Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S4-P03

Volume 46(3-4):163-165, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Comparison of the effects of Pb treatment on thylakoid development in poplar and cucumber plants

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ABSTRACT Pb (10 to 100 μM) effects on the development of thylakoids were investigated in Fe-EDTA and Fe-citrate grown cucumber and poplar to compare the symptoms in different plant species. Increased chlorophyll content of leaves appeared either in PSII core or LHCII depending on the treatment. Lowering of the chlorophyll content, however, was accompanied by decrease in PSI and LHCII, and relatively high stability of PSII core. PSII efficiency and CO_2 fixation were mostly unchanged or decreased moderately, but rise in the non-photochemical quenching was observed in all kinds of treatment. While Pb could not be detected and Mn content decreased in poplar leaves, changes in photosynthetic parameters paralleled with the Pb content in cucumber, and increase in Fe, Mn, and Ca content was measured. The direct effects of Pb or oxidative stress may evoke the changes in cucumber, while Pb induced Mn deficiency seems to play a role in poplar. Alterations in the composition of thylakoids were probably triggered by regulatory processes, which optimise the structure and function under stress conditions.
Acta Biol Szeged 46(3-4):163-165 (2002)

KEY WORDS
chlorophyll-protein
fluorescence induction
heavy metal
lead

The toxicity of heavy metals as Cd, Ni, Cu, Pb for plant metabolism, including photosynthesis, which is one of the most metal sensitive processes, is well known (Krupa and Baszynski 1995; Fodor 2002). They disrupt the physiological processes by binding to protein sulphydryl groups or causing deficiency/substitution of essential metal(s) (van Assche and Clijsters 1990). Pb, a strong reactant to protein N- and S-ligands, was shown to inhibit Chl synthesis (Sengar and Pandey 1996), as well as electron transport (Rashid et al. 1994) and RubisCo activity (Stiborova et al. 1986) *in vitro*. In this study effects of Pb treatment on the development of thylakoids were investigated in cucumber and poplar plants to compare the symptoms of lead treatment and reveal the causes of its effect in different plant species.

Materials and Methods

Cucumber (*Cucumis sativus* L. cv. budai korai) and poplar (*Populus alba*) plants were grown hydroponically (Fodor et al. 1998) with 10 μM Fe-EDTA (FeED) or Fe citrate (Fecit) as iron source. Cucumber having one (1L) or four leaves (4L) and poplar plant at four-leaf stage were treated with 10 and 50 or 100 μM $Pb(NO_3)_2$ for two weeks during which they developed another four to five leaves.

Chlorophyll (Chl) content was measured photometrically in 80% acetone (Porra et al. 1989), CO_2 fixation with isotopic method (Láng et al. 1985), and fluorescence induction using a PAM fluorimeter. Chl-proteins were separated by Deriphat PAGE after the solubilisation of washed thylakoids with glucosidic detergents, and identified by their polypeptide composition (Sárvári és Nyitrai 1994). Their absolute amounts (μg Chl cm^{-2}) were given by dividing the Chl content of one cm^2 leaf material among the complexes according to their relative amounts. Ion content was determined by ICP-AES (Záray et al. 1995) or TXRF (Záray et al. 1997).

Results and Discussion

Leaf growth was hardly inhibited except the case of 1L Fecit cucumber, and it was even stimulated in 4L FeED plants (Table 1).

In accordance, Pb exerted a relatively mild effect on photosynthesis supplied even at relatively high concentration. Chl content increased in poplar plants and in FeED cucumber except at the highest Pb concentration used (Table 1). Strong decrease was observed only in 1L Fecit cucumber treated with 50 mM Pb, where the concentration of Pb inside the leaf might have been high enough to directly inhibit Chl synthesis (Sengar and Pandey 1996). CO_2 fixation was also strongly reduced in the latter plants, while it was only moderately lowered in the other cases. In spite of this, alterations in the development of the photosynthetic apparatus were shown by changes in the Chl *a/b* ratios and in Chl-protein composition of thylakoids. Increased Chl *a/b* ratio was due to the increase in the amount of core complexes, primarily that of PSII (see poplar plants). Its lowering was caused by the increase in the amount of LHCII when an increased Chl content was observed (1L FeED cucumber). However, the lowering of the Chl *a/b* ratio was due to the stronger decrease in the amount of PSI than that of LHCII when the Chl content decreased. In that case the relative (to the controls) stability of PSII was higher than that of the other complexes. These structural

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Table 1. Growth, photosynthetic parameters and ion content of Pb treated poplar and cucumber leaves. Data of 2-5 leaves (1L plants) and 5-6 leaves (4L plants) were taken into consideration. Standard deviations of Chl *a*/*b* and flurescence induction parameters except NPQ were <5% of the given value and it was around 10-15% in all the other cases.

Fe supply	Fe-citrate					Fe-EDTA			
Plant	Poplar		Cucumber			Poplar	Cucumber		
Stage of treatment	4L	4L	4L	1L	1L	4L	4L	1L	1L
Pb concentration (µM)	100	10	10	10	50	10	10	10	50
Growth									
Fresh mass (g, %)	84.9	95.9	97.6	63.3	24.6	107.9	108.1	95.5	91.4
dry mass (g, %)	105.9	93.7	88.5	63.4	22.6	112.1	104.5	107.6	104.3
leaf surface (cm^2, %)	84.3	100.0	91.3	63.2	19.1	93.1	93.4	103.9	105.6
Photosynthesis									
Chl *a+b* (µg cm^{-2}, %)	106.5	105.1	94.0	82.1	24.3	115.7	111.9	106.8	95.4
Chl *a*/*b* (%)	104.5	101.1	100.0	98.5	90.5	100.4	98.7	93.7	97.8
Chl-proteins									
PSI (µg cm^{-2}, %)	62.1	88.4	94.7	57.1	17.2	129.4	99.2	107.9	92.3
PSIICC (µg cm^{-2}, %)	154.2	120.0	98.3	85.7	43.9	118.8	100.7	91.8	101.1
PSIICA (µg cm^{-2}, %)	168.9	136.9	102.4	85.9	58.6	123.7	111.7	103.8	96.9
LHCII (µg cm^{-2}, %)	64.4	88.9	96.6	61.7	26.5	98.6	109.6	105.9	97.2
CO_2 fixation (cpm, %)	120.8	95.3	83.5	75.5	36.2	78.2		95.1	
Fluorescence induction									
F_o	104.8	104.8	105.6				107.5		
F_v/F	101.0	101.7	100.5	104.8	58.4	102.6	100.4	101.3	101.7
F_v'/F_m'	94.6	101.3				96.8			
qP	100.6	101.6	101.0			98.9	100.0		
Φ_{PSII}	94.9	104.6	99.7			101.2	100.2		
NPQ	187.8	126.2	127.6			136.1	106.0		
Ion contents									
Pb (nmol cm^{-2})	0.89	0.00	1.08	2.30		0.00	0.47	0.22	
Fe (nmol $cm^{-2,}$ %)	249.6	97.3	155.2	126.7		105.2	63.6	109.2	
Mn (nmol $cm^{-2,}$ %)	59.9	60.9	110.3	117.5		65.1	84.5	127.1	
K (nmol $cm^{-2,}$ %)	203.0	97.5	80.4	93.8		102.0	70.2	107.2	
Ca (nmol $cm^{-2,}$ %)	108.9	74.2	115.4	127.8		65.0	76.4	150.9	

% - percentage of the control value
PSIICC – photosystem II core complex
PSIICA – connecting antenna of photosystem II

alterations were accompanied with some functional ones in the thylakoids. Maximal efficiency of PSII (F_v/F_m) decreased only 1L Fecit cucumber treated with 50 µM Pb. Yield decline, however, was observed in 4L Fecit poplar treated with 100 µM Pb. Rise in non-photochemical quenching (NPQ) was showed in all kinds of treated plants. As in the case of Cd treatment, these effects could be mostly attributed to regulatory processes induced by the stress (Sárvári et al. 2001).

Heavy metal effects are frequently brought into connection with indirect effects on ion transport in plants (Siedlecka 1995). In this context it is interesting to mention that Pb was detected in poplar leaves only if it was present at high concentration in the nutrient solution (Table 1). Even in the absence of Pb in the leaves, their Mn (and Ca) content decreased significantly. It shows that not only Pb and Mn competition (Rashid et al. 1994) but also Pb induced Mn deficiency may influence the development of PSII. In contrast, changes in photosynthetic parameters paralleled with the Pb content of leaves in cucumber, and increase in Fe, Mn, and Ca content was observed referring to stimulation of ion transport into the leaves. Increased iron concentration in the leaves may induce oxidative damage of membrane components (Becana et al. 1998).

In conclusion, the direct effects of Pb or oxidative stress due to the increased iron concentration may evoke the change in the photosynthetic apparatus of cucumber, while the Pb induced Mn deficiency seems to be connected with the stress induced changes in poplar, where the above mentioned stronger or direct effects come into prominence only at higher lead concentrations. Alteration of the thylakoid composition seems to be mostly indirect response to heavy metal stress triggered by regulatory processes to optimise the structure and function under stress conditions.

Acknowledgments

The authors wish to thank Zsuzsa Ostorics and Mária Dömötör for skilful technical assistance. This work was supported by NATO Science Program (EST.CLG.977480), and financed by Hungarian grants of OTKA (F-021004), and FKFP (0175/1999, 0056/2001).

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