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BRIEF COMMUNICATION

Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants

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Abstract

Fatty acid content and composition of chloroplast membranes, ethylene production associated with thylakoid lipids degradation as well as photosynthetic electron transport involving photosystems 1 and 2 were used to determine the effects of increasing Cd concentrations in the growth medium [0, 14, 28, and 42 mg(Cd) kg^{-1}(sand)] on the photosynthetic performance of barley plants (*H. vulgare* L., cv. CE9704). High concentrations of Cd triggered serious disturbances of the chloroplast membranes. Ethylene production increased whereas a drop of 18:3 fatty acid content occurred, indicating that Cd mediates lipid peroxidation in the thylakoids. The enhanced ethylene production could be used as an early indicator of Cd-induced membrane degradation, yet at very high concentration (42 mg kg^{-1}) Cd decreased ethylene production.

Additional key words: ethylene, fatty acids, *Hordeum vulgare*, photosystems 1 and 2, photosynthesis.

Cd is toxic for photosynthesis (for review, see Clijsters and Van Assche 1985, Krupa and Baszynski 1995). The mechanism of Cd-induced degradation of the chloroplast membrane may be a consequence of *1*) lipoxygenase induction resulting in the oxidation of polyunsaturated fatty acids (Somashekariah *et al*. 1992), *2*) increased galactolipase activity leading to hydrolysis of mono-galactolipid and premature senescence (Krupa and Baszynski 1995), or *3*) oxidative stress (Chien *et al*. 2001, Sandalio *et al.* 2001, Hendry *et al*. 1992) as a consequence of depletion of antioxidative defences (Schützendübel and Polle 2002) that stimulates lipid peroxidation. The degradation of thylakoids mediated by Cd strongly retards electron transport activities of both photosystems (PS) PS2 and PS1 (Baszynski *et al*. 1980, Siedleska and Baszynski 1993).

Cd-induced lipid peroxidation at leaf level has been often detected by the high content of thiobarbituric reactive species (Chien *et al*. 2001, Shah *et al*. 2001), but the available information at thylakoid level is insufficient and controversial. Using chlorophyll (Chl) thermoluminescence techniques, Baryla *et al*. (2001) did not find higher content of lipid hydroperoxides in chloroplasts of oilseed rape plants grown on strongly Cd-polluted soil. Moreover, El-Shintinawy (1999) suggested an enhanced thylakoidal lipid peroxidation of Cd-exposed soybean seedlings on the base of both high ratio of $(C_{18:0} + C_{18:1} + C_{18:2})/C_{18:3}$ fatty acids and several times higher ethylene production of whole seedlings as compared to the control plants. On the contrary, the opposite effect concerning ethylene evolution was observed by Bhattacharjee (1997/98) in Cd-exposed *Amaranthus* seedlings.

Ethylene production associated with thylakoid membrane degradation and mediated by oxy-radicals and H_2O_2 is a final product of acyl lipid peroxidation (Rabinowitch and Fridovich 1983, Lidon and Henriques

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Abbreviations: Chl - chlorophyll; LHC - light harvesting complex; OEC - oxygen evolving complex; PS - photosystem; TFA - total fatty acids.
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1993). Increasing ethylene production and the specific inhibition of $C_{18:3}$ fatty acid by plants under environmental stress are regarded as a monitor of lipid peroxidation (El-Shintinawy and El-Shourbady 1997), yet the coupled metabolism of ethylene synthesis at the thylakoids is still poorly known.

In a previous study we found that Cd retards photosynthetic rate of barley plants due to both stomata and non-stomata limiting factors (Vassilev *et al*. 2002). In order to clarify Cd-induced disorders at thylakoid level we further measured: *1*) ethylene production associated with chloroplast membranes, *2*) their fatty acids content and composition, and *3*) photosynthetic electron transport activities involving PS2 and PS1.

Barley (*Hordeum vulgare* L. cv. CE9704) plants were grown in sand, in a greenhouse under natural irradiation, temperature, and humidity (Vassilev *et al*. 2002). Twenty-day-old plants were exposed for 10 d to Cd treatments through supplying an adequate volume of water Cd solution to final concentrations 0, 14, 28, and 42 mg(Cd) kg^{-1}(sand).

Chloroplast membranes were isolated using 2 g (f.m.) leaf tissue, according to Droppa *et al*. (1987), with minor modifications described by Lidon and Henriques (1992). The lipid fraction was then extracted from the pellet of chloroplast membranes according to Allen *et al*. (1966), in a mixture of chloroform/methanol/water (1:1:1, v/v/v). After evaporation of the chloroform layer, the dry residue was resuspended in 1 cm^3 of a mixture of ethanol/toluene (1:4, v/v) and stored at -20 °C until analysis. After saponification with 0.5 M NaOH in methanol, the fatty acids of lipid extracts were methylated with BF3 (*Merck*, Darmstadt, Germany) according to Metcalfe *et al*. (1966) and analyzed by gas liquid chromatography (*UNICAM 610* gas chromatograph, *Unicam*, Cambridge, UK) (for details, see Ramalho *et al.* 1998).

Ethylene production was measured in 500 mm^3 of the obtained chloroplast membranes incubated at an irradiance of 500-600 µmol m^{-2} s^{-1}, provided by a Björkman lamp in 2 cm^3 flasks. After 2 h of incubation a 1 cm^3 gas sample was withdrawn from the headspace gas of the incubating flask using a gas-tight syringe. Ethylene concentration in this gas sample was assayed by a *Pye Unicam 204* gas chromatograph equipped with a *Porapak Q* (*Waters Millipore Corporation*, Milford, USA) column and a flame ionization detector (FID). Nitrogen, at a flow rate of 0.5 cm^3 s^{-1} was the carrier gas. The temperatures were set to 90 ºC for the oven, room temperature for the injection port, and 150 ºC for the detector. Ethylene was identified and quantified by comparison with the peak area from the gas samples containing a known concentration (29 µmol mol^{-1}) of ethylene standard.

Photosynthetic activities of the chloroplast membranes coupled to PS 2 and PS 1 were measured in a Clark-type oxygen electrode (*LW2*, *Hansatech*, Kings Lynn, UK). The electron transport rates were determined according to Droppa *et al*. (1987) in 1 cm^3 of reaction mixture containing 100 - 150 µg chlorophyll, at 25 °C and irradiance of 3 000 µmol m^{-2} s^{-1}, given by a Björkman lamp (*Hansatech*).

Statistical analysis was performed using a one way *ANOVA* (for $P < 0.05$).

Increasing Cd concentrations, applied for 10 d, retarded photosynthetic capacity (oxygen evolution under saturating irradiance and CO_2) of barley plants up to 20 % (Vassilev *et al*. 2002). At the same time, Cd decreased the total fatty acids (TFA) content of the chloroplast membranes by 23 - 25 % at 14 and 28 mg(Cd) kg^{-1} and by 42 % at 42 mg(Cd) kg^{-1} treatments (Table 1). At 42 mg(Cd) kg^{-1} treatment the content of the major unsaturated fatty acid - linolenic acid ($C_{18:3}$) decreased by 48 % and that of palmitic acid ($C_{16:0}$) by 45 %, but the strongest Cd effect was observed on *trans*-3-hexadecenoic acid ($C_{16:1t}$) - its content was diminished by 66 %. Cd treatment did not cause significant changes in fatty acids percentage, but the level of fatty acid unsaturation tended to decrease as indicated by the double bond index (DBI) although it was not significantly different.

Table 1. Total fatty acid (TFA) and selected fatty acids contents, fatty acid composition and unsaturation (DBI) of thylakoid membrane lipids in barley plants treated with 0 (control), 14, 28 and 42 mg(Cd) kg^{-1}. The determined fatty acids were palmitic ($C_{16:0}$), *cis* and *trans* hexadecenoic ($C_{16:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$). Double bond index (DBI) = $[(C_{16:1c} + C_{16:1t} + C_{18:1} + 2C_{18:2} + 3C_{18:3})/(<C_{16:0} + C_{16:0} + C_{18:0})]$. The values represent the means of triplicates. Within each row, data followed with the same letters are not statistically different ($P < 0.05$).

Parameter	0	14	28	42
[g kg^{-1} (DM)]				
TFA	164.2^{a}	123.3^{ab}	126.5^{ab}	95.2^{b}
$C_{18:3}$	36.8^{a}	24.7^{b}	27.8^{b}	19.2^{b}
$C_{16:1t}$	2.5^{a}	1.2^{b}	1.6^{ab}	0.8^{b}
$C_{16:0}$	12.1^{a}	10.1^{a}	9.5^{a}	6.7^{b}
[mol %]				
$< C_{16:0}$	10.20^{a}	14.87^{a}	11.24^{a}	15.08^{a}
$C_{16:0}$	18.36^{a}	21.45^{a}	19.35^{a}	17.62^{a}
$C_{16:1c}$	3.97^{a}	5.40^{a}	3.61^{a}	4.20^{a}
$C_{16:1t}$	3.74^{a}	2.42^{a}	3.27^{a}	2.19^{a}
$C_{18:0}$	0.65^{a}	0.70^{a}	0.64^{a}	1.26^{a}
$C_{18:1}$	0.74^{a}	0.83^{a}	0.73^{a}	1.60^{a}
$C_{18:2}$	6.22^{a}	6.92^{a}	6.44^{a}	7.87^{a}
$C_{18:3}$	56.13^{a}	47.43^{a}	54.74^{a}	50.19^{a}
DBI	6.68^{a}	5.08^{a}	5.99^{a}	5.17^{a}

Ethylene production associated with chloroplast membranes was [mg (C_2H_4) kg^{-1}(Chl) s^{-1}]: 16.2 ± 1.0 [0 mg(Cd) kg^{-1}], 20.9 ± 1.7 [14 mg(Cd) kg^{-1}], 23.4 ± 1.9 [28 mg(Cd) kg^{-1}] and 11.5 ± 0.9 [42 mg(Cd) kg^{-1}]. Hence, Cd increased significantly ethylene production by 29 and

44 % at the 14 and 28 mg(Cd) kg^{-1} treatments and significantly decreased it by 29 % at the 42 mg(Cd) kg^{-1} treatment. The rise of ethylene production was accompanied by a significant decrease in linolenic acid ($C_{18:3}$) content (Table 1), trend that El-Shintinawy and El-Shourbady (1997) regarded as a monitor of lipid peroxidation. The decrease of ethylene production in the 42 mg Cd kg^{-1} variant was probably due to disruption of the chloroplast membrane, leading to a loss of the ethylene forming enzyme activity, as was suggested by Bhattacharjee and Mukherjee (1996) for salt-damaged leaf tissues of *Amaranthus* seedlings.

The activity of thylakoid electron transport (both PS2 and PS1) was strongly lowered by Cd (Fig. 1). The

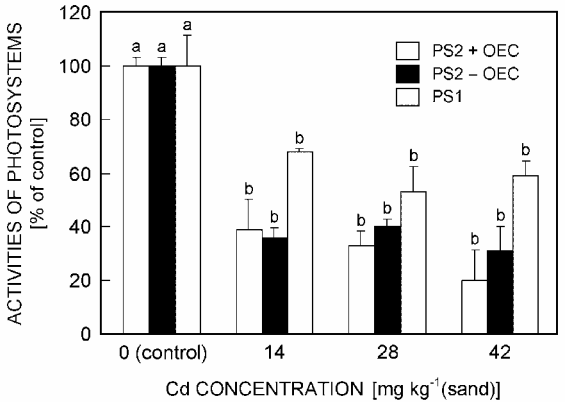

Fig. 1. Rates of photosynthetic electron transport between: *1*) H_2O and 2,6-dichlorophenolindo-phenol (DCPIP) (PS2 + OEC, oxygen evolving complex), *2*) 1,5-diphenyl-carbohydrazide (DPC) and DCPIP (PS2 - OEC), and *3*) reduced $DCPIPH_2$ and methyl viologen MV (PS1) in thylakoid membranes isolated from leaves of barley plants grown for 10 d in Cd-contaminated sand. Control values (representing 100 %) were 17.7, 14.6 and 67.1 mmol(O_2) kg(Chl^{-1}) s^{-1} for PS2 + OEC, PS2 - OEC and PS1, respectively. Means ± SE (n = 3). Within the same parameter, values followed by the same letters are not significantly different ($P < 0.05$).

inhibiting effect of Cd increased gradually with the Cd concentration applied. PS2 was more affected by Cd than PS1. Similar finding has been reported by Tůmová and Sofrová (2002) in experiments with Cd-exposed cyanobacterial cells. After a 10 d exposure to 42 mg(Cd) kg^{-1}, the decrease in PS2 activity with oxygen evolving complex (OEC) was about 80 % and without OEC 69 %, whereas PS1 activity decreased by *ca*. 41 %. Except for 14 mg(Cd) kg^{-1} treatment, the inhibition of PS2 activity with OEC shows that Cd preferably interacts with the donor side of this photosystem. The inhibiting effect of Cd on photosynthetic electron transport confirmed the results of Baszynski *et al*. (1980) and Siedleska and Baszynski (1993) showing that it is linked to the ultrastructural disorders of the thylakoids. Hence we support the hypothesis of Krupa and Baszynski (1995) on the indirect effect of Cd on photosynthetic electron transport: the level of $C_{16:1t}$ fatty acid is positively correlated with light-harvesting complex 2 (LHC2) oligomerization, due to its specifically binding in *sn*-2 position in the chloroplastic phosphatidylglycerol. Decreased $C_{16:1t}$ content may diminish the content of LHC2 oligomer that means less efficient energy collection and distribution between photosystems, finally resulting in decreased rate of photosynthetic electron transport.

The 10-d exposure of barley plants to Cd probably provoked serious disturbances of the chloroplast membranes. The observed increase of ethylene production associated with the thylakoids together with a drop of $C_{18:3}$ fatty acid content gives more precise evidence for Cd-induced lipid peroxidation processes. The 42 mg(Cd) kg^{-1} treatment strongly diminished the total fatty acids content of chloroplast membrane leading probably to its disruption and loss of ethylene forming enzyme activity. Thus, the enhanced ethylene production associated with the thylakoids should be used as an early indicator of Cd-induced membrane degradation.

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