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Interactive effects of selenium and arsenic on growth, antioxidant system, arsenic and selenium species of *Nicotiana tabacum* L.

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ABSTRACT

This paper is aimed to study separate and interactive effects of selenium (Se) (selenite, 0–5 mg L^{-1}) and arsenic (As) (arsenate, 0–5 mg L^{-1}) on the growth and the antioxidant system of flue-cured tobacco (FCT) as well as the contents and species of As and Se in FCT through a hydroponic experiment and clarify the possible mechanism how Se alleviates the toxicity of As. The results were: single addition of Se ($\leq$1 mg L^{-1}) or As (1 mg L^{-1}) by a low dose could stimulate the growth of FCT, but the growth of FCT would be inhibited when Se (5 mg L^{-1}) or As (5 mg L^{-1}) was added by a high dose. Low As levels stimulated the uptake of Se but high levels of As posing the opposite effects with the low Se dosage. However, the addition of As always inhibited the uptake of Se with high Se levels. Moreover, Se showed dual effects on the uptake of As. At the low As dose (1 mg L^{-1}), the addition of Se inhibited the growth of FCT, but significantly promoted the activity of superoxide dismutase (SOD) and peroxidase (POD) enzymes as well as the content of MDA. Meanwhile, the percentages of organic Se and As(III) in the leaves of FCT declined with the increasing Se dose. However, the addition of Se by a moderate dose (0.1 mg L^{-1}) alleviated the toxicity of the high As dose (5 mg L^{-1}) and promoted the growth of FCT by elevating the ability of anti-oxidative stress of FCT and reducing the contents of MDA and As in FCT. The Se species in the leaves of FCT existed in organic ones (SeCys and SeMet) (100%), while the major As speciation was As(III) (75%). Likewise, the addition of As counteracted the toxicity of high Se dose (5 mg L^{-1}) and promoted the growth of FCT slightly as it reduced the formation of organic Se or failed to transform excess inorganic Se species into organic ones and depressed the contents of Se in the roots and leaves of FCT. In a word, the low Se dose (0.1 mg L^{-1}) alleviated of the toxicity of the high As dose and the addition of As counteracted the toxicity of high Se dose (5 mg L^{-1}), as a result of which the promotion of the growth of FCT were realized.

1. Introduction

Arsenic (As) is a severe pollutant which is highly toxic to animals and plants and widely distributes in the environment. Arsenic exists in the forms of arsenate (As(V)), arsenite (As(III)), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), but As(V) and As(III) are the main species (Tripathi et al., 2007; Zheng et al., 2013), which mainly come from mining, coal ash, dust, off gas, pesticides and sewage sluge (Meunier et al., 2011; Saunders et al., 2010). The accumulation of As in soil does not only can affect the growth and development of plants, but also pose a threat to human health via the food chain. Arsenic impedes the photosynthesis and reduces the contents of essential nutrients, thus inhibits the growth of crops and even causes them death (Garg and Singla, 2011). For human, arsenic may lead to cancerous and non-cancerous diseases, including bladder cancer, lung cancer, liver cancer and so on (Farnese et al., 2014).

Although there is no evidence that selenium (Se) is an essential element for plants so far, it is an essential microelement for human and animals. An appropriate dose of Se can improve the antioxidant capacity, scavenge excessive oxygen free radicals,

Abbreviations: Se, selenium; As, arsenic; MDA, malondialdehyde; SOD, superoxide dismutase; POD, peroxidase; AsA, ascorbate acid; GSH, glutathione; As(V), arsenate; As(III), arsenite; ROS, reactive oxygen species; MMA, monomethylarsonicacid; DMA, dimethylarsinic acid; Cd, cadmium; Hg, mercury; Ni, nickel; Pb, lead; GR, glutathione reductase; Se(IV), selenite; Se(VI), selenate; SeCys, selenocysteine; SeMet, selenomethionine; AR, arsenate reductase; PC, phytochelatins.

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decrease lipid peroxidation, defer senescence, promoting the growth of plants (Lin et al., 2012), and detoxify heavy metals (metalloids) in plants, for example arsenic (As) (Malik et al., 2012), cadmium (Cd) (Feng et al., 2012; Lin et al., 2012; Saidi et al., 2014), mercury (Hg) (Zhang et al., 2012a), nickel (Ni) (Gajewska et al., 2013) and lead (Pb) (Mroczek-Zdyrska and Wojcik, 2012). Currently, the researches on the mechanism how Se mitigates the toxicity of As are mainly concentrated on the elimination of reactive oxygen species (ROS), the suppression of lipid peroxidation and the enhancement of the antioxidant capacity of plants (Kramárová et al., 2012; Malik et al., 2012). Se exists mainly in the forms of Se(IV), selenate Se(VI), selenocysteine (SeCys) and selenomethionine (SeMet) in plants (Zhu et al., 2009). After absorbed by plants, Se(IV) or Se(VI) is converted into other forms like selenocysteine (SeCys) and selenomethionine (SeMet) (Zhu et al., 2009). Moreover, Se can replace sulphur in the amino acids as SeMet and SeCys due to their physicochemical similarity. And the organic Se species (SeCys and SeMet) can be incorporated into proteins, replacing cysteine (Cys) and methionine (Met), respectively, which can result in toxicity in plants (Navarro-Alarcon and Cabrera-Vique, 2008; White et al., 2004). However, the toxicity of soluble inorganic As is greater than that of organic As, and the toxicity of As(III) is higher than that of As(V) in the environment (Yamauchi, 1994; Yin et al., 2013). Having taken up by the roots of plants via the phosphate transport pathway (Wu et al., 2011b; Zhao et al., 2010), As(V) can be rapidly reduced to As (III) by arsenate reductase (AR) (Zhao et al., 2009). As(III), which can also be taken up by the roots of plants mainly through silicic acid transport protein (Ma et al., 2008), is chelated with polypeptides like GSH and PCs and finally stored in the vacuoles of roots to detoxify As (Liu et al., 2010; Ye et al., 2011; Zhang et al., 2012c). A previous study showed that Se could mitigate the toxicity of As via antagonistic effects in *Pteris vittata* (Feng et al., 2009a).

However, the changes of As and Se species in plants under the exposure of As and Se are still unclear. We infer that Se can mitigate the toxicity of As by regulating the antioxidant system and simultaneously affecting As species in plants.

Tobacco leaf, which contains abundant and high-quality soluble proteins (Teng and Wang, 2012), is considered as an ideal material to produce Se-rich protein. In this study, *Nicotiana tabacum* L. was chosen as the test material to investigate: (1) the responses of the growth and antioxidant systems of FCT to different doses of Se and As; (2) the relation among the variations of As species and the detoxification of As after Se supplementation.

2. Materials and methods

2.1. Seedling cultivation

The cultivar was *N. tabacum* K326, and the floating cultivation method was adopted for the cultivation of seedlings. Later, the seedlings of similar sizes were transplanted into plastic pots containing 10 L nutrient solution which was composed of 4 mmol L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 5 mmol L^{-1} KNO_3, 1 mmol L^{-1} $NH_4H_2PO_4$, 2 mmol L^{-1} $MgSO_4{\cdot}7H_2O$, 0.01 mmol L^{-1} EDTA-Fe, 0.046 mmol L^{-1} H_3BO_3, 0.0008 mmol L^{-1} $ZnSO_4{\cdot}7H_2O$ and 0.0003 mmol L^{-1} $CuSO_4{\cdot}5H_2O$. The plants grew in a greenhouse with natural light at 25–30 °C.

2.2. Experimental design and implementation

A hydroponic experiment of four Se levels, i.e. 0, 0.1, 1.0 and 5.0 mg L^{-1}, and three As levels, i.e. 0, 1.0 and 5.0 mg L^{-1}, was designed. As and Se were added in the forms of $Na_3AsO_4{\cdot}12H_2O$ and Na_2SeO_3, respectively. A randomized complete block design was employed. There was a total of 12 treatments, namely CK (without the addition of Se and As), Se0.1As0, Se1As0, Se5As0, Se0As1, Se0.1As1, Se1As1, Se5As1, Se0As5, Se0.1As5, Se1As5 and Se5As5, and each treatment was repeated for three times.

Four-leaf FCT seedlings were transplanted to 1/4 strength Hoagland–Arnon nutrient solution at first and three days later, the solution was replaced by 1/2 strength nutrient and the nutrient solution was renewed every five days. After forty days, the seedlings of even size were transplanted to the plastic cases ($22 \times 16 \times 7$ cm) and subject to the above-mentioned Se and/or As treatments with full strength Arnon–Hoagland nutrient solution, and each pot contained 2 plants. The plants were harvested after fourteen days. After rinsed carefully with tap water and deionized water successively, the separated fresh leaves and roots were weighed and divided into two parts. One was immersed in N_2 liquid immediately and stored at −80 °C to determine the Se and As species and the indices of the antioxidant systems later. The other was over-dried at 105 °C for 15 min to de-enzyme at first, and then at 65 °C for 48 h and finally pulverized to determine the contents of As and Se.

2.3. Determination of Se and As contents and species

The contents of Se and As were determined with a hydride generation atomic fluorescence spectrometer (AFS8220, Beijing Titan Instruments Co., China) (Feng et al., 2009a) after the tissues of FCT were digested with concentrated HNO_3–$HClO_4$. The accuracy of elemental analysis was verified by standard reference materials (GBWO7602 (GSV-1)) from the Center for Standard Reference of China.

A methanol:water (1:2) method was used to extract Se species in leaves or roots of FCT plants. The separation of Se^{IV}, Se^{VI}, SeMet and SeCys in the green parts of FCT was carried out with a anion exchange chromatography in which the column was connected to a ultraviolet treatment-hydride generation atomic fluorescence spectrometry (UV-HG-AFS) detection system (Han et al., 2013) online.

The extraction of As species was similar to that of Se species. Because no organic arsenic in FCT were detected in a preliminary experiment, only As(V) and As(III) were measured in this study. The separation of As(V) and As(III) in leaves and roots of FCT plants was conducted with the anion exchange chromatography in which the column was also connected to the HG-AFS detection system (Zhang et al., 2002) online.

The HPLC system consisted of a SHIMADZU 10ATvp Plus liquid chromatography pump (SHIMADZU, Tokyo, Japan), a Rheodyne 7725i injector (Rheodyne, Cotati, USA) and a Hamilton PRP-X100 column (Hamilton, Reno, NV). The mobile phase for HPLC was 15 mmol L^{-1} $(NH_4)_2HPO_4$ (pH 6.0, 1.0 mL min^{-1}). As for the HG phase, the reduction agents were 1.5% KBH_4 (m / v) + 0.5% KOH (m/v) and the carrier solution was 7% HCl (v/v). The detection phase was AFS8220, with the Se hollow cathode lamp current (General Research Institute for Nonferrous Metals, Beijing, China) of 50 mA, the negative high voltage of photomultiplier tube of 270 V, the flow rate of carrier gas of 400 mL min^{-1} and the flow rate of makeup gas of 600 mL min^{-1}.

2.4. Assay of enzymatic and non-enzymatic antioxidants

The activity of superoxide dismutase (SOD) was determined with the method put forward by Zhang et al. (2012b). In brief, 0.5 g fresh FCT leaves was grounded in 5 mL extraction buffer containing 50 mM potassium phosphate (pH 7.8) at first, and then the homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. 3 mL reaction mixture contained 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA and 100 μL enzyme extract. The reaction mixture was illuminated for 15 min. The sample absorbance was