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.

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...
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 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\(^{-1}\), 50 mmol·L\(^{-1}\), 100 mmol·L\(^{-1}\) and mannitol (The concentrations are respectively 0 mmol·L\(^{-1}\), 250 mmol·L\(^{-1}\), 300 mmol·L\(^{-1}\)) 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\(_2\)-column was used for separated. The velocity of mobile phase was 1.0 mL·min\(^{-1}\), 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination rate (%)</th>
<th>Germination potential (%)</th>
<th>Germination index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NaCl (mmol. L⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>98±1.17</td>
<td>55±0.89</td>
<td>28.41±29.08</td>
</tr>
<tr>
<td>AtSPS</td>
<td>92±1.02</td>
<td>10±1.41</td>
<td>21.42±21.33</td>
</tr>
<tr>
<td>50</td>
<td>96±1.33</td>
<td>40±1.72</td>
<td>25.98±1.05</td>
</tr>
<tr>
<td>AtSPS</td>
<td>73±1.02</td>
<td>5±1.02</td>
<td>15.88±0.83</td>
</tr>
<tr>
<td>100</td>
<td>85±1.33</td>
<td>31±1.17</td>
<td>23.59±1.02</td>
</tr>
<tr>
<td>AtSPS</td>
<td>60±1.33</td>
<td>7±0.75</td>
<td>11.30±0.69</td>
</tr>
<tr>
<td><strong>Mannitol (mmol.L⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>96±0.75</td>
<td>55±1.41</td>
<td>28.13±0.18</td>
</tr>
<tr>
<td>AtSPS</td>
<td>91±1.41</td>
<td>42±1.22</td>
<td>20.98±0.81</td>
</tr>
<tr>
<td>250</td>
<td>92±2.24</td>
<td>53±1.50</td>
<td>21.54±0.67</td>
</tr>
<tr>
<td>AtSPS</td>
<td>75±1.12</td>
<td>10±0.75</td>
<td>14.71±0.78</td>
</tr>
<tr>
<td>300</td>
<td>86±1.72</td>
<td>43±1.33</td>
<td>19.06±1.02</td>
</tr>
<tr>
<td>AtSPS</td>
<td>35±2.42</td>
<td>0±1.02</td>
<td>6.21±0.71</td>
</tr>
<tr>
<td><strong>Chilling (°C)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>98±1.41</td>
<td>55±2.04</td>
<td>28.56±1.35</td>
</tr>
<tr>
<td>AtSPS</td>
<td>87±1.60</td>
<td>47±1.02</td>
<td>19.67±1.22</td>
</tr>
<tr>
<td>10</td>
<td>80±1.71</td>
<td>22±1.41</td>
<td>16.60±1.00</td>
</tr>
<tr>
<td>AtSPS</td>
<td>45±1.68</td>
<td>5±1.05</td>
<td>8.46±0.68</td>
</tr>
</tbody>
</table>

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⁻¹ mannitol, leaf area of wild type was 0.41 cm² but leaf area of AtSPS mutant was 0.08 cm². 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 limits the seedling growth of AtSPS mutant, and then impacthes 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.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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