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Silicon alleviates cadmium toxicity by enhanced photosynthetic rate and modified bundle sheath's cell chloroplasts ultrastructure in maize

Marek Vaculík [a,*], Andrej Pavlovič [a,b], Alexander Lux [a]

[a] Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina B2, SK-842 15 Bratislava, Slovakia
[b] Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University in Olomouc, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic

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ABSTRACT

Silicon was shown to alleviate the negative effects of various biotic and abiotic stresses on plant growth. Although the positive role of Si on toxic and heavy metal Cd has been already described, the mechanisms have been explained only partially and still remain unclear. In the present study we investigated the effect of Si on photosynthetic-related processes in maize exposed to two different levels of Cd *via* measurements of net photosynthetic rate (A_N), chlorophyll *a* fluorescence and pigment analysis, as well as studies of leaf tissue anatomy and cell ultrastructure using bright-field and transmission electron microscopy. We found that Si actively alleviated the toxic syndromes of Cd by increasing A_N, effective photochemical quantum yield of photosystem II (ϕ_{PSII}) and content of assimilation pigments, although did not decrease the concentration of Cd in leaf tissues. Cadmium did not affect the leaf anatomy and ultrastructure of leaf mesophyll's cell chloroplasts; however, Cd negatively affected thylakoid formation in chloroplasts of bundle sheath cells, and this was alleviated by Si. Improved thylakoid formation in bundle sheath's cell chloroplasts may contribute to Si-induced enhancement of photosynthesis and related increase in biomass production in C4 plant maize.

1. Introduction

Silicon (Si) and its importance for plant growth and development is documented by several research papers published during the last years. Although this second most abundant element in the earth crust is not easily accessible for plants, some of them, mostly from the family Poaceae, take up this element in relatively high amount (Wiese et al., 2007). In addition to the importance of Si in plant nutrition, optimal growth and development (Guntzer et al., 2012), it plays a significant role in the alleviation of various symptoms of biotic as well as abiotic stresses (Ma, 2004). It was also shown that Si can alleviate iron deficiency (Pavlovic et al., 2013). On the other hand, it is known that Si can decrease the negative effects of elements, including some heavy metals, when present in excess (Liang et al., 2007; Balakhina and Borkowska, 2013; Wu et al., 2013). Formation of aluminosilicates in the presence of Si was suggested as mechanism of detoxification of aluminum (Al) excess in some plants (e.g. Pragabar et al., 2011). Similarly, the mitigation role of Si has been recently described for several other metals or dangerous toxic elements, including chromium (Ali et al., 2013), lead (Li et al., 2012), antimony (Huang et al., 2012; Vaculíková et al., 2014) or arsenic (Fleck et al., 2013; Tripathi et al., 2013).

Previously, it has been reported that Si can mitigate negative influence of dangerous heavy metal cadmium (Cd) on growth of various plants. In maize, the alleviation of Cd toxicity was partially attributed to Si-enhanced cell wall elasticity and plasticity (Vaculík et al., 2009) as well as increased deposition of Cd in the cell walls (Vaculík et al., 2012; Lukačová et al., 2013). Decrease in plant Cd uptake and translocation from root to shoot was suggested as other beneficial role of Si in many species, including maize (Liang et al., 2005, Da Cunha et al., 2008), *Solanum nigrum* (Liu et al., 2013b) or mangrove seedlings (Zhang et al., 2013), although this feature seems to be species and/or cultivar specific and also depends on the concentration of used chemicals (Liang et al., 2005; Vaculík et al., 2009; Lukačová et al., 2013). Additionally, the alleviative effect of Si on Cd toxicity was partially attributed to the changes in the activity of important antioxidative enzymes involved in scavenging of free radicals formed by presence of Cd in pakchoi (Song et al., 2009); maize (Lukačová et al., 2013), *S. nigrum* (Liu et al., 2013b) and other species. There are also some reports that Si influences the development of apoplasmic barriers, root suberization and lignification, as well as modifies leaf and root anatomy in plants exposed to Cd (Da Cunha and do Nascimento

* Corresponding author. Fax: +421 265429064.
E-mail address: vaculik@fns.uniba.sk (M. Vaculík).

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2009; Vaculík et al., 2012; Vatehová et al., 2012; Zhang et al., 2013). Relatively less is known about the influence of Si on the assimilation tissues and processes related with photosynthesis in Cd-treated plants. It was already reported that Si can ameliorate the negative effects of Cd on the content of photosynthetic pigments, gas exchange parameters and photosynthesis in rice (Nwugo and Huerta, 2008), cucumber (Feng et al., 2010) or cotton (Farooq et al., 2013), however the mechanisms are still unclear.

Therefore, to get a better insight in the mechanisms behind the positive effect of Si on the growth of maize-economically important agricultural plant suffered by Cd toxicity, we conducted experiments to investigate the role of Si in relation to the photosynthesis, shoot biomass production and leaf tissue and cell structure, that are shown in this paper.

2. Material and methods

2.1. Cultivation of plants

Caryopses of maize (*Zea mays* L., hybrid Jozefina) were sterilized for 20 min in 5% Savo (Biochemie, Czech Republic) and washed carefully several times with distilled water before the germination. Thereafter, they were imbibed in water for four hours at room temperature and germinated in rolls of wet filter paper for 72 hours at 25 °C in a dark. Seedlings were transferred to 3 L glass containers (10 plants per container) filled with half strength modified Hoagland solution (Hoagland and Arnon, 1950) with or without Cd and/or Si. After two days of the cultivation the medium was changed to full strength Hoagland solution. The solutions were changed every second day. In total, the plants in each treatment were cultivated for 10 days.

Six different treatments were applied:

1. Control (C)-Hoagland solution without Cd and Si;
2. Cadmium 5 (Cd5)-Hoagland solution with 5 μM $Cd(NO_3)\cdot 4H_2O$;
3. Cadmium 50 (Cd50)-Hoagland solution with 50 μM $Cd(NO_3)\cdot 4H_2O$;
4. Silicon (Si)-Hoagland solution with 5 mM Si in the form of sodium silicate solution (27% SiO_2 dissolved in 14% NaOH);
5. Cadmium 5 plus silicon (Cd5+Si)-Hoagland solution with addition of both Cd and Si in the same concentrations as in the Cd5 and Si treatments; and
6. Cadmium 50 plus silicon (Cd50+Si)-Hoagland solution with addition of both Cd and Si in the same concentrations as in the Cd50 and Si treatments.

The pH of each cultivation solution was adjusted to 6.2 using HCl. The Si concentration used in our experiments was based on our previous experiments with the same maize cultivar. It should be noted that no precipitation of Si in the solution was observed.

Young maize plants were cultivated in hydroponics till the second fully developed leaf in a growth chamber with a 12-h photoperiod, a temperature 25/23 °C (day/night), 75% humidity and 200 μmol m^{-2} s^{-1} PAR.

2.2. Evaluation of plant's growth and biomass production

Plant material was harvested at the fully developed second leaf stage at the end of the cultivation (13th day after imbibition, or 10th day of hydroponic cultivation) and processed in followed experiments. The plants were divided into below- and above-ground parts. Fresh weights of below- and above-ground parts of plants were determined. Root and shoot material was dried at 70 °C for 72 h, and the dry weights of below- and above-ground parts were determined.

2.3. Determination of Cd concentration in above-ground plant parts

The concentration of Cd was determined in finely ground dried shoot tissue using atomic absorption spectrometry (AAS) in the Geoanalytical Laboratories of Institute of Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia.

2.4. Determination of changes in photosynthesis and chlorophyll fluorescence

Rates of net photosynthesis (A_N) and chlorophyll a fluorescence were measured simultaneously on the 2nd fully developed maize leaves with a CIRAS-2 (PP-Systems, Hitchin, UK) and a fluorcam FC1000-LC (Photon Systems Instruments, Brno, Czech Republic) attached to the infrared gas analyser. The actinic light of 75 μmol m^{-2} s^{-1} PAR was switched on for induction of photosynthesis. Then the light intensity was increased stepwise with irradiation periods of 3.5 min, steady state fluorescence (F_t) was recorded and subsequent saturation pulses (4000 μmol m^{-2} s^{-1} PAR, 800 ms duration, λ=620 nm) were applied for estimation of maximum chlorophyll fluorescence in the light-adapted state (F_m') at each light intensity, until 1700 μmol m^{-2} s^{-1} PAR was reached. Then the actinic light was switched off and after 10 min, rate of respiration in the dark (R_D) was recorded. The light was provided by blue (λ=455 nm) and red (λ=620 nm) LED diodes. Effective photochemical quantum yield of photosystem II (Φ_{PSII}) was calculated as $\left(F_m'-F_t\right)F_m'$ according to Maxwell and Johnson (2000) and Roháček (2002). Simultaneously, the rate of net photosynthesis (A_N) was recorded at CO_2 concentration 380 μmol mol^{-1}, leaf temperature 25 ± 1 °C and relative air humidity 65–70%.

2.5. Analyses of chlorophyll and carotenoid content

Approx. 50 mg of fresh leaf tissue samples from the mid part of the 2nd fully developed maize leaves were grinded in a mortar with bit of sand and $MgCO_3$, and photosynthetic pigments were extracted with chilled 80% acetone. The suspension was centrifuged at 4 °C for 5 min at 5000*g*. The concentration of chlorophylls and carotenoids in supernatant was determined spectrophotometrically (Jenway 6400, London, UK) and total content of chlorophyll *a*, *b* and carotenoids was calculated according to Lichtenthaler and Wellburn (1983).

2.6. Determination of quantitative changes in the leaf anatomy and cell ultrastructure

For the investigation of anatomical differences of leaf tissues, tissue segments, approx. 2 × 1 mm large, were collected from the mid part of the 2nd fully developed leaf and fixed in 2% glutaraldehyde and 0.2% osmium tetroxide. After dehydration with ethanol and propylene oxide the samples were embedded in Spurr resin (Serva). Approximately 2 μm thick semi-thin sections were prepared using microtome Tesla BS 490 and stained with 0.5% toluidine blue and 0.1% basic fuchsine according to Lux (1981). The sections were analyzed with Zeiss Axioskop 2 plus epifluorescence microscope (Zeiss, Germany), equipped with Olympus DP72 camera. Quantitative anatomical analysis of leaf tissues was performed with the image analysis software Lucia G 4.80 (LIM, Czech Republic).

For the investigation of ultrastructural changes of leaf cells, ultra-thin sections, approx. 80 nm thick, were prepared from previously embedded samples using ultramicrotome Reichert-Jung (Vienna, Austria) and stained with 2% uranium acetate and 2% lead citrate. Sections were analyzed with transmission electron microscope JEM 2000FX (JEOL, Japan). For quantitative