

## **Effects of *AtSPS* on the Growth and Development of *Arabidopsis thaliana* under Abiotic Stress**

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### **Authors' contributions**

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

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### **ABSTRACT**

**Aims:** SPS (Sucrose phosphate synthase) participates in plant growth and yield formation, and plays an important role in plant stress resistance. This study used T-DNA insertion mutant of *AtSPS* in *Arabidopsis* as test material. The growth indexes and soluble sugar contents of *Arabidopsis thaliana* under salt stress, osmotic stress and low temperature stress were determined, which laid the foundation for further understanding the mechanism of SPS in plant growth and development and abiotic stress resistance.

**Study Design:** In order to analyze the mechanism of SPS in plant growth and development and abiotic stress resistance, this study used T-DNA insertion mutant of *AtSPS* in *Arabidopsis* as test material. The growth indexes and soluble sugar contents of *Arabidopsis thaliana* under salt stress, osmotic stress and low temperature stress were determined.

**Place and Duration of Study:** College of Biological Science and Technology, between December 2020 and May 2021.

**Methodology:** The contents of soluble sugar in tomato fruits were measured with HPLC (High performance liquid chromatography). The growth indexes were determined.

**Results:** The results showed that *AtSPS* played positive regulation roles in seed germination and

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seedling growth of *Arabidopsis thaliana*. However, under abiotic stress conditions, *AtSPS* mutant increased the contents of soluble sugar, suggesting that *Arabidopsis thaliana* seedlings might improve resistance through osmotic regulating substances.

**Conclusion:** *AtSPS* played positive regulation roles in seed germination and seedling growth of *Arabidopsis*. Meanwhile, *AtSPS* mutant increased the contents of soluble sugar to increase resistance of *Arabidopsis* under abiotic stresses, and the growth and development were blocked, suggesting that SPS was negative regulatory element to resist abiotic stress.

**Keywords:** *Arabidopsis thaliana*; sucrose phosphate synthase; *AtSPS*; ABIOTIC stress.

## 1. INTRODUCTION

During plant growth, development and metabolism, sucrose is both the product of plant photosynthesis and the substrate of respiration, which provides carbon storage and energy for plant growth and development and enhances plant stress resistance [1-3]. SPS is a key enzyme in plants which controls sucrose synthesis [4]. SPS also participates in plant growth and yield formation, and plays an important role in plant stress resistance [5,6]. Abiotic stress can affect plant photosynthesis, for example, low temperature can break the balance between the chemical energy produced by the photoreaction and the chemical energy consumed by the carbon reaction, and affect the production of sucrose [7,8]. In addition, SPS plays an important role in energy supply, signaling transduction, transcription regulation, starch and fiber synthesis in plants because of its strong influence source and library [9,10].

In order to study on the action of SPS in plant growth and resistance, the *salk\_148643c* of *AtSPS* knockout mutant in *Arabidopsis* was used as experimental materials for further studying. This study compared the difference in growth and development between wild type and mutant of *Arabidopsis*, and determined influence of deletion of the *AtSPS* gene on sucrose metabolism as well as growth and development of *Arabidopsis* under abiotic stress, and laid a foundation for the study of action mechanism of SPS.

## 2. MATERIALS AND METHODS

### 2.1 Test Material

Col-0 and T-DNA inserts mutants *SALK\_148643c* (*AtSPS*)

### 2.2 Abiotic Stress Treatment

The seeds of Col-0 and *AtSPS* mutant were seeded on 1/2 MS medium containing NaCl (The concentrations were respectively 0 mmol·L<sup>-1</sup>, 50

mmol·L<sup>-1</sup>, 100 mmol·L<sup>-1</sup>) and mannitol (The concentrations are respectively 0 mmol·L<sup>-1</sup>, 250 mmol·L<sup>-1</sup>, 300 mmol·L<sup>-1</sup>) under sterile conditions. Low temperature treatment at 10 °C (Sucrose concentration, 3%).

### 2.3 Determination of Soluble Sugar

High-performance liquid chromatography (HPLC) was performed. Agilent 1100 high- performance liquid chromatography system, differential refractometer (Agilent Corporation) were used. NH<sub>2</sub> column was used for separated. The velocity of mobile phase was 1.0 mL·min<sup>-1</sup>, and the temperature of column was 28°C, injection quantity was 15 μL. Proportioning of mobile phase was acetonitrile: water=80:20.

## 3. RESULTS AND ANALYSIS

### 3.1 Effects of *AtSPS* Mutant on Seed Germination of *Arabidopsis* Under Abiotic Stress

Effects of *AtSPS* mutant on seed germination of *Arabidopsis* under abiotic stress were seen the Table 1.

Germination rate, germination potential and germination index of *AtSPS* mutant were significantly decreased during salt stress. And with the increase of NaCl concentration, the degree of reduction was more significant. Mannitol treatment with different concentrations had the same tendency as salt stress treatment.

The germination rate, germination potential and germination index of *Arabidopsis* seed mutated by *AtSPS* were lower than those of Col-0 at normal temperature. Compared with the control group, the germination rate, germination potential and germination index of *AtSPS* mutant decreased by 43.75% , 77.27% and 49.04%, respectively at low temperature treatment. The results indicated that *AtSPS* played positive regulation roles in seed germination of *Arabidopsis*.

**Table 1. Effects of *AtSPS* mutant on seed germination of *Arabidopsis* under abiotic stress**

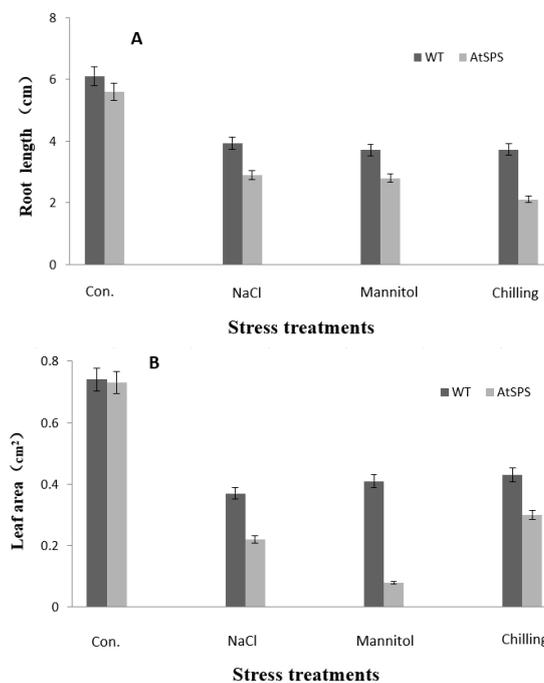
Treatment			Germination rate (%)	Germination potential (%)	Germination index (%)
NaCl (mmol. L <sup>-1</sup> )	0	WT	98±1.17	55±0.89	28.41±29.08
		<i>AtSPS</i>	92±1.02	10±1.41	21.42±21.33
	50	WT	96±1.33	40±1.72	25.98±1.05
		<i>AtSPS</i>	73±1.02	5±1.02	15.88±0.83
	100	WT	85±1.33	31±1.17	23.59±1.02
		<i>AtSPS</i>	60±1.33	7±0.75	11.30±0.69
Mannitol (mmol.L <sup>-1</sup> )	0	WT	96±0.75	55±1.41	28.13±0.18
		<i>AtSPS</i>	91±1.41	42±1.22	20.98±0.81
	250	WT	92±2.24	53±1.50	21.54±0.67
		<i>AtSPS</i>	75±1.12	10±0.75	14.71±0.78
	300	WT	86±1.72	43±1.33	19.06±1.02
		<i>AtSPS</i>	35±2.42	0±1.02	6.21±0.71
Chilling (°C)	22	WT	98±1.41	55±2.04	28.56±1.35
		<i>AtSPS</i>	87±1.60	47±1.02	1.97±1.22
	10	WT	80±1.71	22±1.41	16.60±1.00
		<i>AtSPS</i>	45±1.68	5±1.05	8.46±0.68

### 3.2 Effects of *AtSPS* Mutant on the Growth and Development of *Arabidopsis* under Abiotic Stress

#### 3.2.1 Effects of *AtSPS* mutant on root length and leaf area of *Arabidopsis* under abiotic stress

The *AtSPS* mutant inhibited the root elongation of *Arabidopsis* (Fig.1-A), but had no significant

effect on leaf area (Fig.1-B). *AtSPS* mutant inhibited elongation growth of roots and area growth of leaves under salt stress, osmotic stress and low temperature stress. Especially, osmotic stress had great effect on leaf area. Osmotic stress of 300 mmol·L<sup>-1</sup> mannitol, leaf area of wild type was 0.41 cm<sup>2</sup> but leaf area of *AtSPS* mutant was 0.08 cm<sup>2</sup>. The results indicated that *AtSPS* played positive regulation roles in *Arabidopsis* seedling growth and development.

**Fig. 1. Changes on root length and leaf area of wild type and mutant under abiotic stress**

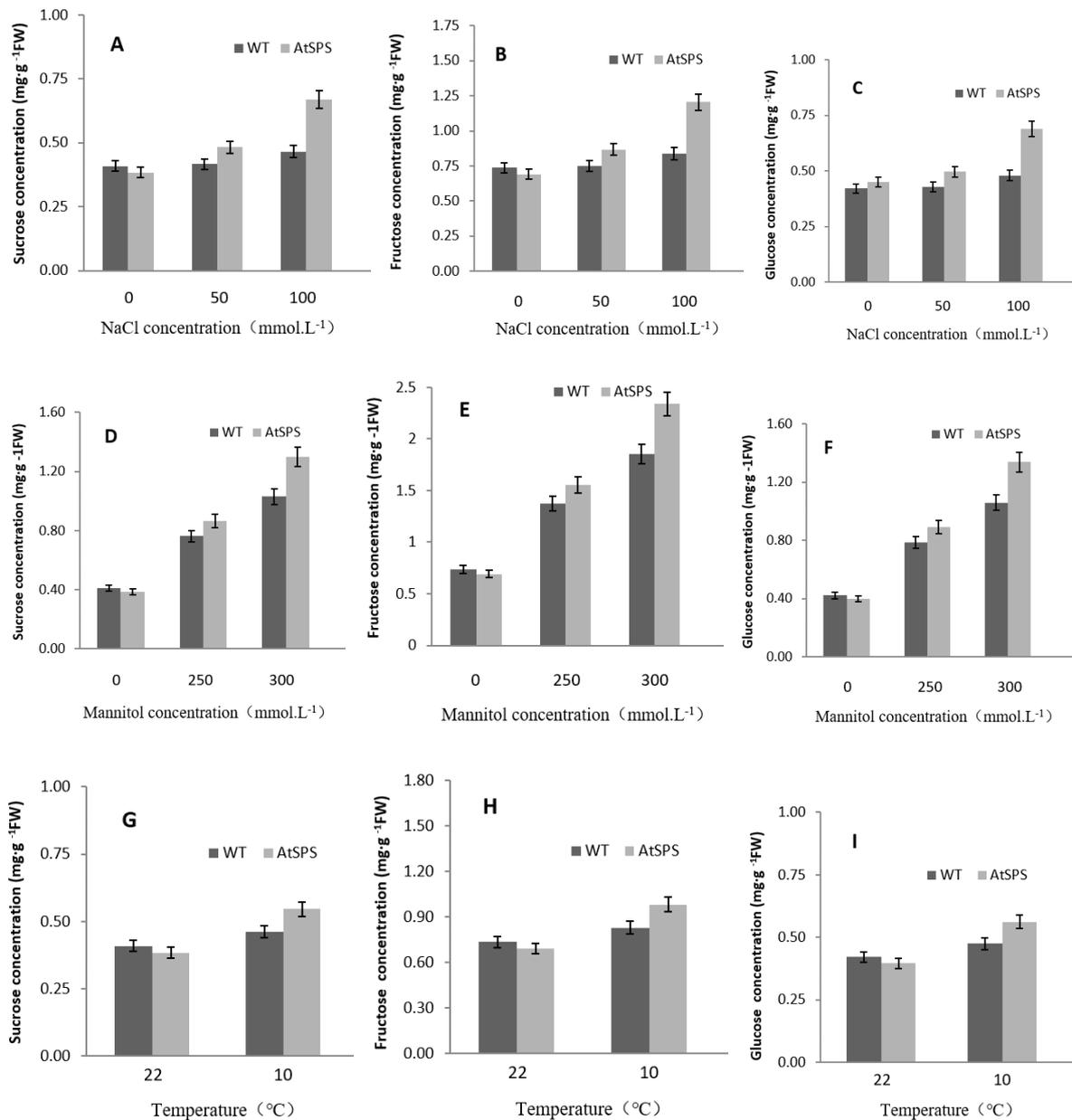
### 3.2.2 Effect of *AtSPS* mutation on sugar contents in *Arabidopsis* seedlings under abiotic stress

The contents of sucrose, fructose and glucose decreased slightly after the mutation of *ATSPS* under normal conditions (Fig. 2). Under salt stress, osmotic stress and low temperature stress, the higher the stress degree was, the more contents of soluble sugar in wild type was. While *AtSPS* mutant could further increase the contents of soluble sugar (Fig. 2), and the higher

the stress degree was, the more contents of soluble sugar was.

### 4. DISCUSSION

In nature, plants are constantly challenged by adverse abiotic conditions, such as drought, low temperatures, excessive salt in the soil, and so on. These abiotic stresses limit the global use of arable land, and have negative impacts on crop productivity. Therefore, it is essential for understanding the regulation of plant adaptation to adverse environmental conditions [11].



**Fig. 2. Changes on soluble sugar contents in leaves of wild type and mutant plants under abiotic stress 4**

Sucrose phosphate synthase is a key enzyme in plants which controls sucrose synthesis [12]. It participates in plant growth and yield formation, and plays an important role in plant stress resistance [13]. But current researches primarily focus on the formation of yield and quality, the role of stress resistance is unclear.

Studies show that, *AtSPS* is expressed almost throughout the plant, meanwhile expression is also found in the embryo [14,15]. So, in order to study on the action of SPS in plant growth and resistance, the salk\_148643c of *AtSPS* knockout mutant in *Arabidopsis* was used as experimental materials in this research, The results indicated that germination rate, germination potential and germination index of *AtSPS* mutant were significantly decreased and elongation growth of roots and area growth of leaves were suppressed under abiotic stress conditions, further evidence was that *AtSPS* played positive regulation roles in seed germination and seedling growth of *Arabidopsis*. Maybe, deletion of *AtSPS* impedes the supply of sucrose to the embryo, and limites the seedling growth of *AtSPS* mutant, and then impactes the growth and development of seedlings [16,17].

Soluble sugar is the feedstocks of plant growth and development, also a class of important osmotic regulating substance, including sucrose, fructose and glucose, and so on. Osmotic adjustment can completely or partially maintain the membrane transport and the electrical properties of the cell membrane, which are directly controlled by the expansion pressure [18-20]. In this study, the contents of soluble sugar in *AtSPS* mutants were increased to enhance the resistance of *Arabidopsis* under abiotic stresses, and the growth and development were blocked.

The results indicated that SPS was negative regulatory element about resisting abiotic stress. So, in production practice, *SPS* gene was silenced by gene knockout technique in order to increase the soluble sugar contents of plants in adversity to resist adverse environment. It would provide a theoretical basis for breeding the varieties with strong stress resistance.

## 5. CONCLUSION

Under abiotic stress conditions, germination rate, germination potential and germination index of *AtSPS* mutants were significantly decreased. Besides, elongation growth of roots and area growth of leaves were suppressed. *AtSPS*

played positive regulation roles in seed germination and seedling growth of *Arabidopsis*. Meanwhile, *AtSPS* mutant increased the contents of soluble sugar to increase resistance of *Arabidopsis* under abiotic stresses, and the growth and development were blocked, suggesting that SPS was negative regulatory element to resist abiotic stress.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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