

## Physiological Response of Salicylic Acid on Physiological Characteristics of *Orychophragmus Violaceus* Seeding under Salt Stress

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**Abstract:** The effects of salicylic acid treatment on physiological properties of *orychophragmus violaceus* seedlings were studied under different salt stress. The results showed that the salt concentration was 0.03%, 0.20%, 0.35% and 0.53%, the soluble sugar content of *orychophragmus violaceus* seedlings treated with salicylic acid were increased by 20.44%, 13.47%, 11.67% and 10.66% respectively compared with the control group. The soluble protein in *orychophragmus violaceus* seedlings treated with salicylic acid increased significantly compared with the control group. Under salt content of 0.2% and 0.35% by the salicylic acid treatment, the content of proline in *orychophragmus violaceus* seedlings increased significantly compared with that of untreated ones. Salicylic acid could significantly reduce malondialdehyde content in the *orychophragmus violaceus* seedlings under salt stress. Under salt content of 0.35% and 0.50%, salicylic acid significantly increased superoxide dismutase activity in the *orychophragmus violaceus* seedlings. Exogenous salicylic acid could significantly change the physiological characteristics in *orychophragmus violaceus* seedlings under salt stress, and alleviate the salt stress.

### 1. Introduction

Soil salinization will cause soil hardening and reduce the available nutrients in the soil, and seriously endangering the growth of plants. It is one of the main stress factors affecting agricultural production [1-2]. Half of the world's irrigated land was affected by salinization to some degree in 2008[3]. The saline soil in China accounts for one third of the country's arable land area [4]. It is one of the effective methods to improve salinized soil for screening and planting salt-tolerant plants.

*Orychophragmus violaceus* is a biennial herb. It has good flower shape and color, high ornamental value, and plays a very important role in improving urban greening environment [5]. *orychophragmus violaceus* can be used as green manure in the dry land of North China, because it can safely overwinter in North China[6]. As green fertilizer, *orychophragmus violaceus* can reduce soil salt content and increase soil organic matter content. It has good effect of fertilizer application on salinized soil [7]. It has certain resistance to water stress [8], and also has certain salt tolerance [9]. It is of great significance to study the salt-tolerance of *orychophragmus violaceus* and the measures to enhance salt-tolerance for making full use of salinized soil.

Salicylic Acid (SA) is considered as a phenolic compound that can activate plant allergic reaction and obtain systemic resistance in response to environmental stress. It can induce the expression of related protein genes in plants to produce salt resistance, drought resistance, heat resistance and so on [10-11]. The effects of salt stress on seed germination and seedling physiological characteristics of *orychophragmus violaceus* have been reported [12-13]. The effect of SA on plant seedlings under salt stress has also been reported mostly on alfalfa [14], maize [15],

oat [16], wheat [17], Chinese cabbage[18], pepper[19] and cauliflower[20]. The effect of salicylic acid on the seed germination of *Orychophragmus violaceus* under NaCl stress was also observed[21], and effects of other exogenous substances on seed germination of *Orychophragmus violaceus* under sodium stress[22-23]. The effects of salt stress on physiological characteristics of *Orychophragmus violaceus* seedlings are fewer studied.

With *Orychophragmus violaceus* seed as test materials, the effects of SA on related enzyme activities and related osmotic substances in its seedlings were studied under different salt content. It provides theoretical basis and technical support for the cultivation of *Orychophragmus violaceus* on salinized soil.

## **2. Materials and Methods**

### **2.1 Experimental Materials**

The test material of *Orychophragmus violaceus* seed was provided by Tianjin Institute of Agricultural Resources and Environment.

### **2.2 Experimental Method**

Soak the seeds in 1% sodium hypochlorite solution for 10 minutes and rinse with 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 in the basin, and each basin was evenly seeded with 15 grains and repeated for 3 times. The salt content of the five soils was 0.03%, 0.20%, 0.35%, 0.50% and 0.65%, respectively, regulated by 0, 40, 80, 120 and 160 mmol/L NaCl solution. When the seedling emergence height reached 2 cm, 3 ml of 1 mmol/L SA 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 8 cm, and the activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) in the leaves were measured.

### **2.3 Determine Items and Measurement Methods**

The content of soluble protein was determined by coomassie bright blue method, activity of POD was determined by guaiacol method [24], activity of CAT was determined by ultraviolet absorption method, activity of 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.

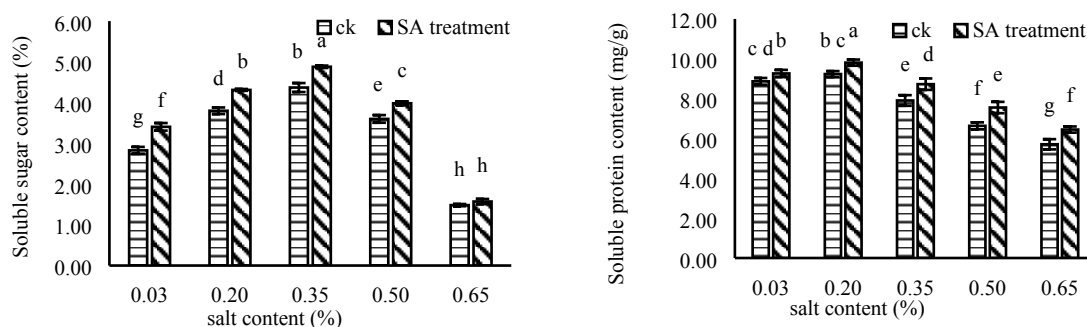
## **3. Results and Analysis**

### **3.1 Changes of Soluble Sugar and Protein Content in *Orychophragmus Violaceus* Seedlings Treated with SA under Different Salt Stress**

As can be seen from figure 1, both the control group and the treatment group showed the soluble sugar content in *Orychophragmus violaceus* seedling increased significantly with the increase of salt content in the salt range of 0.03% to 0.35%. The soluble sugar content decreased significantly with the increase of soil salt content in the salt range of 0.35%-0.65%. After SA treatment, The soluble sugar content of *Orychophragmus violaceus* seedlings increased significantly under salt stress after SA treatment. When the salt concentration was 0.03%, 0.20%, 0.35% and 0.50%, the soluble sugar content of *Orychophragmus violaceus* seedlings treated with SA were increased by 20.44%, 13.47%, 11.67% and 10.66% respectively compared with the control group. The high salt content of 0.65%

also increased by 5.92%, but it did not reach the significant level.

The soluble protein content in *orychopragmus violaceus* seedlings was increased significantly at the low salt content of 0.20%, and decreased significantly with the further increase of soil salt content. The content of soluble protein in the seedlings treated with SA was significantly increased compared with that without SA.



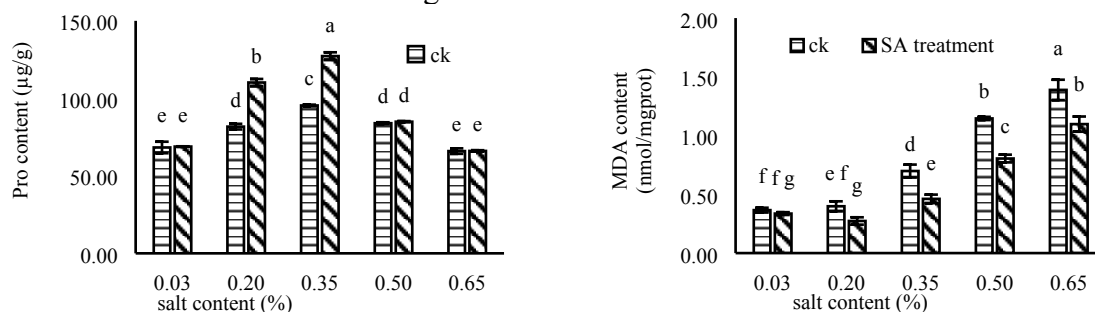
**Fig 1.** Changes of soluble sugar content and soluble protein content in *orychopragmus violaceus* seedlings treated with SA under different salt stress

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

### 3.2 Changes of Proline and MDA Content in *Orychopragmus Violaceus* Seedlings Treated with SA under Different Salt Stress

As can be seen in figure 2, at the range of soil salt content from 0.03% to 0.35%, the proline content in *orychopragmus violaceus* seedlings increased significantly with the increase of salt content, and showed a trend of significant decrease with the further increase of salt content. SA treatment had no significant effect on proline content in *orychopragmus violaceus* seedlings at low salt content of 0.03% and high salt content of 0.50%. At the salt content of 0.20% and 0.35%, the content of proline in *orychopragmus violaceus* seedlings treated with SA increased significantly compared with the control. Under certain salt content, SA can increase the content of proline to improve the salt resistance of *orychopragmus violaceus* seedlings.

The 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, the content of MDA in *orychopragmus violaceus* seedlings treated with SA did not decrease significantly compared with the control. Under other salt contents, the MDA content in *orychopragmus violaceus* seedlings treated with SA was significantly lower than the control. Plant tissues affected by salt stress will increase the content of MDA and reduce its protective ability. SA treatment could decrease the content of MDA and increase the salt tolerance of the seedlings.



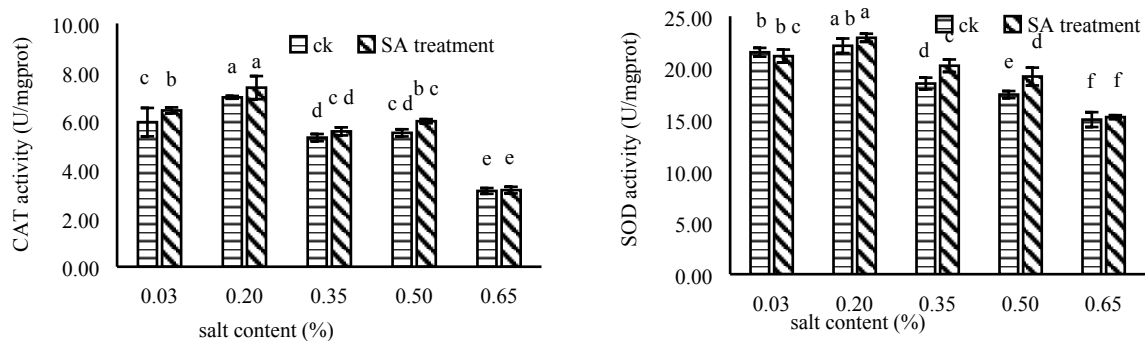
**Fig 2.** Changes of proline content and MDA content in *orychopragmus violaceus* seedlings treated with SA under different salt stress

### 3.3 Changes of CAT, SOD and POD Activities in *Orychopragmus Violaceus* Seedlings Treated with SA under Different Salt Stress

As can be seen from figure 3, the variation trend of CAT activity in control and treatment was

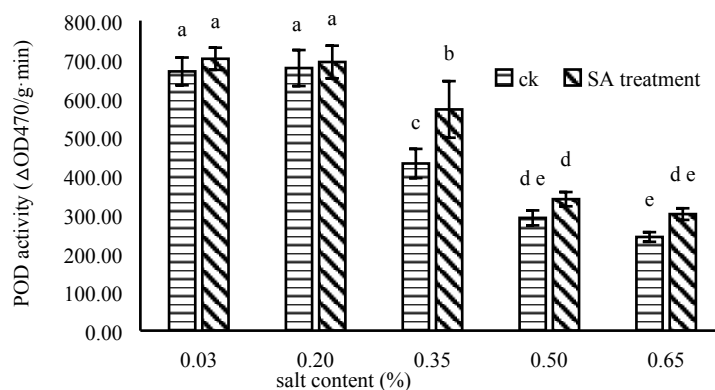
the same, and CAT activity decreased significantly under high salt content. Under the condition of 0.03% salt content, the CAT activity in *orychophragmus violaceus* seedlings treated with SA was significantly higher than that without SA treatment. Compared with the control treatment, the activity of CAT was increased by salicylic acid treatment under other salt content conditions, but it was not significant.

The activity of SOD in *orychophragmus violaceus* seedlings treated with SA was not significantly increased compared with the control under the salt content of 0.20% and 0.65%. SOD activity in the seedlings treated with SA was significantly increased compared with the control under the salt content of 0.35% and 0.50%.



**Fig 3.** Changes of CAT and SOD activities in *orychophragmus violaceus* seedlings treated with SA under different salt stress

As can be seen from figure 4, the variation trend of POD activity in control and treatment was the same, the decrease of POD activity was not significant in *orychophragmus violaceus* seedlings under the salt content of 0.20%. In the range from 0.20% to 0.65% salt content, POD activity decreased significantly with the increase of salt content. The SOD activity of the seedlings treated with SA increased compared with the control under the salt content of 0.20%, 0.50% and 0.65%, but not significantly. Under the salt content of 0.35%, SOD activity with SA treatment was significantly increased by 32.35% compared with the control.



**Fig 4.** Changes of POD activities in *orychophragmus violaceus* seedlings treated with SA under different salt stress

#### 4. Discussion

Salt stress can enhance osmotic stress and inactivate antioxidant enzymes in plant tissues[25]. The results showed that the activities of SOD, POD and CAT in *orychophragmus violaceus* seedlings were significantly decreased under high salt stress, and SA treatment significantly increased the activities of SOD and POD in *orychophragmus violaceus* seedlings under medium salt stress, which was consistent with the results of treating lettuce with exogenous SA[26]. Exogenous SA can significantly increase SOD activity in buckwheat leaves under salt stress[27], and improve SOD and POD activity in leaves of *solanum nigrum* seedlings[28]. These results are also similar to the results of this study. In this study, SA treatment increased CAT activity in *orychophragmus*

violaceus seedlings under salt stress, but not significantly. CAT, POD and SOD are important members of the antioxidant enzyme system in plants. They 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. SA treatment could induce the activity of cell protective enzymes in the seedlings, reduce the membrane peroxidation level caused by salt stress, and improve the salt tolerance of *orychopragmus violaceus* seedlings.

Salt stress leads to oxidation of membrane lipids of the cell membrane, and oxidize the unsaturated bonds in membrane fatty acids to form MDA and increasing the content of MDA[29], which further affects protein synthesis[30]. The content of MDA in *orychopragmus violaceus* seedlings was significantly reduced with SA treatment, and the degree of oxidative damage was obviously alleviated. In this experiment, the contents of soluble sugar, soluble protein and proline in *orychopragmus violaceus* seedlings were significantly increased with SA treatment. These results are consistent with the study on the physiological indexes and antioxidant activity of sweet clover seedlings with HS under NaCl Stress[31], and also consistent with the results of the study on the effects of exogenous BABA on *orychopragmus violaceus* seedlings under NaCl stress[32]. The results showed that the synthesis and accumulation of soluble sugar, soluble protein and proline in *orychopragmus violaceus* seedlings could be promoted with SA treatment under salt stress. These osmotic regulatory substances can reduce the osmotic potential of cells, enhance the water absorption capacity of plant cells, and alleviate the damage of salt stress to *orychopragmus violaceus* seedlings.

## 5 Conclusion

Soluble sugar content in *orychopragmus violaceus* seedlings was significantly increased under SA stress. The content of soluble protein in the seedlings treated with SA was significantly higher than that without SA. Under certain salt content, the content of proline in *orychopragmus violaceus* seedlings treated with SA treatment was significantly increased compared with the control without SA treatment. The content of MDA in *orychopragmus violaceus* seedlings could be significantly reduced with SA treatment under salt stress. The activities of SOD and POD in *orychopragmus violaceus* seedlings could be significantly increased with SA treatment under a certain degree of salt stress. The physiological characteristics of *orychopragmus violaceus* seedlings could be significantly changed by exogenous SA under salt stress. Exogenous SA could alleviate the effect of salt stress on *orychopragmus violaceus* seedlings by increasing the content of osmotic substance, reducing the content of MDA and increasing the activity of antioxidant enzyme. It was suggested that exogenous SA treatment was one of the feasible methods to improve the salt tolerance of *orychopragmus violaceus* seedlings.

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## References

- [1] Zhang Yongfeng, Liang Zhengwei, Sui Li, Cui Yanru. Effect on Physiological Characteristic of *Medicago Sativa* under Saline-alkali Stress at Seeding Stage [J]. *Acta Prataculturae Sinica*, 2009, 18(4):230-235. (in Chinese)
- [2] Zhou D, Lin Z L, Liu L M. Regional Land Salinization Assessment and Simulation through Cellular Automaton-Markov Modeling and Spatial Pattern Analysis [J]. *Science of the Total Environment*, 2012, 439:260-274.
- [3] Khan M H, Panda S K. Alterations in Root Lip Peroxidation and Antioxidative Responses in

- Two Rice Cultivars under NaCl-salinity Stress[J]. *Acta Physiologiae Plantarum*, 2008(30):81-89.
- [4] Guo Chengyuan, Kang Junshui, Wang Haisheng. Coastal Saline-alkali Land Suitable for Plants[M]. Beijing: China Building Industry Press, 2012. (in Chinese)
- [5] Shi Yuqiang, Shi Yingcai. The Application of *Orychophragmus Violaceus* in Urban Greening in Fuxin, Liaoning[J]. *Chinese Horticulture Abstracts*, 2014, (12):104-105. (in Chinese)
- [6] Liu Jia, Cao Weidong, Rong Xiangnong, Jin Qiang, Liang Jinfeng. Nutritional Characteristics of *Orychophragmus Oiolaceus* in North China[J]. *Soil and Fertilizer Sciences in China*, 2012, (1):78-82. (in Chinese)
- [7] Zhao Qiu, Gao Xianbiao, Wu Di, Cao Weidong. Salt Tolerance of Winter Green Manure *Orychophragmus Violaceus* and Its Fertilizer Effect in the Saline-alkaliarable Land[J]. *Soil and Fertilizer Sciences in China*, 2010, (4):65-68. (in Chinese)
- [8] Li Longmei, Wang Yicheng, Yan Haiou. Physiological and Biochemical in Water Stress of *Orychophragmus Violaceus*[J]. *Journal of Inner Mongolia Agricultural University*, 2012, 33(2):34-36. (in Chinese)
- [9] Zhu Pengfang, Pan Zhichao, Zhang Jiahui. Studies on Viability and Salt Tolerance in *Orychophragmus Violaceus* Seeds[J]. *Seed*, 2016, 35(9):46-50. (in Chinese)
- [10] Wang Lijun, Zhan Jicheng, Huang Wwidong. Salicylic Acid and Response to Stress in Plants[J]. *Plant Physiology Communications*, 2002, 38(6):619-624. (in Chinese)
- [11] Meng Xuejiao, Di Kun, Ding Guohua. Progress of Study on the Physiological Role of Salicylic Acid in Plant[J]. *Chinese Agricultural Science Bulletin*, 2010, 26(15):207-214. (in Chinese).
- [12] Dong Yaru, Yin Pengfei, Zhao Dongxiao, Geng Bing, Sun Jingshi, Wang Xiangyu. Seed Germination and Seedling Physiological Characteristics of *Orychophragmus Violaeus* under Seawater Stress[J]. *Shandong Agricultural Sciences*, 2019, 51(11):40-43. (in Chinese).
- [13] Li Yongjin, Liu Yuyan. Influence of Salt Stress on Seed Germination of *Orychophragmus violaceus*[J]. *Molecular Plant Breeding*, 2017, 15(6):2368-2374. (in Chinese).
- [14] Zhou Wanhai, Shi Shangli, Kou Jiangtao. Exogenous Salicylic Acid on Alleviating Salt Stress in Alfalfa Seedlings[J]. *Acta Prataculturae Sinica*, 2012, 21(3):171-176. (in Chinese)
- [15] Peng Hao, Song Wenlu, Wang Xiaoqiang. Effects on Seed Germination and Seedling Growth of Maize under Salt Stress with Salicy Acid and Abscisic Acid[J]. *Journal of Maize Sciences*, 2016,24(6):75-78+87. (in Chinese)
- [16] Yan Yanhua. Effects of Salicylic Acid on Seed Germination of Oat under Salt Stress[J]. *Seed*, 2020,39(1):159-162. (in Chinese)
- [17] Zhang Qian, He Mingrong, Chen Weifeng, Dai Xinglong, Wang Zhenlin, Dong Yuanjie, Zhuge Yuping. Effects of Extraneous Nitric Oxide and Salicylic Acid on Physiological Properties of Wheat Seedlings under Salt Stress[J]. *Acta Pedologica Sinica*, 2018,55(5):1254-1263. (in Chinese)
- [18] Li Runzhi, Jin Qing, Li ZhaoHu, Wang Ye, Peng Zhen, Duan LiuShen. Salicylic Acid Improved Salinity Tolerance of *Glycyrrhiza Uralensis* Fisch during Seed Germination and Seedling Growth Stages[J]. *Acta Agronomica Sinica*, 2020,46(11):1810-1816. (in Chinese)
- [19] Jia Luqi, Xiang Chunyang, Chen Peijing, Shang Tianqin, Du Jin, Cao Gaoyi. Effects of Salicylic Acid on Physiological Characteristics of Linear Pepper Seedlings under Salt Stress[J]. *Journal of Tianjin Agricultural University*,2020,27(3):39-42+48. (in Chinese)
- [20] Wang Yuping, Dong Wen, Zhang Xin, Yang Qian, Zhang Feng. Effects of Salicylic Acid on Seed Germination and Physiological Characters of Cauliflower Seedlings under Salt Stress[J]. *Acta Prataculturae Sinica*, 2012,21(1):213-219. (in Chinese)

- [21] HAN Jian-qiu. Effects of exogenous GA<sub>3</sub> on germination of *Orychopragmus violaceus* seeds under NaCl stress[J]. *Acta Agriculturae Shanghai*, 2012,28(2):59-62. (in Chinese)
- [22] Xu Lingxin, Yan Junxin, Deng Yanan, Hu Pengmin, Mu Yuanjun. Effects of CaSO<sub>4</sub> and GA<sub>3</sub> on Seed and Seedling of *Orychopragmus Violaceus* under NaHCO<sub>3</sub> Stress [J]. *Journal of Northeast Forestry University*, 2018,46(3):24-28. (in Chinese)
- [23] Xu Qian, Guo Shangjing, Wei Huitian, Su Yanyan, Ma Qingping, Ji Lusha. Effects of Exogenous BABA on Growth and Physiological Characteristics of Wheat Seedlings in *Orychopragmus violaceus* Under NaCl Stress[J]. *Northern Horticulture*, 2020,(12):75-81. (in Chinese)
- [24] Zhang Zhiliang, Zhai Weifu. *Experimental Guidance of Plant Physiology*[M]. Beijing: Higher Education Press, 2003. (in Chinese)
- [25] Zribi O T, Labidi N, Slama I, Debez A, Ksouri R, Rabhi M, Smaoui A, Abdely C. Alleviation of phosphorus deficiency stress by moderate salinity in the halophyte *Hordeummaritimum* L.[J]. *Plant Growth Regul.*, 2012,66(1) :75 -85.
- [26] Wu Jianhua, Wu Zhongxia, Wang Yuanhua, Feng Yingna, Yan Zhiming, Cai Shanya, Wang Quanzhi, Sun Ying. Effect of Salicylic Acid and Proline on Gene Expression Profiles in Response to Salt Stress in Lettuce[J]. *Acta Agriculturae Zhejiangensis*, 2017,29(9):1489-1497. (in Chinese)
- [27] Yang HongBing, Sun Ping. Effects of Exogenous Salicylic Acid and Jasmonic Acid on Physiological Traits of Salt Tolerance in Buckwheat (*Fagopyrum esculentum* Moench) Seedlings[J]. *Plant Physiology Journal*, 2012,48(8):767-771. (in Chinese)
- [28] Chang Yunxia, Xu Kedong, Yang Tongwen, Mo Jiayong, Chen Long. Mitigative Effects of Exogenous Salicylic Acid on the Inhibiti on of Drought Stress to Wild *Solanum Nigrum* Seedlings[J]. *Agricultural Research in the Arid Areas*, 2014,32(4):43-46+64. (in Chinese)
- [29] Yang S L, Chen K, Wang S S, Gong M. Osmoregulation as a key factor in drought hardening-induced drought tolerance in *Jatropha curcas*[J]. *Biologia Plantarum*, 2015,59(3):529-536.
- [30] Jiao H. Signaling Mechanism of Hydrogen Sulfide Regulating Seed Germination and Seedling Growth in Plant under Cadmium and Drought Stresses. Master Thesis, Hefei:Hefei University of Technology, 2010. (in Chinese)
- [31] Dong Jing, Li Hongli, Dong Zhi, Bai Wenhua. Effect of HS on Physiological Indexes and Antioxidant Activity of Sweet Clover Seedlings under NaCl Stress[J]. *Pratacultural Science*, 2018, 35(10):2430-2437. (in Chinese)
- [32] Xu Qian, Guo Shangjing, Wei Huitian, Su Yanyan, Ma Qingping, Ji Lusha. Effects of Exogenous BABA on Growth and Physiological Characteristics of Seedlings in *Orychopragmus Violaceus* under NaCl Stress[J]. *Northern Horticulture*, 2020, (12):75-81. (in Chinese)