

Effects of Melatonin on Photosynthetic Characteristics of Waxy Corn Seedlings under Different Salt Stress

Shengwei Guo^{1,a}, Chunyang Xiang^{1,b*}, Qiu Zhao^{2,c*}, Xiuping Tian^{1,d} and Lingli Liu^{1,e}

¹College of Agronomy and Resources and Environment, Tianjin Agricultural University, Tianjin, China.

²Tianjin Institute of Agricultural Resources and Environment, Tianjin, China.

^aguo8871@sina.com, ^bxiang5918@sina.com, ^cqiuzhao_2008@163.com, ^dtian5918@sohu.com, ^e605309021@qq.com

*Corresponding author

Keywords: Melatonin Treatment; Salt Stress; Waxy Corn; Photosynthetic Characteristics

Abstract: In order to study the effect of melatonin on photosynthetic characteristics of waxy corn seedlings at different salt stresses, Runnuo 73 was used as the test material. The seeds and seedlings were treated with exogenous melatonin through pot experiments in soils with different salt content. The photosynthetic characteristics, chlorophyll and nitrogen content of seedlings were measured at the seedling stage. The results showed that compared with the control group, the net photosynthetic rate of waxy corn seedlings at the salt concentration of 0.04%, 0.20%, 0.35% and 0.52%, the treatment group increased significantly by 16.19%, 29.12%, 36.45% and 38.92%, respectively. Compared with the control group, the transpiration rate of waxy corn seedlings in the treatment group was significantly increased at 0.20% salt stress, other treatment conditions were increased but not significantly. Compared with the control group, the waxy corn seedlings stomatal conductance of treatment group significantly increased at 0.04%, 0.20% and 0.35% salt stress, the high-salt treatment conditions were increased but did not reach the significant level. Compared with the control group, the intercellular CO₂ concentration of waxy corn seedlings in the treatment group was significantly reduced at 0.20% and 0.35% salt stress. Compared with the control group, the chlorophyll content of waxy corn seedlings in the treatment group was significantly increased at 0.20% and 0.35% salt stress. Compared with the control group, the nitrogen content of waxy corn seedlings in the treatment group was significantly increased by 7.7% and 6.5% at 0.20% and 0.35% salt stress, respectively. Indicating that melatonin treatment could increase the chlorophyll and nitrogen content, improve photosynthetic characteristics at salt stress and increase the salt tolerance of waxy corn seedlings.

1. Introduction

Corn (*Zea mays* L.) is the largest food crop in China, and it is also an important feed and economic dual use [1]. Therefore, corn has always occupied a high position in the field of agricultural planting in China. How to further increase output is a crucial subject of continuous research by scholars. With the destruction of the ecological environment, the area of saline-alkali land in China has continued to expand, and soil salinization has become an important factor restricting the increase in crop yields. The salt tolerance of corn is relatively poor [2]. High soil salinity inhibits the emergence of seedlings, obstruct the normal absorption of nutrients and water by the roots of plant seedlings [3], reduce the survival rate, destroy the structure of chlorophyll and inhibit its photosynthesis, further seriously reduce the yield of corn [4].

Adversity stresses such as drought and saline-alkali in the external environment are difficult to avoid. In the current research on mitigating plant abiotic stress, various plant growth regulators have outstanding advantages such as easy absorption, high efficiency and no pollution [5], which is consistent with sustainable development strategy. In recent years, based on the requirements of the

development of agriculture, scholars have conducted studies on the photosynthetic and physiological characteristics of corn seedlings such as jasmonic acid [4], salicylic acid [6], betaine [7] and melatonin [8]. The results showed that such exogenous substances have a positive effect on improving crop resistance.

Melatonin (N-acetyl-5-methoxytryptamine, MT) as an animal hormone was first discovered from the pineal gland of animals to regulate many physiological activities [9]. It has been found to exist in plants in 1993 [10]. Melatonin has the effects of regulating plant root growth [11], promoting photosynthesis, increasing seed germination rate [12] and delaying leaf senescence [13]. As a plant growth regulator, an appropriate amount of melatonin could alleviate a variety of adversity stresses such as drought [14], high temperature [15], low temperature [16], saline-alkali [17]. And it could also play a defensive role in various diseases such as rice stripe disease [18], rice blast [19], apple spot brown disease [20], banana wilt [21]. The application of exogenous melatonin significantly reduces the incidence of plant diseases and significantly improves the disease resistance of plants. Current researches mostly focus on ordinary corn. With the rapid development of waxy corn in recent years, the growing area of planting has expanding, which has become a new research hotspot. Taking waxy corn that has rarely been studied as an example. Test materials to explore the effects of melatonin on photosynthetic characteristics of waxy corn seedlings under different salt stresses, to provide theoretical basis and technical support for waxy corn planting on saline soil. This study has important ecology for the improvement of saline soil significance.

2. Materials and Methods

2.1 Test Materials and Processing

The test material was the waxy corn variety Runnuo 73, which was provided by Zhongtian Runnong Technology Co., Ltd. Select uniform seeds, sterilize them with 1% sodium hypochlorite solution for 10 minutes, rinse them with clean water. Then soak them with water (control group) and 1 mmolL⁻¹ melatonin (treatment group) for 12 hours at 20°C. The seeds of the two treatments are sown separately in flowerpots with various saline soil as the substrate, 10 holes are sown evenly in each pot. Repeat 3 times. The experiment set up 5 increasing salt concentrations of 0.04%, 0.20%, 0.35%, 0.52% and 0.68%, adjusted with 0, 40, 80, 120 and 160 mmolL⁻¹ NaCl solution [22].

2.2 Item Measurement and Method

Chlorophyll content (SPAD value) and nitrogen content (mg/g) were measured with a plant nutrition analyzer (TYS-3N); CI-340 ultra-light portable photosynthesis analyzer (CID, USA) (CI-340 Hand-Held Photosynthesis System) (USA CID, Inc.) In the morning when the light is good, from 11: 00 to 12: 00, the middle part of the fully expanded leaf of the corn seedling was selected to determine the net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO₂ concentration.

2.3 Methods Data Statistics and Analysis

The DPS7.05 software system Duncan's new multiple range method was used for multiple comparative analysis (P<0.05), and Excel 2016 was used for data sorting and plotting.

3. Results and Analysis

3.1 The Effect of Melatonin on the Net Photosynthetic Rate and Transpiration Rate of Waxy Corn Seedlings under Different Salt Stresses

As shown in figure 1, control group and treatment group were significantly decreased by 69.15% and 72.94% with the increase of soil salt concentration from 0.04% to 0.68%, respectively. Indicating that salt stress could significantly inhibit the net photosynthetic rate of waxy corn seedlings. In addition, compared with the control group, the net photosynthetic rate of waxy corn seedlings at the salt concentration of 0.04%, 0.20%, 0.35% and 0.52%, the treatment group

increased significantly by 16.19%, 29.12%, 36.45% and 38.92%, respectively. Indicating that the waxy corn seedlings treated with melatonin could enhance its salt tolerance. At the high-salt treatment conditions the net photosynthetic rate of waxy corn seedlings was increased but did not reach the significant level.

It can be seen from figure 2 that with the increase of salt concentration, the transpiration rate of waxy corn seedlings gradually decreased. Compared with the control group, the transpiration rate of waxy corn seedlings was higher in the treatment group. Compared with the control group, the transpiration rate of waxy corn seedlings in the treatment group was significantly increased by 13.33% at 0.20% salt stress, other treatment conditions were increased but not significantly.

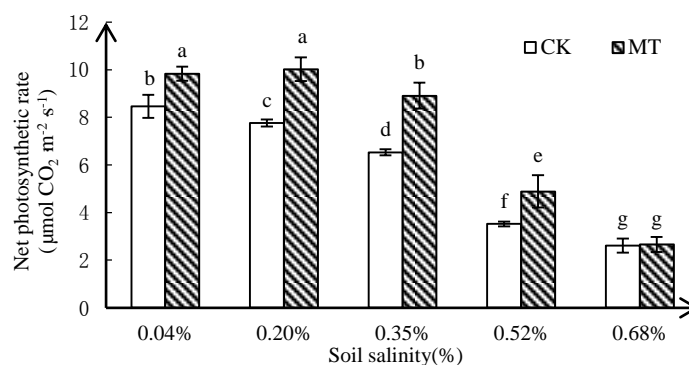


Fig 1. Effect of exogenous melatonin(MT) on net photosynthetic rate of waxy corn seedlings under different salt concentrations

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

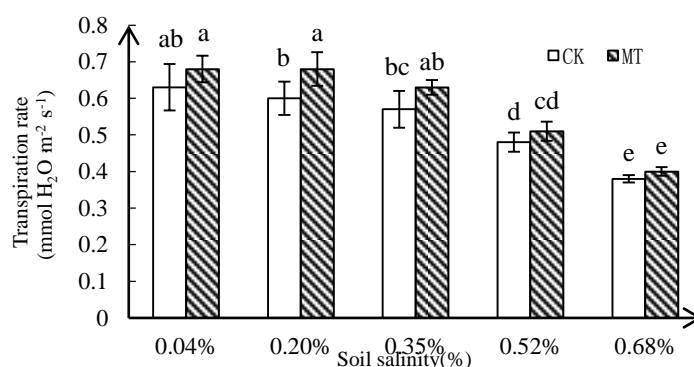


Fig 2. Effect of exogenous melatonin(MT) on transpiration rate of waxy corn seedlings under different salt concentrations

3.2 Effects of Melatonin on Stomatal Conductance and Intercellular CO₂ Concentration of Waxy Corn Seedlings under Different Salt Stresses

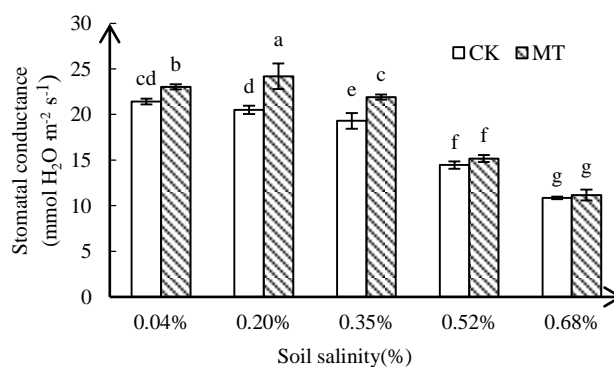


Fig 3. Effect of exogenous melatonin(MT) on stomatal conductance of waxy corn seedlings under different salt concentrations

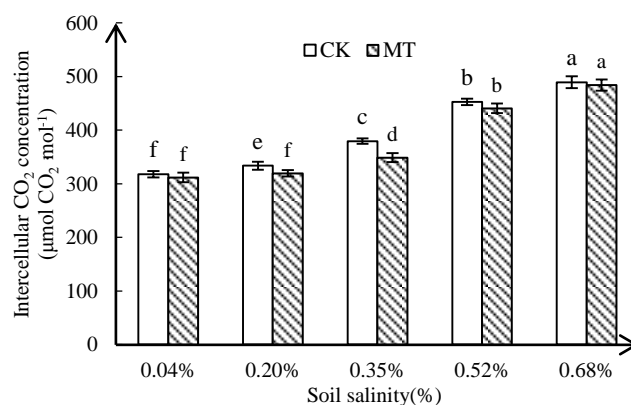


Fig 4. Effects of exogenous melatonin(MT) on the intercellular CO₂ concentration of waxy corn seedlings under different salt concentrations

According to figure 3, it can be seen that the stomata conductance of waxy corn seedlings in the treatment group is higher than that of the control group at each salt concentration. The stomatal conductance of the control group waxy corn seedlings decreased with the increase of salt stress, while the stomatal conductance of the treatment group waxy corn seedlings increased first and then decreased. Compared with the control group, the stomatal conductance of waxy corn seedlings in the treatment group reached the highest at 0.20% salt stress, which was significantly increased by 17.94%. Compared with the control group, the stomatal conductance of waxy corn seedlings treatment group was significantly increased by 13.52% at 0.35% salt stress. The stomatal conductance of waxy corn seedlings was increased at 0.52% and 0.68% salt stress, but did not reach a significant level.

The intercellular CO₂ concentration is opposite to the stomatal conductance. As shown in figure 4, as the salt stress increased from 0.040% to 0.68%, the intercellular CO₂ concentration of waxy corn seedlings increased significantly by 53.93%. At the same soil salt stress, the intercellular CO₂ concentration of the treatment group was lower than that of the control group. Compared with the control group, the intercellular CO₂ concentration of waxy corn seedlings treatment group was significantly reduced by 4.20% and 8.09% at 0.20% and 0.35% salt stress.

3.3 Effects of Melatonin on SPAD Value and Nitrogen Content of Waxy Corn Seedlings under Different Salt Stress

It can be seen from figure 5 that with the increase of soil salt concentration in the treatment group, the SPAD value first increased and then decreased. Compared with the control group, the SPAD value of waxy corn seedlings in the treatment group at 0.20% and 0.35% the salt stress significantly increased by 11.73% and 12.88%, respectively. The SPAD value decreased at 0.52% and 0.68% salt stress, but it still increased by 1.57% and 4.61%, respectively, compared with the salt stress at 0.04%.

Figure 6 is similar to figure 5. The nitrogen content of waxy corn seedlings in the treatment group increased first and then decreased with the increase in soil salt concentration. Compared with the control group, the nitrogen content of waxy corn seedlings in the treatment group was significantly increased by 7.7% and 6.5% at 0.20% and 0.35% salt stress, respectively. The nitrogen content of waxy corn seedlings in the control group and the treatment group was the same at 0.52% salt stress. The above results indicate that melatonin treatment could increase the chlorophyll and nitrogen content, improve photosynthetic characteristics at salt stress and increase the salt tolerance of waxy corn seedlings.

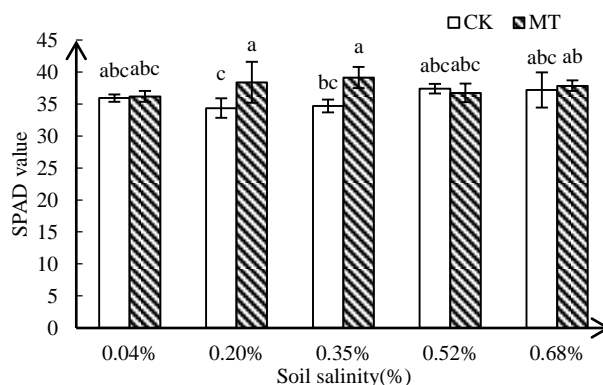


Fig 5. Effect of exogenous melatonin(MT) on SPAD value of waxy corn seedlings under different salt concentrations

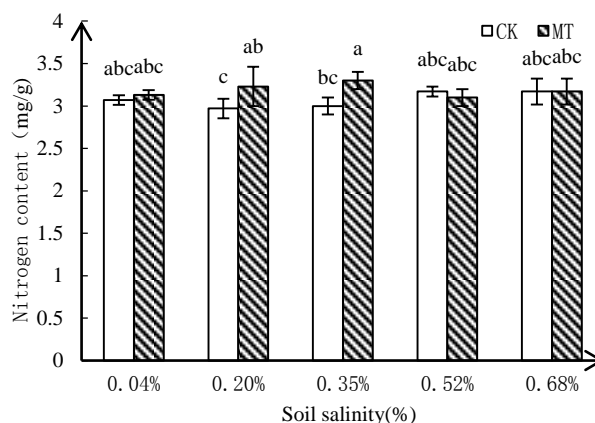


Fig 6. Effect of exogenous melatonin(MT) on nitrogen content of waxy corn seedlings under different salt concentrations

4. Discussion and Conclusion

It is inevitable that crops will encounter various environmental stresses during the growth process [23]. In order to ensure the yield of crops, it is the most effective method to select and breed superior varieties with strong resistance, but the breeding is difficult, and the long breeding cycle is also a problem in the research process. Therefore, the application of exogenous substances to alleviate the inhibition of crop growth due to environmental stress is currently a better way that can be immediately used for production and meets the sustainable development of agriculture which has a good development prospect.

The photosynthesis of crop seedlings is an important factor that affects its own growth and development. It is greatly affected by adversity stress, and has a significant impact on yield. Gas exchange and CO₂ fixation are two important reaction processes in photosynthesis. At salt stress, the photosynthetic rate, transpiration rate, and stomatal conductance of corn all decreased, destroying the osmotic balance of plant leaves and inhibiting photosynthesis [4]. The results of this study indicate that the net photosynthetic rate, transpiration rate and stoma conductance of waxy corn seedlings gradually decrease at five different salt stress, while waxy corn treated with melatonin had improve the above three indicators at the same salt concentration. The improvement effect of exogenous melatonin on corn seedlings at 0.20% and 0.35% salt stress was significantly, it reduces the damage of light and mechanism, and relieves salt stress. It is similar to the research results of Li Wenyang [24], Wang Mingquan [25]. Besides, the latter also compared different resistant and susceptible corn varieties. Salt stress will damage the photosynthetic system, and the structure of the photosynthetic system of the strong salt-tolerant inbred line is relatively more stable. Han Zhiping also obtained the conclusion that photosynthetic rate, transpiration rate and stomatal

conductance all showed a downward trend under salt stress in experiments on watermelon seedlings [26]. Contrary to the first three indicators, the intercellular CO₂ concentration increases with the increase of salt stress. The waxy corn treated with melatonin lower than the control group, which also corresponds to a positive response to salt stress. The SPAD value and nitrogen content in waxy corn seedlings increased first and then decreased after the application of exogenous melatonin. The improvement is significant when the soil salt concentration is 0.20% and 0.35%, the increase was not significant under high-salt treatment in the later stage, it may be because of the salt that the concentration has exceeded the adaptability of waxy corn seedlings. In summary, this study shows that salt stress will directly affect the normal photosynthesis and growth of crops. Seed soaking with exogenous melatonin can effectively alleviate the damage to waxy corn photosynthesis and normal growth caused by excessive salt concentration, thereby improving its growth and salt tolerance.

Acknowledgements

Tianjin Agricultural Scientific and technological Achievement transformation Project (202101090), National Green fertilizer industry technology system project (CARS-22).

References

- [1] Chen Yadong, Guo Shumin, Kou Yuantao, Liu Xianwu. (2018) Research on the Construction of My Country's Corn Industry Policy and Regulation System[J]. *Corn Science*, 26(2):166-172. (in Chinese)
- [2] Xie Yao, Tang Yan, Liu Xia, Meng Jing, Wang Lei. (2020) Research Progress Analysis of Maize Adversity Stress Mechanism Based on Bibliometrics[J]. *Journal of Agricultural Big Data*, 2(2):112-124. (in Chinese)
- [3] Chen Xian, Yang Yong, Liu Fengquan. (2020) Research Progress of Plant Melatonin[J]. *Jiangsu Agricultural Sciences*, 48(24):17-24. (in Chinese)
- [4] Zhou Xiaofu, Wang Yixuan. (2019) Effects of Exogenous Jasmonic Acid on Photosynthetic Characteristics of Corn under Salt Stress[J]. *Journal of Jilin Normal University (Natural Science Edition)*, 40(4):80-86. (in Chinese)
- [5] Du Zhuo, Hou Wen, Wang Li, Li Lin, Zhang Kai, Lu Yuncai. (2020) The Effect of Exogenous Melatonin on Maize Seedlings under Drought Stress[J]. *Chinese Agricultural Science Bulletin*, 36(27):14-19. (in Chinese)
- [6] Cao Shanshan, Du Jin, Lin Jianhua. (2020) Effects of Salicylic Acid on the Germination of Corn Hybrids under Salt Stress[J]. *Tianjin Agriculture and Forestry Science and Technology*, (5):3-5. (in Chinese)
- [7] Xinghong Yang, Congming Lu. (2005) Photosynthesis is Improved by Exogenous Glycinebetaine in Salt-stressed Maize Plants[J]. *Physiologia Plantarum*, 124(3):343-352.
- [8] Zhao Chengfeng, Wang Chenguang, Li Hongjie, Zheng Xuehui, Yang Mei, Zhang Renhe. (2021) Effects of Exogenous Melatonin on Photosynthesis of Maize Leaves under Drought and Rewatering Conditions[J]. *Acta Ecologica Sinica*, 41(4):1-9. (in Chinese)
- [9] Carey Pratt McCord, Floyd P. Allen. (1917) Evidences Associating Pineal Gland Function with Alterations in Pigmentation[J]. *Journal of Experimental Zoology*, 23(1):207-224.
- [10] David L. Van Tassel, Nicholas Roberts, Alfred Lewy, Sharman D. O'Neill. (2001) Melatonin in Plant Organs[J]. *Journal of Pineal Research*, 31(1):8-15.
- [11] Chen Qian, Qi Wen-bo, Reiter Russel J., Wei Wei, Wang Bao-min. (2009) Exogenously Applied Melatonin Stimulates Root Growth and Raises Endogenous Indoleacetic Acid in Roots of Etiolated Seedlings of *Brassica juncea*[J]. *Journal of Plant Physiology*, 166(3):324-328.

- [12] Junpeng Li, Chen Zhao, Mingjing Zhang, Fang Yuan, Min Chen. (2019) Exogenous Melatonin Improves Seed Germination in *Limonium Bicolor* under Salt Stress[J]. *Plant Signaling & Behavior*, 14(11):1-10.
- [13] Arnao M. B., Hernández-Ruiz J.. (2009) Protective Effect of Melatonin Against Chlorophyll Degradation during the Senescence of Barley Leaves[J]. *Journal of Pineal Research*, 46(1):58-63.
- [14] Guo Yanyang. (2020) Effects of Exogenous Melatonin on Photosynthetic and Physiological Characteristics of Maize under Drought Stress[D]. Northwest Sci-Tech University of Agriculture and Forestry. (in Chinese)
- [15] Xu Xiangdong, Sun Yan, Guo Xiaoqin, Sun Bo, Zhang Jian. (2010) Effects of Melatonin on the Ascorbic Acid Metabolic System of Cucumber Seedlings under High Temperature Stress[J]. *Chinese Journal of Applied Ecology*, 21(10):2580-2586. (in Chinese)
- [16] Wu Xuexia, Zhu Zongwen, Zhang Aidong, Xu Shuang, Yao Jing, Zha Dingshi. (2017) Effects of Exogenous Melatonin on the Growth, Photosynthesis and Antioxidant System of Eggplant Seedlings under Low Temperature Stress[J]. *Northwestern Journal of Botany*, 37(12):427-2434. (in Chinese)
- [17] Liu Zheng, Hu Suntian, Shen Xiaofei, Zhu Pei, Chu Xudong, Li Zhuoya, Luo Ping. (2020) Alleviating Effects of Exogenous Melatonin Treatment on Salt Stress of Rose Seedlings[J]. *Journal of Zhejiang A and F University*, 37(5):957-962. (in Chinese)
- [18] Rongfei Lu, Zhiyang Liu, Yudong Shao, Feng Sun, Yali Zhang, Jin Cui, Yijun Zhou, Wenbiao Shen, Tong Zhou. (2019) Melatonin is Responsible for Rice Resistance to Rice Stripe Virus Infection Through a Nitric Oxide-dependent Pathway[J]. *Virology Journal*, 16(1):141.
- [19] Xu Qiuyi, Chen Zhaohui, Ji Fuyan, Shi Haojie. (2018) Melatonin Enhances the Resistance of Rice Seedlings to Rice Blast under Salt Stress[J]. *Anhui Agricultural Sciences*, 46(9):126-128. (in Chinese)
- [20] Lihua Yin, Ping Wang, Mingjun Li, Xiwang Ke, Cuiying Li, Dong Liang, Shan Wu, Xinli Ma, Chao Li, Yangjun Zou, Fengwang Ma. (2013) Exogenous Melatonin Improves *Malus* Resistance to Marssonina Apple Blotch[J]. *Journal of Pineal Research*, 54(4):426-434.
- [21] Shi Haitao, Chen Yinhua, Tan Dun-Xian, Reiter Russel J., Chan Zhulong, He Chaozu. (2015) Melatonin Induces Nitric Oxide and the Potential Mechanisms Relate to Innate Immunity Against Bacterial Pathogen Infection in *Arabidopsis*[J]. *Journal of Pineal Research*, 59(1):102-108.
- [22] Xiang Chunyang, Tian Xiuping, Du Jin, Cao Gaoyi, Zhao Fei, Liu Jian. (2020) An Adjustment Method of Soil Salt Gradient Suitable for Physiological Research of Corn under Salt Stress [P]. Tianjin:CN111869359A. (in Chinese)
- [23] Wang Fang, Liu Yan, Wang Tiebing, Wang Peng. (2020) Study on the Mitigation Effect of Exogenous Melatonin on Salt Stress of Maize Seedlings[J]. *Chinese Journal of Grassland*, 42(5): 14-21. (in Chinese)
- [24] Li Wenyang, Hu Xiujuan, Wang Changjin, Han Kunlong. (2019) Effects of Salt Stress on Seedling Growth and Leaf Photosynthetic Characteristics of Different Maize Varieties[J]. *Ecological Sciences*, 38(2):51-55. (in Chinese)
- [25] Wang Mingquan, Fu Lixin, Li Guoliang, Hu Guanghui, Ren Honglei, Hu Shaoxin, Yang Jianfei, Liu Chang, Gong Shichen. (2021) Studies on the Photosynthesis Mechanism of Salt Tolerance of Maize Resistant Germplasm at Seedling Stage[J]. *Chinese Agricultural Science Bulletin*, 37 (5):8-14. (in Chinese)
- [26] Han Zhiping, Guo Shirong, Jiao Yansheng, Fan Huaifu, Li Jun. (2008) Effects of NaCl Stress on Watermelon Seedling Growth and Photosynthetic Gas Exchange Parameters[J]. *Northwestern Journal of Botany*, 28(4):745-751. (in Chinese)