

Effects of Exogenous Salicylic Acid on Photosynthetic Characteristics in *Orychophragmus Violaceus* Seeding under Salt Stress

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Abstract: The effects of salicylic acid treatment on photosynthetic characteristics of *orychophragmus violaceus* seedlings were studied under different salt stress. The results showed that the content of nitrogen and chlorophyll, net photosynthetic rate, transpiration rate and stomatal conductance were significantly reduced in *orychophragmus violaceus* seedlings under salt stress. The intercellular carbon dioxide concentration was increased under salt stress. Under salt concentration of 0.03%, 0.20%, 0.35% and 0.50%, net photosynthetic rate of seedlings treated with salicylic acid were increased by 17.29%, 13.37%, 21.34% and 21.48% respectively compared with those without SA. The stomatal conductance and transpiration rate were increased with salicylic acid treatment under salt stress, and the intercellular carbon dioxide concentration was significantly reduced with salicylic acid treatment under certain salt stress degree. The content of nitrogen and chlorophyll could be significantly increased by salicylic acid treatment under low salt stress. Exogenous SA could change the photosynthetic characteristics and increase the salt tolerance of the *orychophragmus violaceus* seedlings under salt stress.

1. Introduction

In 2000, the area of salinized farmland and salt wasteland in China was 8.67×10^7 hm², accounted for to 25% of the arable land area [1]. Under the influence of human cultivation and natural conditions, the area of soil salinization and secondary salinization is increasing year by year [2], which seriously affects the growth of plants and crop production. Salt stress can further affect the growth and development of plants by harming their photosynthetic system [3]. Improving photosynthetic capacity and salt resistance of plants in salinized soil is one of the main research contents of scientific researchers at present [4].

Orychophragmus violaceus is a biennial herb. It has good flower shape, color and high ornamental value. It plays a very important role in improving urban greening environment [5]. It 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 salt content in soil, and increase organic matter content in soil. It has better effect on fertilizing salinized soil [7]. Therefore, 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. And SA can induce the expression of related protein genes in plants to produce salt resistance and drought resistance [8]. Exogenous SA soaking can effectively regulate the photosynthetic structure and stomatal opening, promote the accumulation of photosynthetic pigment, and alleviate the damage of salt stress to cotton [9]. Studies have been

reported that SA can improve the salt tolerance of alfalfa [10], maize[11], wheat[12], Chinese cabbage[13] and pepper[14]. Exogenous SA can also promote photosynthetic efficiency, and thus improve salt tolerance of plants [9, 15-17]. The effect of SA on the seed germination and seedling physiological characteristics of *orychopragmus violaceus* under NaCl stress was also reported [18]. However, the effects of exogenous SA on photosynthetic characteristics of *orychopragmus violaceus* seedlings under salt stress have not been reported.

In this experiment, the effects of SA on photosynthetic characteristics of *orychopragmus violaceus* seedlings in soils of different salt content were studied. The effect of SA on salt tolerance of *orychopragmus violaceus* seedlings were probed from the perspective of photosynthetic characteristics, in order to provide theoretical basis for studying the regulation mechanism of SA on crop growth and development, and provide technical support for planting *orychopragmus violaceus* on salinized soil.

2. Materials and Methods

2.1 Experimental Materials

The test material of *orychopragmus 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 then rinse with clean water. The test was divided into control group and treatment group. Seeds were soaked with water for 12 h in the control group and seeds were soaked with 1 mmol/L SA for 12 h in the treatment group. Two kinds of treated seeds were sown in five salt content soil in the basin, and each basin was evenly seeded with 15 grains and repeated for 3 times. The salt contents of the five soils were 0.03%, 0.20%, 0.35%, 0.50% and 0.65%, which were adjusted with 0, 40, 80, 120 and 160 mmol/L NaCl solutions, respectively. When the seedling height reached 2 cm, each basin of the treatment group was sprayed with 3 ml of 1 mmol/L SA, each basin of the control group was sprayed with 3 ml of water. The contents of chlorophyll and nitrogen, net photosynthetic rate, stomatal conductance, intercellular carbon dioxide(CO₂) concentration and transpiration rate of the leaves were measured when the seedlings grew to 12 cm.

2.3 Determine Items and Measurement Methods

Chlorophyll content (SPAD value) and nitrogen content (mg/g) were measured using plant nutrition analyzer(TYS-3N). Net photosynthetic rate (P_n), intercellular CO₂ concentration (INT_CO₂), transpiration rate (E) and stomatal conductance (C) were measured using CI-340 Hand-Held System (USA CID, Inc.) from 9:00 am to 11:30 am. Each test was repeated ten times.

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 Effects of SA on the Net Photosynthetic Rate and the Stomatal Conductance of *Orychopragmus Violaceus* Seedlings under Different Salt Stress

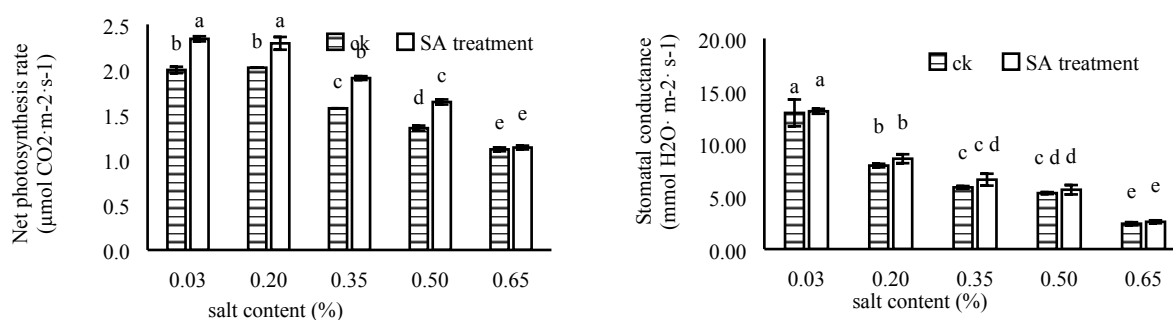


Fig 1. Changes of the net photosynthetic rate and the stomatal conductance in oryctophragmus violaceus seedlings treated with SA under different salt stress

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

As can be seen in figure 1, the net photosynthetic rate of both the control group and the treatment group did not change significantly under the low salt content (within 0.20%), and the net photosynthetic rate of oryctophragmus violaceus seedlings decreased significantly with the increase of salt content in the range of 0.20%-0.65%. In the range of 0.03%-0.50% soil salt content, the net photosynthetic rate of oryctophragmus violaceus seedlings could be increased significantly after SA treatment. Under the salt concentration of 0.03%, 0.20%, 0.35% and 0.50%, net photosynthetic rate of oryctophragmus violaceus seedlings treated with SA were increased by 17.29%, 13.37%, 21.34% and 21.48%, respectively, compared with the control group. The net photosynthetic rate also increased by 1.79% at high salt content of 0.65%, but it did not reach the significant level.

The stomatal conductance of oryctophragmus violaceus seedlings in both the control group and the treatment group decreased significantly with the increase of salt content under salt stress. Under the salt concentration of 0.03%, 0.20%, 0.35%, 0.50% and 0.65%, the stomatal conductance of oryctophragmus violaceus seedlings were increased by 1.20%, 9.20%, 12.46%, 6.02% and 7.47% after SA treatment, respectively, compared with the control group, but did not reach the significant level.

3.2 Effects of SA on the Intercellular CO₂ Concentration and the Transpiration Rate of Oryctophragmus Violaceus Seedlings under Different Salt Stress

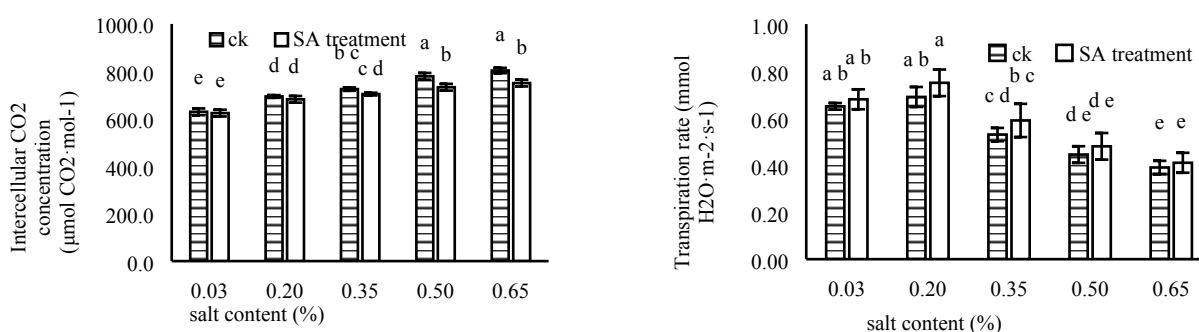


Fig 2. Changes of the intercellular CO₂ concentration and the transpiration rate in oryctophragmus violaceus seedlings treated with SA under different salt stress

As can be seen from figure 2, with the increase of salt stress, the intercellular CO₂ concentration of oryctophragmus violaceus seedlings showed a trend of gradual increase. Under salt content of 0.03%, 0.20% and 0.35%, intercellular CO₂ concentration of oryctophragmus violaceus seedlings treated with SA decreased by 0.87%, 1.73% and 2.90%, respectively, compared with the control group, but no at a significant level. Under the high salt content of 0.50% and 0.65%, the intercellular CO₂ concentration of oryctophragmus violaceus seedlings treated with SA was significantly reduced by 5.92% and 6.59%, respectively, compared with that without SA treatment.

With the increase of salt stress, the transpiration rate of both control group and treatment group showed a trend of increasing first and then decreasing. The transpiration rate was significantly

decreased at salt content of 0.20% and 0.35% in the control group, and at 0.20%, 0.35% and 0.50% in the treatment group. The transpiration rate of *orychophragmus violaceus* seedlings were increased after SA treatment compared with that without SA treatment at all kinds of salt stress, but it did not reach the significant level. SA increased the transpiration rate by 11.32% compared with the control group at 0.35% salt content.

3.3 Effects of SA on the SPAD Value and the Nitrogen Content of *Orychophragmus Violaceus* Seedlings under Different Salt Stress

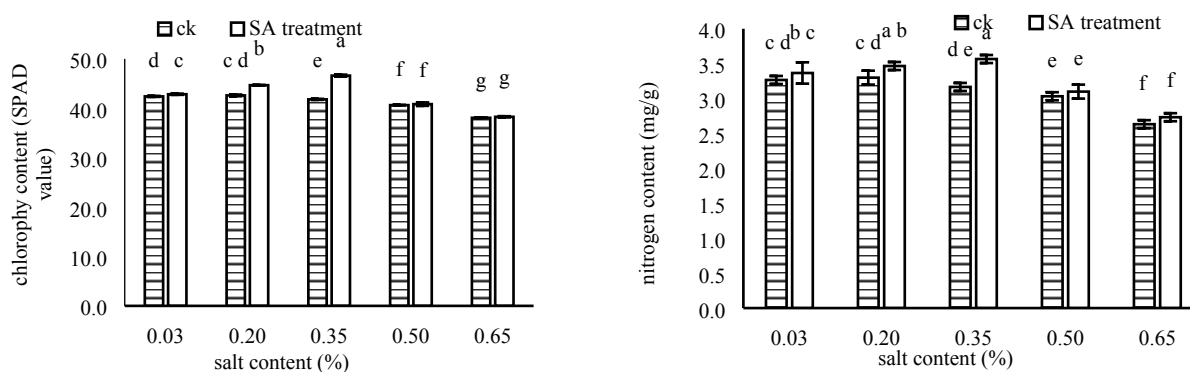


Fig 3. Changes of the SPAD value and the nitrogen content in *orychophragmus violaceus* seedlings treated with SA under different salt stress

The chlorophyll content with SA treatment was increased compared with the control (figure 3). With SA treatment, the chlorophyll content were significantly increased by 1.02%, 4.85% and 11.48% at the salt content of 0.03%, 0.20% and 0.35% respectively compared with those without SA. Under the high salt stress of 0.50% and 0.65%, the chlorophyll content of seedlings treated with SA increased by 0.49% and 0.52%, respectively, compared with control, but did not reach the significant level. The results showed that SA treatment could alleviate the effect of low salt stress on chlorophyll formation of *orychophragmus violaceus* seedlings, and then reduce the inhibition of salt stress on photosynthesis of seedlings.

As can be seen from figure 3, the nitrogen content of *orychophragmus violaceus* seedlings showed a trend of gradual decrease along with the increase of salt content in the control group, while it showed a trend of first increase and then decrease along with the increase of salt content in the SA treatment group. With SA treatment, the nitrogen content were significantly increased by 5.05% and 12.63% at salt content of 0.20% and 0.35% respectively compared with the control group. The nitrogen content were increased compared with the control group at the other salt contents, but not significant. The results indicated that SA treatment could alleviate the effect of salt stress on photosynthesis of *orychophragmus violaceus* seedlings, and also had a stable effect on nitrogen accumulation in seedling.

4. Discussion

Chlorophyll directly affects the rate of photosynthesis and the formation of photosynthetic products in plants, and the reduction of its content will have a significant impact on the photosynthesis of plants [19]. Salt stress will reduce the chlorophyll content of grape rootstocks, thus affecting their growth [20]. The results of this study showed that SA treatment could alleviate the effect of salt stress on chlorophyll formation of *orychophragmus violaceus* seedlings, which was similar to the result that exogenous H₂S could inhibit the decrease of chlorophyll content in tomato seedlings under salt stress [21]. The main way to obtain nitrogen in plants is to absorb nitrogen from soil. Salt stress affects the absorption of nitrogen from soil, and reduces the content of nitrate nitrogen (NO₃-N) in plants. Some studies [22] have reported that exogenous nitric oxide (NO) can alleviate the inhibitory effect of NaCl Stress on nitrogen metabolism of *reaumuria soongorica* seedlings, and this result is similar to the results of this study. SA treatment can alleviate the effect

of nitrate nitrogen absorption on *orychopragmus violaceus* seedlings under salt stress. Compared with control treatment, SA treatment can ensure the accumulation of nitrogen content in the *orychopragmus violaceus* seedlings under salt stress, which is beneficial to the growth of seedlings under salt stress.

Photosynthesis is an important physiological process of plants. The decrease of photosynthesis can reflect the environmental stress on plants during their growth. Net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO₂ concentration are all important parameters of photosynthetic characteristics. The net photosynthetic rate reflects the ability of plants to assimilate CO₂ and the growth status of plants. The transpiration rate reflects the transpiration of plants. Stomatal conductance reflects the difficulty of CO₂ and water vapor passing through leaf stomata. The intercellular CO₂ concentration affects the photosynthesis of plants. Salt stress can inhibit the photosynthetic rate and transpiration rate of rice leaves [23]. The results of this study showed that salt stress inhibited the net photosynthetic rate, stomatal conductance and transpiration rate of *orychopragmus violaceus* seedlings, which was consistent with the results of rice [23] and cucumber [24]. The results of increasing intercellular CO₂ concentration of February orchid seedlings under salt stress are different from those of cucumber [24], but consistent with the results of He Jun-yu et al. [15] and Bao Ying et al. [25]. That means it's complicated that the effect of salt stress on photosynthetic characteristics. Exogenous SA treatment increased the net photosynthetic rate, stomatal conductance and transpiration rate of lettuce seedlings under salt stress, and reduced the intercellular CO₂ concentration of lettuce seedlings under salt stress [15], which was basically consistent with the results of this study. Exogenous SA increased the net photosynthetic rate, stomatal conductance and transpiration rate of *orychopragmus violaceus* seedlings under salt stress, and decreased the intercellular CO₂ concentration. These test results indicate that exogenous SA could improve the photosynthesis of *orychopragmus violaceus* seedlings under salt stress, and could alleviate the damage of salt stress to seedlings. Salt stress can reduce the water absorption capacity of plants and lead to the loss of water, energy and electron transport of photosynthesis will be inhibited. Salt stress will further cause stomatal closure, stomatal conductance decrease, CO₂ diffusion is limited, and transpiration rate will decrease, thus leading to the decrease of plant photosynthesis rate [26]. Exogenous SA improves salt tolerance of *orychopragmus violaceus* seedlings, mainly by increasing stomatal conductance and chlorophyll content, and then increasing net photosynthetic rate. The mechanism of effect of SA on the photosynthesis of *orychopragmus violaceus* under salt stress remains to be further studied.

5. Conclusion

Salt stress significantly reduced nitrogen and chlorophyll content, net photosynthetic rate, transpiration rate and stomatal conductance in *orychopragmus violaceus* seedlings. Salt stress increased intercellular CO₂ concentration. SA treatment could significantly increase the net photosynthetic rate of *orychopragmus violaceus* seedlings under salt stress, and which could increase stomatal conductance and transpiration rate of *orychopragmus violaceus* seedlings under salt stress. SA treatment could significantly reduce the intercellular CO₂ concentration at a certain degree of salt stress. SA treatment could significantly increase the nitrogen and chlorophyll content of *orychopragmus violaceus* seedlings at low salt stress. Exogenous SA can change the photosynthetic characteristics of *orychopragmus violaceus* seedlings at salt stress, and increase the content of chlorophyll and nitrogen, and reduce the damage of salt stress on photosynthesis, and thus increase the photosynthesis of seedlings under salt stress. The results showed that the salt tolerance for seedling could be improved by treating seed and seedling with exogenous SA.

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References

- [1] Wang Baoshan, Zou Qi. Effects of NaCl Stress on the Tonoplast ATPase and PPase Activity in Roots, Sheaths and Blades of Sorghum Seedlings[J]. *Acta Phytophysiologica Sinica*, 2000, 26(3):181-188. (in Chinese)
- [2] Rozema J, Flowers T. Crops for a Salinized World[J]. *Science*, 2008, 322:1478-1480.
- [3] Zhu Xinguang, Zhang Qide. Advances in the Research on the Effects of NaCl on Photosynthesis[J]. *Chinese Bulletin of Botany*, 1999, 16(4):332-338. (in Chinese)
- [4] Wang Cong, Yang Hengshan, Dong Yongyi, Jia Jnuyin, Bao Jinhua, Lv Degui, Shen Xiangjun. Effects of Exogenous Ehitosan on Photosynthesis and Fluorescence Characteristics of Vegetable Soybean under NaCl Stress[J]. *Acta Botanica Boreali-Occidentalia Sinica*, 2015, 35(6):1198-1205. (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 Violaceus* 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 Violaes* and Its Fertilizer Effect in the Saline-alkaliarable Land[J]. *Soil and Fertilizer Sciences in China*, 2010, (4):65-68. (in Chinese)
- [8] 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).
- [9] Wang Lihong, Li Xingxing, Sun Yingying, Amanguli·Maimaitiali, Lazhati·Nuerbulati, Zhang Jusong. Effects of Soaking Seeds with Salicylic Acid on Cotton Seedling Growth and Photosynthetic Characteristics under Salt Stress[J]. *Agricultural Research in the Arid Areas*, 2017, 35(2):114-120. (in Chinese)
- [10] 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)
- [11] Peng Hao, Song Wenlu, Wang Xiaoqiang. Effects on Seed Germination and Seedling Growth of Maize under Salt Stress with Salicylic Acid and Abscisic Acid[J]. *Journal of Maize Sciences*, 2016, 24(6):75-78+87. (in Chinese)
- [12] 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)
- [13] 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)
- [14] 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)
- [15] He Junyu, Ren Yanfang. Effects of Exogenous Salicylic Acid on Growth and Photosynthetic Characteristics of Lettuce (*Lactuca sativa* L.) Seedlings under NaCl Stress[J]. *Journal of Hunan Agricultural University (Natural Sciences)*, 2009, 35(6):628-631. (in Chinese)
- [16] Sun Dezhi, Han Xiaori, Peng Jing, Fan Fu, Yang Hengshan, Ma Yulu, Hou Mihong. Protective Effect of Exogenous Nitric Oxide and Salicylic Acid on the Photosynthetic Apparatus of Tomato

- Seedling Leaves under NaCl Stress[J]. *Chin J Appl Environ Biol*, 2018, 24(3):0457-0464. (in Chinese)
- [17] Shao Changan, Zhang Yingjuan. Effects of Exogenous Salicylic Acid on Photosynthesis of Oats in Two Stages under Salt-alkali Stress[J]. *Journal of Inner Mongolia Normal University (Natural Science Edition)*, 2019, 48(3):257-262. (in Chinese)
- [18] 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)
- [19] Wang Zenghang, Wu Xianshan, Chang Xiaoping, Li Runzhi, Jing Ruilian. Chlorophyll Content and Chlorophyll Fluorescence Kinetics Parameters of Flag Leaf and Their Gray Relational Grade with Yield in Wheat[J]. *Acta Agronomica Sinica*, 2010, 36(2):217-227. (in Chinese)
- [20] Niu Ruimin, Xu Zehua, Shen Tian, Chen Weiping. Effects of Salt Stress on Growth and Chlorophyll Fluorescence Characteristics of Grape Rootstocks[J]. *Northern Horticulture*, 2018(21):85-89. (in Chinese)
- [21] Zheng Zhouyuan, Lin Hairong, Cui Huimei. Effect of Exogenous Hydrogen Sulfide on Photosynthesis Parameters and Chlorophyll Fluorescence Characteristics of Processing Tomato (*Lycopersicon esculentum* Mill ssp. *subspontaneum* Brezh) Seedlings Under NaCl Stress[J]. *Journal of Nuclear Agricultural Sciences*, 2017,31(7):1426-1435. (in Chinese)
- [22] Jia Xiangyang, Chong Peifang, Lu Wentao, Tian Yan. Effect of Foliar-spraying Nitric Oxide on the Nitrogen Metabolism Enzyme Activities and Nutrients in Leaves and Roots of *Reaumuria soongorica* Seedlings under NaCl Stress[J]. *Acta Bot. Boreal.-Occident. Sin.*, 2020,40(10):1722-1731. (in Chinese)
- [23] Wang Xuming, Zhao Xiaxia, Zhou Hongkai, Chen Jingyang, Mo Junjie, Xie Ping, Ye Changhui. Effects of NaCl Stress on Some Physiological and Biochemical Indices and Photosynthetic Physiology Characteristics of Rice Cultivars with Different Salt Tolerance[J]. *Chinese Journal of Tropical Crops*,2019,40(5): 882-890. (in Chinese)
- [24] Li Shuhao, Li Man, Zhang Wendong, Li Yiman, Ai Xizhen, Liu Binbin, Li Qingming. Effects of CO₂ Enrichment on Photosynthetic Characteristics and Reactive Oxygen Species Metabolism in Leaves of Cucumber Seedlings under Salt Stress[J]. *Acta Ecologica Sinica*, 2019,39(6): 2122-2130. (in Chinese)
- [25] Bao Ying, Wang Jiabin, Chen Chao, Yu Xinmiao. The Effects of NaCl and NaHCO₃ Stress on Photosynthesis and Chlorophyll Fluorescence Characteristics of *Hemerocallis Hybridus Stella de Oro*[J]. *Jiangsu Agricultural Sciences*, 2020, 48(3):133-140. (in Chinese)
- [26] Arbona V, Marco AJ, Iglesias DJ, López-Climent MF, Talon M, Gómez-Cadenas A. Carbohydrate Depletion in Roots and Leaves of Salt-stressed Potted Citrus Clementine L[J]. *Plant Growth Regulation*, 2005, 46(2):153-160.