Plant Physiology and Biochemistry 49 (2011) 499–505

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Impact of moderate Fe excess under Cd stress on the photosynthetic performance of poplar (*Populus jacquemontiana var. glauca* cv. Kopeczkii)

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

[a] Department of Plant Physiology and Molecular Plant Biology, Institute of Biology, Eötvös University, Pázmány P. sétány 1/C, Budapest 1117, Hungary
[b] Department of Botany, Faculty of Sciences and Technology, Debrecen University, Debrecen, Egyetem tér 1., Debrecen 4032, Hungary
[c] Division of Agricultural Botany and Crop Physiology, Centre of Agricultural Sciences, Debrecen University, Böszörményi út 138., Debrecen 4032, Hungary

ARTICLE INFO

Article history:
Received 15 October 2010
Accepted 10 February 2011
Available online 24 February 2011

Keywords:
Cadmium
Chlorophyll–protein complexes
Iron nutrition
Photosynthesis
Poplar

ABSTRACT

Cadmium interference with Fe nutrition has a strong impact on the development and efficiency of the photosynthetic apparatus. To shed more light on the interaction between Fe and Cd, it was studied how iron given in moderate excess under Cd stress affects the development and functioning of chlorophyll–protein complexes. Poplar plants grown in hydroponics up to four-leaf stage were treated with 10 μM $Cd(NO_3)_2$ in the presence of 50 μM $Fe^{[III]}$-citrate as iron supply (5xFe + Cad) for two weeks. Though leaf area growth was inhibited similarly to that of Cad (10 μM $Cd(NO_3)_2$ + 10 μM $Fe^{[III]}$-citrate) plants, chlorophyll content, $^{14}CO_2$ fixation and quenching parameters calculated from PAM fluorescence induction measurements were control-like in 5xFe + Cad leaves. Increased chloroplast iron content (measured photometrically by the bathophenanthroline disulfonate method) without changes in the iron and cadmium content of leaves (determined by inductively coupled plasma mass spectrometry) pointed out that a key factor in the observed protection of photosynthesis is the iron-excess-induced redistribution of iron in the leaf. However, the chlorophyll *a*/*b* ratio and the chlorophyll–protein pattern obtained by Deriphat PAGE remained similar to that of Cad leaves. The decreased amount of PSII core and PSI in mature and developing leaves, respectively, refers to developmental stage-dependent remodelling of thylakoids in the presence of Cd. The results underline not only the beneficial effect of iron excess under Cd stress, but also refer to the importance of a proper Fe/Cd ratio and light environment to avoid its possible harmful effects.

1. Introduction

Toxicity of Cd to plant metabolism including photosynthesis is well documented [1,2]. Cd may alter the conformation and activity of proteins owing to its strong affinity to sulfhydryl groups or its ability to replace essential metals in enzymes [3]. In addition, an important *in vivo* effect of Cd is the disturbance of iron metabolism of plants [4]. In poplar, iron translocation into the shoot was the most retarded process [5]. It may be related to a non-competitive type inhibition of the translocation of iron, where Cd may have an impact on the availability of chelators and/or signalling pathways and gene expression leading to the biosynthesis of transporters or chelators [6,7].

Many symptoms of Cd stress are reminiscent of those induced by iron deficiency. As under iron deficiency, root ferric-chelate reductase activity increases under Cd stress. Both treatments reduce the amount and activity of Calvin cycle enzymes, including Rubisco, and inhibit the photosynthetic electron transport, which all contribute to Cd and iron deficiency induced decrease in the overall photosynthetic activity in developing leaves [2,8]. Decrease in the amount of photosynthetic pigments, increase in the ratio of carotenoids to chlorophylls (Chls) and the de-epoxidation of xanthophyll cycle pigments, changes in Chl fluorescence parameters induced by Cd treatment are all typical of Fe-deficient plants, and can be accounted for by Cd-induced Fe deficiency, though direct effects of Cd cannot be excluded [9]. Reduction of Chl synthesis by Cd toxicity [10] and by Cd-induced iron deficiency [9] could decrease the amount of all Chl–protein complexes remarkably. Both activity and activation of water oxidation in photosystem (PS)II were shown being inhibited by Cd [11,12], while PSII activity

Abbreviations: BPDS, bathophenanthroline disulfonate; Cad, Cd treated; Chl, chlorophyll; PS, photosystem; ICP-MS/OES, inductively coupled plasma mass/optical emission spectrometer; LHC, light-harvesting complex; VAZ, violaxanthin + antheraxanthin + zeaxanthin.

* Corresponding author. Tel.: +36 1 3812163; fax: +36 1 3812164.
E-mail address: e_sarvari@ludens.elte.hu (É. Sárvári).

0981-9428/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2011.02.012

was also strongly reduced by Fe deficiency [8]. The biogenesis of PSI core and LHCI antennae was affected by both Cd treatment and iron deficiency [13–16]. The sensitivity of the LHCs under Fe deficiency and Cd stress [14,17,18] may be caused by the inhibition of both Chl synthesis and *Lhc* gene expression [19,20].

Given the demonstrated interaction of Cd with iron metabolism in plants, excess iron may be protective against Cd stress. Under Cd treatment, Cd and Fe content were decreased and increased, respectively, by application of Fe-EDTA to rice grown in hydroponics [21]. When Fe was supplied at moderate excess over the normal level together with Cd to *Phaseolus vulgaris* seedlings, it relieved the Cd-induced inhibitory effects on growth to a certain extent [22]. It also abolished the inhibition of pigment accumulation and photosynthetic activity [23]. In *Brassica juncea*, the presence of iron protected thylakoid complexes against 125 μM Cd compared to iron deficient circumstances [24]. Most photosynthetic symptoms were recovered in plants suffering from Cd stress, together with a moderate to strong increase in the iron content of leaves, when plants were re-supplied with iron in the presence and absence of Cd, respectively [25]. However, in *Nicotiana plumbaginifolia* cuttings, excess iron was found particularly toxic to photosynthetic metabolism shown by higher thylakoid energisation, the increased reduction state of photosystem II (PSII), and the induced photoinhibition [26]. Photodamage of PSII was light dependent, and it derived from the excessive production of singlet oxygen by the increased amount of cyt b_6/f complex in thylakoids under iron excess in pea plants [27].

In an effort to understand better the interaction between Fe and Cd, and the protective effect of iron excess against Cd stress, the goal of the present study was to reveal how iron supplied in a moderate excess together with Cd affects the development of Cd toxicity symptoms with an emphasis on the development and functioning of chlorophyll–protein complexes.

2. Materials and methods

2.1. Plant material

Experiments were performed on poplar, *Populus jacquemontiana var. glauca* (Haines) Kimura, 1982, cv. Kopeczkii, grown in growth chamber with 14/10 h light (120 μmol m^{-2} s^{-1})/dark periods, 24/20 °C and 70/75% relative humidity. Micropropagated plants having several minor leaves were cultivated in hydroponics [28] with 10 μM Fe-citrate as iron source up to the emergence of the fourth normal-sized leaf. At this stage, plants were marked under the fifth leaf. Then, plants were either grown under control conditions (Ctrl) or treated with 10 μM $Cd(NO_3)_2$ (Cad) or were exposed to excess-iron nutrition (50 μM) in the absence (5xFe) or the presence of 10 μM $Cd(NO_3)_2$ (5xFe + Cad) for two weeks. Leaves below and above the mark were designated by negative and positive numbers starting from the mark. Data were usually collected from −3 to +3 leaves. When data of different leaf storeys are not given separately, we refer to lower or upper leaves showing the mean of the data obtained either from −3,−2 (less treated) or +2,+3 (strongly treated) leaves (see Figs. 1 and 4). Experiments were repeated three times with three plants per treatments.

2.2. Determination of element concentration

Dried plant materials were digested by HNO_3 for 30 min at 60 °C and then in H_2O_2 for 90 min at 120 °C. Ion contents were measured by ICP-MS (Inductively Coupled Plasma Mass Spectrometer, Thermo-Fisher, USA) for microelements and by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer, Perkin-Elmer, USA) for macroelements. Determinations were repeated 3 times. Samples in a given experiment were mixed from the three plants of a treatment.

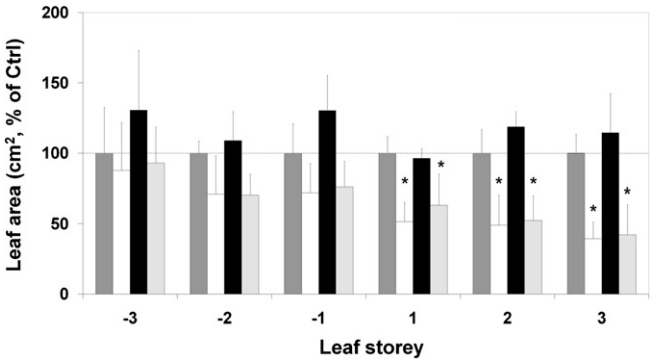

Fig. 1. Influence of different treatments on leaf area growth: dark grey – control (10 μM Fe), white – Cad, black – 5xFe, light grey – 5xFe + Cad. Dashed line: control level. Control values of leaves −3 to +3 were 37 ± 12, 59 ± 5, 67 ± 14, 90 ± 11, 84 ± 14, 102 ± 13 cm^2, respectively. Stars indicate significant changes compared to the control ($n = 6$, $p < 0.01$).

Iron content of chloroplasts was measured photometrically at 535 nm in the form of $Fe^{[II]}$-bathophenanthroline disulfonate ($Fe^{[II]}$-BPDS) complex (extinction coefficient: 22.14 mM^{-1} cm^{-1}) following solubilisation in 50 mM HEPES-KOH pH 7.0, 330 mM sorbitol, 2 mM $MgCl_2$, 1% SDS and 1% dithiothreitol. To calibrate the method, iron content of control chloroplasts was also determined by ICP-MS (see above). Chloroplasts were isolated in 50 mM HEPES-KOH, pH 7.0, 330 mM sorbitol, 2 mM EDTA, 2 mM $MgCl_2$, 0.1% (w/v) BSA, 0.1% (w/v) Na-ascorbate by centrifugation with 1500×g for 5 min (SW rotor). The pellet was resuspended in isolation buffer without EDTA, BSA and ascorbate. Intact chloroplasts were purified on 20/45/60% stepwise sucrose gradient in resuspension buffer (15 min, 2000×g), and pelleted after dilution with resuspension buffer (2500×g, 5 min). The number of chloroplasts was determined in a Bürker chamber by light microscopy (Nikon Optiphot-2, equipped with Nikon D70 camera).

2.3. Determination of pigment content

Chl content was measured spectrophotometrically in 80% acetone, and calculated using the extinction coefficients of Porra et al. [29]. Carotenoid content was determined as in Tóth et al. [30] using a Nucleosil C18 column in an HPLC-system equipped with an UV/VIS detector (JASCO Int. Co., Japan). Samples in a given experiment were mixed from the three plants of a treatment.

2.4. Measurement of photosynthetic activity

Fluorescence induction measurements were performed using a PAM 101-102-103 Chlorophyll Fluorometer (Walz, Effeltrich, Germany) on intact leaves. Leaves were dark adapted for 30 min. F_0 level of fluorescence was determined by switching on the measuring light (modulation frequency of 1.6 kHz and photosynthetic photon flux density (PPFD) less than 1 μmol m^{-2} s^{-1}). The maximum fluorescence yields, F_m in the dark-adapted state and F_m' in the light-adapted state, were measured by applying a 0.7 s pulse of white light (PPFD of 3500 μmol m^{-2} s^{-1}, light source: KL 1500 electronic, Schott, Mainz, Germany), which saturated PSII electron transport by closing all PSII traps. Maximal and actual efficiency of PSII centres were determined as $F_v/F_m = (F_m - F_0)/F_m$ and $\Delta F/F_m' = (F_m' - F_t)/F_m'$, respectively. For quenching analysis, actinic white light (PPFD of 100 μmol m^{-2} s^{-1}, KL 1500 electronic) was provided. Simultaneously with the onset of actinic light the