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Physiological characteristics of *Ficus tikoua* under antimony stress

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Abstract: A greenhouse culture experiment was used to evaluate the effects of antimony (Sb) stress on *Ficus tikoua* (*F. tikoua*). The results showed that the growth of *F. tikoua* leaves was significantly inhibited when Sb concentration was higher than 30 μmol/L, and no significantly inhibitory effect of Sb on the roots and stems of *F. tikoua* was found in all the treatments, implying that leaves were more sensitive to Sb toxicity than roots and stems. Antimony concentration in the roots was higher than that in the stems and leaves. To reduce reactive oxygen species (ROS) level in the *F. tikoua*, the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) increased with Sb treatments, but the SOD and CAT were more early active than POD. Although the decrease of chlorophyll content with high Sb treatments (450 μmol/L) was observed at the end of the experiments, the positive impact on chlorophyll content was observed with all the Sb treatments at the early period. No significant difference of the maximum quantum efficiency of PSII and quantum yield of PSII electron transport values with different Sb treatments was observed at the end of this experiment, suggesting that the photosynthesis was not inhibited with Sb concentration below 450 μmol/L. The results implied a certain tolerance to Sb stress for *F. tikoua*. This meets the essential condition for utilization in Sb contamination environments.
Key words: antimony; biomass; antioxidant enzyme activity; *F. tikoua*; chlorophyll fluorescence

1 Introduction

Antimony (Sb) is a toxic element with adverse effects on humans and the environment. An increase in Sb mining and smelting processes has resulted in the release of large quantities of Sb, causing serious Sb contamination in local environment [1,2]. Even at low concentration, Sb can cause irreparable damage to the environment [3]. Antimony is known to suppress germination and growth, inhibit photosynthesis, affect membrane structure and permeability, and damage the structure and function of photosystem II (PSII) in plants [4,5]. Research has indicated that the inhibition of photosynthesis is a primary damage mechanism in plants exposed to Sb even at a lower concentration [4,6]. Under Sb stress, the chlorophyll synthesis was inhibited and plastoquinone pool was diminished in plants, with a significant reduction in the total area between the fluorescence induction curves [7]. Also, Sb exposure resulted in more strong inhibition of electron transport both on PSII donor side and acceptor side than the oxygen-evolving complex and light-harvesting pigment-protein complex II [8].

A typical symptom of Sb toxicity is oxidative damage. In addition, Sb can induce the production of reactive oxygen species (ROS), including superoxide radicals (O^{-2}), hydroxyl radicals (•OH) and hydrogen peroxide (H_2O_2), which react very rapidly with DNA, lipids and proteins to cause cellular damage [9]. The excessive ROS reacts with lipids, proteins and pigments, which results in membrane damage and enzyme inactivation [9]. Antimony toxicity leads to the generation of ROS by inhibiting the electron transport chain in the chloroplast and mitochondria [9,10]. To counter ROS, plants produce antioxidants and antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT).

Ficus tikoua (*F. tikoua*), an endemic species in China belonging to the Moraceae family, has been recognized as a valuable restoration species and a medicinal source [11,12]. *Ficus tikoua* has been found wildly growing in the active and abandoned Sb mining areas. During the past decade, research focused on the

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evaluation of biological activities of *F. tikoua* [13]. Few research studies have investigated the photosynthetic response of *F. tikoua* to metal/metalloid stress [14]. Moreover, little is known about the response of *F. tikoua* to Sb treatment. Thus, the objective of this study is to assess the Sb resistance capacity of *F. tikoua* in preparation for eventual application to the re-vegetation of Sb-polluted soils.

2 Experimental

2.1 Plant cultivation and Sb treatment

Cuttings of *F. tikoua* from an antimony mine area in Lengshuijiang city, Hunan province, China (111°29′E, 27°45′N) were grown on water-saturated perlite in a growth chamber in a greenhouse. The cuttings were illuminated with three 25 W fluorescent lamps; the photoperiod was set as 14 h/10 h and the light intensity was 150 μmol/(m^2·s) photosynthetically active radiation (PAR). Day/night temperatures of 25 °C/18 °C were applied, and the humidity was 60%−80%. Cuttings were supplied with half-strength Hoagland nutrient solution until all six or seven leaves were completely unfolded. Thereafter, four plants of *F. tikoua* were carefully transferred to plastic pots that were filled with perlites. Four Sb concentrations (0, 30, 150 and 450 μmol/L) were tested in this study. Antimony was supplied as $C_4H_4KO_7Sb·1/2H_2O$. Each pot was supplied with 50 mL of Sb-containing half-strength Hoagland solution at 5 d intervals. All conditions were the same as those described above.

2.2 Observation of biomass and antimony in plant tissue

After 78 d, the Sb-exposed plants were collected and then rinsed three times with deionized water. The roots, stems and leaves were separately collected, dried at 70 °C and weighted. The plant samples were ground and sieved through a 1 mm sieve and digested with $V(HNO_3):V(HClO_4)$=4:1. The Sb concentration in the digested solution was quantified by atomic fluorescence spectrometry (AFS) (Titan AFS−810).

2.3 Assay of antioxidant enzymatic activities and MDA content

Approximately 0.2 g of fresh tissue was homogenized in a pre-cooled mortar with 5 mL precooled sodium phosphate buffer (50 mmol/L, pH 7.8) solution. And then the supernatant was collected for detection of enzyme activity by centrifugation for 20 min at 11000*g* and 4 °C [10]. The supernatant was used to determine SOD, POD, CAT, MDA and soluble sugar concentration according to the following photo-chemical method.

1) SOD assay was performed according to the method of TANG et al [15], with some modifications. Briefly, 3 mL reaction mixture that contains 0.3 mL of 750 μmol/L nitroblue tetrazolium (NBT), 0.3 mL of 20 μmol/L riboflavin, 0.3 mL of 130 mmol/L methionine, 0.3 mL of 100 μmol/L EDTA-Na_2, 1.5 mL of sodium phosphate buffer (50 mmol/L pH 7.8), 0.2 mL of deionized water and 0.1 mL of enzyme extract was placed under light with an average photon flux density of 78 μmol/(s·m^2) for 20 min at 25 °C, and the absorbance of the reaction mixture was recorded at 560 nm. Reaction solution placed in the dark was used as the control. One unit of enzyme activity was defined as the amount of the enzyme that resulted in 50% inhibition of the rate of NBT reduction.

2) POD activity was determined by measuring the absorbance changes at 470 nm and 25 °C [10]. 0.1 mL of enzyme extract was added into a mixture solution of 1 mL 0.3% H_2O_2, 0.9 mL 0.2% guaiacol, 1 mL sodium phosphate buffer (50 mmol/L, pH 7.0) to start enzymatic reaction. One unit of POD enzyme activity was defined as the amount of the enzyme that caused an increase in absorbance at 470 nm of 0.1 per minute.

3) CAT activity was measured by monitoring the decrease of H_2O_2 at 240 nm for 1 min at 25 °C [16]. 0.1 mL enzyme extract was added in a mixture solution containing 1 mL 0.3% H_2O_2 and 1.9 mL sodium phosphate buffer (50 mmol/L, pH 7.0) to initiate the reaction. One unit of CAT activity was calculated as the amount of enzyme that caused a reduction in absorbance at 240 nm of 0.1 per minute.

4) The Sb-induced oxidative damage (membrane liquid peroxidation) was estimated by measuring the MDA concentrations [17]. The MDA content was assayed using a solution containing 3 mL of 20% (*w*/*V*) trichloroacetic acid, which included 0.5% (*w*/*V*) thiobarbituric acid and 1 mL enzyme extract. The solution was kept in boiling water bath for 20 min and then quickly cooled. After refrigeration, the homogenate was centrifuged at 5000*g* and 25 °C for 10 min. The absorbance of the supernatant was recorded at 450, 532 and 600 nm, respectively. The content of MDA (C_1) (μmol/L) was calculated from the following formula: $C_1=[6.45(A_{532}-A_{600})-0.56A_{450}]$, where A_{450}, A_{532}, A_{600} represent the absorbance values at 532, 600 and 450 nm, respectively. The content of soluble sugar (C_2) (mmol/L) was calculated by the following formula [18]: $C_2=11.71\times A_{450}$.

2.4 Chlorophyll estimation and chlorophyll a fluorescence measurement

To determine chlorophyll and carotenoid contents in leaves, the homogenate of fresh leaves (0.2 g) was extracted for 24 h in 25 mL of 80% acetone in darkness.