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The Physiological Response of Three *Narcissus pseudonarcissus* under NaCl Stress

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Abstract

In order to explore the salt-tolerance of *Narcissus pseudonarcissus* and enrich its cultivation and application forms. In this experiment, "Manly", "Dutch" and "Wilden" daffodils were used as test materials. NaCl stress was applied at different concentrations, and the corresponding physiological indexes were measured at three time nodes: bolting stage, early flowering stage and blooming stage. The results showed that all three showed some resistance under low concentration salt stress, in which, SOD, POD enzyme activity, chlorophyll proline and soluble sugar content increased, while MDA content decreased. The physiological metabolism of the three was disturbed under high concentration of salt stress, in which, SOD, POD enzyme activity and chlorophyll content decreased, while MDA, proline and soluble sugar content increased. With the extension of stress time, the injury was gradually deepened and the salt tolerance of the three varieties is "Dutch", "Wilden" and "Manly" in order.

Keywords

Narcissus pseudonarcissus, NaCl Stress, Antioxidase, MDA, Proline, Soluble Sugar

1. Introduction

Soil salinization is a common problem in the world, and it is one of the abiotic stress factors that seriously restrict global agricultural production [1], according to statistics, more than 800 million hm^2 of land in the world is affected by salinization to varying degrees [2]. China has 27 million hm^2 of salinized land, among which the coastal areas are seriously affected by salinization, about 5.0106 hm^2, which restricts the local agricultural economic development [3]. Therefore, the screening and cultivation of salt-tolerant plants and the study of salt-tolerant mechanism are of great significance to the development of saline-alkali land and

the local greening and ecological construction.

Narcissus pseudonarcissus is a perennial bulb flower of Amaryllidaceae *Narcissus*, which originating from Spain, Britain and other Mediterranean coastal areas [4]. It is one of the most eminent commercial bulbous flowers welcomed in the world flower market because of its unique flower type and flower color [5]. *Narcissus pseudonarcissus* has a very high ornamental value, as a world famous bulb flower, it is widely used in Chinese landscape [6], but whether it can grow normally in saline and alkaline land has not been studied. Therefore, in order to fully develop and utilize saline-alkali land, enrich the cultivation of *Narcissus pseudonarcissus*, in this experiment, three commonly used varieties of *Narcissus pseudonarcissus* were used as test materials to study their physiological responses under different flowering stages and different concentrations of salt stress, and to explore their tolerance under salt stress, so as to provide reference for future cultivation and application.

2. Materials and Methods

2.1. Plant Materials and Treatment

Three imported varieties of *Narcissus pseudonarcissus* "Manly", "Dutch" and "Wilden" were used as experimental materials, the substrate was mixed with peat, coconut bran, vermiculite and perlite in a ratio of 4:4:1:1, commercial seed balls with consistent specifications were selected and cultivated in a double-color basin with diameter of 10 cm and height of 8 cm, the upper covering matrix was 2 - 3 cm, which was cultured in a solar greenhouse until root and germination. Sunlight greenhouse always stay day and night temperature of 12˚C/7˚C. And shade the bulbs properly before budding.

NaCl solution with a concentration of 0 (CK), 100, 300, 500 mmol/l was used to treat the small buds when they were exposed to soil, and 30 pots of plants were used for each treatment, and 100 ml was poured from 7:00 to 9:00 in the morning, and once every two days until moss extraction. The physiological indexes of the middle of functional leaves of the same parts were measured in the three stages of bolting, early flowering and blooming.

2.2. Determine Items and Methods

Soluble sugar content was determined by anthrone colorimetry [7]. The content of free proline was determined by sulfosalicylic acid extraction method [7]. Chlorophyll content in leaves was determined by 80% acetone extraction and spectrophotometry [7]. The content of malondialdehyde (MDA) was determined by thiobarbituric acid method [8]. Superoxide dismutase (SOD) activity was determined by NBT photochemical reduction method, and POD activity was determined by guaiacol method [7]. All the experiment was repeated 3 times.

2.3. Statistics and Analysis

SPSS 22.0 software was used to process the test data, LSD method was used to

analyze the significance of the difference, and the data was expressed as the mean value of three times.

3. Results and Analysis

3.1. Effects of Salt Treatment on Chlorophyll Content in Leaves of *Narcissus pseudonarcissus*

Plant chlorophyll is involved in the absorption, transmission and transformation of light energy, and its content is an important indicator reflecting the strength of photosynthesis [9] [10]. As can be seen from **Figure 1**, the chlorophyll content of all three varieties increased with the extension of time. The growth of "Manly" was the most obvious, which increased from 7.815 $mg \cdot g^{-1} \cdot FW$ (bolting stage) to 17.041 $mg \cdot g^{-1} \cdot FW$ (blooming period), followed by "Dutch" and "Wilden". With the increase of salt content, all of three varieties showed a trend of first increasing and then decreasing. At a low concentration of 100 mmol/l NaCl salt stress, it had a maximum value, indicating that the low concentration of NaCl salt stress conditions would promote the synthesis of chlorophyll in *Narcissus pseudonarcissus*, and improve the stress resistance of *Narcissus pseudonarcissus* to resist the damage of salt stress. After NaCl concentration exceeded 300 mmol/l, the chlorophyll content of the three varieties began to decline significantly in each period, indicating that chlorophyll in the body of the *Narcissus pseudonarcissus* was seriously degraded with the increase of salt injury level, thus affecting its physiological functions such as photosynthesis. However, the blooming periods of "Manly" and "Wilden" both showed a continuous decline, the chlorophyll content was always lower than the control level. It may be that with the extension of salt injury time, the chlorophyll degradation in their bodies was greater than the synthetic amount. The Photosynthesis effect began to weaken, and the plants were subjected to a certain degree of stress damage [11]. From the perspective of chlorophyll content and change trend, "Dutch" is the most resistant to salt damage among the three varieties, followed by "Wilden" and "Manly".

3.2. Effects of Salt Treatment on MDA Content in Leaves of *Narcissus pseudonarcissus*

Malondialdehyde (MDA) is a product of membrane lipid peroxidation, and its content can directly reflect the degree of cell membrane damage [12]. At the same time, MDA can also bind to membrane proteins, affect the structure of membrane proteins, trigger the polymerization between proteins, change the membrane permeability, affect the transport of osmotic regulatory ions, and thus affect the osmotic potential [13].On the other hand, MDA also affects the structure of thylakoid membrane, causing chlorophyll degradation and thereby affecting plant photosynthesis [14].At the same time, MDA, as a membrane lipid peroxide product, also has a feedback effect on the antioxidant protection system of cells, affecting its antioxidant enzyme activity [15].

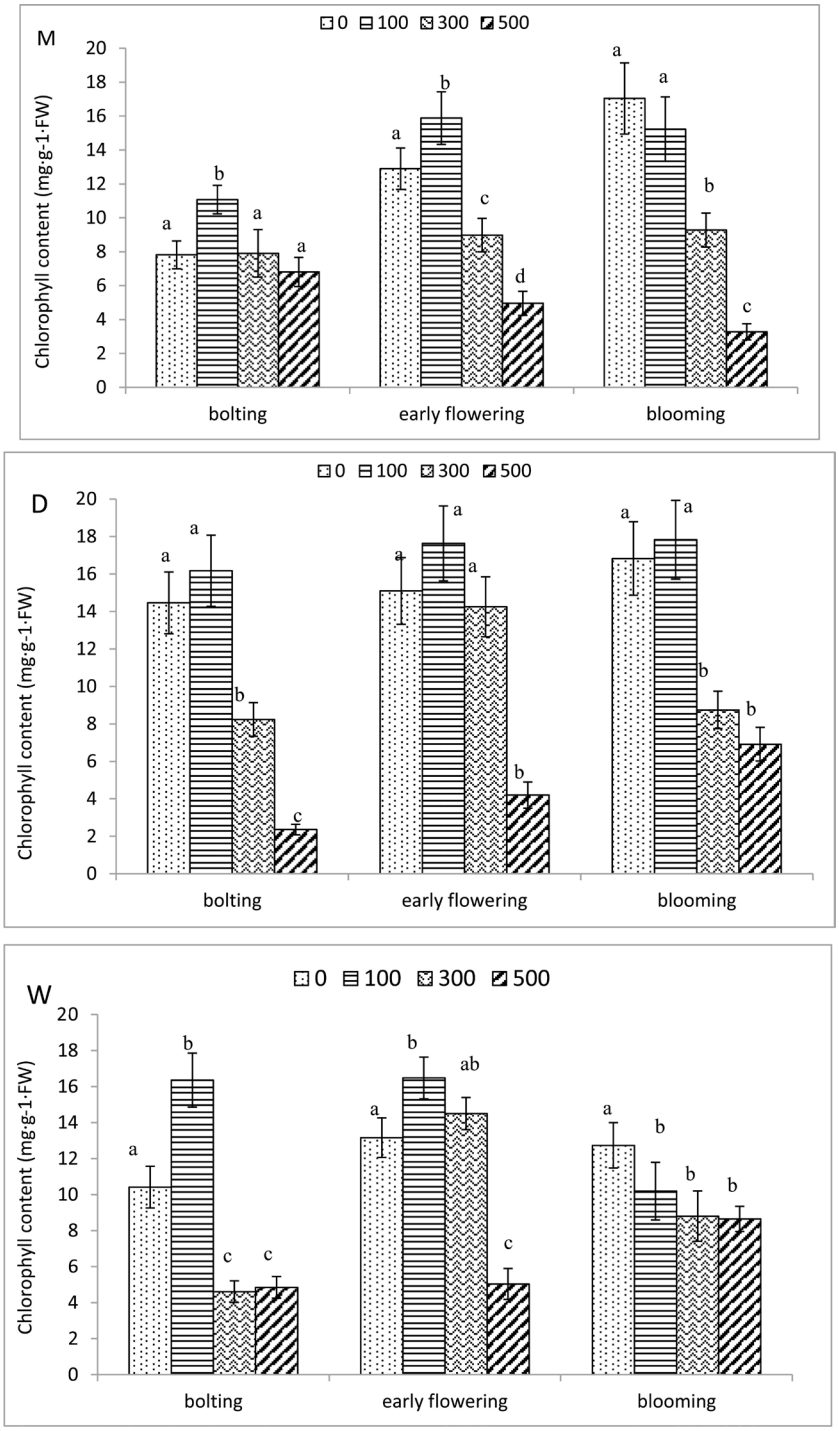

Figure 1. Changes of chlorophyll content in *Narcissus pseudonarcissus* leaves under salt treatment (M stands for "Manly"; D stands for "Dutch"; W stands for "Wilden". Different lowercase consonants indicate significant differences. The same below.)

Under low concentration salt stress (100 - 300 mmol/L NaCl) (**Figure 2**), MDA content in the three *Narcissus pseudonarcissus* varieties in this experiment increased slowly, even lower than that in the control. It maybe that the low concentration of salt stress promoted the activity of antioxidant enzymes in the

plants of narcissus, which accelerated the metabolism of superoxide, peroxide and other harmful substances and reduced the damage to the cell membrane system. And when NaCl concentration tendency for more than 300 mmol/L, the

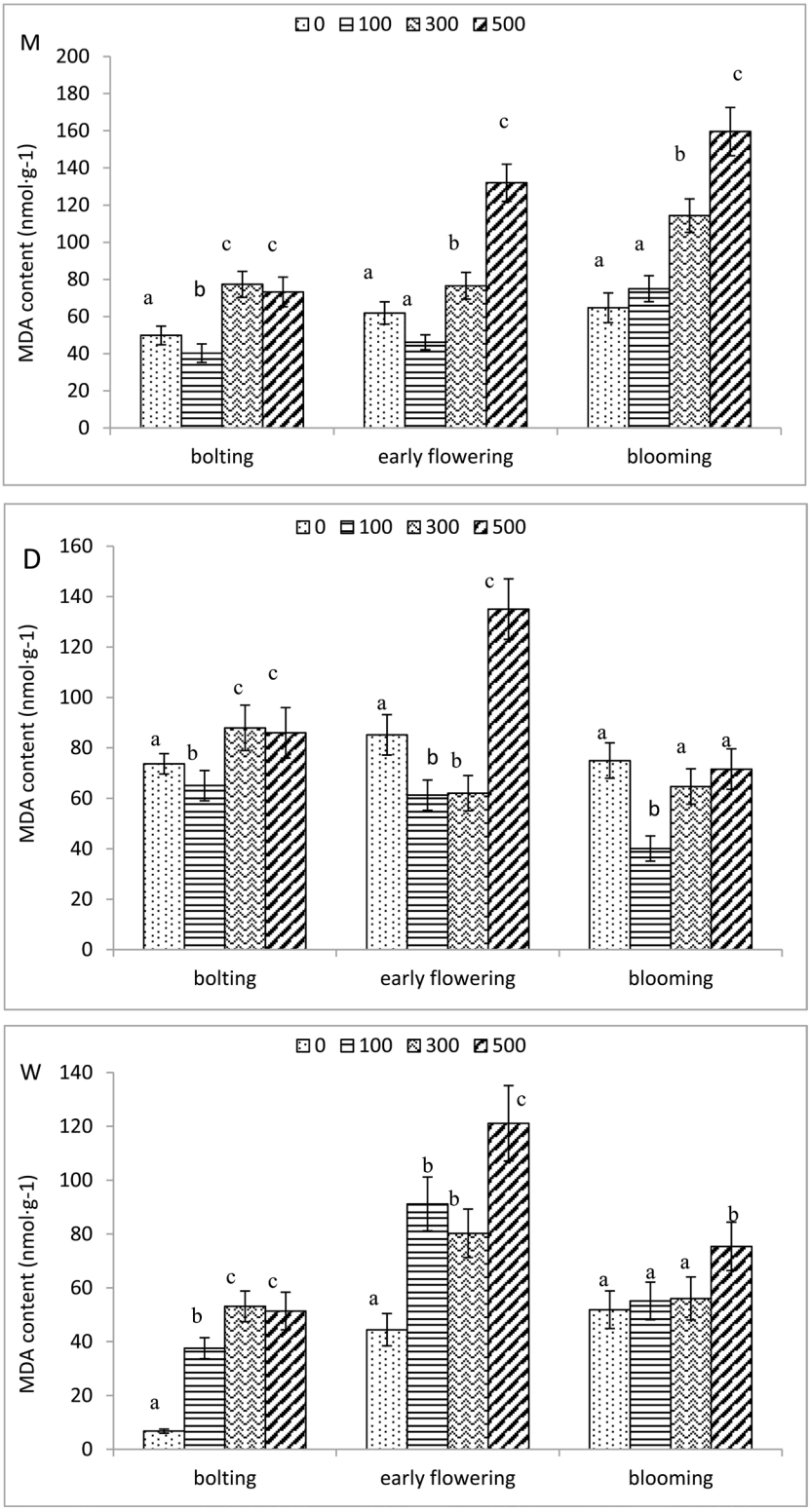

Figure 2. Changes of MDA content in *Narcissus pseudonarcissus* leaves under salt treatment.

MDA content in general increase higher than their respective control, of which the most early flowering changes significantly. When the concentration is tendency for 500 mmol/L, MDA content are approximately twice the contrast, it shows that the high NaCl concentration makes the *Narcissus pseudonarcissus* has already happened the cell membrane lipid peroxidation, antioxidant system function of cell membrane is abate, difficult to balance the stress damage, severely damaged cell membranes, resulting in a decline in cell function and affected the physiological metabolism process of *Narcissus pseudonarcissus.*

MDA content of the same variety varies in different periods, and the blooming period of "Manly" is significantly higher than that of bolting stage and early flowering stage, while the "Dutch" and "Wilden" is the highest in the early flowering stage. At the same time, it can be seen that the tolerance degree of the three varieties is different. Among them, the "Dutch" is the best. In the three periods, MDA content first decreases and then increases with the increase of salt concentration, and the content is the lowest under the concentration of 100 mmol/L NaCl. The Change trend of "Manly" decreased first and then increased in the bolting stage and the early flowering stage, while it continued to increase in the blooming stage. The relative content of "Wilden" was always lower than that of "Manly" in each period, although it showed a continuous increasing trend in the three periods. This shows that the salt damage resistance of the three is "Dutch" greater than "Wilden" greater than "Manly".

3.3. Effects of Salt Stress on Antioxidant Enzyme System of *Narcissus pseudonarcissus*

The antioxidant enzyme system in plants can eliminate peroxides such as reactive oxygen species produced during metabolism to maintain a dynamic balance in the body. Under the external salt stress, reactive oxygen species accumulate free radicals, while SOD and POD in the antioxidant enzyme system play an important role in scavenging free radicals [16] [17]. For the purpose of this test data (**Figure 3**), SOD activity of the same variety was not significantly different in different periods, while NaCl treatment with different concentrations showed significant differences. Under low NaCl salt stress, the activity of SOD enzyme in the body of 3 varieties was generally increased, which was significantly higher than that of the control, at this point, SOD enzyme decomposes the superoxide anion into singlet oxygen and H_2O_2, and H_2O_2 still has strong oxidability, POD enzyme is required to further decompose into O_2 and H_2O. When NaCl concentration exceeded 300 mmol/L, the activity of SOD enzyme in the body of *Narcissus pseudonarcissus* "Manly" began to decline, which was lower than that of the control group. This indicated that stress injury in the body had affected the function or degradation of SOD enzyme, reduced its content or activity, and weakened its antioxidant capacity. The SOD enzyme activity in *Narcissus pseudonarcissus* of "Dutch" and "Wilden" varieties also decreased, but it was still higher than the control, indicating that the SOD enzyme was less damaged by stress and had stronger tolerance to salt stress.

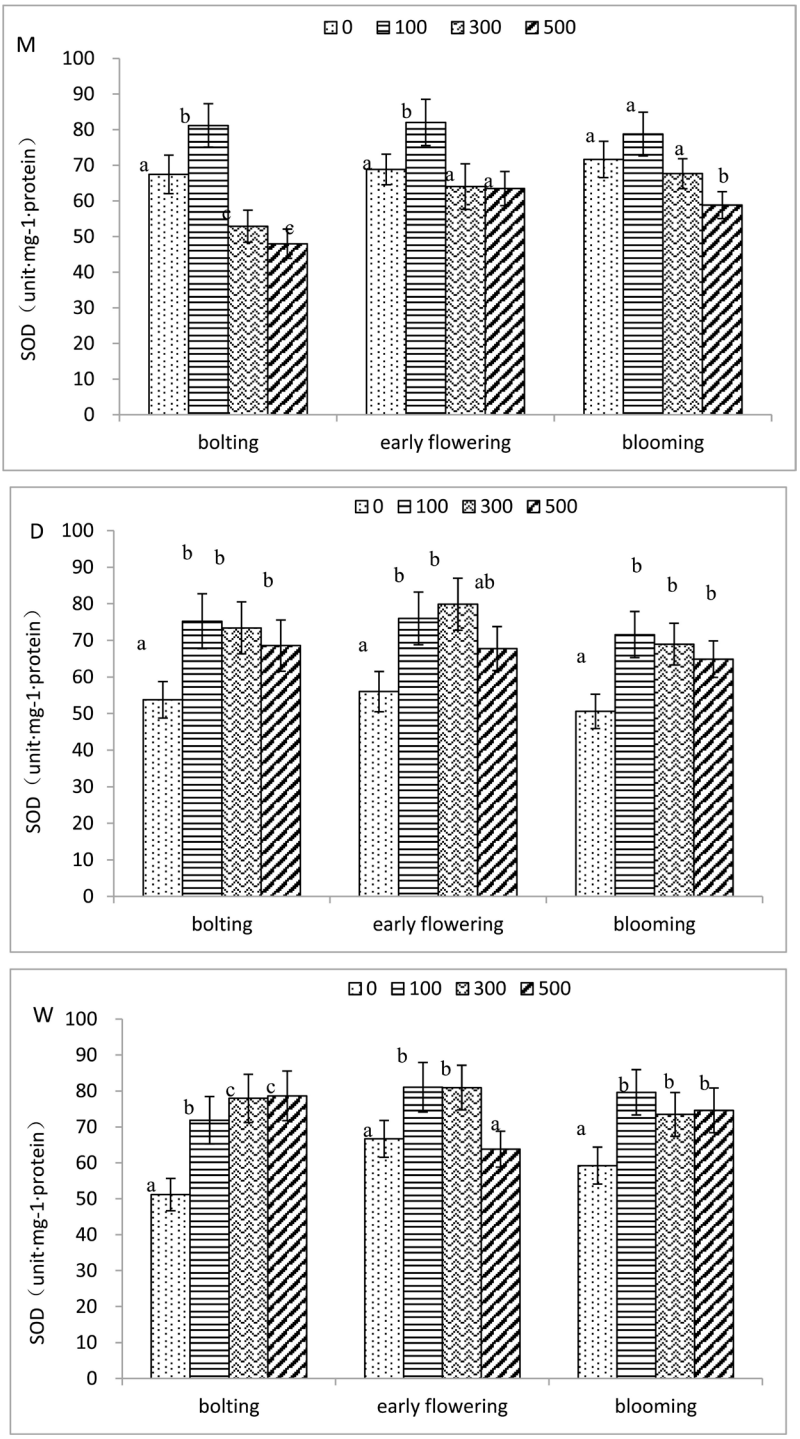

Figure 3. Changes of SOD enzyme activity in *Narcissus pseudonarcissus* leaves under salt treatment.

The change trend of POD activity in the three varieties of *Narcissus pseudonarcissus* was consistent with that of SOD enzyme activity (**Figure 4**), which also showed a trend of increasing first and then decreasing. This indicated that POD enzyme activity was activated under low concentration NaCl salt stress, and the H_2O_2 generated by SOD metabolism was further degraded to produce O_2 and

H_2O, so as to maintain the metabolic balance of peroxide in the body of *Narcissus pseudonarcissus* and reduce the oxidative damage to the membrane system. Under the stress of NaCl concentration over 500 mmol/L, POD activity of all

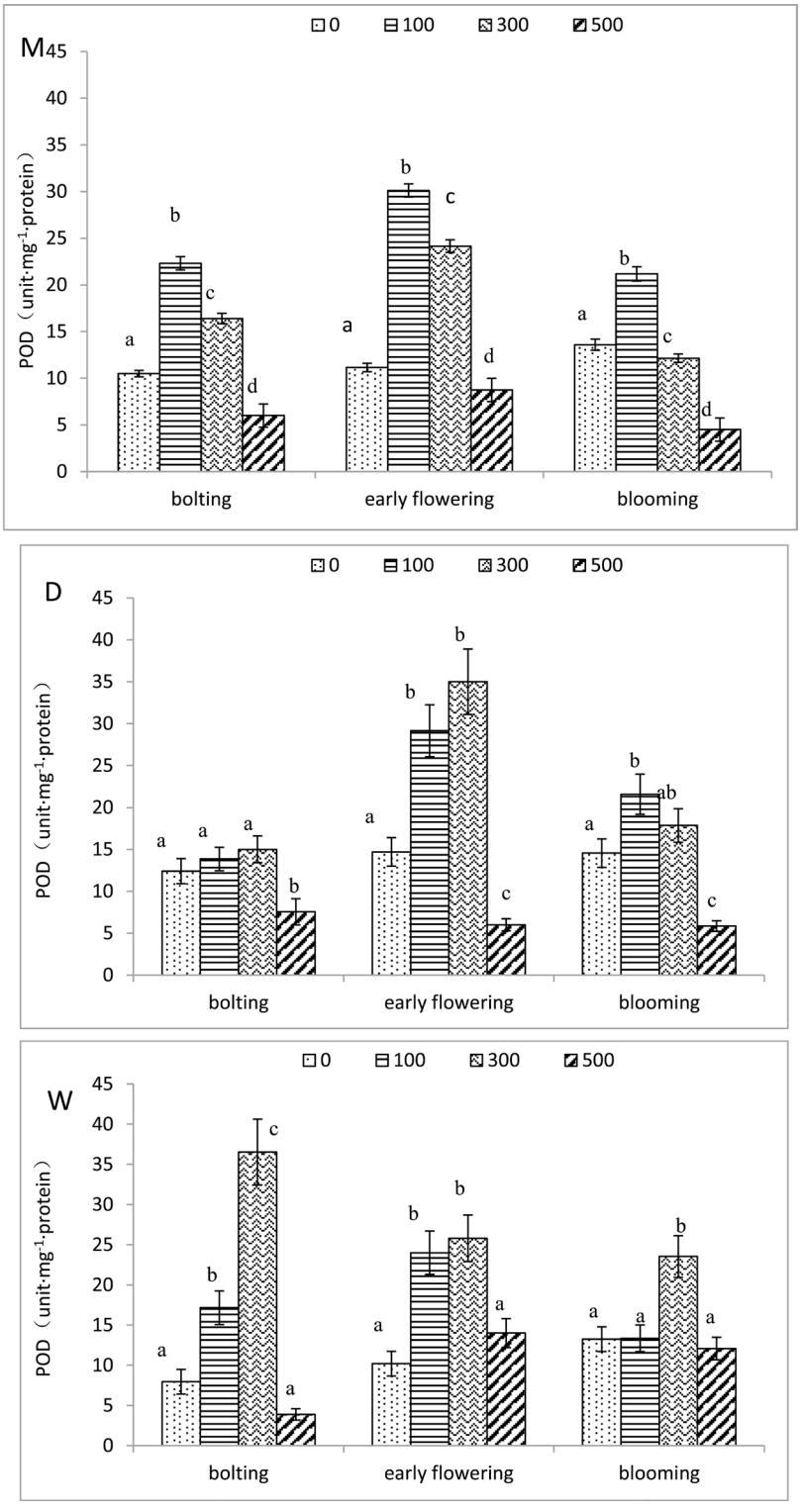

Figure 4. Changes of POD enzyme activity in *Narcissus pseudonarcissus* leaves under salt treatment.

Narcissus pseudonarcissus showed a significant decrease *in vivo*, which was lower than their respective control levels, indicating that POD activity was more significantly affected by stress under the condition of high concentration of salt. The changes of POD content in different periods of the same cultivar were also different. The POD content of "Manly" and "Dutch" was the highest in the early flowering stage, while the POD content of "Wilden" was the highest in the moss extraction period, indicating that the salt tolerance of the same cultivar was different in different periods. The salt stress was more sensitive in the early stage, but gradually decreased in the later stage. At the same time, it can be seen from the figure that different varieties have different degrees of salt tolerance. "Dutch" and "Wilden" usually reach the maximum at 300 mmol/L, while "Manly" at all three periods reach the maximum at 100 mmol/L, which shows that "Dutch" and "Wilden" have strong tolerance.

3.4. Effects of Salt Stress on Proline and Soluble Sugar Content in *Narcissus pseudonarcissus*

Proline, as an important osmotic regulatory substance in plants, can be used as a reference index for the degree of salt and alkali stress in plants. It is essentially a highly water-soluble free amino acid in the cytoplasm, which can protect the cell membrane system, maintain the structure and function of enzymes and reduce the degradation of Intracellular protein [18]. In this experiment, the contents of proline in the three varieties increased at different levels of salt stress in different periods, which was significantly higher than the control level. In addition, with the increase of NaCl concentration, the contents of proline basically showed an upward trend (**Figure 5**). This indicates that proline, as an osmotic regulatory substance in the cells of *Narcissus pseudonarcissus*, can sensitively respond to the external salt stress, reduce the osmotic potential in the cells through the increase of its own content, slow down the damage caused by cell water loss to plants, and play a stabilizing and protective role in the biomacromolecules in the cells. The relative content of proline in the same cultivar was not significantly different in different stages, but was slightly higher in early flowering and blooming stages. In terms of the relative content of proline among the three varieties, "Dutch" is higher than the other two varieties, reflecting stronger salt tolerance.

On the one hand, soluble sugar can be used as osmotic regulatory substance in plants to regulate the change of osmotic potential in cells; on the other hand, it can also be used as a carbon framework for intracellular macromolecules and an energy source in plants [19]. As can be seen from **Figure 6**, the soluble sugar content in the body of "Manly" at three stages increased with the increase of NaCl salt stress, showing a trend of increasing at first and then decreasing. It reached the maximum value at 100 mmol/L at the early flowering stage (28.67 $mg \cdot g^{-1} \cdot FW$), and reached the maximum value at 300 mmol/L at the bolting (31.44 $mg \cdot g^{-1} \cdot FW$) and blooming (34.00 $mg \cdot g^{-1} \cdot FW$) stages. It showed that the soluble sugar content of "Manly" species increased rapidly under the condition

of medium and low concentration NaCl stress, so as to maintain the appropriate level of osmotic potential in cells, slow down cell water loss and reduce stress injury. However, under the condition of relatively high concentrations of NaCl stress, the soluble sugar in the body appear a large number of decomposition,

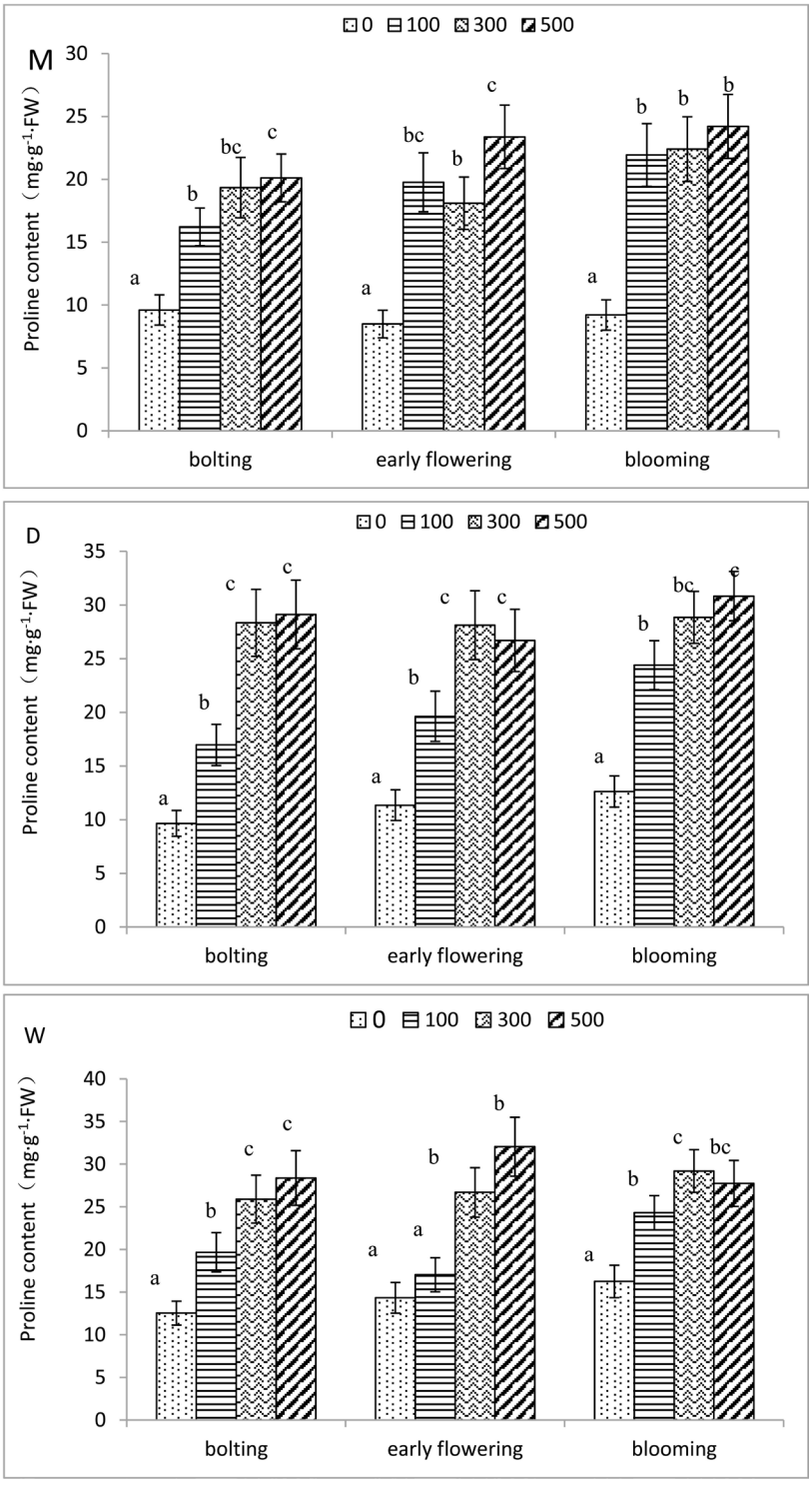

Figure 5. Changes of proline content in *Narcissus pseudonarcissus* leaves under salt treatment.

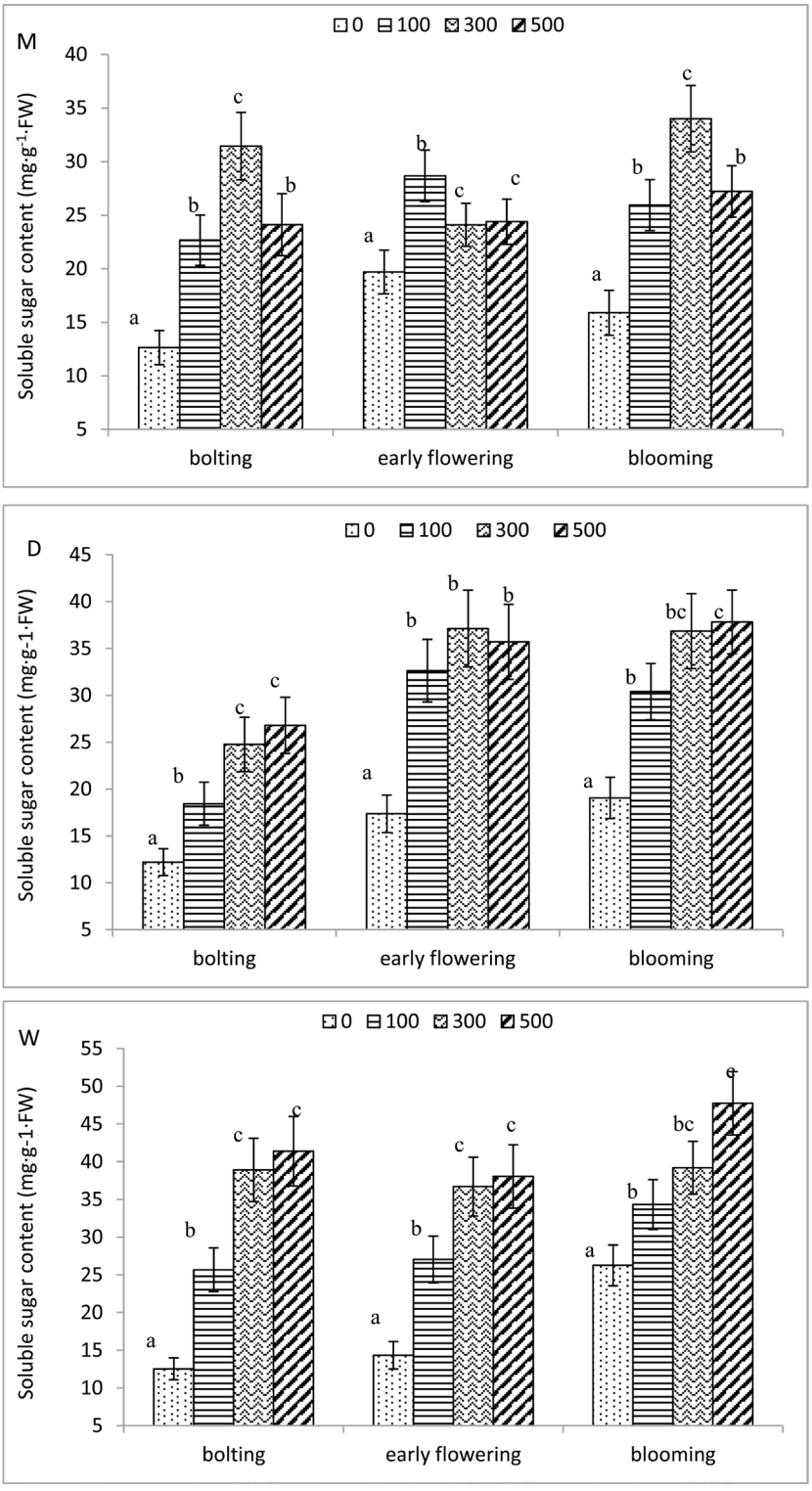

Figure 6. Changes of soluble sugar content in *Narcissus pseudonarcissus* leaves under salt treatment.

decomposition quantity is greater than the amount of synthetic, results in the decrease of total soluble sugar, the plant resistance is abate, the damage is aggravating, reducing salt stress resistance ability. And "Wilden" and "Dutch" on the

soluble sugar content increased with the increase of NaCl concentration in the body gradually on the rise, shows the two varieties of soluble sugar in the body under the condition of high concentration of NaCl stress decomposition quantity is limited, and decomposition of synthetic quantity is always greater than the amount, to maintain the high level state of soluble sugar in the body, thus reducing the damage of salt stress on plant. In terms of the soluble sugar content of the same variety in different periods, all three showed that the blooming period was larger than the early flowering and the bolting period, which may be caused by the increase of the soluble sugar accumulated by the extension of the growth period.

4. Discussion

Salt stress conditions can induce a series of physiological and biochemical reactions in plants, and the comprehensive expression of these reactions affects the salt tolerance of plants [20]. In this experiment, under the condition of low concentration of NaCl, the three varieties of *Narcissus pseudonarcissus* showed a certain degree of salt resistance in each period. First, the increase of chlorophyll content promoted the absorption, transmission and transformation of light energy, and improved the light contract ability, providing material and energy basis for plants to resist salt stress. The increase of harmful superoxide anions under low salt stress induces the activity of SOD and POD enzymes in the ROS system, enhances the ability to eliminate peroxide substances in plants, and reduces the damage to the membrane lipid structure in cells, which is reflected in the decrease of MDA content [21]. *Narcissus pseudonarcissus* osmotic regulation substances in the body also play a role of the corresponding, proline and soluble sugar content increased obviously, in order to reduce cell osmotic potential, prevent cell water loss and damage, and to maintain the structure stability of cell membrane and protein and the activity of the enzymes in the cell, thus ensuring the *Narcissus pseudonarcissus* various physiological metabolic processes in the body function.

Under the stress of high concentration of NaCl, salt-tolerant physiological and biochemical reactions in the body of 3 varieties of *Narcissus pseudonarcissus* were confused, and the antioxidant enzyme activities of SOD and POD were reduced. Although the content of proline and soluble sugar continued to rise, it was still difficult to resist the damage caused by high salt stress, which caused serious damage to the plasma membrane and resulted in a large accumulation of MDA content. High concentration of MDA can promote the destruction of chloroplast membrane structure and accelerate the degradation of chlorophyll. At the same time, high concentration of salt stress can also lead to the change of lamellar structure in chloroplasts, reduce the light contract ability of *Narcissus pseudonarcissus* and reduce the plant function [15] [22].

In this experiment, with the extension of salt stress time, MDA content decreased first and then increased to an increasing trend, This suggests *Narcissus*

pseudonarcissus with the increase of stress time hurt gradually deepened, on its own, of course, also by certain way to resist the persecution, performance of chlorophyll, soluble sugar and proline content increased, the content was higher in early flowering and flowering, both based on the extension of its own growth period accumulated materials such as chlorophyll, proline and soluble sugar increased, reflecting the plant adaptability of ego to protect a stress [21] [23].

This study has some shortcomings, such as small sample size and not doing chemical analysis. The next step is to expand the determination range and combine physiological changes with chemical changes, so as to explain the influence of salt stress on *Narcissus pseudonarcissus* from many aspects, and to lay a sufficient theoretical foundation for the popularization and application of *Narcissus pseudonarcissus* on saline-alkali land.

5. Conclusion

In this experiment, 3 varieties of *Narcissus pseudonarcissus* all showed some resistance to salt stress environment, SOD and POD of two varieties "Dutch" and "Wilden" still show stronger activity than the control under high concentration of NaCl stress, and play a role in scavenging peroxide. At the same time, the soluble sugar content in the plants of Dutch and Wilden varieties did not significantly decrease under the condition of high salt stress, but maintained a high level, which reduced the osmotic stress brought by high salt environment. This indicated that "Dutch" and "Wilden" varieties showed better salt resistance compared with "Manly" varieties. However, all three showed physiological and metabolic dysfunction in the high-salt environment, indicating that their tolerance to salt stress injury was limited. Based on the above six physiological indicators, it is considered that the salt tolerance of the three is" Dutch ", "Wilden" and "Manly" in order.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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