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ARTICLE

The effect of selenium (Se) on development and nitric oxide levels in *Arabidopsis thaliana* seedlings

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ABSTRACT Selenium (Se) is an essential element for many organisms, but its excess leads to toxicity symptoms such as growth inhibition or chlorosis. Since nitric oxide (NO) is a multifunctional signal molecule in plants acting during several physiological plant responses, we examined the effect of selenite treatment on nitric oxide status of *Arabidopsis* plants during their growth. The effect of selenite on the development and cell viability of seedlings proved to be concentration and time-dependent and it can be explained by the disturbance of protein synthesis, structure and function. Selenite treatment modified the endogenous NO status in the root and the cotyledon of *Arabidopsis* plants. During the first days after germination the effect of Se was shown to be rather inhibitory on NO content, while during the late developmentin Se-treated plants NO levels increased, which may contribute to growth inhibition.
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KEY WORDS

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selenium
morphological parameters
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viability

Selenium (Se) is an essential trace element, which is originally contained in soils and waste water through natural and anthropogenic processes (Minorsky 2003). The accumulation of it in relatively high concentration is toxic for plants, animals and even humans. Plants are able to take up Se in different forms such as selenate (SeO_4), selenite (SeO_3) or organic Se (Zayed et al. 1998). Selenium is chemically similar to sulphur (S), as it was shownin *Arabidopsis thaliana* L., where the selenate directly competes with sulphate for uptake causing S starvation symptoms (chlorosis and reduced growth) (Ellis and Salt 2003). Selenium is metabolized through the sulphate assimilation pathway, which leads to selenocystein and selenomethionine formation. The incorporation of these selenoanalogs in proteins disturbs the synthesis, structure and functions of proteins (Tamaoki et al. 2008) However, it is well-known from the literature that some species of *Astragalus* and *Stanleya* are Se-hyperaccumulators, which means they have the capacity to take up the Se of thousands or hundreds of mg/kg (Ellis and Salt 2003).

Nitric oxide (NO) is an ubiquitous free radical gas, which acts as a general plant signal during growth and development and stress responses. In concurrence with plant hormones NO was shown to induce e.g. seed germination, lateral root formation, root hair development, pollen tube growth (Beligni and Lamattina 2003; Correa-Aragunde et al. 2004; Lombardo et al. 2006; Prado et al. 2008). During copper-induced morphological responses NO proved to be the negative regulator of auxin (Pető et al. 2011). Nitric oxide is able to modulate gene expression via S-nitrosylation or tyrosine nitration of transcription factor proteins (Astier et al. 2011).Until now there was no literature data about the relationship between NO and selenium, thus our aim was to investigate the effect of selenium treatment on morphological parameters, viability and endogenous nitric oxide level of *Arabidopsis thaliana.*

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Materials and Methods

Plant material

During the experiments wild type (Col-0) *Arabidopsis thaliana* L. were used. Seeds were surface sterilized with 5% (v/v) sodium hypochlorite for 20 minutes and rinsed five times with sterile distilled water before being transferred to half-strength MS (Murashige and Skoog 1962) medium [1% (w/v) sucrose and 0.8% (w/v) agar] supplemented with sodium selenite (Na_2SeO_3) at 0, 10, 20 or 40 μM concentrations. The Petri dishes were placed vertically in greenhouse at photo flux density of 150 μmol m^{-2} s^{-1} (12/12 day/night period) at a relative humidity of 55-60% and 25 ± 2°C. The experiments were carried out at the 2^{nd}, 3^{rd}, 4^{th}, 7^{th} and 14^{th} day after germination (DAG).

Morphological measurements

On the 2nd, 3rd and 4th DAG primary root length (mm), hypocotyl length (mm) and cotyledon diameter (mm) were measured under a Zeiss Axiowert 200M microscope (Carl Zeiss, Jena, Germany). Primary root length of one-week-old plants was measured manually using a scale,the hypocotyl length and cotyledon diameter was determined under the microscope and radii were calculated ($r^2\pi$). In two-week-old

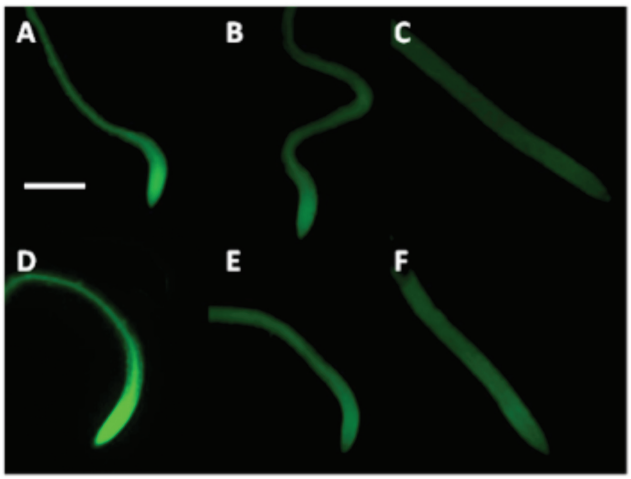

Figure 1. Cell viability of *Arabidopsis* primary roots on the 3rd (A-C) and 7th (D-F) DAG. Control (A, D), 20 μM Se (B,E), 40 μM Se (C,F). Plants were stained by 10 μM FDA as described in Materials and Methods. Bar=1 mm.

plants petiole length (mm) was measured under the microscope; the number of leaves was counted manually.

Fluorescence microscopy

For the fluorescent detection of NO and cell viability Zeiss Axiowert 200M microscope equipped with a high resolution digital camera (Axiocam HR, HO CCD, Carl Zeiss, Jena, Germany) and filter set 10 (exc.: 450-490 nm, em.: 515-565 nm) was used. Nitric oxide levels were visualized by a NO-sensitive fluorescent dye, 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA) according to Pető et al. (2011). Whole seedlings were vacuum infiltrated for 10 minutes and incubated for 30 minutes in 10 μM DAF-FM DA (in 10mM Tris-HCl, pH 7.4) solution in the dark at 25 ± 2°C and were washed twice within 30 minutes with Tris-HCl. Fluorescein diacetate (FDA) was applied for the investigation of cell viability (Lehotai et al. 2011). Seedlings were vacuum infiltrated for 10 minutes and incubated in small Petri dishes containing 2 ml FDA (in MES/KCl buffer, 10^{-3} M, pH 6.15) at room temperature for half an hour. After dyeing the samples were washed 4 times in 20 minutes with MES/KCl buffer. Fluorescent intensities were measured on digital images using Axiovision Rel. 4.8 software.

Statistical analysis

Significant differences were determined using the Student's test applying Microsoft Excel 2007 software. All the experiments were carried out three times. Statistically significant differences among means (n=9-15) are indicated by one(*P≤0.05), two (**P≤0.01) or three (***P≤0.001) asterisk(s).

Results and discussion

Selenite affects on seedling development and viability

According to primary root elongation, 10 μM Se did not cause any changes on the 2nd and 3rd DAG, but it significantly reduced PR length on the 4th, 7th and 14th day. Higher Se concentrations reduced PR length during both the early and the late developmental phases. Hypocotyl growth is another developmental process driven by cell elongation. On the first days (2nd and 3rd) the hypocotyl length was not affected by 10 μM Se, while this concentration resulted in a significant decrease during the further development. During the whole growth period hypocotyls of 20 or 40 μM Se-treated seedlings were shorter compared to control, although the inhibition of elongation was milder than in PRs. The root system is the first site of selenite accumulation, which explains its enhanced sensitivity (Tamaoki et al. 2008). In case of cotyledon expansion, 10 μM Se was ineffective during the early seedling development, while more serious Se excess (20 or 40 μM) resulted in a decrease already on the 2nd DAG. In case of two-week-old plants, petiole length and leaf number was also determined. Every selenite concentrations caused serious decrease in both morphological parameters of *Arabidopsis* plants. In primary roots of two-day-old seedlings, 40 μM Se decreased cell viability, while in cotyledons no changes were observed. On the 3rd and 4th DAG, viability loss was detected in cotyledons of 20 and 40 μM Se-treated seedlings and during the late development (one and two weeks) every concentrations of Se treatment resulted in viability reduction in both organs (Fig. 1).

The effect of selenite on the development and cell viability proved to be concentration and time-dependent and it can be explained by the disturbance of protein synthesis, structure and function triggered by selenocystein and selenomethionine formation (Tamaoki et al. 2008).

Endogenous NO status of *Arabidopsis* seedlings is disturbed by selenite

In the first stages of seedling development Se exposure did not affect NO levels of cotyledons, although in case of primary roots 20 μM Se enhanced and 40 μM Se decreased endogenous NO. After one-week-treatment, 10 and 20 μM selenite caused an increase in NO formation within the primary root and 40 μM Se significantly reduced NO levels in both organs. On the 14th DAG endogenous NO content of cotyledons and roots significantly increased as the effect of 10, 20 or 40 μM Se exposure.

Selenite treatment was able to influence the endogenous NO status within the root and the stem system of Col-0 *Arabidopsis* plants. During the early seedling development the effect of Se was shown to be rather inhibitory, while later (when all the growth parameters were reduced) NO levels

increased in Se-treated plants. In the future, further research is needed to elucidate the involvement of nitric oxide in selenium-induced developmental changes.

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