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Incorporation of iron into chloroplasts triggers the restoration of cadmium induced inhibition of photosynthesis

Ádám Solti[a,*], Éva Sárvári[a], Brigitta Tóth[b], Ilona Mészáros[c], Ferenc Fodor[a]

[a] Department of Plant Physiology and Molecular Plant Biology, Institute of Biology, Faculty of Sciences, Eötvös Loránd University, Pázmány Péter sétány 1/C, Budapest, 1117, Hungary
[b] Department of Agricultural Botany, Crop Physiology and Biotechnology, Institute of Crop Sciences, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi út 138, Debrecen, 4032, Hungary
[c] Department of Botany, Institute of Biology and Ecology, Faculty of Sciences and Technology, University of Debrecen, P.O. Box: 14 Debrecen, 4010 Hungary

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ABSTRACT

Photosynthetic symptoms of acute Cd stress can be remedied by elevated Fe supply. To shed more light on the most important aspects of this recovery, the detailed Fe trafficking and accumulation processes as well as the changes in the status of the photosynthetic apparatus were investigated in recovering poplar plants. The Cd-free, Fe-enriched nutrient solution induced an immediate intensive Fe uptake. The increased Fe/Cd ratio in the roots initiated the translocation of Fe to the leaf with a short delay that ultimately led to the accumulation of Fe in the chloroplasts. The chloroplast Fe uptake was directly proportional to the Fe translocation to leaves. The accumulation of PSI reaction centers and the recovery of PSII function studied by Blue-Native PAGE and chlorophyll *a* fluorescence induction measurements, respectively, began in parallel to the increase in the Fe content of chloroplasts. The initial reorganization of PSII was accompanied by a peak in the antennae-based non-photochemical quenching. In conclusion, Fe accumulation of the chloroplasts is a process of prime importance in the recovery of photosynthesis from acute Cd stress.

1. Introduction

Many regions all over the world suffer from heavy metal pollution due to anthropogenic activities. Areas with high industrial or agricultural uses have to cope with increased soil cadmium (Cd) concentration (Nagajyoti et al., 2010). Cd has a known toxicity to the environment and to all plants (Sanità di Toppi and Gabbrielli, 1999), and Cd contamination thus has increasing importance. Poplars (*Populus* spp.), which are able to tolerate Cd exposure, are economically important species from the point of view of phytoremediation.

In plants, Cd disturbs, among other things, the homeostasis of several metals by competing with essential metal uptake and translocation (Gallego et al., 2012). Cd stress leads to strong Fe deficiency in the shoot (Siedlecka and Krupa, 1999; Fodor et al., 2005; Solti et al., 2008). Root-to-shoot Fe transport requires citrate for Fe(III)-citrate complex formation (Rellán-Álvarez et al., 2010). While Cd is supposed to translocate in non-chelated form, it reduced the expression of *AtFRD3* citrate transporter in *Arabidopsis* (Yamaguchi et al., 2010), leading to diminished Fe translocation and Fe deficiency in the shoot. In addition, the signaling pathways of the expression are disrupted and altered by the presence of Cd both in the roots (Besson-Bard et al., 2009; Wu et al., 2012) and in leaf tissues (Li et al., 2014). In contrast to the effects of Cd on root Fe uptake and translocation, little is known about its effects on the uptake of Fe across different membrane systems in the mesophyll cells.

In the shoot, Cd toxicity and Cd induced Fe deficiency deeply influence the development and activity of the photosynthetic apparatus (Siedlecka and Krupa, 1999). Inhibition of the chlorophyll (Chl) biosynthesis is one of the causes of the retarded thylakoid development. Although Cd inhibits δ-ALA dehydratase directly, the main reason for the Cd-induced inhibition of Chl accumulation is the inhibition of Mg-protoporphyrin-IX-monomethyl-ester oxidative cyclase, which is an enzyme operating with Fe cofactor (Padmaja et al., 1990). Inhibition of Chl biosynthesis decreases the accumulation of all Chl-protein complexes (Fagioni et al., 2009; Basa et al., 2014). Cd induced alterations in the photosynthetic structures are in many ways similar to those caused by Fe deficiency. As photosystem I (PSI) is the major Fe containing complex

* Corresponding author.
E-mail address: adam.solti@ttk.elte.hu (Á. Solti).

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in the photosynthetic apparatus, Fe deficiency strongly retards the accumulation of PSI in the thylakoid membranes (Andaluz et al., 2006; Timperio et al., 2007; Basa et al., 2014). Strong inhibition of the photosynthetic electron transport is a general response to Cd stress. While PSI activity was less affected, Cd was shown to inhibit photosystem II (PSII) at molecular level (Sigfridsson et al., 2004). Functional disturbances of photochemical reaction centers lead to the generation and accumulation of reactive oxygen species (ROS) (Gallego et al., 2012). In the chloroplasts, one of the most important targets of ROS is the D1 protein of PSII. Damages in the PSII reaction center leads to inactivation. Non-photochemical quenching (NPQ) pathways are essential to eliminate the surplus excitation energy and thus prevent the generation of ROS. Among the variable quenching mechanisms, heat dissipation in antenna complexes and quenching by inhibited PSII centers can significantly contribute to NPQ (Hendrickson et al., 2005). Using internal non-photochemical quenching routes, the inactive reaction centers protect the neighboring active PSIIs (Chow et al., 2005).

An elevated level of Fe was shown to provide protection against many toxic effects of Cd. In the presence of Cd, increased Fe supply helped in retention of growth, pigments, and photosynthetic activity in bean and poplar seedlings (Siedlecka and Krupa, 1996; Sárvári et al., 2011). In *Brassica juncea*, the presence of Fe was found to protect thylakoid complexes against Cd compared to Fe-deficient circumstances (Qureshi et al., 2010). In addition, it was also shown that a five-fold higher Fe supply was able to recover the acute Cd toxicity symptoms of photosynthesis (Solti et al., 2008), which also caused Fe accumulation in the leaves independently of the presence of Cd in the nutrient solution. However, the exact reasons for this recovery, i.e. how and why this Fe accumulation starts and how it contributes to the physiological restoration are not yet clear. Thus, our aim was to find out the determining processes in this recovery. Therefore, the detailed dynamics of the Fe trafficking and accumulation processes as well as the changes in the status of the photosynthetic apparatus were investigated in recovering poplar plants.

2. Materials and methods

2.1. Plant material and treatments

Experiments were performed on micropropagated poplars (*Populus jacquemontiana* var. *glauca* [Haines] Kimura cv. 'Kopeczkii'). Plants were grown in climatic chamber [14/10 h light (100 μE m^{-2} s^{-1})/dark periods, 24/22 °C and 70/75% relative humidity] in hydroponics of quarter-strength Hoagland solution [1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, 0.08 μM $CuSO_4$, 4.6 μM $MnCl_2$, 0.19 μM $ZnSO_4$, 0.12 μM Na_2MoO_4, 11.56 μM H_3BO_3, and 10 μM $Fe^{(III)}$-citrate as iron source] for three weeks (four-leaf stage). Low growth irradiance was necessary for the survival of Cd treated plants (Solti et al., 2011). Then, non-treated control (Ctrl) plants were further grown under the same conditions. A set of plants were treated with 10 μM $Cd(NO_3)_2$ for one week (Cad plants). Nutrient solution was changed in every two days. To induce regeneration processes, Cd treated plants were transferred to Cd-free nutrient solution containing a five-fold elevated Fe supply (50 μM $Fe^{(III)}$-citrate; Cad/Ctrl50 plants). Recovery processes were followed on 6th leaves, which developed entirely under Cd treatment and before the regeneration period.

2.2. Determination of chloroplast iron content

Intact chloroplasts of poplar leaves were isolated using a stepwise sucrose gradient, as mentioned in Sárvári et al. (2011). Fe content of solubilized chloroplasts was measured in reduced form as ferrous-bathophenantroline complex: $[Fe(BPDS)_3]^{4-}$ at 535 nm (ε = 22.14 mM^{-1} cm^{-1}) by UV–vis spectrophotometer (Shimadzu, Japan). In order to normalize the Fe content on a chloroplast number basis, images were taken on suspensions in a Bürker chamber in Nikon Optiphot-2 microscope (Zeiss Apochromatic 40/0.95 160/0.17 objective) equipped with Nikon *D*70 DSLR camera. Chloroplasts were counted using ImageJ software (rsbweb.nih.gov/ij/) with Cell Counter plugin.

2.3. Determination of Fe concentration in the nutrient solution

Samples of 1 mL volume were taken from the 400 mL nutrient solution of the plants. After reducing the whole available Fe content into Fe^{2+} form by adding 100 μM ascorbic acid, 300 μM BPDS was added to determine the Fe concentration according to the above mentioned bathophenantroline method.

2.4. Determination of element concentrations

Leaves were dried for a week at 60 °C, powdered and digested using HNO_3 for 30 min at 60 °C, and then bleached by H_2O_2 for 90 min at 120 °C. After filtration through MN 640 W paper, element contents were measured by ICP-MS (Inductively Connected Plasma Mass Spectrometer, Thermo-Fisher, USA).

2.5. Measurements of photosynthetic pigments

Chlorophyll content of leaves was determined in 80% (v/v) acetone extracts by a UV–vis spectrophotometer (Shimadzu, Japan) using the absorption coefficients of Porra et al. (1989).

For the quantification of xanthophyll cycle components, leaf discs were adapted to darkness or to an actinic light of 100 μmol m^{-2} s^{-1} for 30 min, and stored in liquid nitrogen. Discs were powdered in liquid nitrogen and extracted with 80% (v/v) acetone containing 0.1% (v/v) NH_4OH at 4 °C. Carotenoid components were separated by HPLC method (Goodwin and Britton, 1988) using a Nucleosil C18 column in HPLC-system equipped with an UV/VIS detector (JASCO Int. Co., Japan). The eluents were (i) acetonitrile:water mixture (9:1, 0.01% (v/v) triethylamine) and (ii) ethyl acetate. Zeaxanthin standard was used for the identification of peaks and calculation of pigment concentrations (Tóth et al., 2002). The de-epoxidation state of xanthophyll cycle pigments (DEEPS) was calculated as DEEPS = (Z + 0.5A)/(V + A + Z), where V refers to the amount of violaxanthin, A to antheraxanthin and Z to zeaxanthin, respectively.

2.6. Separation and estimation of chlorophyll-protein complexes

Thylakoid membranes were isolated then solubilized, separated, identified and quantified as described by Sárvári et al. (2014). Shortly, after solubilization with 2% (w/v) *n*-dodecyl-β-D-maltoside on ice for 30 min, 1st D electrophoresis was run using BlueNative PAGE (Kügler et al., 1997) in 5–12% (w/v) acrylamide gradient gels (Mini-Protean, BioRad) with 10–20 μL solubilized thylakoids (0.5 mg Chl ml^{-1}) applied per lane. Electrophoresis was carried out with constant voltage of 40 V (15 min), then 150 V and a maximum of 5 mA per gel at 6 °C for approximately 6 h. To analyze the polypeptide composition of the different complexes, thin slices of native gels were transferred to the top of denaturing gels, and run in second dimension by the method of Laemmli (1970) with a modification by adding 10% glycerol to the stacking (5%) and separating (10–18% linear gradient) gels. Complexes were identified by their characteristic polypeptide patterns as in Basa et al. (2014). Gels were scanned using an Epson Perfection V750 PRO gel scanner. The quantities of Chl-protein complexes were assessed according