Changes of antioxidant enzyme activities in chloroplast of ‘717’ poplar under NaCl treatment

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Abstract. In order to study the salt tolerance of ‘717’ hybrid poplar under salt treatment, we studied the changes of antioxidant enzyme activities in chloroplasts treated with 0, 25,75mmol/L Knack. The results showed that: (1) Knack treatment can increase the content of hydrogen peroxide (H2O2) and malondialdehyde (MDA) in chloroplast. The H2O2 increased the highest at 1d, and reached 39.5%. The content of MDA in chloroplast increased by 38.3% at the low concentration of day 5, and increased to 74.4% at day 10 after high concentration of Knack treatment. (2) The activity of acerbate peroxidase (APX) in chloroplast increased at low concentration and decreased at high concentration. The activity of peroxidase (POD) in chloroplast was not significantly changed at low concentration, but at high concentration, the POD activity decreased by up to 35% at day 1. The activity of glutathione reductive (GR) in chloroplast was increased by 15.9% under low Knack treatment. Increased by 53.6% at 1 d on high concentration of Knack. (3) Knack treatment, the activities of ascorbic acid (As A) and (reduced glutathione) GSH in chloroplast were increased. At low concentration, the activity of ASA in chloroplast increased by 43.8% at 10 d, and the content of GSH was an increase of 72.2% at 10 d. In the high concentration of Knack treatment, the activity increased significantly. In conclusion, the low concentration of Knack (25mmol / L) could promote the activity of antioxidant enzymes in chloroplast of 717 and maintain the growth, while the antioxidant enzyme activity was inhibited when the Knack was treated with high concentration (75mmol / L). Hydrogen peroxide and MDA content increased, the cells were subjected to Knack stress.

Keywords. Salt stress; 717 hybrid yang; tissue culture seedlings; antioxidant system.

1. Introduction

At present, about one-tenth of the world's arable land is under the threat of salinization, about 1 billion hm2. Due to the salinity of the soil is too high, resulting in the land salinization, but salt plays an irreplaceable role in plant growth and development [1-2]. The study found that, in order to be more effective in improving saline-alkali soil, we can plant salt-tolerant plants in salt-alkali soil to play the role of desalination [3].

In recent years, many scientists have conducted an in-depth study of the characteristics of different plants under salt stress. Tong Shi Bo [4] confirmed Arabidopsis transgene, the Pro content was significantly increased, the resistance of the salt environment was significantly strengthened. Sheng et
al. [5] found that the activities of SOD, POD and CAT reached the maximum when the salt concentration reached a certain level. However, as the Nalco concentration continued the activities of SOD, POD and CAT decreased gradually, which indicated that the activity of SOD, POD and CAT could not be maintained at a high level, which would enhance the lipid peroxidation and damage the cell membrane. Li [6] found that the activity of SOD and POD in potato virus-free seedling leaves was significantly affected by salt concentration. However, the interaction between salt concentration and salt concentration had no significant effect on the activity of SOD and POD in potato virus-detoxified leaves. Other studies used foreign aid Si, salicylic acid, chitosan, and selenium to plant plants so that salt-treated plants have reduced MDA levels and are not affected by salt stress.

Salt has two sides’ effects on plants. Low salt (less than 0.2%) is good for plant growth and development. High salt (more than 0.3%) is harmful to plants and significantly inhibits plant growth. Salt stress is similar to other types of stress and is also a factor that limits plant growth and produces ROS such as superoxide, H2O2 and hydroxyl radicals. ROS through the oxidation of lipids, proteins, nucleic acids to damage the normal metabolism of broken ring plants [7]. Plant ROS mainly exist in the applet, chloroplast, mitochondria and cytosol. ROS accumulation has been observed in many polyhydroxylated plants, including Arabidopsis thaliana, apple, etc. [8]. Studies using transmission electron microscopy found that H2O2 was localized in the cell wall and intercellular space in explant tissue, indicating oxidative damage to lipids, proteins and nucleic acids. Therefore, in order to prevent verification of plant body organelles, it is necessary to remove ROS at the subcellular level. Photosynthesis of plants to produce reactive oxygen species mainly in chloroplasts, under normal circumstances, the removal and production of reactive oxygen species in chloroplasts are in a state of dynamic equilibrium. The higher the antioxidant enzyme activity, plant adaptability to adversity is stronger. In Liu’s study of aluminum stress, the content of chlorophyll in tobacco leaves significantly decreased, resulting in the accumulation of ROS. At present, anti-oxidase activity has been detected in various organelles of various plant species. Antioxidant enzymes are found in various subcellular organelles of plant leaf cells, including chloroplasts [9].

Populous spp. is one of the main species of afforestation and shelterbelt currently in the world. It has the advantages of rapid growth and high biomass yield, and is easy to grow in saline-alkali, arid and barren areas. Poplar not only can quickly restore vegetation, prevent soil erosion and rehabilitate saline soil, but also can be used as woody fiber energy plant for biomass development and utilization, which has great potential value. At the same time, poplar also has the characteristics of fast-growing, adaptable, easy to reproduce, windbreak, etc., but also a good species of afforestation, water conservation and soil and water conservation. Populous tremolo L. × P. Alba L. (abbreviated as 717 hybrid poplar) is a good variety bred from poplar and populous tormentors hybrids. Quick, straight branches, good material, from Europe and the United States and other countries. 717 hybrid poplar has strong tolerance, has the potential application value of vegetation restoration, soil erosion prevention and saline-alkali recovery, and is also a high-quality woody fiber energy plant in the development of biomass energy.

In order to further understand the mechanism of salt tolerance in hybrids of 717, the physiological and biochemical changes of 717 hybrids under salt stress, the content of oxidation products in chloroplasts, the activity of antioxidant enzymes in chloroplasts and the activity of antioxidants in chloroplasts were compared the study. Through the comparative study of salt-tolerance mechanism of hybrid poplar 717, we laid the foundation for the research on salt-tolerance of poplar in the follow-up, provided the theoretical basis for cultivating transgenic salt-tolerant poplars, and introduced the poplar varieties with high salt tolerance for coastal areas to improve the salinization Regional vegetation coverage provides a theoretical reference.

2. Materials and Methods
Populous tormentors 'INRA 717-1B4' hybrid poplar (Populous tremolo L. × P. Alba L.) sterile seedlings from Nanjing Forestry University tissue culture room.
Nalco concentration was 0 (control), 25 mmol/L, 75 mmol/L. When the tissue culture seedlings grow to about 10 cm, Nalco is directly added to the culture medium of the tissue culture seedlings that grow more consistently. Poplar seedlings after salt treatment for 20 days were taken fresh weight of each leaf of 2 g, 3 times repeated, sealed with a sealed bag, placed in a refrigerator (-70 °C) standby.

The experiment was repeated 3 times for each concentration. The average and standard errors were obtained from the data of three experiments. The analysis of variance was carried out by SPSS19.0 program.

3. Results and analysis

3.1. Effect of Salt Stress on H₂O₂ in Chloroplast
Hydrogen peroxide (H₂O₂) is an indispensable member of reactive oxygen species. Hydrogen peroxide plays an important role in environmental stress. For example, in the face of defensive reaction to adverse conditions, it participates in stomata movement of guard cells and regulates plants Growth and development and many other physiological processes [36].

As shown in Fig. 1, the content of H₂O₂ in chloroplasts increased under salt treatment, and at 1d, 5d and 10d, the content of H₂O₂ in chloroplast was higher than that at low concentration and increased at 1d Up to 1.46 times the low concentration. As can be seen, the content of H₂O₂ in chloroplasts increased more significantly at higher concentrations.

![Figure 1. The content of H₂O₂ in chloroplast at different salt concentration](image)

3.2. Effect of Salt Stress on Content of Malondialdehyde (MDA) in Chloroplast
Plants that survive adversity often undergo membrane lipid peroxidation during organ aging and their product MDA can severely damage the biofilm. We often use MDA as a membrane lipid peroxidation indicators to show the extent of lipid peroxidation of the cell membrane and plant aging indicators and the extent of the reaction to adverse conditions [14].
As can be seen from Fig. 5, the content of MDA in the chloroplast was increased as compared with the control under salt treatment. At low concentrations, the contents increased by 38.3% and 30.9% at 5d and 10d, respectively, but increased by 66.8% and 74.4% at high concentration of salt treatment. These results indicated that the ability of 717 hybrid poplar to adapt to low salt stress was better than high salt stress and adaptable to the stress of salt stress.

3.3. Effects of Salt Stress on Antioxidant Enzymes in Chloroplasts

3.3.1. Effect of Acerbate Peroxidase (APX) Activity in Chloroplasts under Salt Stress. As shown in FIG. 3, APX activity increased by 49% and 14.9%, respectively, at 5 days and 10 days as compared with the control. However, APX activity in chloroplasts continued to decrease in high concentration salt solution and decreased by 32.4% at 10 days. The results showed that the APX activity in chloroplasts became stronger when low salt concentration was applied. However, low stimulation stimulated the APX activity in chloroplasts to be stronger, while the salt concentration was too high.

3.3.2. Effect of Salt Stress on Peroxidase (POD) Activity in Chloroplast. As shown in FIG. 4, the POD activity of chloroplasts in the tissue culture seedlings changed after salt treatment. It can be seen that the POD activity in the chloroplast decreased by 35% at 1 day under high salt treatment. However, the
POD activity in chloroplasts at low concentrations did not decrease or even slightly increase compared with the control, and increased by 22.4% at 5 days.

![Figure 4. Determination of POD Activity in Chloroplasts at Different Concentration](image)

3.3.3. Effect of Glutathione Reductive (GR) Activity under Salt Stress. From Fig. 5, the activity of GR in the chloroplast is increased in the case of salt treatment. At low concentrations, they increased by 6%, 8% and 15.9% respectively. At high concentration, it increased by 53.6% at 1 day, and the change of activity increased more significantly.

![Figure 5. Determination of GR Activity in Chloroplasts at Different Concentration](image)

3.4. Effect of Antioxidants on Chloroplast under Salt Stress

3.4.1. Effect of ascorbic acid (As A) content in chloroplasts under salt stress. As shown in Fig. 6, under the condition of low concentration of salt concentration, it increased by 6%, 22.8% and 43.8% at 1d, 5d and 10d, respectively, and increased by 43.8% at 10d. However, under the treatment of high concentration salt solution, it increased most at 10 days, but only increased by 11.5%. Thus, at low concentrations, changes in as an activity in chloroplasts were more pronounced than at high concentrations.
3.4.2. Effect of Salt Stress on Content of Reduced Glutathione (GSH) in Chloroplast. As shown in Fig. 7, GSH activity in chloroplasts increased compared with the control at a low concentration of external conditions, and the magnitude of increase was large. At 5d and 10d, they reached 1.72 and 1.51 times of the control respectively. Although chlorophyll GSH activity was also increased in chloroplasts on day 5 and day 10 with high concentration salt solution treatment, they were not as significant at low concentrations as 1.26 and 1.11 folds, respectively.

4. Discussion and conclusion
According to the results of this study, the phenomenon is as follows:
(1) The content of H2O2 increased under salt treatment. It can be seen that under salt stress, the content of hydrogen peroxide increased in the chloroplast of leaves, but the increase range was not as high as that of salt solution with high concentration obviously under. The content of MDA in the leaf of chloroplast of poplar was also increased compared with that of the control group, which was similar to that of Li Holyan et al. [15].
(2) DHAR and APX activities in chlorophyll of 717 hybrids increased under Nalco stress compared with the control at 25mmol / L salt concentration, while at 75mmol / L salt concentration Teach the control group decreased. The activity of POD in chloroplast decreased with the increasing of salt concentration, but not obvious at low concentration. Under high concentration of salt treatment, the activity of POD activity was weakened in the control group. The activity of GR in chloroplast is not obvious with the increasing extent under salt treatment.
(3) As A and GSH activity in chloroplasts of hybrid poplar 717 increased under salt stress compared with the control, but at low concentrations, the increase larger, but under the condition of high concentration salt solution, the activity intensity increases little. Among them, the activity of AsA increased by 43.8% compared with the control, and reached 11.5% at the highest. The GSH content increased by a maximum of 51.4% at low concentrations and only a maximum of 26.8% at high concentrations.

Salt stress causes oxidative stress and produces ROS, mainly including H2O2, •OH and so on. H2O2, •OH content in plants significantly increases under salt stress [16]. This experiment also showed that salt stress increased the production of H2O2 in the chloroplast of hybrid poplar 717. In this study, we focused on the changes of antioxidant enzyme activity, the content of oxidized products and the antioxidant activity of 717 hybrid poplar leaf chloroplasts under different salt concentrations to observe their resistance.

It can be shown from this point that the plant itself will produce a resistance mechanism under adverse conditions and continuously increase the content of soluble protein in the leaves to osmotic ally regulate the physiological processes of the plant and resist the harm caused by the adverse environment. Previously, Dong et al. [17] found that under alkaline salt stress, the soluble protein content of roots and leaves of Achnatherum spenders was higher than that of the control. It also shows that 717 hybrid poplar has some salt tolerance.

The activity of DHAR and APX in chloroplasts not only did not weaken but increased slightly under salt treatment of low concentration, but the activities of DHAR and APX were changed under high concentration of salt solution weak. The activity of POD is weakened in the presence of high concentrations of salt solution and is almost unaffected at low concentrations. GR activity has been enhanced, even more pronounced at high concentrations. Oxidized products in chloroplasts tended to increase after salt treatment, but were more pronounced at high concentrations. The activity of other antioxidants generally showed an upward trend under salt treatment but more pronounced at low concentrations. The chloroplast protein content in the case of high concentrations increased significantly. These results show that hybrid 771 has some adaptability to salt stress and is more adaptive and salt tolerant under low salt stress.

In the process of experiment, due to the sampling time, the determination of sample loading error, illumination and other factors, it is easy to lead to experimental error, so in order to more accurately understand the different concentrations of salt treatment of hybrid poplar 717 Metabolism of chloroplasts, the need to accurately grasp the experimental time, timely observation of changes in their physiological indicators, to minimize manual errors.

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