Effect of Plant Growth Promoting Rhizobacteria on Medicago Falcate Seeding under the Salt Stress

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Abstract: Medicago falcate is treated by the growth-promoting rhizobacteria sowing on different concentrations of the germination of salt solution in the culture box. Seed germination in the process of determination of the SOD, POD, CAT, as well as changes in amylase activity. The results showed that: In the 0 mmol/L, 50 mmol/L, 100 mmol/L concentration of the three SOD activity under salt stress increased at first, then dropped, POD the optical density decreased after the first rise, CAT in the optical density value of 0 mmol/L, 50 mmol/L in line with changes in POD. In the salt concentration to 50 mmol/L when the strain after the adoption of Medicago falcata antioxidase are low in the untreated group, but not salt stress strain treatment group were higher than untreated antioxidase Group. Amylase α and amylase β in the 0 mmol/L, 50 mmol/L concentration did not change significantly.

Keywords: Growth-Promoting Rhizobacteria; Medicago Falcate; Anti-Oxidant Enzyme; Amylase, Salt Stress

1. Introduction

Plant Growth-Promoting Rhizobacteria (PGPR) are defined as bacteria that live freely in soil, rhizosphere, rhizoplane, and phyllosphere and are beneficial to plant growth under certain conditions. At present, most studies on PGPR focus on rhizospheric PGPR, while studies on phyllospheric PGPR are scarce; meanwhile, it has been found that endophytic PGPR also exist in plants. PGPR generally promote plant growth in two distinct ways: (1) They directly affect plant metabolism by providing substances that are deficient in plants. These bacteria can fix nitrogen, solubilize phosphorus and iron, and produce plant hormones such as auxins, gibberellins, cytokinins, and ethylene. In addition, they can enhance plant stress resistance, including resistance to drought, high salt, heavy metal toxicity, and pesticides; (2) Biocontrol PGPR (Biocontrol-PGPR) indirectly promote plant growth while preventing infection by harmful plant pathogenic microorganisms. The substances they produce can inhibit other microorganisms (but not plants) by restricting pathogenic bacteria from obtaining available iron or altering the metabolism of host plants to enhance the host's ability to resist pathogenic infection. Biocontrol PGPR may also promote plant growth through mechanisms such as nitrogen fixation or hormone secretion [1].

The salt tolerance of plants mainly depends on the stability of the membrane system, i.e., maintaining the integrity of the membrane system under salt stress to preserve the selective absorption of ions and other functions. When plants are in a saline environment, the high osmotic potential caused by excessive salt concentration exerts osmotic stress on various membrane systems of plants. At the same time, the specific toxic effect of high-concentration charged monovalent ions will inevitably affect the stability of biological macromolecules, enzymes, proteins, and membrane structures. Under stress conditions, the production rate of O_2^-in plants increases with the intensification of stress, leading to the imbalance of O_2^-metabolism in cells and the accumulation of excess O_2^-. These excess O_2^- will induce a free radical chain reaction in cells, generating more free radicals and reactive oxygen species (ROS) such as $H_2O_2$and OH^-, which oxidize the double bonds of unsaturated fatty acids in membrane cells, causing breakage, decomposition, and damage to the membrane. Peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) are protective enzymes in the enzymatic defense system of plants against membrane lipid peroxidation [2].

Therefore, this study investigated the changes in the activities of several related enzymes in Medicago falcata under the combined effects of PGPR and salt stress.

2. Materials and Methods

2.1 Materials

2.1.1 Test materials

Medicago falcata: Collected from the Grassland Ecology Research Station of Northeast Normal University.

PGPR strains: Provided by the laboratory.

Analytical grade $NaHCO_3$and Na_2CO_3 (molar ratio of 1:9).

2.1.2 Main instruments and reagents

12×12 cm germination boxes; electronic balance; illuminated incubator; T6 Xinyue Visible Spectrophotometer; Beijing Puxi UV-Visible Spectrophotometer.

2.2 Methods

2.2.1 Preparation of test bacterial suspension

Refer to the method in Reference [3]. The strains were transferred to YPG liquid medium (composed of 5 g yeast extract, 5 g glucose, 5 g tryptone, 1 g sodium chloride, and finally dissolved and made up to volume in 1000 mL of water) and cultured with shaking at 25℃ for 24 h. After the strains grew sufficiently, the optical density (OD) value of the fermented bacterial solution was determined using a 721-type spectrophotometer. When the OD value reached 0.5 (at a wavelength of 660 nm) — indicating that the number of cells in the fermented bacterial solution was 10^9 cells/mL — each fermented bacterial solution was adjusted to the same OD value with sterile water. Under sterile conditions, the fermented bacterial solution was injected into a sterilized glass bottle, sealed, and stored at low temperature.

2.2.2 Seed treatment

Medicago falcata seeds were divided into 54 packages of 100 seeds each using sulfuric acid paper. The packaged seeds were disinfected by soaking in 0.1% mercuric chloride solution for 10~15 minutes. After discarding the mercuric chloride solution, the seeds were rinsed with distilled water three times to remove residual mercuric chloride solution on the surface of the sulfuric acid paper packages. The seeds were then divided into two groups: one group was soaked in the bacterial suspension, and the other group was soaked in sterile water, with each soaking lasting 3 hours.

2.2.3 Preparation of salt solutions

Mixed salt solutions with concentrations of 50 mmol/L, 100 mmol/L, 150 mmol/L, and 200 mmol/L were prepared using sterile water, respectively.

2.2.4 Germination test

The treatment used analytical grade $NaHCO_3$ and Na_2CO_3 at a molar ratio of 1:9, with sterile water as the control. Three replicates were set for each concentration. The soaked Medicago falcata seeds were evenly placed in germination boxes lined with 3 layers of filter paper, with 100 seeds per box. Different concentrations of salt solution (10 mL) were added to each box to saturate the filter paper. The boxes were then covered, labeled, and placed in an illuminated incubator. The culture conditions were 25℃/15℃ (day/night) and 14 h/10 h (light/dark). Recording was initiated on the day the seeds were placed in the boxes, with germination defined as the plumule protruding 2 mm above the seed coat. During the culture period, evaporated water was appropriately supplemented to maintain the treatment concentration constant. Relevant enzyme indicators were determined during seed germination.

2.3 Determination Methods

2.3.1 Determination of SOD activity

The Nitroblue Tetrazolium (NBT) method was used [4], with one enzyme activity unit defined as the amount of enzyme required to inhibit the photoreduction of nitroblue tetrazolium (NBT) by 50%.

2.3.2 Determination of amylase activity

The 3, 5-Dinitrosalicylic Acid method was used [5].

2.3.3 Determination of POD activity

The Guaiacol Colorimetric method was used [6].

2.3.4 Determination of CAT activity

Ultraviolet Spectrophotometry was used [7].

3. Results and Analysis

3.1 Effect of PGPR on the Activity of Protective Enzymes in Medicago Falcata under Salt Stress

Under normal conditions, a reactive oxygen species (ROS) scavenging system exists in plants, maintaining a dynamic balance of ROS content in cells, thereby preventing damage to plants. The scavenging of ROS in plant cells is mainly achieved through related enzymes and some antioxidants. The main protective enzymes in cells include superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), among which SOD is the most important [8].

3.1.1 Changes in SOD (Superoxide Dismutase) activity

SOD is the most important enzyme for plants under stress; it can promptly scavenge free radicals and ROS, thereby improving the antioxidant capacity of plant tissues. SOD, CAT, and POD work synergistically to defend against damage to the membrane system caused by ROS or other peroxides, and the activity of SOD is closely related to plant stress resistance [9]. As shown in Figure 1, without the addition of PGPR, the SOD activity at 0 mmol/L salt concentration was significantly higher than that at 50 mmol/L. In the group treated with PGPR, the SOD activity of Medicago falcata first increased and then decreased under the three salt concentrations, but the change was not significant. Among them, the SOD activity at 50 mmol/L salt concentration was the highest, which was 26.3% higher than that at 0 mmol/L and 52.6% higher than that at 100 mmol/L.

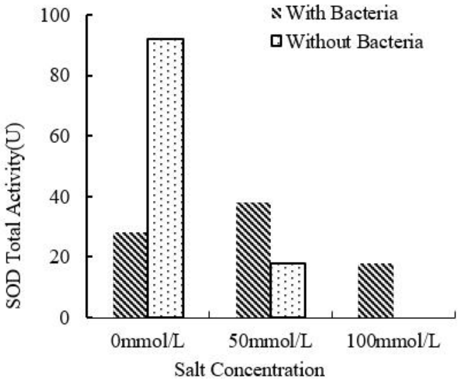

Figure 1. Changes in SOD Activity

3.1.2 Changes in POD (Peroxidase) activity

POD is a type of protective enzyme in plants, which can decompose excess peroxides in plants [10]. As shown in Figure 2, regardless of whether PGPR was added or not, the optical density value of POD first decreased and then increased. However, the optical density value of samples treated with PGPR was higher than that of samples without PGPR treatment. After adding PGPR, the change in the optical density value of the samples was not significant. The optical density value at 0 mmol/L was the highest, which was 31.5% higher than that at 50 mmol/L and 24.9% higher than that at 100 mmol/L.

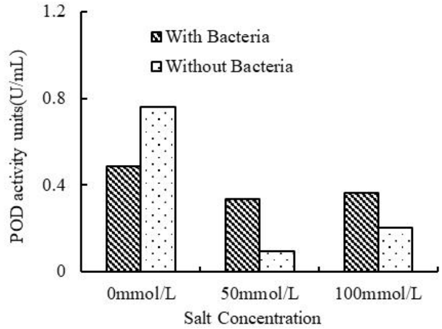

Figure 2. Changes in POD Activity

3.1.3 Changes in CAT (Catalase) activity

Catalase is widely present in plant tissues, and its activity is related to the metabolic level of plants as well as their drought and disease resistance, so it is often determined. As shown in Figure 3, in the control group without PGPR addition, the optical density value of CAT decreased with the increase in salt concentration. However, in the group treated with PGPR, the optical density value of CAT increased with the increase in salt concentration. The optical density value of CAT at 50 mmol/L salt concentration was 81.7% higher than that at 0 mmol/L. It can be seen that the CAT content decreased after adding PGPR.

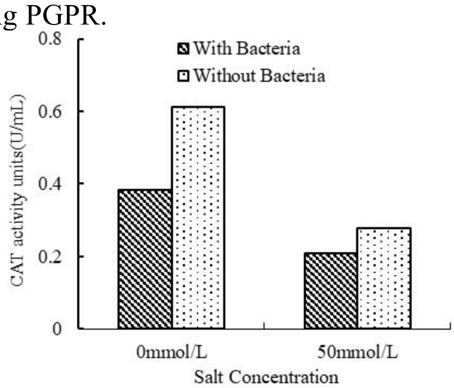

Figure 3. Changes in CAT Activity

3.2 Effect of PGPR on Amylase Activity in Medicago Falcata under Salt Stress

Amylase in plants can hydrolyze stored starch into maltose. Amylase exists in almost all plants, with the highest activity in cereal seeds. There are two types of amylase in plants: α-amylase and β-amylase, and their activities vary with the growth and development stages of plants. As shown in Figure 4, under single salt stress (without PGPR), the activity of α-amylase ranged from 7.5 to 8 [mg/(g·min)], while the activity of α-amylase in the PGPR-treated group was above 8 [mg/(g·min)].

At both salt concentrations, the activity of α-amylase in the PGPR-treated group was higher than that in the control group: it was 7.3% higher at 0 mmol/L and 3.5% higher at 50 mmol/L. This indicates that the presence of PGPR enhances the activity of α-amylase. As shown in Figure 5, under single salt stress, the activity of β-amylase ranged from 23 to 24.5 [mg/(g·min)], while the activity of β-amylase in the PGPR-treated group decreased to approximately 22 [mg/(g·min)] and slightly increased with the increase in salt concentration.

Superoxide dismutase (SOD) is the main protective enzyme in the membrane lipid peroxidation defense system; it can catalyze the dismutation reaction of reactive oxygen species (ROS) to produce non-toxic molecular oxygen and hydrogen peroxide, thereby preventing plants from being damaged [11]. The experiment found that the SOD activity at a salt concentration of 50 mmol/L was higher than that in the control group, indicating that PGPR exerted the strongest growth-promoting effect on Medicago falcata at this concentration.

Peroxidase (POD) is a type of protective enzyme that mainly works with CAT to eliminate hydrogen peroxide produced by SOD, maintaining hydrogen peroxide at a low level [12]. The experiment showed that the optical density value of POD at salt concentrations of 50 mmol/L and 100 mmol/L was higher than that in the control group, and it was 71.4% higher at 50 mmol/L. This indicates that PGPR can promote the activity of POD in Medicago falcata at these two concentrations.

Catalase is an iron-containing enzyme commonly found in plant tissues; it mainly exists in peroxisomes and sometimes in chloroplasts. Since catalase can decompose H_2O_2 into H_2O and O_2, it reduces the production of singlet oxygen (O_2^-) and certain free radicals induced by H_2O_2, thereby avoiding damage to membrane structures and biological macromolecules such as DNA and proteins. In this sense, catalase can protect plants under stress and delay senescence. The experiment found that the change trend of CAT was similar to that of POD, but the overall activity was lower than that in the control group, indicating that PGPR had no significant promoting effect on the CAT activity of Medicago falcata under salt stress.

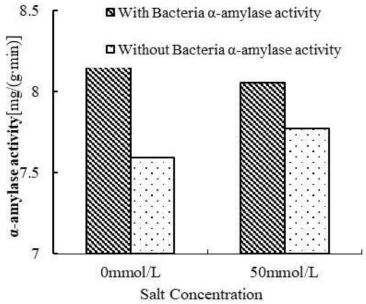

Figure 4. Changes in α-Amylase Activity

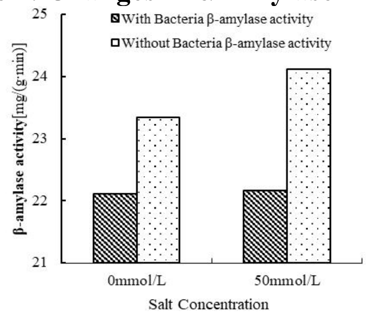

Figure 5. Changes in β-Amylase Activity

The activity of amylase in plants varies with their growth and development stages; therefore, the growth and development status of plants can be judged by determining amylase activity. The experiment showed that PGPR promoted the activity of α-amylase in Medicago falcata under different salt concentrations; it had no promoting effect on the activity of β-amylase, but the change in β-amylase activity was not significant.

4. Conclusion

PGPR can alleviate salt-induced damage to Medicago falcata seedlings by regulating the activities of antioxidant enzymes (SOD and POD activities are significantly enhanced, while CAT shows no significant long-term promotion) and α-amylase. The 50 mmol/L salt concentration is the optimal condition for PGPR to exert its effects, effectively improving the salt tolerance of seedlings, although it has no promoting effect on β-amylase. This study provides a basis for the application of PGPR in enhancing the salt resistance of Medicago falcata in saline-alkali environments.

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