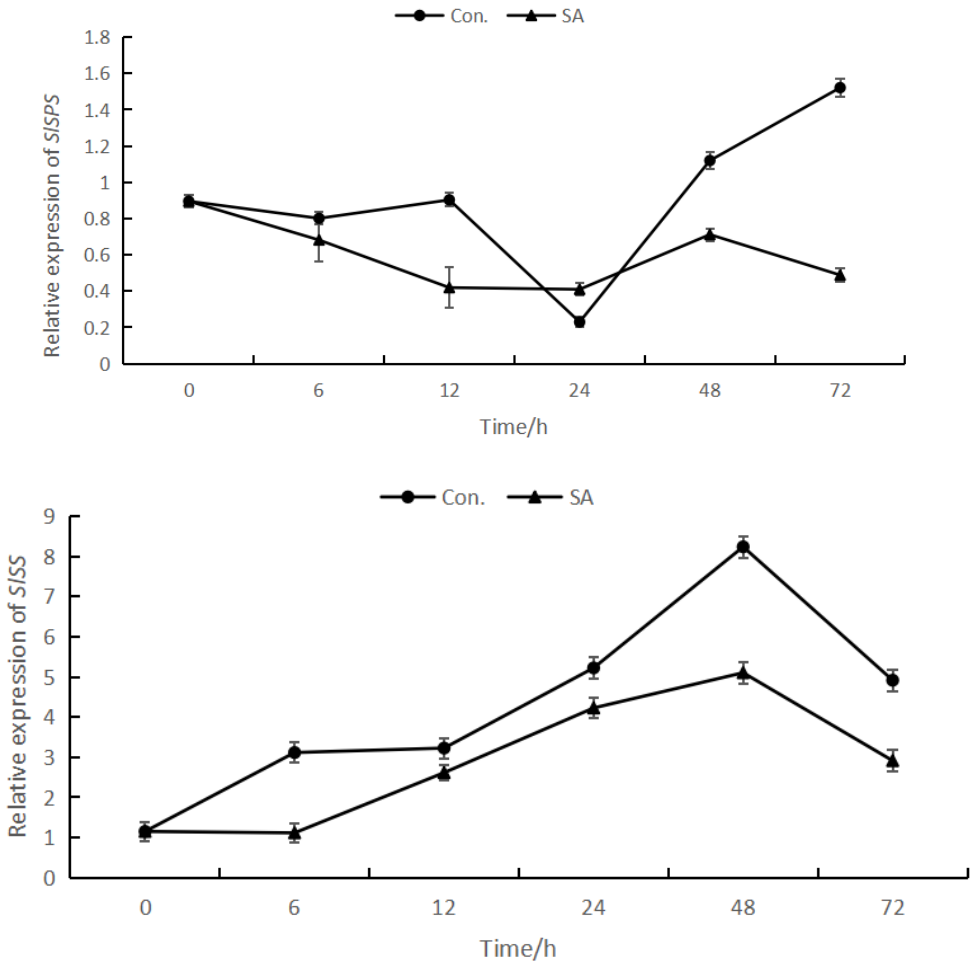


Fig. 2. Changes of enzyme activities related to sugar metabolism by SA treatment

Note: Con., control; SA, salicylic acid treatment



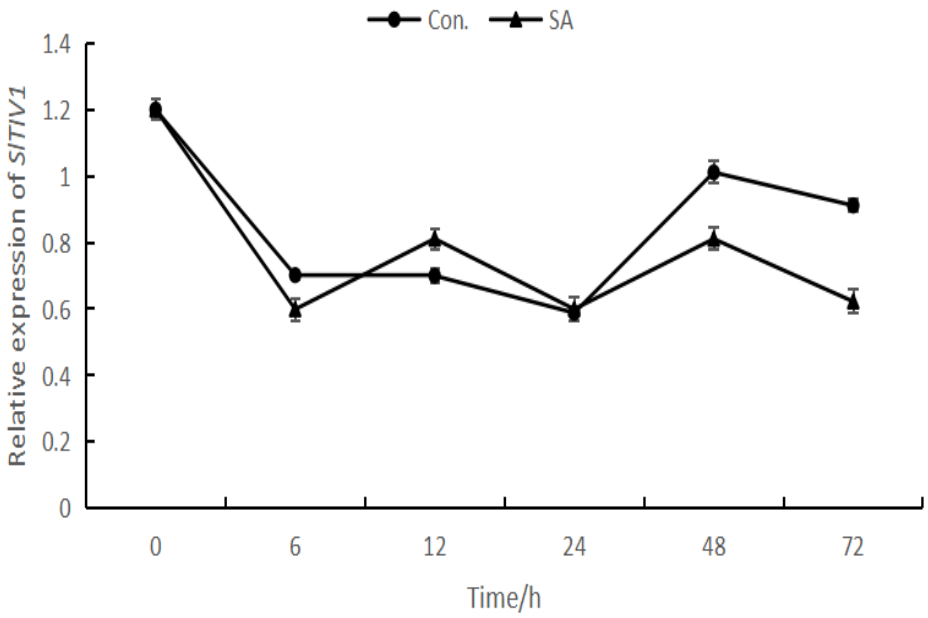


Fig. 3. Changes of gene expressions of enzymes related to sugar metabolism by SA treatment
Note: Con., control; SA, salicylic acid treatment

Photosynthesis is an important physiological process in crops, which directly provides nutrition and capacity for the growth and development of crops. Part of the organic matter produced by photosynthesis is loaded into phloem and transported to the reservoir organs for growth and development [15]. However, some of them are left in the leaves for the metabolism of the leaves themselves. Therefore, sugar metabolism directly affects the growth and development of crop seedlings [16]. Studies have shown that exogenous SA spraying can affect the sugar metabolism of tomato fruit, increase the contents of soluble sugar in fruit, and participate in the formation of fruit flavor and quality [10]. However, the effect of exogenous SA treatment on sugar metabolism in tomato seedlings is unclear. In this study, tomato seedlings were sprayed with SA. The results showed that the contents of soluble sugar and enzyme activities related to sugar metabolism changed greatly after 6 h of SA treatment. That meant SA treatment could increase the contents of soluble sugar and the activities of enzymes related to sugar metabolism in tomato seedlings.

SA treatment could increase the contents of fructose, glucose and sucrose in tomato seedlings. The enzyme activities of SlAI, SlNI and SlSS were higher than those in the control group between 6 and 24 h, and the enzyme activities of SlSPS had little change, indicating that SA treatment mainly affected the activities of SlAI, SlNI and SlSS, thus promoting the accumulations of soluble sugar, and providing more nutrition for the growth and development of tomato seedlings. The effect of SA on the gene expressions of sugar metabolism related enzymes were inconsistent with the enzyme activities, indicating that there might be existing the post transcriptional regulation.

The study on the effect of SA on sugar metabolism at seedling stage laid a foundation for further study on the regulation of SA on plant growth and development, and also provided a theoretical basis for using SA to promote tomato growth in production practice. With the in-depth study of the physiological effects of SA and the continuous revelation of its mechanism of action, SA will be more widely used in plants. This will increase the production of plants, reduce the use of toxic pesticides and reduce the damage to the environment, which can bring both environmental and social benefits.

5. CONCLUSION

The contents of soluble sugar, enzyme activities related to sugar metabolism had changed

considerably by SA treatment. Especially from 6 h to 24 h after SA treatment, the contents of fructose and glucose were significantly higher than those of the control group, and the changes of sugar contents were mainly related to the increases of enzyme activities of AI, NI and SS, suggesting that SA treatment mainly affected the activities of SlAI, SlNI and SlSS to promote the accumulations of soluble sugars. Thus, SA treatment can be carried out to promote the formation of soluble sugar and provide nutrition for the growth and development of tomato seedlings in the production practice of tomato cultivation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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