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Short communication

Effects of indole-3-acetic, kinetin and spermidine assisted with EDDS on metal accumulation and tolerance mechanisms in ramie (*Boehmeria nivea* (L.) Gaud.)

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ABSTRACT

The effects and mechanisms of indole-3-acetic (IAA), kinetin (KN) and spermidine (Spd) assisted with ethylenediamine disuccinic acid (EDDS) on Cd and Pb accumulation in ramie were investigated by a pot experiment. In the first stage, the optimum concentrations of IAA, KN, and Spd were determined. And the effects of IAA, KN, and Spd at their optimum concentration on the antioxidant enzymes, non-enzymatic antioxidants, and metal accumulation in ramie were evaluated in the secondary stage. The results show that the translocation factor (TF) of Cd and Pb were increased by approximately 47 and 112%, respectively, at the presence of KN in combination with EDDS. In comparison with IAA treatment, Cd and Pb in root symplast were increased, approximately 2.02 and 2.62 times upon the combined application of IAA and EDDS. In addition, the contents of antioxidant enzymes and non-enzymatic antioxidant were increased dramatically through the addition of IAA, KN, and Spd plus EDDS. On the whole, IAA, KN, and Spd assisted with EDDS could significantly alleviate the oxidative stress induced by Cd and Pb in ramie.

1. Introduction

Cadmium (Cd) and lead (Pb) are highly toxic heavy metals, which can be taken up by plants, and further enter into the food chain (Ben Rejeb et al., 2013). Compared to the traditional physical and chemical methods, phytoextraction is proposed as an environmentally friendly in situ remediation technique to maintain soil fertility and structure (Zhang et al., 2013b). However, the efficiency of phytoremediation is low due to limited metal immobilization in soil. Chelate-enhanced phytoremediation has been proposed by using chelant to improve the efficiency of phytoextraction. Ethylenediamine disuccinic acid (EDDS) has been proposed as a potential chelant which is biodegradable and thus producing little secondary pollutants (Mühlbachová, 2011).

Excessive Cd and Pb can induce the formation of reactive oxygen species (ROS), such as the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$), which can cause the breakdown of proteins, lipid peroxidation in membranes, and DNA injury (Liu et al., 2007). Plants have evolved a variety of defense mechanisms to deal with the oxidative stress, including antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) as well as non-enzymatic compounds such as glutathione (GSH) and ascorbate (AsA) (Groppa et al., 2001).

IAA and KN are phytohormones involved in different developmental processes: IAA can promote cell division and coleoptile elongation (Ouni et al., 2014), and KN can stimulate cell division, leaf expansion, and chlorophyll synthesis (Zhao et al., 2011). Spd involves in regulatory processes such as promotion of growth, DNA replication, cell division and differentiation (Groppa et al., 2001).

Ramie is widely planted in Asian countries such as China, Philippines, India, and Thailand (Liu et al., 2008). Mature ramie has the high potential for the remediation of metal-contaminated soil, due to their large biomass and root system. Despite some

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literatures concerning the response of ramie to Cd toxicity in hydroponic condition (Liu et al., 2007), little information is available on the heavy metals accumulation and tolerance mechanism of ramie in the presence of phytohormones and EDDS. The present study aims (1) to determine the optimum concentration of IAA, KN, and Spd for ramie growth; (2) to evaluate the effects of IAA, KN, and Spd assisted with EDDS on metals accumulation in ramie under Cd and Pb compound stress; (3) to evaluate the roles of IAA, KN, and Spd assisted with EDDS in alleviating Cd and Pb induced oxidative stress by regulating the antioxidant defense system.

2. Materials and methods

2.1. Plant materials and treatments

Uncontaminated control soil was collected in Yuelu Mountain, which located in Changsha, China. The basic properties were listed as follows: organic C 18.5 g kg^{-1}; total N 0.870 g kg^{-1}; total P 0.254 g kg^{-1}; total K 15.6 g kg^{-1}; CEC 16.7 cmol kg^{-1}; Cd undetected (<0.025 mg kg^{-1}); Pb undetected (<0.4 mg kg^{-1}). The soil was air dried, ground, and was applied with 30 mg kg^{-1} Cd and 500 mg kg^{-1} Pb (from solutions of $Cd(NO_3)_2{\cdot}4H_2O$ and $Pb(NO_3)_2$, respectively). The mixed soil was incubated for 4 weeks and then put into plastic pots (1.5 kg per pot). One seeding of ramie (from Chinese Academy of Agricultural Sciences, China) in each pot was planted. The ramie seedings were cultivated in Cd and Pb compound-contaminated soil for 40 days.

In the first stage, IAA was used at 0, 1, 10 and 100 μM, while KN or Spd was used at 0, 10, 100 and 1000 μM. The sprays of IAA, KN, and Spd took place at 17:00 each day. After harvest, each plant was measured from the main root apex to the crown and from the crown to the main shoot apex to determine the effects of IAA, KN, and Spd on ramie growth.

In the secondary stage, the leaves were sprayed with 10 μM IAA, 100 μM KN or Spd. These concentrations were based on the first experimental stage. The application of 5 mmol kg^{-1} soil EDDS to soil significantly increased concentrations of Cd and Pb in shoots compared to the treatment of 2.5 mmol EDDS kg^{-1} soil (Luo et al., 2005). Therefore, the soil was supplied with EDDS at 5 mmol kg^{-1} in our study. The sprays of IAA, KN, and Spd also took place at 17:00, while EDDS was applied to the soil one week before the plants were harvested. The treatments were set as T0 (without Cd/Pb, controls), T1 (Cd/Pb), T2 (Cd/Pb/EDDS), T3 (Cd/Pb/IAA), T4 (Cd/Pb/KN), T5 (Cd/Pb/Spd), T6 (Cd/Pb/IAA/EDDS), T7 (Cd/Pb/KN/EDDS), and T8 (Cd/Pb/Spd/EDDS). The ramies were kept in a controlled room with 14 h light period at light intensity of 300 μmol m^{-2} s^{-1}, 25 °C/20 °C day/night temperature and 60–70% relative humidity. After harvested, fresh samples were frozen immediately in liquid nitrogen and stored at − 80 °C for further analysis.

2.2. Metal analysis

Upon harvest the samples were washed with deionized water and the roots were then rinsed with 5 mM $CaCl_2$ for approximately 5 min to displace the metals absorbed (Zhao et al., 2010). The plants were separated into roots, stems, and leaves. The samples were dried, ground, and digested with HNO_3–$HClO_4$ (3:1). The resulting solutions were determined by atomic absorption spectroscopy (Analyst 700, Perkin Elmer, USA).

2.3. Metals distribution in root apoplast and symplast

The metal distribution in root apoplast and symplast were measured using a desorption procedure (Zhao et al., 2010). The entire root system was rinsed using 5 mM $CaCl_2$ for approximately 5 min to displace the metals adsorbed to the root surface. Then the roots were desorbed in 30 mL of 5 mM $CaCl_2$, and the solution was changed every 10 min for 4 times. Then the roots were rapidly frozen in liquid nitrogen to destroy cell membranes, and desorption was continued for 40 min. The metals desorbed in the first 40 min plus the metals remaining in the roots were considered apoplastic fraction. The metals released following the freeze-thaw was considered as symplastic fraction.

2.4. Enzyme and non-enzymatic antioxidant analysis

The activity of antioxidant enzyme (POD, SOD) and GSH content were determined with an assay kit purchased from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China.

APX activity was determined by estimating the rate of ascorbate oxidation (Nakano and Asada, 1987). Leaves (0.2 g fresh weight) were homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100, 1% (w/v) polyvinylpyrollidone (PVP) and 2 mM AsA. The leaves were applied at proportions 1:4 (w/v). The homogenate was centrifuged at 4000 rpm for 20 min at 4 °C and the supernatant was used for enzyme assays. APX activity was determined immediately, using a reaction mixture (3 ml) containing 50 mM potassium phosphatebuffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM AsA and 0.1 mL enzyme liquid. Decrease in absorbance at 290 nm was measured at 25 °C for 3 min (E = 2.8 mM^{-1} cm^{-1}).

2.5. Statistical analysis

Data were analyzed with Origin 8.0 or SPSS 19.0 for windows. The significant differences were detected by the LSD test, taking $P < 0.05$ as the significant level. The results are presented as mean values ± S.E. of three replications.

3. Results and discussion

3.1. Effects of IAA, KN, and Spd on ramie growth

As shown in Fig. 1, the length of ramie roots and shoots were increased by the application of IAA at the presence of Cd/Pb. The probable reason was that IAA promoted cell division and coleoptile elongation (Ouni et al., 2014). Besides, both the root length and shoot height (6.4 and 22.0 cm) exposed to 10 μM IAA were longer than those of other treatments. On the other hand, the length of the root was between 3.5 and 4.5 cm in all treatments (Fig. 1b), indicating that KN did not have a significant effect on the elongation of ramie roots, which was in accordance with the previous study (Zhao et al., 2011). Additionally, the shoots were longer (18.2 cm) at 100 μM and shorter (13.8 cm) at 1000 μM KN, compared to the treatment (15.8 cm) with Cd/Pb alone (Fig. 1b). As shown Fig. 1c, the optimum concentration of Spd was 100 μM, with the root length 6.7 cm and shoot height 25.9 cm. On the whole, the optimum concentrations of IAA, KN, and Spd were 10, 100, and 100 μM, respectively.

3.2. Cd and Pb uptake and distribution in ramie

The effects of IAA, KN, and Spd, alone and combined with EDDS, on Cd and Pb uptake and translocation in ramie are listed in Table 1. Cd and Pb contents in different tissues of ramie decreased following the order of roots > stems > leaves. Lower Cd and Pb in the roots of ramie were determined upon the application of EDDS. On the contrary, the concentrations of Cd and Pb in leaves and stems were increased with EDDS. The result demonstrated that EDDS acting as a chelating agent was useful to facilitate Pb and Cd movement