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ARTICLE

Significance of antioxidative defence under long-term Cd stress

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ABSTRACT Cadmium is a highly toxic heavy metal which causes strong oxidative stress thereby inactivating PSII and the photosynthetic electron transport. However, plants acclimate to moderate Cd stress under longer treatment. Here, the role of antioxidative defence was studied during this acclimation. Micropropagated poplar plants were treated with 10 µM $Cd(NO_3)_2$ from their four-leaf-stage for four weeks. Increase in the malondialdehyde content and in the ratio of inactive, quenching PSII reaction centres (Φ_{NF}) was observed in the first two weeks of the treatment. Starting from the third week both parameters decreased in parallel to the rise in the ascorbate peroxidase activity and β-carotene content, both are important in the antioxidative defence in chloroplasts. Therefore, an acute and an acclimation phase were identified as a consequence of the delay in activation of antioxidative defence mechanisms, the protective role of which is important in the acclimation to moderate Cd stress. **Acta Biol Szeged 55(1):151-153 (2011)**

KEY WORDS

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Cadmium is a highly toxic heavy metal for all livings. In plants, it retards the synthesis of chlorophyll (Chl) and Chl-protein complexes mainly as a consequence of Cd-induced iron deficiency (Fagioni et al. 2009). Modification of thylakoid membranes and the direct inhibition of the photosynthetic electron transport activity by Cd (Sigfridsson et al. 2004; Faller et al. 2005) cause strong oxidative stress (Romero-Puertas et al. 2004; Domínguez et al. 2010). One of the most important targets of reactive oxygen species (ROS) in the chloroplasts is the D1 protein of PSII. Inactivated reaction centres are strong quenchers that can protect their active neighbours by dissipating excess energy under stress circumstances (Öquist et al. 1992; Lee et al. 2001; Chow et al. 2005). Such reaction centres can form oligomers in the thylakoid membrane as a response to Cd stress (Solti et al. 2009). Usually, oxidative stress also activates other components of the antioxidative defence. Smeets et al. (2008) showed that the expression of glutathion reductase, peroxidases, catalase and ascorbate peroxidase (APX) genes increased under moderate Cd stress. Here, we studied some protective components, which exert effects in the chloroplast, to clear up their time-dependent changes in poplar during long-term Cd treatment.

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Materials and Methods

Micropropagated poplar (Populus jacquemontiana var. glauca [Haines] Kimura, 1982 cv. 'Kopeczki') plants were grown in climatic chamber [14/10 hours light (100 µE m^{-2} s^{-1})/dark periods, 24/22°C and 70/75% relative humidity] in hydroponics of quarter-strength Hoagland solution with 10 µM $Fe^{(III)}$-citrate as iron source. Plants were treated with 10 µM $Cd(NO_3)_2$ from their four-leaf-stage for four weeks. Second leaves emerged during the treatment were tested.

Fluorescence induction was measured as in Solti et al. (2009) using PAM 101-102-103 Chlorophyll Fluorometer (Walz, Effeltrich, Germany). The energy dissipation of inactive PSII reaction centres (Φ_{NF}) was calculated according to Hendrickson et al. (2005): ,

$$\Phi_{NF} = 1 - \left(\frac{F_v / F_m}{F_{vM} / F_{mM}}\right)$$

where F_{vM} and F_{mM} parameters refer to the (definitely not inhibited) control's values.

Carotenoid content of leaves was determined by a HPLC separation technique as described in Láposi et al. (2009).

Malondialdehyde (MDA) content was measured according to Heath and Packer (1968) at 532 nm (ε = 155 mM^{-1} cm^{-1}) by UV-VIS spectrophotometer (Shimadzu, Japan).

APX (L-ascorbate: H_2O_2 oxidoreductase, EC 1.11.1.11) activity was measured as in Nakano and Asada (1981). Leaves were homogenized in 50 mM Na-K-phosphate buffer [pH

Table 1. Changes in the β-carotene and MDA content, APX activity and value of Φ_{NF} under Cd stress. β-carotene and MDA content and APX activity are shown as the percentage of control values (41.2±8.1 mmol β-carotene mol^{-1} Chl, 82.6±12.2 nM MDA g^{-1} fresh weight and 9.0±4.1 nmol ascorbate μg $protein^{-1}$ min^{-1}), respectively. Φ_{NF} in control plants was definitely 0 with an average standard deviation of SD=0.013.

day of treatment	β-carotene	Φ_{NF}	APX	MDA
9.	110.6± 6.7	0.284±0.063	63.2±23.0	123.7± 7.9
14.	132.5±11.4	0.150±0.083	86.0±22.4	241.0±19.8
21.	144.4± 1.3	0.049±0.032	169.3±21.9	182.3±12.3
29.	144.4±19.2	0.019±0.020	157.9± 1.7	84.9± 4.5

7.0], 1.0 mM EDTA, 0.1% (w/v) Triton X-100, 2 mM PVP and centrifuged at 15000 g for 15 min. Reaction mixture contained 50 mM Na-K-phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM ascorbic acid, 0.1 mM EDTA, and 100 μl crude enzyme extract. APX activity was followed photometrically at 290 nm (ε = 2.8 mM^{-1} cm^{-1}).

Results

In the first two weeks of the treatment, Cd stress caused acute damage evidenced by the increased MDA content and the elevated ratio of quenching by inactive PSII reaction centres (Φ_{NF}; Table 1). In parallel to the development of oxidative damage, antioxidative defence mechanisms started to activate as it was shown by the slight increase in β-carotene content and APX activity on the 14th day of treatment. By the end of the third week, neither Φ_{NF}, nor MDA content differed significantly from the control values, the MDA content of stressed plants was even lower than that of the controls. Nevertheless, both of the β-carotene content and APX activity remained elevated in the fourth week, though they reached their maxima in the third week.

Discussion

Photosynthetic activity decreases under acute Cd stress (Kučera et al. 2008). At the beginning of 10 μM Cd treatment, the parallel increase in MDA content and Φ_{NF} indicates the impact of oxidative damage of PSII reaction centre. Despite the effective defence mechanisms present in the chloroplasts (Asada 2006), increase in APX activity was delayed which also contributed to the development of oxidative damage. β-carotene may also have role in ROS elimination (Okamoto et al. 2001). Similarly to green algae (Shariati and Yahyaabadi 2006), its content also increased in poplar leaves as a physiological response to continuous Cd stress. After the activation of defence mechanisms, Φ_{NF} started to decrease which is a good sign of the restoration of photochemical efficiency (Hendrickson et al. 2005). Decrease of Φ_{NF} together with increase in β-carotene content may also indicate the synthesis of new PSII reaction centres, which is inhibited when ROS are accumulating (Takahashi and Murata 2008). The elevation of APX activity caused antiparallel changes in the level of damage. The elimination of ROS via water-water cycle in chloroplasts (Asada 2006) is a self-enhanced process: a decrease in the amount of ROS allows an increase in the ratio of non-inhibited PSII centres, and the rising photosynthetic activity produces new capacity for ROS elimination. Therefore, antioxidative defence, which reacted to oxidative damage with some delay but remained elevated even after the elimination of acute oxidative stress, has important role in the acclimation to moderate Cd exposure.

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