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The impact of selenium treatment on some physiological and antioxidant properties of *Apium repens*

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ABSTRACT

Studies are scarce regarding the physiological effects of selenium treatment on antioxidant capacity of plants. In the presented *in vitro* experiment, *Apium repens* was used to investigate its ability to absorb Se(IV) at different concentrations (0.01, 0.1, 1, 10 and 50 mg L^{-1}) and its physiological response to treatment. These plants possessed a high affinity to take up Se(IV), since Se concentration measured in plants reached 730 μg g^{-1} DM in 50 mg L^{-1} treated plants. Lower Se(IV) concentrations (0.01–1 mg L^{-1}) promoted growth, while higher Se(IV) concentrations (10 and 50 mg L^{-1}) affected it negatively. The photochemical efficiency decreased significantly in plants treated with higher Se(IV) concentrations compared to control plants. In the roots, total cysteine and glutathione content increased gradually from 0.01 to 10 mg L^{-1} Se treatment, when compared to the control. Enhanced total glutathione levels were also determined in the above ground parts when the plants were treated with lower Se(IV) concentrations (0.01–1 mg L^{-1}). In both roots and above-ground parts, 50 mg L^{-1} Se(IV) treatment caused a significant degradation of total cysteine and glutathione, which was accompanied by a significantly larger oxidized glutathione pool. By applying five different concentrations of Se(IV), it was possible to identify a threshold Se content for *A. repens*, above which the nature of the effects induced changes from antioxidant to pro-oxidant.

1. Introduction

Selenium (Se) is an essential trace element for many organisms, but its essentiality for plants is still debatable, although in moderate concentrations it can exert positive effects on plants (Hawrylak-Nowak, 2008; Feng et al., 2013). Elevated concentrations of Se are toxic for most plants and lead to inhibition of plant growth and accumulation of reactive oxygen species (ROS) in plants (Freeman et al., 2010; Mroczek-Zdyrska and Wójcik, 2011). Feng et al. (2013) proposed that the increased production of ROS at high Se levels may be partially related to an imbalance in the levels of glutathione (GSH), thiols, ferredoxins and/or NADPH, which can play vital roles in the assimilation of Se. Se can control the production and quenching of ROS either directly or indirectly via the regulation of antioxidants. Three possible mechanisms have been proposed for the decrease in O_2^- levels when the appropriate doses of Se were added, including the spontaneous dismutation of O_2^- into H_2O_2 (without catalysis by the SOD enzyme), the direct quenching of O_2^- and $OH^•$ by Se compounds, and the regulation of antioxidative enzymes. Several studies (Hawrylak-Nowak, 2008; Feng et al., 2013) confirmed that, when plants were exposed to different stressors (drought, salinity, trace metals), the H_2O_2 levels were greatly diminished by the proper doses of Se, possibly owing to the reactivation of antioxidants by Se, especially of $H_2O_2^-$ quenchers (e.g., glutathione, GSH-peroxidase).

Among antioxidants, GSH plays the most important role in ROS interception. In addition, it acts directly in detoxifying metals by forming nontoxic complexes with the metals and facilitating their sequestration away from sensitive sites in cells (Noctor et al., 2012; Shahid et al., 2014). As an antioxidant, GSH interacts with ascorbate in the Halliwell–Asada cycle (ascorbate–glutathione), in which hydrogen peroxide is reduced to water by ascorbate peroxidase at the expense of reduced ascorbate. Subsequently, dehydroascorbate (DHA) is formed, which is reduced to ascorbate by the action of DHA

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reductase, using GSH as the reducing substrate. During this reaction, glutathione disulfide (GSSG) is generated, which is reduced by GSH reductase to GSH by using NADPH (Noctor et al., 2012). Thus, the concentration of GSH and ascorbate, as well as the activity of the antioxidant system enzymes, may be altered by excess Se exposure. Furthermore, the GSSG/GSH redox balance transmits specific information in order to fine tune cellular signalling pathways and responses under environmental stress conditions (Noctor et al., 2012; Shahid et al., 2014). Besides its role as a primary antioxidant and signalling molecule, GSH plays an important role in the detoxification of metals by directly forming nontoxic complexes with the metals and facilitates their sequestration away from sensitive sites in cells (Feng et al., 2013; Shahid et al., 2014).

Worldwide, we can find areas with toxic Se concentrations that pose a problem. Therefore, research into aquatic plants suitable for use in the cleanup of Se-contaminated water is underway (Dhillon and Dhillon, 2009; Basile et al., 2012). Plants mostly absorb the soluble forms of Se, selenite and selenate. Once Se is taken up by a plant, it can bioaccumulate and be transferred along the food chain. In the plant, Se undergoes certain changes: Se(VI) is reduced to Se(IV) and later to selenide, involving reduced GSH in the process. Selenide is incorporated into Se-cysteine, Se-methionine and proteins (Terry et al., 2000). Plants that accumulate Se transform Se-cysteine into less harmful compounds (SeMeSeCys, Se-cystathione, etc.), which are not toxic.

Apium repens (Jacq.) Lag., Apiaceae, is a small creeping perennial which grows in flood-prone pastures (McDonald and Lambrick, 2006). New shoots and wide spreading stolons, with roots at the nodes, extend from rosettes. From these, it can clonally propagate and spread in the natural environment (Burmeier and Jensen, 2009). This plant also regenerates in tissue culture on a simple, solid Murashige and Skoog (1962) medium (MS) lacking growth regulators. Therefore, tissue culture of *A. repens* is suitable for cultivation for several research purposes.

Despite considerable progress in understanding the biochemistry of ROS overproduction and scavenging, we still lack in-depth studies on GSH associated with semimetals such as Se and its tolerance capacity in plants. It is still unclear to what extent Se exposure alters the distribution of thiols in plant roots and aboveground parts, especially in aquatic plants suitable for the cleanup of Se-contaminated water. Therefore the aim of this study was to examine the affinity of *A. repens* for uptake of Se (0.01, 0.1, 1, 10, 50 mg L^{-1}) and the effect of Se(IV) on physiological parameters and antioxidants properties of *A. repens* grown under *in vitro* conditions in order to identify concentrations that exert an antioxidative effect while promoting plant growth and development.

2. Material and methods

2.1. Plant material and growth conditions

To investigate the response of *Apium repens* L. to selenite (Se(IV)) exposure, new shoots were placed on 20 mL of solid Murashige and Skoog (1962) medium (MS) without growth regulators. The MS medium was supplemented with 0.8% Difco Bacto agar, with 3% sucrose, and adjusted to pH 5.7–5.8 before autoclaving. Two shoots, each approximately 90 mg of fresh weight, were placed on the surface of the MS medium in a culture vessel for root induction. After two weeks, when the plants had developed on average 0.7 g FW roots, 20 vessels per treatment were filled with an additional 20 mL of sodium selenite (Na_2SeO_3) (98%, Sigma–Aldrich®, Taufkirchen, Germany) aqueous solutions at concentrations of 0.01, 0.1, 1, 10 and 50 mg L^{-1} for another three weeks. Controls consisted of plants that were treated with water. The vessels were incubated under controlled conditions at 23 ± 2 °C, with a photoperiod of 16 h at 38–50 μmol m^{-2} s^{-1} (Osram L 58W/77 – Fluora) and at 50% relative humidity. The experiment, with 6 replicates per treatment, was repeated twice.

2.2. Growth and developmental parameters

In order to monitor the growth and developmental parameters, the dry weight, the length of shoots and roots, number of stolons and shoots were monitored weekly.

The average relative growth rate (RGR) was calculated for each treated plant group (0, 0.01, 0.1, 1, 10 and 50 mg L^{-1}) as RGR = (lnM2 − lnM1)/(t2 − t1), where M2 is the value (dry weight or height of plants) measured on days 7, 14 and 21 after Se(IV) treatment, and M1 is the initial value (day 0); t2 − t1 is the time interval (days).

2.3. Physiological parameters – photochemical efficiency

Chlorophyll fluorescence was measured weekly *in situ* on eight plants from each Se concentration treatment, using a fluorometer (Handy PEA, Hansatech, Kings Lynn, UK). The measurements of chlorophyll fluorescence were made after 10 min of darkness, provided by dark-adaptation clips. Fluorescence was excited with a saturating beam of 'white light' (PPFD = 8 000 μmol m^{-2} s^{-1}, 0.8 s) (Schreiber et al., 1995).

2.4. Biochemical parameters

2.4.1. Determination of total selenium

For Se analysis, the samples were lyophilized (LIO–5 P Kambič, Slovenia) and ground (Fritsch pulverisette 14), and the amount of Se was measured using Perkin Elmer Elan DRC-e. Each sample from the experiment was analyzed twice. The detection limit (LOD) of the method was 0.050 mg kg^{-1}. The accuracy of the method was checked against the reference material Fapas T07223QC (The Food and Environment Research Agency, Sand Hutton, York, United Kingdom).

2.4.2. Determination of photosynthetic pigments

The content of chlorophylls *a* and *b* and carotenoids was measured each week in three plants from each Se concentration treatment. The lyophilized samples were extracted with acetone, and the amount of pigment was determined using a UV/VIS spectrometer (Lichtenthaler and Buschmann, 2001). For anthocyanins, the lyophilized samples from each Se concentration treatment were extracted in the media (methanol:HCl = 99:1) and amount of pigment was measured as described by Drumm and Mohr (1978).

2.4.3. Determination of thiols

Eight samples from each Se treatment were used to determine thiol content. The experiment was repeated twice with the same number of samples. Each sample consisted of the above-ground parts and roots of three *in vitro* plants, which were separated and immediately frozen in liquid nitrogen after harvesting. Later, the material was freeze dried and ground (Fritsch pulverisette 14).

A quantitative analysis of thiol compounds (total glutathione, oxidized glutathione, total cysteine and cystine) was done according to Tausz et al. (2003). The separation and determination of the derivatized thiols were done using a gradient high-pressure liquid chromatography system (HPLC), consisting of the Waters 2695 HPLC system, the Waters 2475 Multi Fluorescence Detector (excitation: 380 nm wavelength; emission: 480 nm wavelength), the column Spherisorb S5 ODS2 250 × 4.6 mm. Solvent A: 0.25% (v/v) acetic acid in water containing 5% methanol, pH 3.9. Solvent B: 90% (v/v) methanol in water; gradient: 5% solvent B to 15% solvent B