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Stimulating effect of low-dose stressors on the development of maize and bean chloroplast

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Introduction

Different kinds of harmful compounds (stressors), as heavy metals and herbicides used in low concentrations (low-dose stressors) have a beneficial effect on plants (Zatykó 1973, Mishra and Kar 1973; Beaumont *et al*. 1980; Wójcik and Tukendorf 1999). The stimulating effect can be shown in the delaying or inhibition of senescence or causing rejuvenation of senescing plants or plant organs. It was assumed, that these low-dose stressors may influence ion uptake and/or change hormonal balance (i.e. increase cytokinin synthesis). To investigate this stimulation effect of low-dose stressors, we chose developing maize and bean seedlings and for monitoring the inhibition of senescence we used detached bean leaves.

Materials and methods

Maize and bean seedlings were grown in Hoagland solution of ¼ strength supplied with microelements up to 3 weeks in 16/8 hours light/dark period at 24/18°C. Treatment of greening seedlings was carried out with Cd (5.10^{-8} -10^{-7} M), Pb, Ni and DCMU (10^{-7}-10^{-6} M) and Ti (10^{-6}-10^{-5} M) in nutrient solution (with lower concentrations) or spraying leaves on every second day (with higher concentrations). Three-week old detached bean first leaves were partially immersed in the above solutions for 3 weeks. Chl content and Chl a/b ratio of leaves was determined according to Porra *et al.* (1989). Photosynthetic activity ($^{14}CO_2$ fixation) of leaves was measured as described by Láng *et al.* (1985). Samples for electron microscopy were prepared according to Böddi *et al*. (1997).

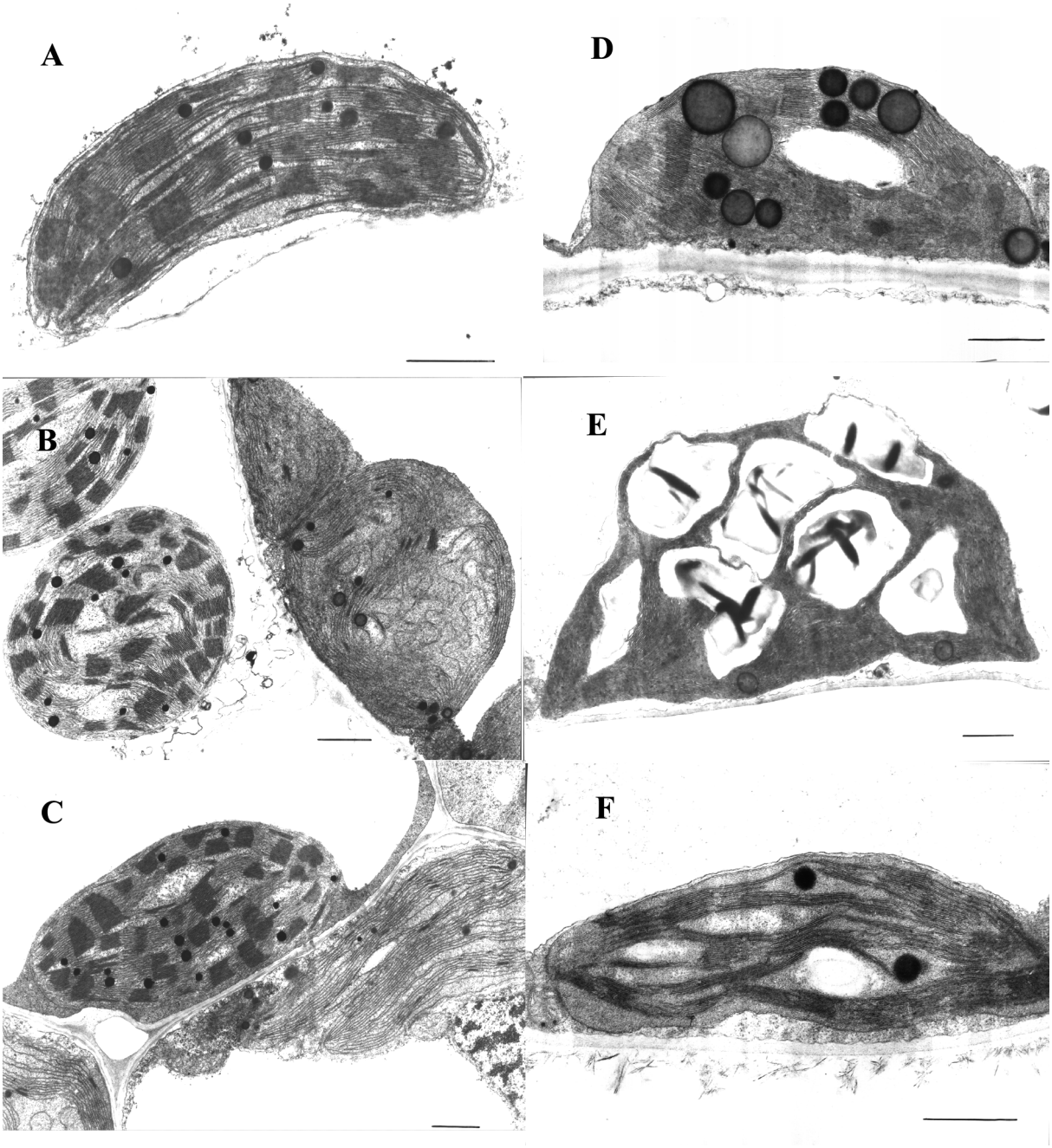

Fig. 1. Electron micrograph of: chloroplast in developing first leaf of maize (A-C) and detached first leaf of bean (D-F). A: control mesophyll and bundle sheath chloroplasts, B: mesophyll and bundle sheath chloroplasts after Pb treatment, C: mesophyll and bundle sheath chloroplasts after Ni treatment, D: senescing chloroplast in the control, E: effect of Pb treatment, F: effect of Ti treatment. Bars= 1 μm.

Results

Chl content of maize leaves greened up to three weeks increased considerably (Table 1.). Chl a/b ratios altered (decreased) only in DCMU treated leaves. Similar tendency was also found in bean leaves (not shown). Also CO_2 fixation capacity increased in maize leaves (Table 2.). Electron micrographs did not show significant differences between the lamellar system of control and treated chloroplasts (Fig. 1. A, B, C), except a slight increase in the proportion of grana after DCMU treatment. Changes in the shape of plastids are connected to the status of the plasmalemma and tonoplast, which is improved by Ni, but impaired by Pb treatment.

Detached three-week old bean leaves standing in nutrient solution showed, that every treatment decreased the loss of Chl and even increased the Chl content (rejuvenation), which was significant especially in Pb, Ni, Ti and DCMU treated samples (Table 3.). CO_2 fixation capacity increased considerably in all cases (Table 4.). Meantime, the intact control leaves left on the plants lost 70% of their Chl content and 77% of their CO_2 fixation capacity. Detached leaves developed roots during the three-week treatment. Electron micrographs showed, that the number of plastoglobuli was reduced in treated samples. Ni and Pb treatment caused large accumulation of starch (Fig. 1. D, E, F).

Table 1. Chl content of control (Ctr) and treated first and second leaves of maize seedlings in the course of greening. Standard deviations are within 10%.

	Chlorophyll content (µg Chl/g fr w)											
	11 day				14 day				21 day			
	leaf 1	%	leaf 2	%	leaf 1	%	leaf 2	%	leaf 1	%	leaf 2	%
Ctr	1675	100	1846	100	1807	100	2004	100	1665	100	2330	100
Cd	2064	123	2131	115	2167	120	2407	120	2003	120	2611	112
Pb	1841	110	2404	130	2383	132	2337	117	1993	120	2648	114
Ni	2261	135	3047	165	2201	122	2504	125	2047	123	2549	109
Ti	1934	115	2553	138	2332	129	2654	128	2162	130	2560	110
DCMU	2788	166	3059	166	2225	123	2132	106	1706	102	2413	104

Table 2. Photosynthetic activity ($^{14}CO_2$ fixation) of control (Ctr) and treated first and second leaves of maize greened up to 3 weeks. Standard deviations are within 10%.

	leaf 1 (cpm)	%	leaf 2 (cpm)	%
Ctr	80301	100	61703	100
Cd	96324	120	75660	123
Pb	95857	120	64788	137
Ni	99725	124	94533	153
Ti	100927	126	79916	130
DCMU	95871	111	67107	109

Table 3. Chl content first leaves of bean detached then treated for 3 weeks. (Ctr=control). Standard deviations are within 10%.

	Chl content (μg Chl/g fr w)	%
Ctr intact	656	31
Ctr 0 day	2120	100
Ctr 21 day	1732	82
Cd	1928	91
Pb	2615	123
Ni	2824	133
Ti	3305	156
DCMU	2920	138

Discussion

In our experiments, various low-dose stressors facilitated the Chl synthesis and CO_2 fixation capacity of maize and bean seedlings at different stage of their greening and caused agent-specific side-effects shown in increasing or decreasing amount of ultrastructural artefacts. In detached bean leaves used as a model of senescence, these low-dose stressors decreased the loss of Chl, moreover, the Chl content of Pb, Ni, Ti and DCMU treated leaves was higher than the Chl content of control ones measured at the beginning of treatment. So these stressors

Table 4. Photosynthetic activity ($^{14}CO_2$ fixation) of first leaves of bean detached then treated for 3 weeks. (Ctr=control). Standard deviations are within 10%.

	leaf 1%	(cpm)
Ctr intact	9980	23
Ctr 0 day	42587	100
Ctr 21 day	39357	92
Cd	48803	115
Pb	64471	151
Ni	60530	142
Ti	47002	110
DCMU	65771	154

not only slowed down the senescence of detached bean leaves, but turned it back and caused rejuvenation marked by a smaller amount of plastoglobuli (Fig. 1. F). The starch content

seemed to change in a compound-specific manner (Fig. 1. E). Inhibition of senescence may be due to the cytokinin synthesis of newly formed roots (Ctr 21 day minus Ctr intact) and rejuvenation due to the treatments (treated samples minus Ctr 21 day) (Table 3.). Experiments are in progress to show whether these low-dose stressors may rejuvenate leaves by changing the hormonal balance.

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