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ARTICLE

On the mechanism of stimulation by low-concentration stressors in barley seedlings

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ABSTRACT Beneficial effect of low-concentration chemical stressors like Cd salt and a herbicide DCMU have been investigated previously in different plant model systems. The symptoms of stimulation are known from earlier studies, but information about the mechanism is limited. In this work, the mechanism of stimulation of low-concentration Cd (5.10^{-8} M) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 10^{-7} M) was investigated in barley seedlings. In treated plants, the amount of cytokinins increased in roots and, after being transported to the leaves, they caused stimulation of chlorophyll accumulation, photosynthetic activity, and delayed senescence. To identify the signal transduction pathway(s) involved in the primary stimulation of cytokinin synthesis in roots, specific phosphatidylinositol-4,5-bisphosphate – inositol-1,4,5-triphosphate/diacylglycerol (PIP_2-IP_3/DAG) and mitogen activated protein kinase (MAPK) signaling pathway inhibitors were added to the nutrient solutions. Both of them proved to be effective, eliminating the stimulation of the stressors
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KEY WORDS

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Harmful effects of of chemical stressors like heavy metals and herbicides have been widely investigated in the presence of their high concentrations (Krupa and Baszyński 1995; Sanitá di Toppi and Gabrielli 1998). Chemical stressors applied in low-concentration may have a beneficial effect (Zatykó 1973, Karavaev et al. 2001). These stimulative effects include increase of chlorophyll (Chl) content, changes in the composition of Chl-protein complexes, elevation of CO_2 incorporation, and changes in the ultrastructure of chloroplasts (Nyitrai et al. 2003, 2004, 2007).

In the present work, our aim was to explore the mechanism of stimulation. The role of cytokinins (CKs) could be expected on the basis of our earlier results with a rooting, detached bean leaf model (Nyitrai et al. 2004). Specific inhibitors for PIP2-IP3/DAG pathway and also for the mitogen activated protein kinase (MAPK) pathway were applied to examine whether these signaling pathway(s) were involved in the stimulation of plants.

Materials and Methods

Barley (*Hordeum vulgare* L. cv. Omega) were grown in ¼ strength Hoagland solution under standardized conditions: 14/10 h light/dark periods, at 24/18°C, photosynthetically active radiation of 100 µmol $m^{-2}s^{-1}$ (Fodor et al. 1998). After 10 days of germination, hydroponic treatments were started under the same conditions with 5.10^{-8} M $Cd(NO_3)_2$ or 10^{-7} M DCMU. Barley seedlings were also treated with signalling pathway inhibitors (10^{-5} M LiCl, 10^{-5} M stearoylcarnitine chloride – SCC, or 5.10^{-5} M 2-(2'-amino-3'-methoxyphenyl)-oxanaphtalen-4-one – PD) added to control or together with the above-mentioned stressors in the nutrient solutions. Samples were taken after 0, 1 and 2 weeks of treatment from roots, and the first leaves.

Chl content were determined according to Porra et al. (1989).

Amount of active CKs were measured according to Biddington and Thomas (1973) with a modified *Amaranthus* betacyanin bioassay.

Results and Discussion

The Cd content of roots raised considerably, but its amount increased very little in leaves during the treatment (not shown). One week treatment with Cd already lead to the increase of the CK level in roots and it remained at a high level throughout the experiment (Fig. 1 A) compared to the control. During the second week, its amount increased almost three times in leaves (Fig. 1 B). After one and two weeks of DCMU treatment, the amount of CKs were considerably higher than the control level in roots and similarly in leaves (Fig. 1 A and B).

As a consequence of higher the level of CK in Cd and DCMU-treated leaves, Chl accumulation was facilitated slightly after one week of treatment, and more pronounced up to the end of treatment (Fig. 2). The signalling pathway inhibitors (Li, SCC, and PD) applied together with the stres-

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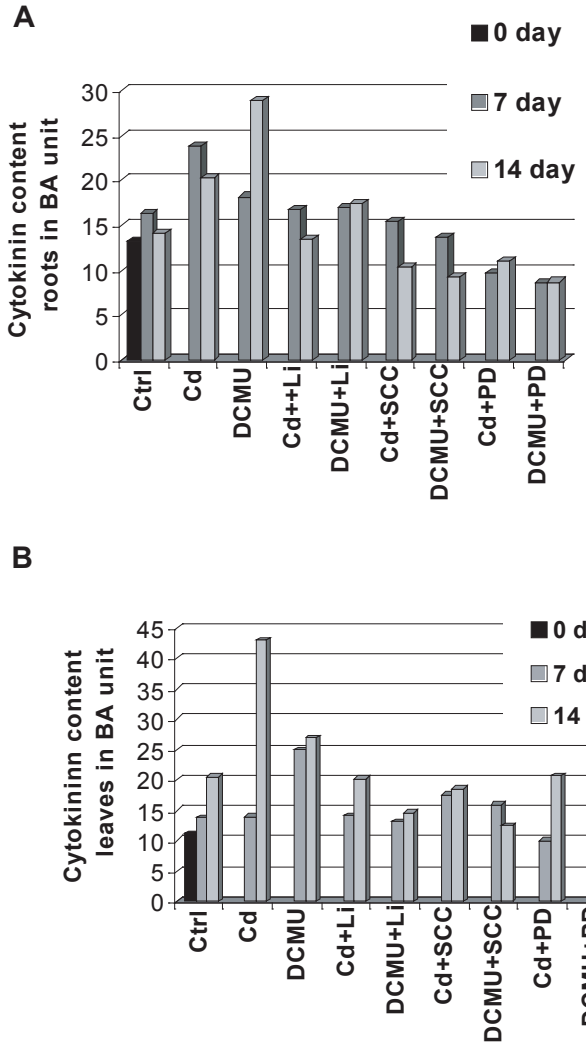

Figure 1. Amount of CKs in roots (A) and the first leaves (B) of control (Ctrl), stressor (Cd, DCMU) treated or stressor plus inhibitor (Li, SCC, PD) treated barley seedlings. SDs are within 10%.

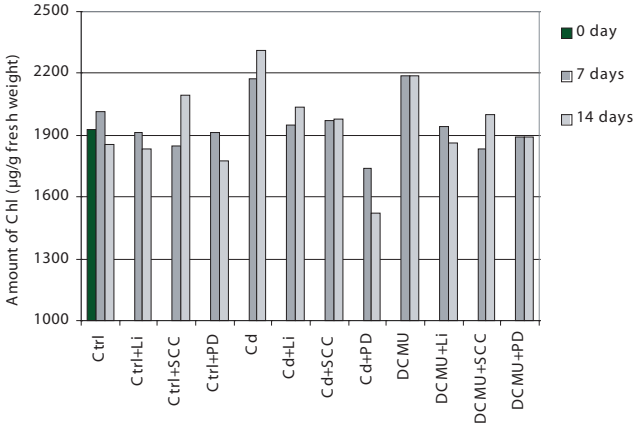

Figure 2. Chl content in the first leaves of control (Ctrl), stressor (Cd, DCMU) treated or stressor plus inhibitor (Li, SCC, PD) treated barley seedlings. SDs are within 5%.

sors caused a decrease in the amount of of active CKs in their roots and leaves compared to the stressor-treated plants (Fig. 1 A and B). Similarly, the stressor plus inhibitor treated plants contained lower amount of Chl compared to the stressor-treated plants (Fig. 2).

Electron microscopy of control and treated chloroplasts supported the rejuvenating effect of stressors, mainly on the basis of the difference in total amount of plastoglobuli.

In summary, the signal from the low-concentration stressors is transmitted through PIP_2-IP_3/DAG and protein kinase C to MAPK pathway, and it causes the synthesis (and/or activation) of CKs in roots. CKs are then transported to the leaves and facilitate Chl accumulation via cytokinin transporters and the cytokinin signaling pathway extending the CK signal to the leaves.

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