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Influence of light intensity on thylakoid development under Cd stress in poplar

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Introduction

The high toxicity of divalent heavy metal ions for plants is well known. They generally inhibit overall physiological processes (van Assche and Clijsters 1990) photosynthesis being particularly susceptible (Krupa and Baszynski 1995). Multiple inhibitory effects as leaf chlorosis, inhibition of photosynthetic electron transport and the carbon reduction cycle have been observed. However, the main factors affecting thylakoid organisation under Cd stress still remain to be an area to learn more about. In an accompanying paper (Sárvári *et al*. 2001) we found that iron- and manganese deficiency may play important role in the differential inhibition of the accumulation of chlorophyll (Chl)-protein complexes and in the attenuation of the photosynthetic activity. However, another possibility is that changes in thylakoid organisation could be the result of regulatory processes working under excess light. It was supported by the strong non-photochemical quenching together with the decreasing values of the actual efficiency of photosystem (PS)II (Φ_{PSII}) under strong Cd stress. Light can be in excess even at low ambient irradiance if photosynthesis is inhibited (Horton *et al*. 1996). Therefore, the combined effect of visible light and Cd treatment was studied to find out the main factor influencing the thylakoid organisation under Cd stress.

Materials and methods

Micropropagated poplar plants (*P. glauca* var. Astria and *P. alba*) were grown hydroponically in Hoagland solution of ¼ strength with 10 µM Fe-citrate as iron supply. Four-week old plants grown at 90 µmol photon m^{-2} s^{-1} had 4 leaves. They were treated with 10 µM $Cd(NO_3)_2$ through their roots at 40, 90, and 140 µmol photon m^{-2} s^{-1} light intensity up to six-week age during which they developed another 4-5 leaves. Chl content (µg cm^{-2} leaf area) and Chl-proteins solubilised by mainly glucosidic detergents, separated by Deriphat PAGE, identified on the basis of their polypeptide patterns, and expresssed in µg Chl cm^{-2} leaf area were determined according to Sárvári and Nyitrai (1994). Fluorescence induction parameters were measured with a PAM fluorimeter (Walz). $^{14}CO_2$ fixation was studied according to Láng *et al.* (1985). Experiments were repeated two times.

Results

Growth and Cd sensitivity of *P. glauca* and *P. alba* plants did not differ significantly. Poplar plants grew well at moderate light intensity, but the growth of the newly emerged leaves was strongly retarded even in the control plants at 40 µmol photons cm^{-2} s^{-1}. However, growth inhibition by Cd did not significantly varied as a function of light intensity (not shown).

Concerning the photosynthetic parameters in control plants, the Chl content was at about the same level in the different leaf storeys, and it was a little higher at moderate to higher light intensity. Chl *a/b* ratio rose at higher leaf storeys, and with the increasing light intensity being 3.52, 3.67, and 3.85 in the upper leaves at 40, 90, and 140 μmol photon m^{-2} s^{-1}. The changes in the Chl *a/b* ratio were due to variations in the ratios of the different Chl-protein complexes. The relative amount of PSI and PSII increased, and that of the light-harvesting complex (LHC)II decreased with the increasing light intensity (not shown). CO_2 fixation rose at higher light intensity, where an increased stomatal conductance was also observed (not shown).

Unfortunately, we could not grow plants at so high light intensity that we could see the clear effect of photoinhibitory light on Chl-protein pattern. Instead we compared the changes in the inhibitory effect of Cd treatment as a function of light intensity. The Chl accumulation decreased with the increasing light intensity in leaves developed during the treatment (Table 1). The Chl *a/b* ratio slightly increased also in Cd-treated plants as the light intensity rose, and was 3.30, 3.32, and 3.41 in the upper leaves at 40, 90, and 140 μmol photon m^{-2} s^{-1}. However, it became lower and lower compared to that of control leaves (Table 1). Both the amount and the relative proportion of Chl-proteins changed in the treated leaves (Fig. 1). PSII was the most susceptible complex at low irradiance (mild stress). At higher irradiance, we got the usual pattern: PSI being the most sensitive, LHCII also decreased, while PSII was the most stable component. Further increase in the light intensity the sensitivity of PSII started to increase again, and LHCII more and more became the most stable complex. The PSI-related long wavelength component of 77K fluorescence emission spectra showed gradual decline in Cd treated plants with increasing light intensity (not shown). Inhibition of CO_2 fixation was similar to that of the Chl content (Table 1). Elevating the light intensity, the Cd induced decrease in Φ_{PSII} and F_v/F_m became stronger, as did the increase in F_o and the non-photochemical quenching parameter (NPQ).

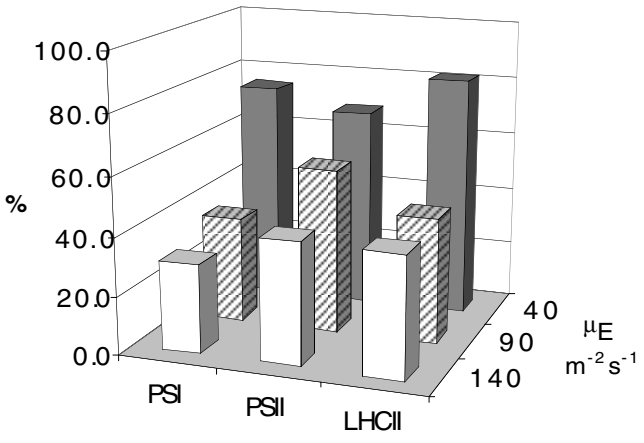

Fig. 1. Changes in Chl-protein content (μg Chl cm^{-2}) of Cd treated plants expressed as the percentage of the control values.

Table 1. Light intensity dependence of different photosynthetic parameters in Cd treated *P. alba* plants.

Mean values of upper three leaves, which developed during the treatment, are given. All values are expressed as the percentage of the control. Standard deviations are <5% of the measured values in the case of Chl *a/b* ratios, and F_o, F_v/F_m, and Φ_{PSII} values in the control plants, and 10-15% in all the other cases.

Light intensity	Chl *a+b*	Chl *a/b*	CO_2 fix.	F_o	F_v/Fm	Φ_{PSII}	NPQ
40	81.2	93.5	79.5	113.4	98.1	90.7	94.7
90	45.0	90.6	39.8	161.1	81.1	81.2	289.3
140	35.5	88.5	41.5	205.7	73.1	77.0	516.8

Discussion

The changes in Chl *a/b* and Chl-protein ratios observed in control plants under varying light environment were in agreement with the literature data (Anderson *et al*. 1995).
The Cd treated plants differed in their thylakoid development depending on the irradiance. The higher the light intensity the stronger stress effects were observed. While the mild stress effect, the relative susceptibility of PSII was observed at low light intensity, the superficial stability of PSII was characteristic around 100 μmol photons cm^{-2} s^{-1}. This can be connected with the accumulation of stable, inactive PSII centres which are thought to take part in the dissipation of excess light under sustained light stress (Öquist *et al*. 1992, Anderson and Aro 1994). It is in agreement with the strong increase in the NPQ and the decrease in CO_2 fixation activity and the F_v/F_m and Φ_{PSII} values. Nevertheless, it has to be mentioned that the maximal photochemical efficiency of Cd treated plants must have been underestimated due to the iron deficiency related rise in apparent F_o as a result of dark reduction of PQ (Belkhodja *et al.* 1998). PSII stability was accompanied with the decrease in the amount of PSI and LHCII, which may be caused by their decreased synthesis or increased degradation. Concerning PSI, either its biogenesis can be inhibited due to iron deficiency (Sárvári *et al*. 2001) or its degradation can be enhanced (Gallego *et al*. 1996). However, the reduction in the amount of LHCII, which is the most stable component even during scenescence (Humbeck *et al.* 1996), must be caused by its active degradation under excess light (Yang *et al*. 1998). At later stages of development with Cd or at higher light intensity, the onset of senescence-like processes could be observed when LHCII became the most stable component (Humbeck *et al*. 1996).
In conclusion, the strong enhancement of Cd effectivity with rising light intensity refer to the essential role of excess visible light in the organisation of thylakoids in treated plants.

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