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Sodium chloride alleviates cadmium toxicity by reducing nitric oxide accumulation in tobacco

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ABSTRACT

Nitric oxide (NO) is involved in regulating the response of plants to Cd toxicity. In this study, we examined possible involvement of NO in the alleviation of Cd toxicity by NaCl in tobacco plants. Two independent experiments were conducted to investigate the changes of NO accumulation and Cd concentration in tobacco plants after the addition of a NO donor, sodium nitroprusside dehydrate (SNP), or a NO inhibitor, nitro-L-arginine methyl ester (L-NAME) in the solution containing NaCl and Cd. NO accumulation in tobacco roots was enhanced when plants were exposed to Cd, but reduced in the treatments of NaCl or L-NAME. NO production was not enhanced even when SNP (NO donor) was added to the solution containing Cd and NaCl. Root number was reduced in plants exposed to Cd, and increased by the addition of NaCl and reduced by the addition of SNP. Addition of NaCl or L-NAME to the Cd-containing solution reduced Cd concentration in plant tissues, with L-NAME having a more dramatic effect. It can be concluded that alleviation of Cd toxicity by NaCl contributed to reduction of NO accumulation in plants.

1. Introduction

Cadmium (Cd) is a toxic heavy metal for both human and plants. Excessive Cd level in soils will inhibit plant growth through limiting root growth and reducing leaf photosynthesis, disordering ion balance and causing nutrient deficiency (Li et al., 2012). The accumulated Cd in plants enhances the production of nitric oxide (NO) (Besson-Bard et al., 2009; Vescovi et al., 2013) resulting in programmed cell death of an Arabidopsis suspension culture (De Michele et al., 2009) and oxidative stress in rice (Panda et al., 2011). However, it is also reported that Cd stress suppresses NO synthesis (Xiong et al., 2009; Xu et al., 2011). It is well documented that NO metabolism is closely associated with Cd tolerance of crop plants (Xiong et al., 2010; Gill et al., 2013). Recently NO was found to be involved in Cd toxicity alleviation by gibberellic acid in Arabidopsis (Zhu et al., 2012) and by hydrogen sulfide in alfalfa (Li et al., 2013). Although NO accumulation was enhanced in plants when exposed to Cd stress, application of exogenous NO, in the form of sodium nitroprusside dehydrate, could alleviate Cd toxicity on root growth of *Medecago truncatula* (Xu et al., 2010a,b), wheat seedlings (Hasanuzzaman and Fujita, 2013) and rice (Zhao et al., 2013). The conflicting results about the effect of NO on Cd toxicity might vary with plants species and NO level in plant tissues (Xiong et al., 2010), indicating the complex of NO in affecting Cd toxicity of plants.

NaCl is commonly known as a stress factor for plant growth (Zhu, 2001). However, several studies have revealed the function of NaCl in alleviating Cd toxicity (Ghnaya et al., 2007; Lefèvrea et al., 2009; Xu et al., 2010a). NaCl alleviated the Cd-induced growth reduction of the halophyte *Sesuvium portulacastrum* (Ghnaya et al., 2007), and reduced Cd accumulation in the Mediterranean halophyte species *Atriplex halimus* (Helal et al., 1999; Lefèvrea et al., 2009; Xu et al., 2010a). In a previous study, we found NaCl could alleviate Cd toxicity in tobacco by reducing Cd accumulation and ROS (reactive oxygen species) formation (Zhang et al., 2013). Thus, it is imperative to determine the underlying mechanisms of Cd toxicity alleviation by NaCl.

In this study, we addressed the possibility of NO involvement in Cd toxicity alleviation by NaCl in tobacco plants, and examined the function of NO in Cd tolerance development of tobacco plants.

2. Materials and methods

2.1. Plant culture

Tobacco seeds (Yunyan85, provided by Guizhou Tobacco Science Institute) were germinated on dishes filled with quartz sand, after sterilized with 0.5 percent H_2O_2

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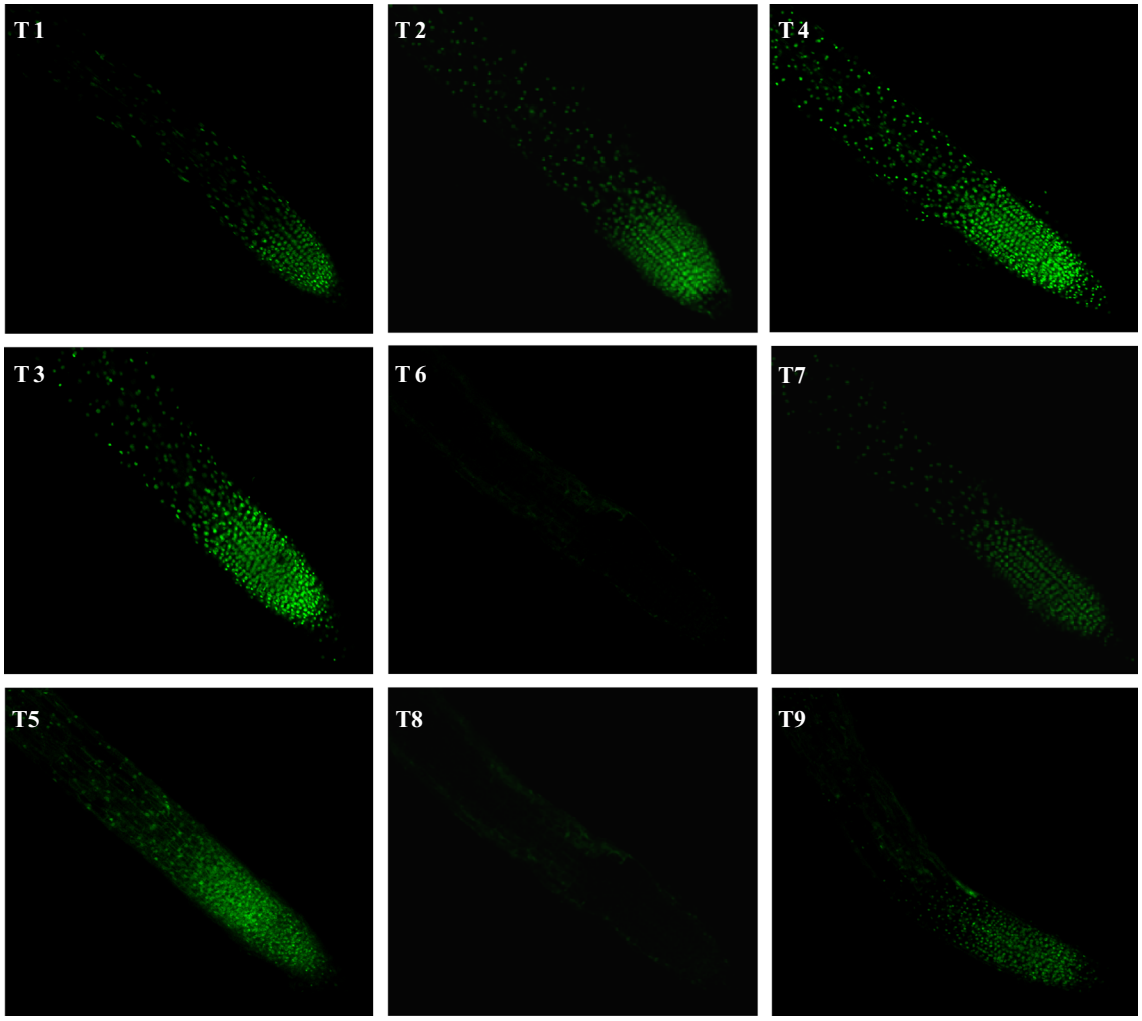

Fig. 1. NO accumulation in root tips of tobacco seedlings for experiment one. T1, control; T2, 1 μM $CdCl_2$; T3, 5 μM $CdCl_2$; T4, 10 μM $CdCl_2$; T5, 5 μM $CdCl_2$+0.5 mM L-NAME; T6, 5 μM $CdCl_2$+10 mM NaCl; T7, 50 μM $CdCl_2$; T8, 50 μM $CdCl_2$+0.5 mM L-NAME; and T9, 50 μM $CdCl_2$+10 mM NaCl. The florescence photos were taken at 50-folds enlargement.

for 10 min. When the seedlings had grown three leaves, the plants with uniform size were selected and transplanted into 5 L pots, which were filled with 1/4 Knops nutrient solution. The nutrient components were as follows: KNO_3, 0.375 mM; $CaNO_3 \cdot 4H_2O$, 0.75 mM; $MgSO_4 \cdot 7H_2O$, 0.312 mM; KH_2PO_4, 0.25 mM; $CuSO_4 \cdot 5H_2O$, 0.025 μM; H_3BO_3, 6.25 μM; $MnCl_2 \cdot 4H_2O$, 0.5 μM; $ZnSO_4 \cdot 7H_2O$, 0.5 μM; H_2MoO_4, 6.25 μM; and ferric citrate, 10 μM. After two weeks, the solution was changed to 1/2 Knops solution with the same composition mentioned above. The plants were cultivated for another two weeks, and then the treatments were applied. All tobacco plants were cultivated in a growth chamber with a mean temperature of 25 °C, 70 percent relative humidity (RH) and 14 h/10 h (day/night) photoperiod.

2.2. Chemicals

L-NAME (N^G-nitro-L-arginine methyl ester, hydrochloride), as an inhibitor of NO production, and SNP (sodium nitroprusside dehydrate), as a NO donor, were used to test the responses of plants to Cd stress. L-NAME and SNP were purchased from Sigma-Aldrich, and all the other chemicals were from Sinopharm Chemical Reagent CO., Ltd.

2.3. Treatments

To investigate how NaCl influences NO production, Exp 1 was conducted with the treatments as follows: T1, control; T2, 1 μM $CdCl_2$; T3, 5 μM $CdCl_2$; T4, 10 μM $CdCl_2$; T5, 50 μM $CdCl_2$; T6, 5 μM $CdCl_2$+0.5 mM L-NAME; T7, 5 μM $CdCl_2$+10 mM NaCl; T8, 50 μM $CdCl_2$+0.5 mM L-NAME; and T9, 50 μM $CdCl_2$ +10 mM NaCl. The treatments were lasted for 72 h, and then root tips were taken for use of measuring NO production. Meanwhile, root and shoot samples were collected to measure Cd concentration.

For determining the response of tobacco seedlings to Cd, NaCl and NO, Exp 2 was conducted with treatments as follows: P1, control; P2, 5 μM $CdCl_2$; P3, 5 μM $CdCl_2$+5 mM NaCl; P4, 5 μM $CdCl_2$+0.2 mM L-NAME; P5, 5 μM $CdCl_2$+10 μM SNP; P6, 5 μM $CdCl_2$+10 mM NaCl+10 μM SNP; P7, 5 mM NaCl; P8, 10 μM SNP; and P9, 0.2 mM L-NAME. At 48 h after treatments, root tips were taken for use of measuring NO production. In addition, at five days after treatment, photos were taken to count lateral root number and length.

2.4. Measurements of Cd and NO accumulation

The sampled plants were separated into shoots and roots, washed with deionized water, dried at 80 °C in an oven to a constant weight, and then weighed. The dried plant tissues were then prepared for measurement of Cd concentration. Approximately 0.1 g dry sample was mixed with HNO_3 and digested in a microwave digestion instrument (Microwave300, Anton PAAR, Graz, Austria). The solution was used to measure Cd concentration with ICP-MS (inductively coupled plasma mass spectrometry, Elan DRC-e, PerkinElmer, USA).

NO accumulation in roots was measured by DAF-FM florescence approach. Root tips of tobacco seedlings, about 2 cm length, were cut and immerged into a solution (20 μM DAF-FM, 50 mM Tris–HCl, pH 7.5) after washed with deionized water for three times, then placed for 2 h under dark condition at room temperature to load dyes. After dying, the samples were washed off the residual dye and observed under a confocal laser scanning microscope (ZEISS LSM780, Germany).

2.5. Statistical analysis

Data were statistically analyzed using ANOVA. The difference among treatments was determined using the least significant difference (LSD) test.