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Selenium uptake, speciation and stressed response of *Nicotiana tabacum* L.

Dan Han[a,b], Xihong Li[c], Shuanglian Xiong[a,b,*], Shuxin Tu[a,b], Zhenguo Chen[c], Jinping Li[c], Zhijian Xie[a,d]

[a] College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China
[b] Microelement Research Center, Huazhong Agricultural University, Wuhan 430070, China
[c] Tobacco Research Institute of Hubei, Wuhan 430030, China
[d] Institute of Soil & Fertilizer and Resources & Environment, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China

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ABSTRACT

The research on the function and mechanism of selenium (Se) is of great significance for the development of Se-enriched agricultural products. In this paper, uptake, speciation distribution, the effects on the flue-cured tobacco growth and antioxidant system of Se at different levels (0–22.2 mg Se kg^{-1}) were studied through a pot experiment, aiming to clarify flue-cured tobacco's response to Se stress and the relationship between Se speciation and antioxidant system. The results showed that the leaf area and number, the biomass and the chlorophyll content reached the maximum at 4.4 mg kg^{-1} of Se treatment. Selenium at low levels ($\leq$4.4 mg kg^{-1}) stimulated the growth of flue-cured tobacco by elevating the capability of antioxidant stress and reducing the malondialdehyde (MDA) content to 0.6–0.8 times of that of the control. However, high Se levels ($\geq$11.1 mg kg^{-1}) depressed the capability of antioxidant stress and raised the MDA content to 1.5-fold of that of the control, and meanwhile the biomass of the aboveground parts and underground parts declined notably. The Se content in different parts of flue-cured tobacco significantly increased with the growth of Se levels. The range of Se content in roots, leaves and stems at 2.2–22.2 mg kg^{-1} of Se treatment were 16.7–58.6 mg kg^{-1}, 2.6–37.3 mg kg^{-1} and 2.2–10.3 mg kg^{-1}, respectively. According to the detection of different Se speciation, only selenocysteine (SeCys) was detectable in leaves at 2.2 mg kg^{-1} Se treatment; SeCys, selenite [Se(IV)]and selenate [Se(VI)] were detected in flue-cured tobacco leaves at Se treatment ($\geq$4.4 mg kg^{-1}), which accounted for 4.6–10%, 9–18.7% and 71–86% respectively; SeCys, selenomethionine (SeMet) and Se(IV) were detected in roots, and organic selenium(66–84%) was the main Se species at Se $\leq$ 11.1 mg kg^{-1} treatment; four Se species [SeCys, SeMet, Se(IV) and Se(VI)] were detected in flue-cured tobacco roots, and the main Se species was inorganic Se (60%) at 22.2 mg kg^{-1} Se treatment. That was to say, the percentage of organic Se species (SeCys and SeMet in flue-cured tobacco leaves and root) declined, whereas the ratio of inorganic Se species [Se(IV) and Se(VI)] increased with the growth of Se levels. The correlation analysis showed that the superoxide dismutase (SOD) activity as well as the glutathione (GSH) and MDA contents were positively correlated with the Se(IV) and Se(VI) contents at $P < 0.01$ and excessive inorganic Se might destruct the reactive oxygen species (ROS) balance and enhance the MDA content, thus causing damage to the plant growth. In a word, the present study suggested that the ratio of inorganic Se [Se(IV) and Se(VI)] was closely related with the growth and the antioxidant capacity of flue-cured tobacco and the excessive application of Se led to the higher proportion of inorganic Se and poorer antioxidant capacity, which ultimately inhibited the growth of flue-cured tobacco.

1. Introduction

Selenium (Se) is an essential microelement for human beings and animals. Se deficiency can cause health disorders, such as Keshan disease, Kashin-Beck disease, cardiovascular disease, muscle syndrome, and even death (Rayman, 2012). It was reported that approximately 72% of the land in China was Se deficient and the average dietary intake of Se was only 26–32 μg per day by Chinese adults (Chen et al., 2002), which was far lower than the reference intake of 40–200 μg per day specified by World Health

Abbreviations: Se, selenium; Se(IV), selenite; Se(VI), selenate; SeCys, selenocysteine; SeMet, selenomethionine; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde; GSH, glutathione; AsA, ascorbate acid; ROS, reactive oxygen species; WHO, World Health Organization.

* Corresponding author at: College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China. Tel.: +86 027 87282137; fax: +86 027 87288618.
E-mail address: xsl@mail.hzau.edu.cn (S. Xiong).

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Organization (WHO) (Cao et al., 2001). Application of Se in the soil is highly recommended as an approach to produce Se-rich food and eliminate Se deficiency in human diet. As a result, lots of Se-rich agricultural products appear at present, such as Se-rich rice, Se-rich maize, Se-enriched tea, Se-enriched vegetables and Se-enriched protein of tobacco leaves (Molan et al., 2009; Pyrzynska, 2009; Carey et al., 2012).

No evidence has demonstrated that Se is an essential element for plants so far. Se often exerts a dual effect on plant growth. That is to say, Se can stimulate the growth of plants at low doses but can bring damage to plants at high dosages. For example, Se treatment (0–2.0 mg kg^{-1}) promoted the biomass of wheat (*Triticum aestivum* L.) seedlings under drought stress (Yao et al., 2009), and 2 μM Se treatment (sodium selenite) also improved the growth of ryegrass (*Lolium perenne* L.) under Al stress (Cartes et al., 2010). Exogenous application of Se significantly increased chlorogenic acid, chlorophyll a and b, carotenoids in plant leaves (Dong et al., 2013), but dramatically depressed reactive oxygen species (ROS: H_2O_2 and $O_2^{\bullet -}$) and malondialdehyde (MDA) accumulation, thus enhancing the anti-oxidation of enzymatic and non-enzymatic system and improving the ability of plant resistance to abiotic stresses (Kumar et al., 2012; Lin et al., 2012). However, excess Se (≥6.0 μM Se in form of sodium selenite) would lead to ROS accumulation and the increase of $O_2^{\bullet -}$ and MDA contents in *Vicia faba* L., undermined the antioxidant system and enhanced lipid peroxidation, which inhibited the plant growth (Mroczek-Zdyrska and Wójcik, 2012).

Selenium existed in inorganic [Se(IV) and Se(VI)] and organic forms in plant tissues. After absorbed by plants, Se(IV) or Se(VI) could be converted into other forms, such as selenocysteine (SeCys) and Selenomethionine (SeMet) (Afton et al., 2009; Zhu et al., 2009). The beneficial or toxic effect of Se on human being and animals was not only dose-dependent, but also related to the chemical form and the bioavailability of Se (Thiry et al., 2012). Compared with inorganic Se, organic Se was safer and more efficient (Platis and Labrou, 2006). Therefore, it is of great significance to reveal Se uptake and chemical speciation transformation in Se-rich plants after application of Se into soil for our understanding of Se metabolism mechanism in plants as well as its impacts on human health. Previous studies have clearly reported Se species in different crops and vegetables, such as rice (*Oryza sativa* L.) (Carey et al., 2012), maize (*Zea mays* L.), alfalfa (*Medicago sativa* L.), soybean (*Glycine max* L.) (Yu et al., 2011), onion (*Allium cepa*), carrot (*Daucus carota* ssp. *Sativus*) (Kapolna et al., 2012), and so on. However, the speciation of Se in plants in different dosages of Se is seldom investigated.

Selenium plays an important role to stimulate the antioxidant system of plants at low levels but acts as a prooxidant at high levels (Feng et al., 2013). We presumed that the effect of Se on the antioxidant capacity of plants might depend on Se species and its concentration in plants. Tobacco leaves contain abundant and high-quality soluble protein (Teng and Wang, 2012), which is an ideal material for the production of Se-rich protein. In this study, with *Nicotiana tabacum* chosen as the test material, Se uptake, bioaccumulation and speciation as well as its influence on the growth and the antioxidant system of plants under various Se levels were studied, aiming to (1) obtain the optimal dose of Se for the flue-cured tobacco growth, (2) investigate Se transformation and distribution in plants under different Se levels, (3) reveal the relationship between Se species and the antioxidant capacity of plants.

2. Materials and methods

2.1. Experimental materials

The plant used was *N. tabacum* Yunyan87, a popular cultivar in China. The seeds were provided by Tobacco Scientific Research Institute of Hubei, China.

The soil tested was yellow brown soil taken from Xiangyang, Hubei province, China. Soil samples were air-dried at room temperature for 2 weeks, and passed through a 2 mm sieve prior to laboratory analysis. Soil pH (1:2.5 soil: water ratio), total organic C (the Walkley and Black method), total nitrogen (the Kjeldahl method), total P (the colorimetric method), total K (atomic absorption spectrometry), hydrolyzable N (alkali diffusion solution), extractable P (the Olsen's method), and available K (with ammonium acetate) were determined following procedures described in Page et al. (1982). Main physical and chemical soil properties were as follows: pH 6.95 (soil water ratio of 1:2.5), organic matter 18.08 g kg^{-1}, total N 0.21 g kg^{-1}, total P 0.45 g kg^{-1}, total K 2.68 g kg^{-1}, alkaline hydrolysis N 29.17 mg kg^{-1}, Olsen-P 7.71 mg kg^{-1}, available K 86.06 mg kg^{-1}, total Se 0.288 mg kg^{-1}.

2.2. Experimental design

A soil pot experiment was conducted in the greenhouse of the Micro-element Research Center in Huazhong Agricultural University in Wuhan, China (N 31°28′26″, E 114°20′15″) from May to July 2011. Five levels of Se (sodium selenite) treatment, i.e. 0 (control), 2.2, 4.4, 11.1 and 22.2 mg kg^{-1} Se were performed in the experiment. The size of pot was 30 cm × 30 cm and each pot was filled with 10 kg of air-dried and 2 mm-sieved soil. The mixtures of 0.24 g kg^{-1} N, 0.36 g kg^{-1} P_2O_5, 0.6 g kg^{-1} K_2O [in the form of $CO(NH_2)_2$, KH_2PO_4 and K_2SO_4 respectively] and 1 mL Arnon's microelement solution for each kilogram soil were applied as basal dressing at 14 days before transplanting. Each treatment was replicated for 3 times.

After sterilized with 2% (v/v) NaOCl for 10 min, the seeds of tobacco were thoroughly rinsed in tap water and then sown in floating nursery. Tobacco seedlings (at four-leaf stage) were transplanted into pots on May 18, 2011, with one plant for each pot. During the growth period, the roots were irrigated with 75% Thiophanate-methyl WP for 2 times to cure black roots of tobacco, and additionally mancozeb and Bilken Virusicide of 600–800 times were used for foliar spray to prevent climate spot and brown spot diseases.

Two months after transplanting, the plants were harvested. The plant samples were separated into 5 parts, i.e. upper leaves (6 leaves in the upper), middle leaves (6 leaves in the middle), lower leaves (6 leaves in the low), stem and roots. All tissues were washed with tap water and rinsed with Milli-Q water (Millipore, USA). After the water on the surface was dried with gauze, the second and fourth of middle leaves were collected and separated into two parts: one was immersed in N_2 liquid immediately and afterwards stored at −80 °C until the analysis of the antioxidant system; and the other as well as half of roots were immersed in N_2 liquid immediately, then triturated with an agate mortar and pestle and freeze–dried, finally stored at −80 °C until the extraction of Se species. Other leaves, stem and half of roots were over-dried at 105 °C for 15 min to deenzyme at first and then at 65 °C for 48 h, weighed and pulverized for Se determination.

2.3. Plant Se determination and speciation

The content of Se were determined with a hydride generation atomic fluorescence spectrometer (AFS8220, Beijing Titan Instruments Co., China) after the plant tissues were digested with concentrated HNO_3-$HClO_4$ (Feng et al., 2009b). The accuracy of the elemental analysis was checked by standard reference material GBW07602 (GSV-1) (branches and leaves of shrubs certified at 0.184 ± 0.013 μg Se g^{-1}) purchased from the Center for Standard Reference of China. The standard reference material was run in parallel with the samples, and the value obtained was found in good agreement with the certified value.