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Research article

Effects of salicylic acid, Fe(II) and plant growth-promoting bacteria on Cd accumulation and toxicity alleviation of Cd tolerant and sensitive tomato genotypes

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ABSTRACT

In this study, we investigated the ameliorative effects of salicylic acid (SA), metal ion (Fe(II)), and plant growth-promoting bacteria *Burkholderia* sp. D54 (B) on two tomato genotypes with different Cd tolerances under Cd stress, viz. Liger (Cd tolerant) and Tabd (Cd sensitive). The plant biomass, Cd accumulation, antioxidative response, pigment content and photosynthetic performance were determined. According to the results, exogenous application of SA, Fe(II) and *Burkholderia* sp. D54 or their complex effectively reduced Cd accumulation and increased biomass of root, stem and leaves in both Cd sensitive and Cd tolerant genotypes. Among all treatments, SA+Fe+B exerted the best performance. *Burkholderia* sp. D54 effectively alleviated Cd-induced oxidative toxicity in both tomato genotypes, while SA ameliorated oxidative stress in Cd sensitive genotype. Photosynthetic pigment content and photosynthetic rate of Cd tolerant genotype was increased by all treatments, but only SA and *Burkholderia* sp. D54 treatment increased pigment contents and photosynthetic performance in Cd sensitive genotypes. All treatments significantly decreased Cd accumulation in both tomato genotypes. The effect of Cd reduction was Fe+SA+B>SA>Fe>B. Taken together, our results indicated that exogenous application of SA, Fe(II) and *Burkholderia* sp. D54 could alleviate the Cd toxicity in both Cd sensitive and Cd tolerant genotypes, although the extent varies.

1. Introduction

Cadmium (Cd) is a widespread heavy metal and toxic contaminant in the environment (Nazar et al., 2012). Due to sewage wastewater irrigation, chemical fertilizer application as well as rapid industrialization, increasing of Cd contaminated agriculture area as well as Cd concentration in the soil become more and more evident. Cd exhibits toxic, mutagenic and carcinogenic properties to most organisms (Adams et al., 2012). Cd stress usually results in a series of physiological, biochemical and structural changes in plants, including inhibition of seed germination (Kuriakose and Prasad, 2008), reduction of growth rate (Dias et al., 2013), induction of oxidative response (Pinto et al., 2017), inhibition of stomatal opening (Fasahat and Fasahat, 2014), disruption of photosynthetic apparatus (Parmar et al., 2013), inhibition of plant metabolism, disturbance of the uptake and translocation of mineral nutrients (Nazar et al., 2012). These changes ultimately lead to the reduction of both quality and yield of the crop. Various substances have been used to alleviate heavy metal stress in plants, including exogenous application of phytohormones, beneficial metal elements as well as plant-growth promoting rhizobacteria (PGPR).

Salicylic acid (SA), a well-known endogenous defense hormone, is involved in multiple physiological processes in plants. Its function against heavy metal stress has been extensively characterized (Wani et al., 2016). Salicylic acid plays an important role in both local resistance and in systemic acquired resistance in plants (Zhang et al., 2010), it has been applied in many plant species to alleviate Cd toxicity by regulating plant growth, reducing Cd uptake, altering Cd distribution, protecting membrane integrity, scavenging reactive oxygen species and improving photosynthetic capacity (Wani et al., 2016; Liu et al., 2016). It is reported that exogenous application of SA decreased the adverse effects of Cd on

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photosynthesis in maize (Krantev et al., 2008). Pretreatment of flax seeds with SA significantly decreased the absorption and transportation of Cd but increased the chlorophyll, malondialdehyde (MDA), total lipid content as well as other nutrient elements in Cd-affected flax plants (Belkhadi et al., 2010).

Metal ion (Fe(II)) is a well-known nutrient that interacts with Cd. Studies have shown that an increased exogenous supply of Fe(II) leads to decreased Cd uptake by plants, such as Arabidopsis and rice (He et al., 2017; Shao et al., 2007). Exogenous application of Fe alleviated Cd-induced leaf chlorosis, growth inhibition and oxidative damage in Arabidopsis, it also decreased Cd level in Arabidopsis via competition between the uptake of Fe and Cd (He et al., 2017).

Burkholderia sp. D54 (B) is a heavy metal resistant bacteria with plant-growth promoting properties, it produces indole acetic acid, siderophores and 1-aminocyclopropane-1-carboxylate deaminase, it also has the ability of solubilizing inorganic phosphate (Guo et al., 2011). *Burkholderia* sp. D54 inoculation significantly increased the growth of *Sedum alfredii* Hance grown on metal contaminated soil. In addition, *Burkholderia* sp. D54 exhibits Fe(II) and Mn(II) oxidation ability, which could stimulate the formation of iron plaques and prevent the uptake of heavy metals on the root surface of plants. Inoculation of rice with this bacteria in the presence of Fe(II) significantly decreased the Cd accumulation in rice plants, but the photosynthetic rate of rice leaf was enhanced (Dong et al., 2016).

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable worldwide and it is relatively sensitive to heavy metal stress. Cd stress has severely influenced both the quality and yield of the tomato plants. Due to the biological function of SA, Fe(II) and *Burkholderia* sp. D54 in plants exposed to stressful environment as mentioned above, the present study was aimed to address the following questions: (1) whether exogenous application of SA, Fe(II) and *Burkholderia* sp. D54 as well as their complex could alleviate Cd toxicity in tomato plants. (2) whether the Cd sensitive genotype and the Cd tolerant genotype have similar response to these treatment under Cd stress.

2. Materials and methods

2.1. Tomato seedlings preparation

Tomato seeds (variety Liger and Tabd) were provided by the Chinese Academy of Agricultural Sciences, the surface of the seeds was sterilized with 30% H_2O_2 for 15 min, then rinsed five times with distilled water and sown in trays with vermiculite for germination. After the second leaf emerged, plants were shifted to hydroponic pots with 1/2 Hoagland's nutrient solution and transferred to a controlled plant growth chamber (16 h light, 8 h dark, 60% relative humidity, temperature of 26 °C (day)/20 °C (night)). After three weeks acclimation of hydroponic condition, uniform individuals were selected and transplanted in the hydroponic bowl (volume 1000 ml, 15 × 17 cm) for experiments.

2.2. Experimental design

The following treatments were established: (1) CK: control (no Cd added); (2) Cd: Cd treatment, 5 mg L^{-1} Cd ($CdSO_4$); (3) SA: pretreatment with 100 μmol L^{-1} SA followed by 5 mg L^{-1} Cd stress; (4) Fe: pretreatment with 250 μmol L^{-1} Fe ($FeSO_4$) followed by 5 mg L^{-1} Cd stress; (5) B: inoculation with bacteria *Burkholderia* sp. D54 followed by 5 mg L^{-1} Cd stress; (6) Fe + SA +B: pretreatment with 100 μmol L^{-1} SA, 250 μmol L^{-1} Fe as well as *Burkholderia* sp. D54 followed by 5 mg L^{-1} Cd stress. Salicylic acid, Fe and the *Burkholderia* sp. D54 treatments were performed 3 days before Cd stress. Salicylic acid was exogenous supplied by spraying evenly on tomato leaves in dark. Iron solution was prepared as stock using $FeSO_4$ and EDTA-Na_2 and the final application concentration was 250 μmol L^{-1} *Burkholderia* sp. D54 was grown in LB broth at 28 °C for 24 h. Cells were harvested by centrifuge at 5000 rmp for 15 min and resuspended in 0.01M phosphate buffer (pH = 7.0). Cell concentration was adjusted to 10^6 L^{-1} (OD = 1.0 at 600 nm). Tomato roots were then dipped in bacteria suspension for two hours in order to get successful inoculation, and then transferred to hydroponic bowl. Each treatment has 6 replicates.

2.3. Sample preparation and Cd content analysis

Cd content was determined as previously described with minor changes (Subhashini et al., 2013). Plant samples (each 0.25 g) were grounded and soaked with 10 ml HNO_3 in digestion tube for 12 h, then digested at 80 °C for 1.5 h, 120 °C for 1.5 h, 150 °C for 3 h in digestion furnace and acid-driving at 175 °C (LabTech DigiBlock ED54, China). The liquid was transferred to 50 ml volumetric flasks, followed by dilution with 1% HNO_3 to the volume, the fluid was filtered through a 0.45 μm membrane and Cd content was determined by ICP-MS (Agilent 7500a, USA). Each treatment was repeated 6 times.

2.4. Antioxidant enzyme activity, proline content and malondialdehyde level determination

Activities of antioxidant enzymes, including superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), were determined as described by Arora et al. (2012). Peroxidase (POD) activity and proline (Pro) content was determined according to the method described by Popović (Sun et al., 2011). Evaluation of malondialdehyde (MDA) level was determined as described by Banerjee (Banerjee et al., 2016).

2.5. Photosynthesis and photosynthetic pigment assay

The photosynthesis parameters, including photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr), were determined with a LI-COR 6400 infrared gas analyzer (LI-COR 6400; LI-COR Inc, Lincoln, NE, USA). The water-use efficiency (WUE) was calculated by dividing the Pn with the Tr. Measurements were performed at 9:30 a.m. and 4:30 p.m. Beijing time.

The photosynthetic pigment contents of tomato seedling leaf, including chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car), were determined. Fresh leaf samples were collected from the three-weeks-old tomato plants, 0.2 g sample was grounded in the presence of 5 ml 80% acetone, the homogenate was filtered through Whatman filter paper No.2. The absorbance of the filtered solution was then recorded at 470 nm, 646 nm and 663 nm using a UV/Vis spectrophotometer (Model UV-2102C, UNICO, USA). Acetone (80%) was used as control.

2.6. Statistical analysis

Statistical analysis was performed using one-way ANOVA (SPSS Inc, Chicago, IL, USA). Significant differences between treatments were calculated at 5% probability levels ($p < 0.05$).

3. Results

3.1. Effects of different treatment on Cd accumulation of two tomato genotypes

The influence of SA, Fe, B and Fe+SA+B on Cd accumulation of both Cd tolerant (Tabd) and Cd sensitive (Liger) tomato genotypes