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Effects of salicylic acid, Epi-brassinolide and calcium on stress alleviation and Cd accumulation in tomato plants

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ABSTRACT

Salicylic acid (SA), Epi-brassinolide (EBL) and calcium (Ca) play crucial roles in plant development and mediate plant response to biotic and abiotic stress. This study was aimed to investigate the possible mediatory role of SA, EBL, Ca or their combination in protecting tomato plants from cadmium (Cd) toxicity. According to the results, Cd stress resulted in a significant reduction of plant dry mass, photosynthetic pigment content as well as photosynthetic rate. Exogenous application of SA decreased the malondialdehyde (MDA) level by 39.27% and increased catalase (CAT) activity by 81.17%. SA and EBL treatment significantly increased chlorophyll a (Chl a), chlorophyll b (Chl b) content, photosynthetic rate (Pn) as well as water use efficiency (WUE). SA + EBL (1:1)/Ca + SA + EBL (1:1:1) treatment obviously alleviated Cd-induced growth inhibition, the dry mass of different tomato organs were significantly increased ($p < 0.05$). Especially in Ca + SA + EBL treated plants, the dry mass of roots, stems and leaves increased by 141.18%, 128.57% and 118.52%, respectively. Besides, SA + EBL and Ca + SA + EBL treatments reduced the MDA level, but increased photosynthetic pigment concentration and photosynthetic efficiency. CAT activity was increased by 62.92% in Ca + SA + EBL treated plants, the WUE was increased by 557.76% in SA + EBL pretreated plants. Moreover, exogenous application of SA, SA + EBL and Ca + SA + EBL significantly decreased Cd accumulation in tomato organs ($p < 0.05$) compared with Cd-stressed plants. Taken together, our results indicated that exogenous application of SA, EBL and Ca individually or in combination could alleviate Cd toxicity in tomato plants, although the extent varies.

1. Introduction

Cadmium (Cd), a widespread heavy metal, is well recognized for its toxic, mutagenic and carcinogenic properties (Adams et al., 2012). In 2014, a national soil pollution report disclosed that 16.1% of the soil and nearly 19.4% of the arable land in China are contaminated by heavy metals, such as Cd (Dong et al., 2016). Elevation of Cd in soil is mostly due to industrial pollution, sewage wastewater irrigation and chemical fertilizer application (Wu et al., 2010). Cd does not exhibit beneficial biological functions in plants, in contrast, it is considered as a toxic heavy metal to plants. Cd depresses plant physiological effects, such as inhibition of seed germination, reduction of growth rate and reduction of photosynthesis, respiration and transpiration (Susana et al., 2012). Cd also leads to change of plant hormone homeostasis, damage of cell membranes, disruption of electron transport, alteration of antioxidative enzyme activity (Sghayar et al., 2015; Kapoor et al., 2014). At the subcellular level, Cd interacts with different components of the photosynthetic apparatus, thus inhibiting electron transport efficiency and chlorophyll biosynthesis.

Salicylic acid (SA) is an endogenous signal molecule which is involved in regulating oxidative level in response to environmental stress. It plays a key role in plant stress regulation and may thus be used against heavy metal toxicity. SA could alleviate Cd toxicity by regulating plant growth, reducing Cd uptake, altering Cd distribution, protecting membrane integrity and stability, scavenging reactive oxygen species, enhancing antioxidant defense system and improving photosynthetic capacity (Liu et al., 2016). Guo et al. (2007) reported that SA treatment enhanced the antioxidative ability of rice, thus alleviated Cd-induced oxidative damage and enhanced Cd tolerance. It is also reported that SA could alleviate Cd-induced inhibition of leaf growth and reduction of fresh weight of tomato seedlings (Çanakci, 2012). Calcium (Ca) is a well-known secondary messenger, under environmental stress, cytosolic Ca concentration is transiently elevated, which then delivers this signal to cells and subsequently triggers a variety of cascade responses and lead to the adaption of the environmental stress (Bootman et al., 2012). Ca is also an essential plant

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macronutrient, it is involved in various plant physiological processes, such as plant growth and development, cell division, cytoplasmic streaming and photosynthesis (Huang et al., 2017). Recent studies have shown that Ca could be used as an exogenous substance to protect plants against Cd stress by alleviating growth inhibition, regulating heavy metal uptake and translocation, improving photosynthesis, mitigating oxidative damages and stimulating signal transduction in the plants. Epi-brassinolide (EBL) is an endogenous polyhydroxy steroidal phytohormone which is found throughout the plant development, it is involved in numerous plant processes, including seed germination, rhizogenesis, senescence, cell elongation and stress alleviation (Bajguz and Hayat, 2009; Lukatkin et al., 2011). Exogenous application of EBL significantly reduced chromium accumulation and improved the growth of *Oryza sativa* L seedlings, it also ameliorated the chromium stress by up-regulating the activity of antioxidative enzymes (Sharma et al., 2016). EBL application also alleviated Cd toxicity and significantly reduced Cd concentration in both leaves and roots of tomato plants (Hayat et al., 2010). In addition, 28-HBL, a brassinosteroid, could elevate the activity of antioxidant enzymes and increase the tolerance of radish seedlings to Cd stress (Sharma et al., 2010).

Phytohormone signaling cascades are reported to interplay during abiotic stress. It was reported that EBL-mediated increase in stress tolerance in *Arabidopsis* is integrated with other hormone pathways, such as abscisic acid, ethylene and SA pathways (Divi et al., 2010). Co-application of EBL and SA could effectively ameliorate Pb toxicity in *Brassica juncea* plants via modulating various metabolites (Kohli et al., 2018). In addition, EBL + SA play an imperative role in improving the antioxidative defense gene expression in *Brassica juncea* plants (Kohli et al., 2017). It is also reported Ca + SA treatment could regulate root elongation and Al concentration in soybean, the Al toxicity was alleviated via increasing the citrate secretion and activity of antioxidative enzyme. Meanwhile, Ca could stimulate SA accumulation under Al stress and SA treatment could increase cytosolic Ca content and the expression of Ca-related genes (Lan et al., 2016).

According to the data from FAOSTAT database in 2012, China is one of the three main tomato producers in terms of planting area (1,005,003 $h\,m^2$) and total production (50,125,055 tones). However, heavy metal concentration in soil is increasing year by year (Gil et al., 2004; Martín et al., 2013), thus posing a serious threat to tomato production due to the influence of both quality and the yield. According to Zhu et al. (2006), over 19.4% of the tested tomatoes contain Cd that exceeded the standard rate. Cd is absorbed by plants and consumed by human through the food chain, it could cause serious health problems if it is in an excess amount. Attempts have been made to alleviate heavy metal stress by the application of phytohormones as well as macromolecules. However, limited information regarding SA, EBL and Ca regulating tomato plant growth, detoxification and Cd accumulation has been published, their combined effects are still unknown. Therefore, this study was aimed to investigate the possible mediatory role of SA, EBL, Ca or their combination in protecting tomato plants from Cd toxicity. It will potentially provide new insights into novel strategies for mitigating heavy metal stress in tomato plants.

2. Materials and methods

2.1. Raising of tomato seedlings

Tomato (*Solanum lycopersicum* L.) seeds were surface sterilized with 30% H_2O_2 for 15 min, rinsed thoroughly with distilled water, and sown in trays with vermiculite. After the second leaf emerged, plants were shifted to 1/2 Hoagland's nutrient solution. Seedlings of uniform size were transferred to hydroponics pots in a growth chamber with the daily photoperiod of 16 h, temperature of 26 °C/20 °C (day/night) and relative humidity of 60%. After three weeks' acclimation of the hydroponic condition, uniform individuals were selected and transplanted in the hydroponic bowl with full Hoagland's nutrient solution (volume 1000 mL, 15 × 17 cm) for further experiments.

2.2. Experimental design

The following treatments were established: (1) Control: no Cd added; (2) Cd: treated with 5 $mg\,L^{-1}$ Cd; (3) SA: treatment with 100 $\mu mol\,L^{-1}$ SA followed by 5 $mg\,L^{-1}$ Cd stress; (4) EBL: treatment with 1 $\mu mol\,L^{-1}$ EBL followed by 5 $mg\,L^{-1}$ Cd stress; (5) Ca: treatment with 10 $mmol\,L^{-1}$ Ca ($CaCl_2$) followed by 5 $mg\,L^{-1}$ Cd stress; (6) SA + EBL: treatment with 100 $\mu mol\,L^{-1}$ SA and 1 $\mu mol\,L^{-1}$ EBL (V/V = 1:1) followed by Cd stress; (7) Ca + SA + EBL: treatment with 10 $mmol\,L^{-1}$ Ca, 100 $\mu mol\,L^{-1}$ SA and 1 $\mu mol\,L^{-1}$ EBL (V/V/V = 1:1:1) followed by 5 $mg\,L^{-1}$ Cd stress. The SA, EBL and Ca treatment was done by spraying the solution evenly on leaves of tomato seedlings three days before Cd stress. After two weeks, the root, stem and leaf samples of tomato were collected separately and plant tissue for analysis of antioxidant enzymes and chlorophyll content were transferred to − 80 °C, other samples were dried in an oven at 65 °C for 72 h until constant weight. There were six parallel experiments in each group.

2.3. Sample preparation and Cd content analysis

Plant samples were grounded and weighted accurately (0.25 g each), then soaked with 10 mL HNO_3 in digestion tube for overnight, followed by digestion 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 boiling fluid was transferred to 50 mL volumetric flasks, dilute with 1% HNO_3 to the volume, the mixture was filtered through a 0.45 μm membrane, then stored in 50 mL plastic bottles (Dong et al., 2016). Cd content was determined by ICP-MS (Agilent 7500a, USA). Each treatment has six replicates.

2.4. Antioxidant enzyme activity and malondialdehyde level determination

The activity of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) were determined as previously described (Sharma et al., 2010). The malondialdehyde (MDA) level was determined according to the method of Jambunathan (2010).

2.5. Photosynthesis and photosynthetic pigment assay

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

Photosynthetic pigment, including chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car), were extracted and determined as described (Zlobin et al., 2015). The fresh leaf samples (0.2 g) from three-weeks-old plants were grounded in the presence of 5 ml 80% acetone at 4 °C. The homogenate was then filtered using Whatman filter paper No. 2. The absorbance of the filtered solution was spectrophotometrically 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 by one-way ANOVA in SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant differences between treatments were calculated at 5% probability levels ($p < 0.05$).