Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis, 2005

Volume 49(1-2):105-106, 2005
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Effect of low-concentration stressors on the senescence of detached barley leaves

Mariann Mayer[2], Péter Nyitrai[1]*, Áron Keresztes[2]

[1]Department of Plant Physiology and Molecular Biology, Eötvös Loránd University, Budapest, Hungary, [2]Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary

ABSTRACT Effect of low (sub-micromolar) concentrations of some stress-inducing heavy metals like Cd, Pb, Ni, and Ti salts and the herbicide DCMU on the senescence of chloroplasts was investigated in detached leaves of barley. These agents delayed the loss of chlorophylls and photosynthetic activity ($^{14}CO_2$ fixation). Decrease of the number and size of plastoglobuli in treated chloroplasts also indicated the anti-senescence effect of low-concentration stressors. The active cytokinin content of barley leaves tested by Amaranthus betacyanin bioassay did not show any changes. It is assumed that these stressors may activate a cytokinin-independent signal transduction pathway in the cells. **Acta Biol Szeged 49(1-2):105-106 (2005)**

KEY WORDS

barley
stressors
senescence
heavy metals
DCMU

Harmful effects of some chemical compounds (stressors) like heavy metals and herbicides have been extensively investigated (Kahle 1993; Sanitá di Toppi and Gabrielli 1998). Toxic effects of these agents were observed by applying them at relatively high concentrations (Van Assche and Clijsters 1990; Krupa and Baszyński 1995). When used at low concentrations (~ 10^{-8} – 10^{-6} M), they may cause temporary beneficial effects on plants (Beaumont et al. 1980; Prasad et al. 2001). Acceleration of plant growth (Ernst et al. 1992), facilitation of Chl synthesis (Prasad et al. 2001), stimulation of the photosynthetic activity (Karavaev et al. 2001), and delay of senescence (Mishra and Kar 1973) were observed.

Stimulating effect of these low-concentration stressors in rooting detached bean leaves was attributed to the higher level of the agent-induced cytokinin synthesis (Nyitrai et al. 2004). To test whether these agents have a stimulating, senescence-delaying effect also in leaves, which do not develop roots and where appearence of newly synthesized cytokinins is not expected, we chose detached barley leaves as a model system,

Materials and Methods

Barley (*Hordeum vulgare* L. Mv 245) seedlings were grown in Hoagland solution of ¼ strength supplemented with microelements for one week using 14/10 h light/dark periods at 24/18°C, and a photon flux density of 100 μmol m^{-2} s^{-1}. One-week-old leaves were detached and placed upright in the nutrient solution. They were treated with $5x10^{-8}$ M $Cd(NO_3)_2$, 10^{-7} M $Pb(NO_3)_2$, $NiSO_4$ and 3-(3,4-dichlorophyenyl)-1,1-dimethylurea (DCMU) or 10^{-6} M $TiCl_3$ added to the nutrient solutions. Solutions were changed every three days during the three-week period of treatment. Chlorophyll (Chl) content and Chl a/b ratios of leaves were determined according to Porra et al. (1989). Photosynthetic activity ($^{14}CO_2$ fixation) of leaves was measured as described by Láng et al. (1985). The radioactivity of leaf discs was detected with a liquid scintillation apparatus (Beckman LS 5000 TD). Electron microscopy of leaf pieces was carried out according to Nyitrai et al. (2004). Amaranthus betacyanin bioassay for cytokinins was carried out according to Biddington and Thomas (1973).

*Corresponding author. E-mail: pnyiti@ludens.elte.hu

Results and Discussion

The low-concentration stressors slowed down the loss of Chl in detached barley leaves (Table 1). The difference became pronounced after the first week of treatment. Effect of agents was similar also in case of the photosynthetic activity ($^{14}CO_2$ fixation; Table 2).

Electron microscopy of control chloroplasts showed an increasing amount of plastoglobuli during the first week of experiment referring to the senescence, while treated chloroplasts had a fewer number and smaller size of plastoglobuli. Results of Amaranthus betacyanin bioassay did not show a significant difference in cytokinin content of control and treated barley leaves (*i.e.* we could not observe a redistribution between the inactive and active cytokinin pools). Unlike in detached rooting bean leaves in detached non-rooting

Table 1. Chlorophyll content in μg Chl/g fresh weight of control (Co) and treated detached barley leaves. SDs are within 10%.

	0. day	4. day	7. day	14. day	18. day
Co	1400±138	1288±93	1172±15	456±45	459±41
Cd	1400±138	1248±112	1238±64	968±56	676±42
Pb	1400±138	1143±90	1333±30	877±49	635±46
Ni	1400±138	1035±81	1302±44	786±56	589±31
Ti	1400±138	1362±80	1330±64	868±65	478±43
DCMU	1400±138	1292±35	1290±80	955±47	782±68

Table 2. Photosynthetic activity ($^{14}CO_2$ fixation in cpm/cm^2) of control (Co) and treated detached barley leaves. SDs are within 10%.

	0. day	7. day	12. day	14. day	18. day
Co	276914±5782	142155±5231	37446±2271	53905±3902	10317±430
Cd	276914±5782	118557±3357	65150±6395	79468±4309	37321±3688
Pb	276914±5782	158787±9452	51562±3960	83519±2698	58345±5628
Ni	276914±5782	165127±5737	117663±2954	76739±3370	28337±533
Ti	276914±5782	125215±7829	53850±5175	76820±3580	84601±8170
DCMU	276914±5782	212660±10814	48071±2376	127533±3818	50082±4796

barley leaves the effect of low-concentration stressors can not be explained by the change of the hormonal (*i.e.* cytokinin) level. It is assumed that these agents have a direct influence on the plant cell metabolism using a cytokinin-independent signal transduction pathway.

Acknowledgments

This work was supported by grants T-047243 from OTKA.

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