Effect of Exogenous Oxygen Free Radical H2O2 on SOD Activity in One-year-old Panax notoginseng Plants

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Abstract: The effect of exogenous oxygen radical H2O2 on SOD activity in leaves of one-year-old Panax notoginseng seedlings under salt stress was analyzed. SOD activity is inhibited by H2O2 and decreases with the increase of the concentration of exogenous oxygen radical H2O2, resulting in the increase of superoxide radical. The one-year-old notoginseng plants were soaked in 4mmol/L oxalic acid solution for 8min as treatment and soaked in clean water as control. The plants were stored at room temperature (20℃), and the effects of H2O2 treatment on physiological indexes related to reactive oxygen species were analyzed. The results showed that exogenous H2O2 increased the activities of SOD, POD, CAT and APX enzymes, increased the contents of soluble protein, soluble sugar and proline, and decreased the contents of H2O2 and MDA in the cells under salt stress, which were significantly higher than those of the control. Exogenous oxygen radical H2O2 can enhance the ability of one-year-old notoginseng seedlings to remove ROS in vivo and reduce membrane permeability by increasing SOD activity, alleviate the inhibitory effect of salt stress on the growth of one-year-old notoginseng seedlings, and thus improve the salt tolerance of one-year-old notoginseng plants. The oxidative damage degree of plants under Ca (NO3) 2 stress alone is lower than that under NaCl stress alone..

1. Introduction

Soil salt damage is one of the most important adversities that restrict crop yield and income. It can lead to accumulation of active oxygen in plant cells, destroy photosynthesis in leaves, interfere with protein synthesis, hinder energy metabolism and inhibit growth [1]. Activation of SOD, POD, CAT, APX and other SOD enzyme activities, elimination of reactive oxygen species produced by plants under salt stress and alleviation of membrane peroxidation are important regulatory mechanisms for plants to adapt to and resist salt stress [2]. In recent years, with the in-depth study of reactive oxygen species, the relationship between reactive oxygen species and vitrification of test-tube plantlets has attracted the attention of researchers. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbic acid peroxidase (APX) have scavenging effects on active oxygen, which can maintain the dynamic balance of active oxygen, protect the normal cell membrane structure, and finally enhance the stress resistance of crops [3]. In this study, one-year-old Panax notoginseng plant strains with different salt tolerance were used as experimental materials, and exogenous H2S and H2O2 solutions were sprayed under NaCl stress. Through the changes of seedling growth, osmotic adjustment, substance accumulation and active oxygen metabolism of one-year-old Panax notoginseng plant, the modulation effects of these two signal molecules on physiological mechanisms of relieving salt stress were discussed, providing theoretical basis for improving the salt tolerance of one-year-old Panax notoginseng plant.

2. Materials and Methods

2.1. Test Material

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For one-year-old notoginseng plants with weak salt tolerance, exogenous oxygen radical H2O2 solution was prepared with NaHS.

2.2. Method

When the one-year-old notoginseng seedlings were 30 days old, the seedlings with the same growth were selected and treated with 0. 7% NaCl solution under salt stress (once every 5 days, 100mL/ pot). Soak in saturated detergent solution for 20min, then rinse with tap water for 30min. The fully washed notoginseng plants are placed on a super clean workbench. The cut tubers were mixed with sulfur-containing mancozeb to dry and planted in foam boxes. There are 12 foam boxes with 3 ditches per variety, 2 rows per box and 2 plants per row. When the seedlings grow to three leaves and one heart, the seedlings with uniform growth are selected and transplanted into a nutrition pot with vermiculite as the main substrate for continuous culture, and different treatments are carried out when the one-year-old notoginseng seedlings have four leaves and one heart. The determination of H2O2 content is based on Lin et al. [4]; Superoxide radical content was determined according to Wang et al [5]. The above experiments were repeated 3 times. The biomass and relative plasma membrane permeability were measured at 8 days after salt stress treatment. Meanwhile, the second and third fully developed functional leaves and roots under the growing point of one-year-old notoginseng seedlings were taken for other indexes.

3. Experimental Results

3.1. Effect of H2o2 on Reactive Oxygen Species Content

In the aerobic metabolism of plants, the production of active oxygen is inevitable. However, under the combined action of SOD enzyme activity and antioxidant, active oxygen does not cause harm to cells. The change trend of H2O2 content in fruits treated with H2O2 and control is basically similar, but the H2O2 content in fruits treated with H2O2 keeps relatively stable change. After H2O2 treatment, the stem diameter, seedling fresh mass, root fresh mass, seedling dry mass and root dry mass of the seedlings of the two strains increased to different degrees than those under NaCl stress. That is, when the soil water content is 30% ~ 40% of the maximum water holding capacity in the field, most of the upper leaves of the one-year-old notoginseng plants are curled, while the upper leaves of the plants sprayed with SNP are mostly spread and curled less. However, the difference gradually became significant with the extension of treatment time. at the 20th day after treatment, the glass transition rate of 2.0mmol/LH2O2 treatment increased to 22%, reaching a significant level ($P<0.05$). The results showed that exogenous H2O2 treatment could reduce the damage of salt stress on membrane quality of one-year-old notoginseng seedlings.

3.2. Effect of Exogenous Oxygen Free Radical H2o2 on Mda Content of Seedlings of Panax Notoginseng Under Salt Stress

There are many kinds of SOD activities in plants, among which SOD, CAT, APX, POD and GR are the five main SOD activities in cells. Exogenous oxygen radical H2O2 has an important influence on their physiological functions under salt stress. The superoxide anion content in the control leaves of one-year-old Panax notoginseng plants increased under mild and moderate stress, and the difference was significant under moderate stress. During storage, the MDA content of the fruits treated with H2O2 and the control showed a trend of rising first and then falling, but their changes were significantly different. The MDA content of the fruits treated with H2O2 increased significantly lower than that of the control in the early and late storage period. On the 5th day after treatment, the plant height and fresh weight of the 2.5mmol/L H2O2 treatment increased by 22.1% and 18.8% respectively ($p < 0.05$) compared with the 7.0mmol/L H2O2 treatment (fig. 1).

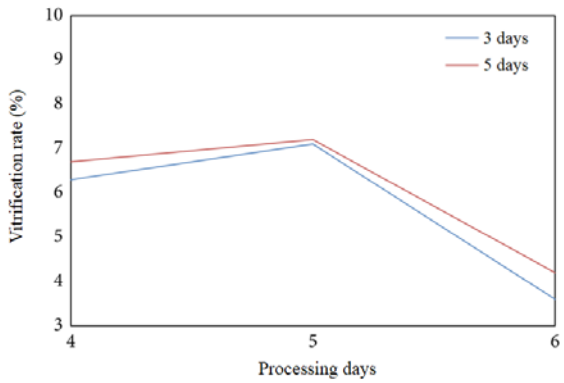

Figure 1 Effects of exogenous H2O2 on the vitrification rate, plant height and fresh weight of Panax notoginseng seedlings

Through salt stress alone, the contents of soluble protein, soluble sugar and proline were significantly increased by 10.3%, 48.2% and 89.6% respectively compared with the control group. From the effect of exogenous H2O2 on the plant height and fresh weight of one-year-old notoginseng seedlings, it can be seen that the plant height and fresh weight of test tube seedlings decreased with the increase of H2O2 treatment concentration when the treatment concentration was 2.0 ~ 6.0 mmol/L. The relative plasma membrane permeability of leaves and roots of one-year-old Panax notoginseng seedlings under stress+H2O2 treatment decreased by 30.55% and 12.08%, respectively.

3.3. Effect of Exogenous H2o2 on Sod Activity In Leaves of Panax Notoginseng Under Salt Stress

Salt stress treatment (T1) can significantly reduce the activities of antioxidant SOD, POD, CAT and APX in leaves of one-year-old Panax notoginseng plants ($P < 0.05$), while adding exogenous H2O2 treatment (T2) can significantly increase the activities of SOD enzyme in leaves of one-year-old Panax notoginseng plants under salt stress ($P < 0.05$), but there is no significant difference between T1 treatment and T2 treatment. APX has higher affinity with H2O2, and can remove lower content of H2O2. When APX removes H2O2, ascorbic acid (AsA) is required as an auxiliary substrate. Under the same soil moisture condition, the superoxide anion content in T2 control leaves was lower than that of T1 control leaves under various degrees of salt stress except the normal moisture condition. The range of change was slow during storage for 0 ~ 3 days, and was large during 3 ~ 5 days after harvest. The CAT activity of H2O2-treated fruits was significantly higher than that of the control ($P<0.05$) during storage for 4 ~ 5 days. The activities of SOD, POD and APX were significantly different. The results showed that exogenous H2O2 could regulate the enzyme activity of antioxidant system in one-year-old Panax notoginseng plants, establish defense system and improve salt tolerance.

4. Discussion

Recent studies have shown that high concentration H2O2 produces oxidative stress on plants, while low concentration H2O2 is an important signal molecule in plant cells and participates in plant responses to abiotic stress [6]. However, long-term or high-intensity stress will cause internal metabolism imbalance of tissue culture seedlings and generate excessive oxygen free radicals. If these oxygen free radicals are not removed in time, membrane system will be attacked and membrane lipid peroxidation will occur. The results of this experiment showed that, compared with the control, after 8 days of isotonic NaCl and Ca (NO3) 2 stress treatment, the biomass of one-year-old notoginseng seedlings was significantly reduced, thus inhibiting the growth of one-year-old notoginseng plants. At the same time, the MDA content in leaves of one-year-old Panax notoginseng plants was reduced. However, H2O2 treatment of one-year-old notoginseng seedlings under salt stress can effectively reverse the protective effect of exogenous H2O2. This indicates that

exogenous H2O2 sharing can effectively alleviate the inhibitory effect of NaCl stress on the growth of one-year-old notoginseng seedlings, thus improving the salt tolerance of one-year-old notoginseng seedlings.

Under normal temperature conditions, exogenous oxygen radical H2O2 can increase POD activity in leaves of one-year-old notoginseng seedlings [7]. H2O2 can also increase POD activity in the system acquired resistance. Water stress resulted in an increase of H2O2 content in leaves of one-year-old Panax notoginseng plants, but the change of H2O2 content was not related to the degree of salt stress. At the same time, a large number of studies show that with the continuous production of reactive oxygen species after harvest, the reactive oxygen species scavenger starts to start, thus maintaining a favorable and harmless free radical concentration with low physiological level and stability [8]. Exogenous spraying of 25μmol/L H2O2 can increase the contents of soluble sugar, soluble protein and proline osmoregulation substances in one-year-old seedlings of Panax notoginseng plants, and further improve the activities of SOD, POD, CAT and APS enzymes. It may be because H2O2, as an oxidative stress factor, is a regulating factor for external environmental stress response when the concentration is low, and is harmful to cells when the concentration is too high. These results indicate that exogenous oxygen radical H2O2 can significantly reduce ROS level by enhancing SOD activity in vivo, and effectively relieve the peroxidation damage of one-year-old Panax notoginseng plants caused by isotonic NaCl and Ca (NO3) 2 stress. Only when harmful free radicals generated in plants can be quickly removed, can MDA production and lipid peroxidation be effectively prevented, and plant cells can be protected from salt stress.

The results of this experiment showed that the contents of free proline and soluble sugar in the seedlings of Panax notoginseng plants increased significantly in the next year under NaCl stress, and the contents of free proline and soluble sugar in the leaves of seedlings after H2O2 treatment increased significantly. At this time, APX plays an important role. The increase of H2O2 content in leaves treated with H2O2 is related to the inhibition of APX activity. H2O2 treatment reduced superoxide anion content of one-year-old Panax notoginseng plants under moderate and severe stress, and increased POD and APX activities of one-year-old Panax notoginseng plants under salt stress. At the same time, it was found that oxalic acid induced plant disease resistance was related to its increased POD activity and induced POD isozyme synthesis. The changes of osmoregulation substance and H2O2 content play a key role in improving the salt tolerance of one-year-old notoginseng seedlings. The increase of soluble sugar, soluble protein and proline content plays a role in osmotic adjustment of one-year-old notoginseng plants. MDA is the final product of membrane lipid peroxidation. Its accumulation can reflect the activity of free radicals in the body and the damage degree of cell membrane to a certain extent. The electrolyte permeability can also represent the damage degree of cell membrane. In the future, in-depth research will be conducted on the differential regulation mechanism of exogenous oxygen free radical H2O2 on the stress relieving effects of NaCl and Ca (NO3) 2 isotonic.

5. Conclusion

To sum up, exogenous H2O2 not only affects membrane lipid peroxidation, SOD enzyme activity and content of non-enzymatic antioxidant substances of one-year-old notoginseng seedlings under salt stress, but also affects physiological and biochemical metabolism of one-year-old notoginseng seedlings. The increase of H2O2 content increases lipid peroxidation, enhances membrane permeability, and significantly inhibits plant growth. The injury degree and injury mechanism of isotonic NaCl and Ca (NO3) 2 stress on one-year-old Panax notoginseng plants are different. If the stress concentration exceeds the adaptive range of the plant and the normal growth of the plant is inhibited, the plant will age ahead of time. It can be seen that exogenous H2O2 with appropriate concentration is an important signal transduction molecule in the salt tolerance process of one-year-old notoginseng plants. The results showed that after H2O2 treatment, SOD and CAT activities of notoginseng plants increased and LOX decreased in one year, resulting in the decrease of H2O2 and MDA levels. Exogenous oxygen radical H2O2 can enhance SOD activity of one-year-

old notoginseng plants under salt stress, thus enhancing the resistance of one-year-old notoginseng plants to salt stress, but the degree of enhancement is related to the variety resistance and stress degree.

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