Effects of Aluminum Stress on Protective Enzyme Activity in Tie Guanyin leaves

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Abstract. The experiment was adopted to study the change of SOD, CAT and POD activity of Tie guanyin (new leaf and old leaf blade of different concentrations of aluminum stress; in this paper, 0 (CK), 40, 200, four gradients of 400mg/L concentration of Al³⁺ in acidic conditions, Tieguanyin tea leaf SOD, cat and POD activity changes. The results showed that high concentrations of aluminum stress on antioxidant enzyme system activity cannot continue to increase; at the same time showed that SOD is sensitive to aluminum toxicity concentration change, its sensitivity is higher than CAT and POD, SOD and CAT activity and the aging and decline of plant There was a positive correlation.

1. Introduction
Iron Buddha, a variety of Oolong tea, is a famous Chinese tea. It helps in staying healthy, preventing arteriosclerosis, diabetes, and tooth decay, keeping fit, comfort emotion, and relieving the effects of cigarette smoking and alcohol consumption. Iron Buddha tea has a high content of amino acids, vitamins, minerals, tea polyphenols, alkaloids, and various nutrients. Moreover, it is medically useful and highly preferred by the public [1].

Tea trees grow widely in aluminium-rich soils, and therefore, they are a kind of aluminium-enriched plant species. In China, tea trees are commonly planted in the southern lateritic region. Due to the deterioration of the global environment in recent years, soil acidification has become a more severe problem. The percentage of acid rain in the Quanzhou region was 100% in the current year. This imposes adverse effects on the yield and quality of different crops in the Quanzhou region. Previous studies suggested that soil acidification due to acid deposition is the main reason for decrease in crop production. In addition, aluminium toxicity is directly caused by soil acidification, and it is considered that soil acidification is actually aluminium toxicity. The reason is that lattice-bound aluminium gradually dissociates and is released to the soil in soluble forms. It is subsequently adsorbed by plants, thereby leading to aluminium stress [2]. Sun et al. has investigated the structure of aluminium and the characteristics of aluminium accumulation processes in tea tree bodies. Their results showed that aluminium is mostly present in organic forms or chelated forms in tea trees, whereas old tea tree leaves show stronger aluminium-accumulating ability [3]. Therefore, the effects of increasing soluble aluminium in soil on the growth of tea trees, aluminium toxicity in tea leaves,
and their quality have received increasing attention. The adverse effects of aluminium stress on plants include damages to micro-structures, inhibition of root and leaf growth, inhibition of nutrient adsorption, disturbance of internal metabolism, inhibition of chlorophyll production, gradual decrease in optical strength, and changes in normal physiological processes and activity of important enzymes in plants, thus leading to large reductions in crop yields [4].

This article uses Iron Buddha tea tree as a model for investigating the effects of aluminium stress on the activity of protective enzymes in Iron Buddha tea tree under acidic conditions. It further reveals the mechanism of aluminium toxicity in Iron Buddha tea tree and provides scientific evidence on the mechanism of aluminium stress resistance of Iron Buddha tea tree.

2. Materials and methods

2.1. Materials

2.1.1. Experimental materials, Seedlings of Iron Buddha tea were used. They were grown by hydroponics in the laboratory of School of Resources and Environmental Sciences, Quanzhou Normal University.

2.1.2. Cultivation medium, approximately 10-cm long, one-year-old seedlings of Iron Buddha tea were collected and planted in 1-L beakers. Three strains of seedlings were grown in one beaker, and one-fourth of Kobayashi Yimao Nutrient Solution [5] was used for cultivation.

2.1.3. Reagents for inducing aluminium stress, Pure AlCl₃·6H₂O was used.

2.2. Methodology

2.2.1. Cultivation of Iron Buddha tea, Seedlings grown under the same conditions were selected after they showed steady growth. The pH values of all cultivation media were adjusted to 4.3 with acids and different concentrations of aluminium. The aluminium concentrations were as follows: 0 (CK), 40 (K1), 200 (K2) and 400 (K3) (mg/L), where CK was the control. Measurements of different parameters were made 30 days after initiation of the treatments.

2.2.2. Measurement of enzyme activity in leaves, Measurement of SOD activity was carried out by tetrazoline colourimetry following Li et al. [6]. Measurement of CAT activity was done by ultraviolet spectrophotometry according to Fan et al. [7] whereas the activity of POD was measured by the guaiacol method according to Hao et al. [8].

2.2.3. Data analysis, Statistical analysis was carried out using Excel 2016.

3. Results

At different concentrations of aluminium, changes in activities of superoxide dismutase, catalase, and peroxidase were fundamentally identical. Their activities increased first, but subsequently decreased with increasing concentrations of aluminium (Figure. 1).

3.1. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) commonly exists in organisms. It can catalyse the quenching of superoxide anion radicals O₂⁻ to protect cells from damage by oxygen ions and related with reactive oxygen species.

The results of the experiments are shown in Figure 1-a. SOD can remove superoxide anion radicals, and its activity increases under adverse conditions. This strengthens the resistance of plants against adverse conditions [9]. Under the experimental conditions, the activity of SOD increased rapidly at an aluminium concentration of 40 mg/L, but rapidly decreased when the aluminium concentration
increased to 200 mg/L. Variation in SOD activity of the old and young leaves were the same, but the activity of the old leaves was obviously higher.

3.2. Catalase (CAT)
Catalase (CAT) can catalyse the decomposition of hydrogen peroxide to oxygen and water [11]. It does not require a special donor in organisms but can protect the bodies from reactive oxygen species. Therefore, it is an essential antioxidase and exists in most living cells.

The degree of variation in CAT activity was significant for balancing oxidation and reduction in the plants [9]. The CAT activities of both old and young leaves showed the same variations with increasing concentrations of Al$^{3+}$ (Figure 1-b), although the CAT activity of the old leaves was significantly higher. When the Al$^{3+}$ concentration exceeded 200 mg/L, the CAT activity of the old leaves dropped gradually.

3.3. Peroxidase (POD)
Peroxidase (POD) [9] is one of the most important enzymes in the antioxidase systems of plants. It plays important roles in physiological processes of plants related to stress resistance, including removal of reactive oxygen radicals and inhibition on lipid peroxidation [10]. In addition, it catalyses the decomposition and removal of H$_2$O$_2$, and coordinates with SOD to effectively remove the reactive oxygen generated during metabolism. It eventually prevents lipid peroxidation and other toxic reactions of reactive oxygen species.

POD, an iron-bearing metallic protein, is a common oxidoreductase in plants. POD catalyses the decomposition of H$_2$O$_2$ and other phenols [10]; therefore, it is an important enzyme for removing reactive oxygen species from cellular systems. In Figure 1-c, the POD activities of both old and young leaves showed similar variation patterns with increasing concentrations of Al$^{3+}$. When the concentration of Al$^{3+}$ was lower than 40 mg/L, the POD activity of the old leaves increased with rising Al$^{3+}$ concentrations. However, the activity gradually decreased with increasing Al$^{3+}$ concentrations higher than 40 mg/L.
Figure 1. Effects of aluminium stresses on enzyme activities in Iron Buddha tea leaves

Under adverse conditions, plants are exposed to poisoning by reactive oxygen. Therefore, the ability of the protective enzyme system to remove radicals is an important criterion in evaluating the resistivity of Iron Buddha tea against aluminium. The present study suggests that the activities of all the three enzymes studied gradually increase with increasing aluminium concentrations applied, thereby relieving the stress caused by free radicals on plant cells. However, when the applied concentration reached 200 mg/L or greater, the production and removal of reactive oxygen species were not balanced, and the activities of all three enzymes decreased. It demonstrated that under aluminium toxicity, all three enzymes could stay at a comparatively high level of activity to accommodate the aluminium stresses and reduce the damage inflicted on cell membranes by lowering the content of reactive oxygen species and radicals. When the concentration of aluminium reaches a certain degree, the activities of enzymes decrease rapidly, indicating that the resistance of the enzyme system in Iron Buddha tea leaves against aluminium toxicity is limited. The harms caused by aluminium on Iron Buddha tea trees become more severe when the aluminium concentration keeps rising.

4. Conclusion
Aluminium stresses at low concentrations can enhance the activity of antioxidase systems, which prevents damage to plants. The activity of antioxidase systems do not continuously increase under high-concentration aluminium inducing compounds. Moreover, SOD is shown to be more sensitive
than CAT and POD to variations in aluminium toxicity, whereas the enzyme activity of old Iron Buddha tea leaves was higher than that of the young leaves.

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