Effects of Salicylic Acid on Physiological Characters of Melilotus of Ficinalis Seedlings under Salt Stress

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Abstract: The effects of salicylic acid treatment on physiological properties of melilotus of ficinalis seedlings were studied under different salt stress. The results showed that salicylic acid significantly increased the proline content in the melilotus of ficinalis seedlings under low salt stress. Salicylic acid could significantly reduce MDA content in the melilotus of ficinalis seedlings and increase the content of soluble sugar and soluble protein under various salt stresses. Salicylic acid significantly increased CAT activity in the melilotus of ficinalis seedlings under low salt stress. The SOD activity in melilotus of ficinalis seedlings treated with salicylic acid increased compared with the control group, but did not reach the significant level. Exogenous salicylic acid could significantly change the physiological characteristics of melilotus of ficinalis seedlings under salt stress, alleviate the salt stress on melilotus of ficinalis seedlings by increasing the content of osmotic substance, reducing the content of malondialdehyde, and increasing the activity of antioxidant enzyme.

1. Introduction

Half of the world's irrigated land was affected by salinization to some degree in 2008[1]. At present, the problem of soil salinization is more prominent, which seriously affects the growth of plants and crop production. mellilotus of ficinalis is a legume herbage and green fertilizer with high economic value, which can be used as high-quality livestock feed [2]. It is also an excellent green fertilizer [3], which can effectively improve soil fertility and has high economic value.

Salicylic acid (SA) can be used as a signaling molecule in plants, which plays a role in improving the salt resistance of plants [4]. Low concentration of SA can effectively improve the salt tolerance of plant seedlings, while high concentration of SA can inhibit the growth of seedlings. The 1mmol/L SA is the appropriate concentration to promote seed germination and seedling growth of Chinese cabbage [5].

Although mellilotus of ficinalis had certain salt tolerance, its seedling growth and yield were still significantly affected under salt stress. It is great significance to study the salt-tolerance and enhance the salt-tolerance of mellilotus of ficinalis for making full use of salinized soil. At present, there have been some reports on the germination and growth of mellilotus of ficinalis seedlings under salt stress [6, 7]. The effects of SA on seedling under salt stress have also been conducted on pepper [8], alfalfa [9], corn [10], cotton [11], oat [12], wheat [13], cauliflower[14] and other plants, but seldom on mellilotus of ficinalis.

With mellilotus of ficinalis seed as test materials, the effects of SA on related enzyme activities and related osmotic substances in mellilotus of ficinalis seedlings were studied under different salt content. It provides theoretical basis and technical support for the cultivation of mellilotus of ficinalis on salinized soil, the improvement of salinized soil and the development of salt-tolerant seed coating. SA treatment can alleviate salt stress and has important ecological significance for the

germination and growth of mellilotus of ficinalis on salinized soil and improving salinized soil.

2. Materials and Methods

2.1 Experimental Materials

The test material of mellilotus of ficinalis seed was provided by Tianjin Institute of Agricultural Resources and Environment.

2.2 Experimental Method

Mellilotus of ficinalis seeds were disinfected with 1% sodium hypochlorite solution for 10 minutes and rinsed with clean water. Soak the seeds in water for 12 h in the control group, and soak the seeds in 1 mmol/L SA for 12 h in the treatment group. Two kinds of treated seeds were planted separately in five salt content soil of the basin, and each basin was evenly seeded with 20 grains and repeated for 3 times. The salt content of the five soils was 0.04%, 0.20%, 0.35%, 0.52% and 0.68%, respectively, regulated by 0, 40, 80, 120 and 160 mmol/L NaCl solution. When the seedling emergence height reached 3 cm, 5 ml salicylic acid was sprayed on each basin of the treatment group. The contents of proline, soluble sugar, soluble protein and malondialdehyde(MDA) in the leaves were measured when the seedlings grew to 10 cm, and the activities of catalase(CAT) and superoxide dismutase(SOD) in the leaves were measured.

2.3 Determine Items and Measurement Methods

The content of soluble protein was determined by Coomassie bright blue method [15], activity of catalase(CAT) was determined by ultraviolet absorption method, activity of superoxide dismutase (SOD) was determined by nitrotetrazolium blue chloride(NBT) method, content of soluble sugar was determined by anthrone colorimetric method, content of MDA was determined by thiobarbituric acid(TBA) method, content of proline was determined by colorimetric method. (Kit: Nanjing Jiancheng Reagent Company)

2.4 Data Processing

Data processing and draw graphs were performed by using Excel 2013, and data statistical analysis was used by SPSS 25 software.

3. Results and Analysis

3.1 Effect of SA on Proline and MDA Content in Mellilotus of Ficinali Seedlings under Salt Stress

The accumulation of proline in plants can effectively improve the ability of cells to absorb water and retain water in adversity. It can be seen from figure 1, the range of soil salt content from 0.04% to 0.35%, proline content in mellilotus of ficinali seedling increased significantly with the increase of salt content, and decreased significantly with the further increase of salt content. SA treatment had no significant effect on proline content in mellilotus of ficinali seedlings at low salt content of 0.04% and high salt content of 0.52% or more. Under salt content of 0.2% and 0.35% by the SA treatment, the content of proline in mellilotus of ficinali seedlings increased significantly compared with that of untreated ones. SA could improve the salt resistance of mellilotus of ficinali seedlings by increasing the content of proline in seedlings.

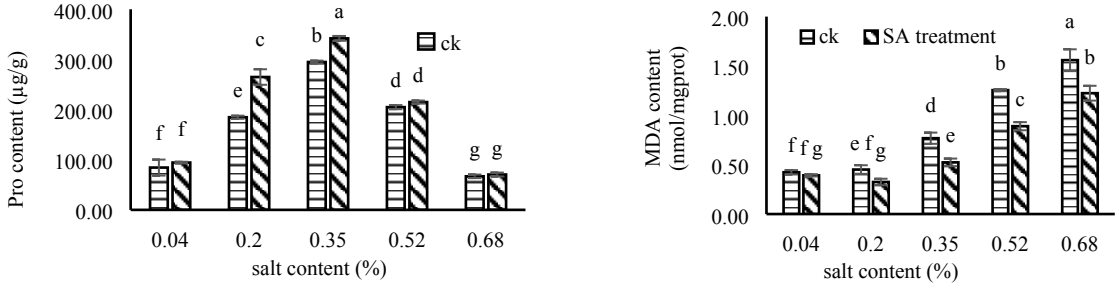

Fig 1. Changes of proline content and MDA content in melilotus of ficinalis seedlings treated with SA under different salt stress

Different lower-case letters above the bar mean significant difference at the 0.05 probability leve1, the same below.

The more severe the damage caused by salt stress, the higher the content of MDA in plant tissues, and the weaker the protective ability of plant tissues[16]. As can be seen from figure 1, content of MDA in both the control group and the SA treatment group increased with the increase of soil salt content. In the absence of salt stress, content of MDA in melilotus of ficinalis seedlings treated with SA did not decrease significantly compared with the control group. MDA content in melilotus of ficinalis seedlings treated with SA was significantly lower than that of the untreated with SA in other soils with different levels of salt concentration. MDA content in melilotus of ficinalis seedlings treated with SA decreased most significantly at 0.35% salt in the soil, reaching 32.47%. The results were 0.20%, 0.52%, 0.68% and 0.04%, which were reduced by 28.89%, 28.80%, 21.79% and 7.14%, respectively. The results showed that SA treatment had little effect on MDA content in melilotus of ficinalis seedlings under the absence of salt stress, while MDA content in melilotus of ficinalis seedlings was decreased by SA treatment under salt stress. It can be seen from the change of MDA content that SA has a significant effect on melilotus of ficinalis seedlings salt resistance in a certain salt content range.

3.2 Effect of SA on Soluble Sugar and Soluble Protein Content in Mellilotus of Ficinali Seedlings under Salt Stress

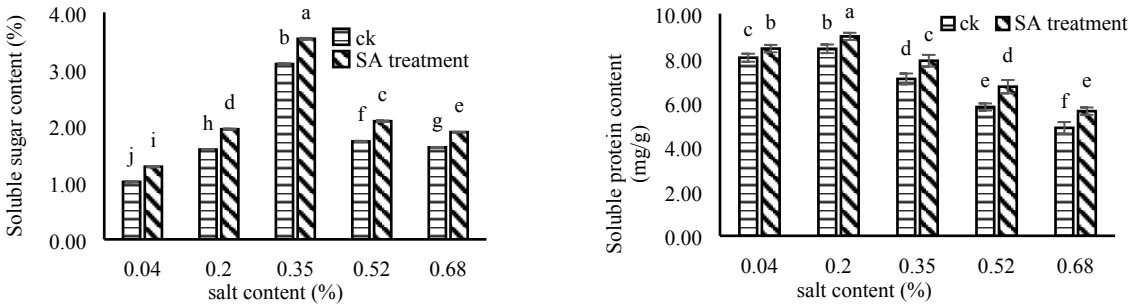

Fig 2. Changes of soluble sugar content and soluble protein content in *melilotus of ficinalis* seedlings treated with SA under different salt stress

Soluble sugar is an important physiological osmotic regulating substance. The increase of soluble sugar content can also improve the water retention ability of plant cells under salt stress. As can be seen from figure 2, the range of soil salt content from 0.04% to 0.35%, the soluble sugar content in mellilotus of ficinali seedling increased significantly with the increase of salt content, and decreased significantly with the further increase of salt content. The soluble sugar content of melilotus of ficinalis seedlings was increased significantly under salt stress after SA treatment. When the salt concentration was 0.04%, 0.20%, 0.35%, 0.52% and 0.68%, the soluble sugar content of melilotus of ficinalis seedlings treated with SA were increased by 16.67%, 22.78%, 14.24%, 20.23% and 26.73% respectively compared with the control group. It is observed that the salt resistance of mellilotus of ficinali was improved by increasing the content of soluble sugar in seedlings after SA treatment.

Soluble protein is not only a nutrient, but also an important osmotic regulator. The increase and

accumulation of soluble protein in plants can also improve the water retention ability of cells. As can be seen from figure 2, the soluble protein content in melilotus of ficinalis seedlings was increased significantly with the increase of soil salt content in the low salinity range from 0.04% to 0.20%, and decreased significantly with the increase of soil salt content in the salinity range from 0.20% to 0.68%. The content of soluble protein in mellilotus of ficinali seedlings increased significantly compared with that of untreated ones. It can be seen that SA could improve the salt-tolerance of mellilotus of ficinali seedlings by increasing the content of soluble protein in seedlings.

3.3 Effect of SA on the Activities of CAT and SOD in Mellilotus of Ficinali Seedlings under Salt Stress

Both CAT and SOD are important members of the antioxidant enzyme system in plants. Both CAT and SOD can effectively eliminate harmful substances produced by organisms in the process of metabolism, prevent the damage caused by plant peroxidation, so as to generate resistance to salt stress environment. As can be seen from figure 3, both the control and SA treatment showed that CAT activity in melilotus of ficinalis seedlings gradually increased with the increase of salt content below 0.35% salt content, and CAT activity in melilotus of ficinalis seedlings gradually decreased with the increase of salt content above 0.35% salt content. CAT activity in melilotus of ficinalis seedlings treated with SA was significantly higher than that without SA at 0.20% soil salt content. Under the 0.04%, 0.35% and 0.52% soil salt content, CAT activity in melilotus of ficinalis seedlings was increased by SA treatment compared with control treatment, but not significantly. The results showed that SA could alleviate salt stress on melilotus of ficinalis seedlings by increasing CAT activity.

As can be seen in figure 3, under the condition of 0.20% low salt content, low salt could significantly promote SOD activity in melilotus of ficinalis seedling. SOD activity in melilotus of ficinalis seedling was decreased significantly with the increase of salt content. Although the salt concentration at 0.04%, 0.20%, 0.35%, 0.52% and 0.68%, the SOD activity in melilotus of ficinalis seedlings treated with SA were increased by 1.40%, 2.56%, 6.97%, 3.76% and 0.95% respectively compared with the control group, but the increase was not significant. The results showed that SA could improve the activity of antioxidant enzyme and further improve salt tolerance of melilotus of ficinalis seedlings. However, SA in enhancing SOD activity needs to be further verified.

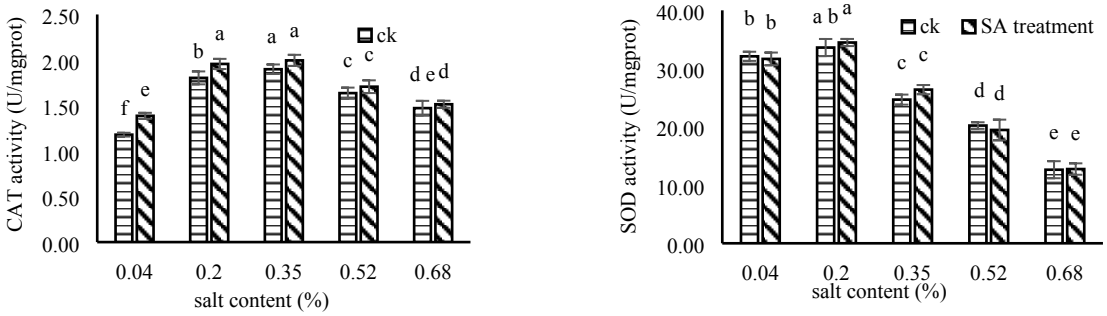

Fig 3. Changes of CAT activities and SOD activities in Melilotus of ficinalis seedlings treated with SA under different salt stress

4. Discussion

In this experiment, SA treatment significantly increased the content of soluble sugar, soluble protein and proline in melilotus of ficinalis seedlings, which were consistent with the results of that effect of HS on physiological indexes and antioxidant activity of sweet clover seedlings under NaCl stress[17].This indicated that SA treatment could promote the accumulation of soluble sugar, soluble protein and proline in melilotus of ficinalis seedlings under salt stress, and the synthesis and accumulation of these osmotic regulatory substances could reduce the osmotic potential of cells, thus enhancing the water absorption capacity of plant cells and alleviating the damage caused by salt stress[18].

Exogenous SA can alleviate salt damage by inducing increased activity of antioxidant enzymes

such as POD in lettuce under salt stress [19]. In this experiment, SA treatment significantly improved the CAT activity in melilotus of ficinalis seedlings under low salt stress, and significantly reduced the content of MDA. That activity of antioxidant enzyme was increased and content of MDA was decreased obviously relieve the oxidative damage degree, these are the physiological index of the resistance to salt stress [20]. Salt stress can stimulate the increase of reactive oxygen species in plant cells, further degradation for biological molecules such as proteins in the plant tissue. It inactivates metabolic enzymes and causes oxidative damage to plant tissues and cells [21]. The content of MDA was increased by membrane lipid oxidation in the cell membrane, and MDA further affects the synthesis of protein [22].

Exogenous SA could significantly increase the SOD activity of buckwheat leaves under salt stress [23], while SA treatment for corn [24] and barley [25] was beneficial to increase the seeding growth and POD activity of seedlings under salt stress. Exogenous SA protect the membrane of seedling tissues and cells, and reduce the content of MDA. The activity of SOD, POD and CAT could were improved by exogenous SA treatment in wild solanum nigrum Seedlings [26]. The results of this experiment indicate that melilotus of ficinalis were treated with appropriate SA could induce significantly activity of protective enzyme in seedlings, and inhibit accumulation of MDA in melilotus of ficinalis seeding, and decrease membrane peroxidation caused by salt stress, and raise the salt resistance of melilotus of ficinalis seeding.

5. Conclusion

Under low salt stress, SA treatment could significantly increase proline content in melilotus of ficinalis seeding. SA treatment had little effect on MDA content in melilotus of ficinalis seeding under salt-free stress, but SA treatment could reduce MDA content in melilotus of ficinalis seedlings under salt stress. The content of soluble sugar and soluble protein were significantly increased with SA treatment in melilotus of ficinalis seedlings. CAT activity in melilotus of ficinalis seedlings was significantly improved with SA treatment at low salt stress, but CAT activity did not increased significantly at high salt stress.

The SOD activity of SA treated melilotus of ficinalis seedlings was increased compared with that of the control group, but it did not reach the significant level, and the improvement of SOD activity needs to be further verified. In conclusion, exogenous SA could significantly change the physiological characteristics of melilotus of ficinalis seedlings under salt stress, and alleviate the effects of salt stress on melilotus of ficinalis seedlings by increasing the content of osmotic substance, reducing the content of MDA, and increasing the activity of antioxidant enzymes. It was shown that exogenous SA treatment was one of the effective methods to improve the salt-tolerance of melilotus of ficinalis seedlings.

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