Effects of Melatonin on the Physiological Characteristics of Maize Seedlings under Different Salt Stress

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Abstract: In order to reveal the effect of melatonin on the anti-salt stress capacity of maize seedlings under different salt stress, the antioxidant enzyme activity, soluble protein, soluble sugar, proline (PRO) and malondialdehyde (MDA) content of maize seedlings treated with melatonin (1mmol/L) at 5 different salty soil content were explored (The soil salt content is 0.04%, 0.20%, 0.35%, 0.52% and 0.68%, respectively). The results showed that when the salt content was 0.20% and 0.35%, the content of MDA in the leaves of the melatonin-treated maize seedlings was significantly lower than that of the untreated, and the content of PRO increased significantly. The soluble sugar content, catalase (CAT) and peroxidase (POD) activity in the leaves of melatonin-treated maize seedlings increased significantly when the salt content in soil was 0.04%, 0.20%, 0.35% and 0.52%, the soluble protein content in the leaves of melatonin-treated maize seedlings will be significantly increased in the case of soil salt content of 0.04%, 0.20%, and 0.35%, and when the salt content was 0.20%, 0.35% and 0.52%, the activity of the superoxide dismutase (SOD) in melatonin-treated maize seedling leaves was also significantly increased, indicating that melatonin can reduce salt damage and improve the salt resistance of seedlings by increasing the antioxidant enzyme activity of maize seedling leaves under different salt stress and osmotic balance regulation.

1. Introduction

It has been the main ecological environment problem facing in China about the aggravation of land salinization at present, the saline soil area accounts for about 20% of the arable area in China [1], and there is an increasing trend of the salinization area in recent years, which makes a serious difference on the normal growth and development of maize, maize yield and quality. Maize is a moderately salt-sensitive maize and the most widely distributed grain maize of the Gramineae family in China [2]. However, salinization to varying degrees exists in the main maize producing areas in China [3], which seriously hinders the growth and development of maize and the sustainable development of agriculture. It has become a hot issue in agricultural production to enhance the salt-resistant coercion ability of maize and promote the effective use of saline land.

The research shows that the growth regulator of exogenous plant can effectively improve the resistance of maize and is an effective way to relieve the biological and abiotic stress of maize. Melatonin belongs to the compound, also known as N-acetyl-5-methoxychromide, is a growth hormone similar to the plant hormone [4], which has a positive effect on improving plant resistance and delaying aging [5]. Melatonin, as an antioxidant, can enhance the antioxidant ability of plants under adversity stress, reduce the level of reactive oxygen and active nitrogen in the organism, thereby improving the resilience of plants [6]. Studies have shown that melatonin can improve the activity and content of antioxidant enzymes in kiwi leaves, eliminate reactive oxygen and relieve

salt damage [7]. With the reduction of lipid peroxidation levels to promote the low temperature adaptability of rape seedlings [8], melatonin can also enhance the salt resistance of apple birch [9], improve the fruit quality of tomatoes under salt stress [10]. But so far, little research has been reported on the use of melatonin in maize, and there has been a lack of in-depth research on the effects of maize on physiological characteristics under different salt stresses. In this study, with the maize variety runnuo 73 as the material, analyzing and determining the physiological index of maize seedling leaves under different salt stress, and exploring the physiological mechanism of melatonin affecting the response of maize salt stress, with a view to providing a scientific basis for the use of exonym melatonin to alleviate salt stress injury, is of great significance to the development of maize industry in arid saline region of China.

2. Materials and Methods

2.1 Experimental Materials

The test material is the maize variety runnuo 73, provided by Zhongtian Runnong Technology Co, Ltd.

2.2 Experimental Method

Select a full and consistent maize seed and disinfect 10min with 10% sodium hypochlorite, rinsed with sterile water and spare. The experiment used potted soil cultivation method, set up water treatment (control group) and melatonin treatment (treatment group) two groups of tests, respectively, at 20℃ immersion 12h, and then two groups of treated seeds are planted in different salt concentration soil as a base pot, each pot evenly sown 10 holes, soil salt content set 5 levels, respectively, with 0 mmol/L, 40 mmol/L, 80 mmol/L, 120 mmol/L, and 160 mmol/L NaCl solutions were adjusted to determine the salinity of the soil at 0.04%, 0.20%, 0.35%, 0.52% and 0.68% respectively, repeating each treatment 3 times. On the 15th day, the control group used water, and the treatment group sprayed the seedlings with melatonin solution for 5 ml to determine the physiological index of the seedlings on the 22nd day.

2.3 Determine Items and Measurement Methods

Take maize seedling leaves, the content of soluble protein was determined by Coomassie bright blue method, activity of CAT was determined by ultraviolet absorption method, activity of SOD was determined by hydroxylamine method, content of soluble sugar was determined by ketone colour method, content of MDA was determined by thiobarbituric acid (TBA) method, content of POD was determined by ultraviolet spectrophotometer method, content of PRO was determined by colorimetric method.

2.4 Data Processing

Data processing and draw graphs were performed by using Excel 2010, and data statistical analysis was used by DPS software.

3. Results and Analysis

3.1 Effect of Melatonin on MDA Content of Maize Seedling Leaves under Different Salt Stress

Plants under the condition of adversity stress, membrane lipid peroxidation will occur, MDA is one of the final decomposition products, its content can reflect the degree of cell membrane lipid peroxide and the strength of the plant's response to adversity conditions, the higher the content, the more serious the stress damage to cells. The results of the MDA content of the control groups of maize (Figure 1) show that, under different concentrations of salt stress treatment, the higher the salt content of the soil, the higher the content of MDA in maize leaves, and the highest MDA content of 0.68% in the soil, the highest MDA content of the control group was 1.07 nmol/mgprot. The MDA content in maize leaves in melatonin treatment group showed a decreasing trend,

compared with the control group, MDA content decreased by 6.45%, 15.38%, 22.86%, 6.90%, 3.74% under 5 salt content, respectively. The content of MDA in the melatonin treatment group decreased significantly at the salt content of 0.20% and 0.35%, which showed that the melatonin-treated seeds could effectively inhibit the content of membrane lipid peroxide in maize leaves under salt stress, thus alleviating oxidation damage.

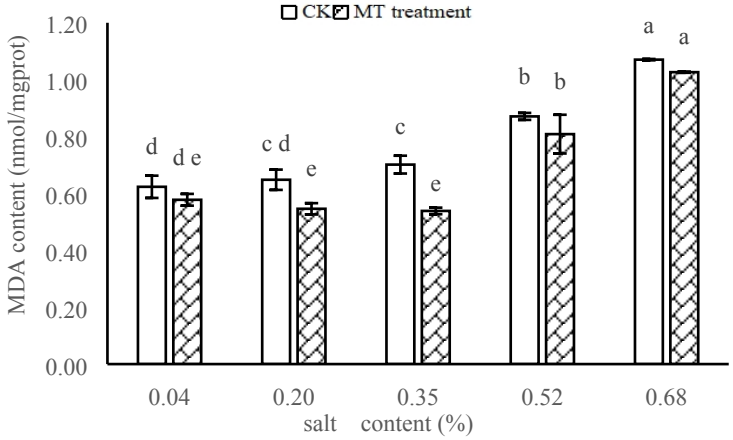

Fig 1. Changes in the MDA content of maize leaves under different treatments

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

3.2 Effects of Melatonin on the Permeation and Regulation of Substance Content in Maize Seedling Leaves under Different Salt Stress

As can be found from figure 2, when the soil salt content was 0.04%-0.52%, the soluble sugar content of melatonin treatment group compared with control group was significantly increased, and the salt content of soil was 0.20%, the soluble sugar content in the treatment group reached the highest, 13.18%, an increase of 24.22% over the control group. PRO is the main organic substance regulating osmotic pressure in plants, and its content is positively related to the plant's ability to withstand stress. As shown in figure 3, under salt stress treatment, the PRO content of the control group decreased with the increase of soil salt content, and when the salt content was 0.2%-0.35%, the PRO content of the melatonin treatment group was significant compared to that of the control group. The increase was the highest percentage increase at 0.35%, at 26.09%. Soluble protein is one of the protective mechanisms of plant resistance to saline stress, and the adversity of salt stress can activate protease to make it active. The data in figure 4 show that when the salt content is 0.04%-0.35%, the soluble protein content of the melatonin treatment group is significantly higher than that of the control group, and the percentage increase at 0.35% is 12.91%, reaching the highest percentage increase, which proves that melatonin-treated seeds can stimulate the activity of enzymes, so that plants are in a more active physiological state, but also can increase the salt stress in the maize seedling leaves osmotic regulation of the content of substances, and thus resist salt stress to plant damage.

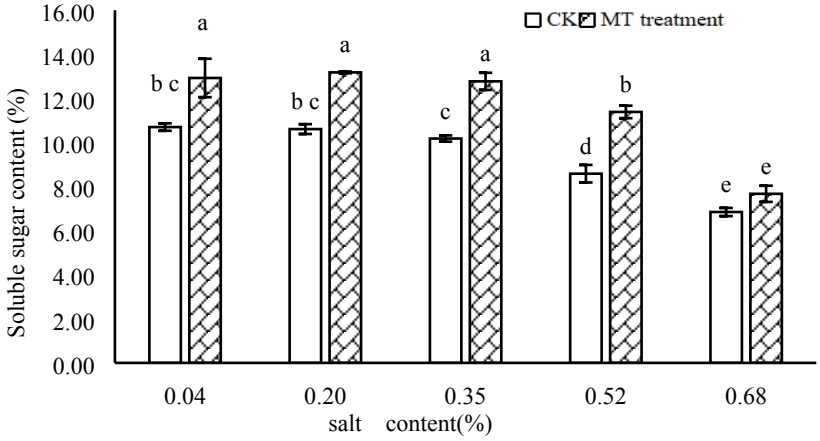

Fig 2. Changes in soluble sugar content of maize leaves under different treatments

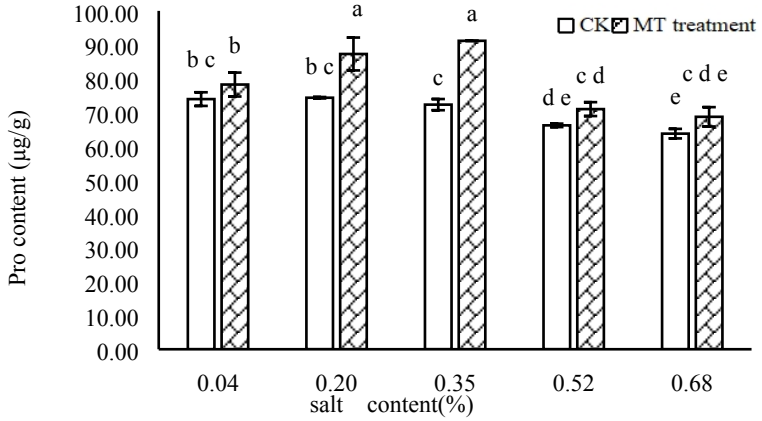

Fig 3. Changes in PRO content of maize leaves under different treatments

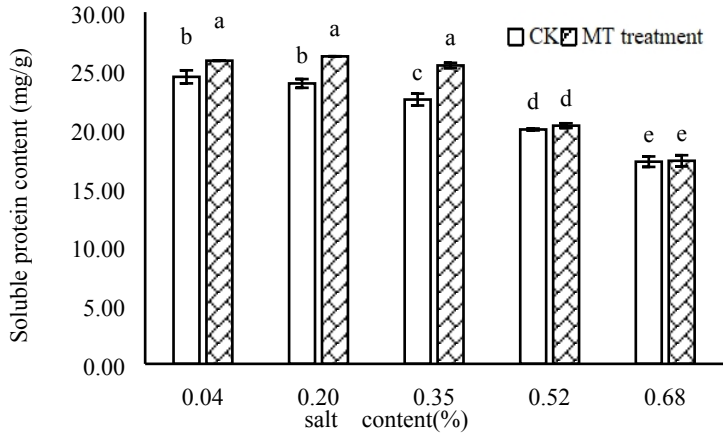

Fig 4. Changes in soluble protein content of maize leaves under different treatments

3.3 Effects of Melatonin on Antioxidant Enzyme Activity in Maize Seedling Leaves under Different Salt Stress

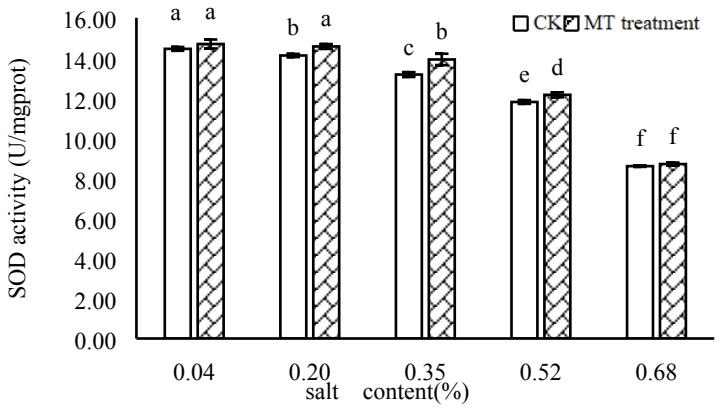

Fig 5. Changes in the activity of maize leaf SOD enzymes under different treatments

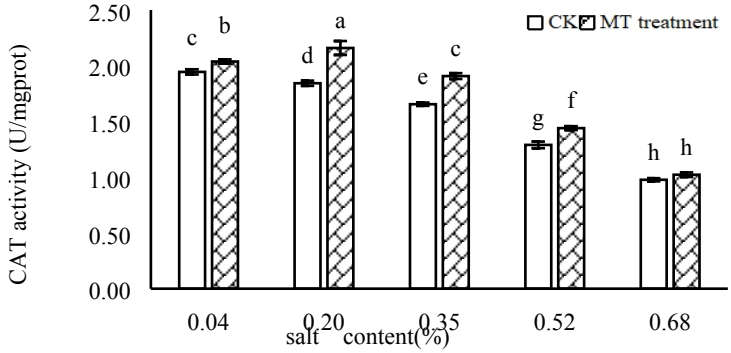

Fig 6. Changes in the activity of maize leaf CAT enzymes under different treatments

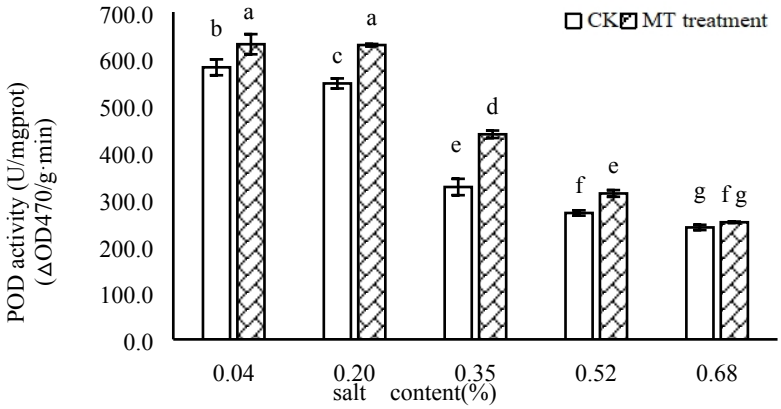

Fig 7. Changes in the activity of maize leaf POD enzymes under different treatments

The antioxidant enzyme activity of seedlings in the control group was significantly reduced under salt stress treatment. Because melatonin had the effect of removing reactive oxygen, the activity of antioxidant enzymes increased in the treatment group compared to the control group. As shown in figure 5, figure 6 and figure 7, the activity of SOD increased significantly by 3.19%, 5.69% and 3.05% at the salt content of 0.20%, 0.35% and 0.52%, respectively, and the enzyme activity also reached the highest percentage increase value of 5.69% when the salt content of soil was 0.35%. The activity of CAT increased significantly by 4.62%, 17.39%, 15.06% and 11.63% at the salt content of 0.04%,0.20%, 0.35% and 0.52%, respectively, with the highest percentage increase in activity at 0.20% of soil salt content, at 17.39%. The activity of POD increased significantly by 8.47%, 15.00%, 34.59% and 15% at the salt content of 0.04%,0.20%, 0.35% and 0.52%, respectively, compared with the control group, and the percentage increase in enzyme activity at 0.35% of soil salt content reached a maximum of 34.59%. The normal metabolic balance of seedlings and the higher antioxidant enzyme activity of melatonin treatment group seeds indicate that melatonin could improve the activity and content of antioxidant enzymes in cells under salt

stress, thereby improving the ability of maize to resist adversity stress.

4. Discussion

Salt stress is one of the main factors affecting the normal growth and development of plants and the yield of quality [11]. Melatonin, as a plant growth regulator, has a positive effect on alleviating the damage plants suffer under the stress of adversity [12]. The study shows that melatonin treatment could effectively reduce the content of MDA in the leaves of maize seedlings under salt stress, but also increase the content of soluble sugars, PRO and soluble proteins, and also have an effect on the enhancement of antioxidant enzyme (SOD, POD, CAT) activity, thereby increasing the resistance of maize seedlings, mitigating the damage of salt stress to maize seedlings.

Under the condition of salt stress, a large amount of reactive oxygen will be produced in the plant[13], and if the reactive oxygen cannot be removed in time, it will induce membrane lipid peroxidation, resulting in a large amount of MDA accumulation in the cell, destroying the permeability of the cell membrane, and affecting the normal metabolism of the plant [14].The results of this study show that melatonin treatment can inhibit the accumulation of MDA in maize seedlings under salt stress conditions and protect the permeability and integrity of cell membranes. Its action may be melatonin as an antioxidant substance, involved in the removal process of in-cell reactive oxygen, so that reactive oxygen maintained at a relatively low level, melatonin also inhibits membrane lipid peroxidation, protect the stability of membrane structure, thereby reducing the damage of salt stress to maize seedlings.

PRO, soluble sugar and soluble protein are important osmotic regulators in plants, and some studies have suggested that the accumulation of PRO can maintain cell puffing and maintain the osmosis balance in plants, thus increasing the resistance of plants to salt stress[15];Soluble proteins can, to some extent, reflect the metabolic levels of plant life activity[16].Fan Haixia[17] found that under the condition of salt stress, the exogenous melatonin treatment of reed seedlings, the activity of antioxidant enzymes and the content of osmotic regulatory substances such as PRO were significantly higher than the control group of added water treatment, the content of MDA was also significantly reduced, so it can alleviate the salt stress on the growth inhibition of reed seedlings. This study shows that in the control group treated with salt, the content of soluble protein, soluble sugar and PRO in maize seedling leaves showed a decreasing trend, while in the melatonin treatment group, the content of osmotic regulatory substances in the leaves increased significantly, which may be because melatonin inhibits the degradation of soluble proteins, or as a signal substance participates in the synthesis of soluble protein. Melatonin also regulates the metabolism of PRO and soluble sugars. The accumulation of osmotic regulatory substances is also conducive to reducing cell penetration, helping cells to absorb water from the outside world and maintain normal metabolic levels. This suggests that melatonin can improve the salt resistance of maize seedlings by regulating cell penetration and enhancing cell metabolic levels.

Plants accumulate large amounts of reactive oxygen under adversity conditions, and SOD, POD, and CAT are key enzymes in plants that remove reactive oxygen and inhibit enzyme oxidation, preventing excessive accumulation of reactive oxygen [18]. In this experiment, the activity of antioxidant enzymes in maize seedlings in melatonin treatment group was significantly higher than in the control group. Liu [19] have shown that external melatonin treatment can improve the activity of SOD, POD, and CAT in cucumber seedlings under salt stress, remove excess reactive oxygen, and alleviate the damage caused by cucumber salt stress. Therefore, it can be inferred that the melatonin-treated seeds, melatonin content increased, activated the enzyme activity in the antioxidant enzyme system, is conducive to further remove excess reactive oxygen, protect membrane lipids from peroxidation damage, so that cells can metabolize normally, and thus alleviate the oxidation damage caused by salt stress on maize, improve the salt resistance of maize.

In summary, melatonin treatment can reduce the degree of membrane lipid peroxidation, reduce the accumulation of MDA, can improve the level of osmotic regulating substances, slow down the degradation of soluble proteins, increase the content of PRO and soluble sugars, but also can improve the activity of antioxidant enzymes, conducive to the removal of reactive oxygen. This test

shows that melatonin can improve the anti-salt damage ability of maize seedlings, presumably because melatonin as an antioxidant involved in the process of reactive oxygen removal, while inhibiting the peroxidation of membrane lipids, by increasing the activity of antioxidant enzymes to enhance the antioxidant capacity of plants, and thus enhance the anti-salt stress capacity of maize seedlings, to make full use of melatonin to alleviate salt stress injury provides a scientific basis for the development of China arid saline region maize industry.

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