

How does a little stress stimulate a plant?

Erika Kovács-Bogdán,^{1,2,*} Péter Nyitrai¹ and Áron Keresztes²

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

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Abbreviations: Chl, chlorophyll; CK, cytokinin; DAG, diacyl-glycerol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ICP-MS, inductively coupled plasma mass spectrometry; MAPK, mitogen activated protein kinase; PKC, protein kinase C

In contrast to the damaging effect of high-concentration chemical stressors, the same agents in very low (submicromolar) concentrations have a positive effect on the treated plants, which is non-specific (independent of the chemical nature of the agent). The direct responses depend on the treated organ. When leaves are treated, the effects include an increase in chlorophyll content, CO₂ fixation and delaying senescence of chloroplasts. When roots are treated, the direct effect is an increased cytokinin synthesis. This hormone, after being transported to the shoot, exerts secondary effects, which are similar to the primary ones in leaves. The signalization routes involved in the primary effects proved to be the phosphoinositide and MAPK pathways in any stimulated organ. In this mini-review we summarize our current knowledge about the effects of low-concentration stressors and their mechanism of action with the help of the four used model systems: detached non-rooting and rooting leaves, hydroponically treated and sprayed seedlings.

by Mattson and Calabrese⁹ where low concentrations cause “hormesis” (make the organism resistant), but higher concentrations are already toxic. Zatykó¹⁰ investigated this positive effect in plants, finding that after weak chemical or mechanical stimuli plants showed a general resistance increment (longer survival in darkness). Based on this work and own experiences, Á. Keresztes and P. Nyitrai with changing coworkers designed and carried out a series of experiments for finding the best conditions and markers of the chemical stimulation, and understanding its mechanism.

In this mini-review we summarize what has been explored about the effects of low-concentration stressors and their mechanism in plants. Studies in this topic can be divided into four subgroups, according to the used plant model: (1) non-rooting, (2) rooting detached leaves, (3) hydroponically treated and (4) sprayed seedlings.

Detached, Non-Rooting Leaves

Introduction

The life processes of a plant are influenced by several environmental factors, which in excess may be stressors. Effects of these biotic or abiotic stressors have been widely investigated: e.g., what happens after wounding, after poisoning with heavy metals, herbicides or after heat stress. Concerning on metals and herbicides the effect is dependent on the concentration, exposure time and of course, on the physiological status of the plant. Using high concentrations (~10⁻⁵–10⁻³ M) induces a strong negative effect, including decreased root growth,^{1,2} decreased photosynthetic activity,^{3,4} inhibition of the Calvin cycle enzymes⁵ and signs of senescence in chloroplast ultrastructure.⁶

Using stressors in low concentration or for shorter time, has interestingly an opposite effect. Selye in his stress-theory^{7,8} described that mild stress (e.g., short cold treatment, small amount of body strange compounds) in mice generated a non-specific positive effect, independently on the used treatment (“general adaptation syndrome”). Recently, the effect of stressors has been described with a biphasic dose response model

Detached leaves are the simplest model system for investigation of the effect of low-concentration stressors on plants. The delayed appearance of senescence is a good indicator for stimulation, as it was observed by Mishra and Kar¹¹ after floating detached rice leaves on solution containing NiCl₂ in millimolar concentration. Romanowska et al.¹² described also the stimulation of respiration in detached pea, barley and maize leaves after the treatment with micromolar concentration of Pb(NO₃)₂.

Nyitrai et al.¹³ treated detached barley leaves hydroponically with different low-concentration stressors [10⁻⁷ M Pb(NO₃)₂, DCMU or 10⁻⁶ M TiCl₃] for testing their effects (Fig. 1A). All of the agents caused a high increase in the Chl amount and in the CO₂-fixation activity, showing a significant rejuvenating effect, whereas the control leaves turned yellowish, indicating senescence. The several plastoglobuli in the chloroplasts of control leaves but not in the treated ones also showed that the treatments delayed aging.

For finding out the way to the rejuvenation Nyitrai et al.¹³ measured the amount of CKs. Although a leaf without roots is not supposed to synthesize CKs, the inactive CK-conjugates could be activated, but in this study this was not experienced. Increased ion-uptake, as a possible elicitor, was also excluded, using CaSO₄ solution versus Hoagland nutrient solution. Accordingly, the stimulation was a direct effect of the agents, that accumulated in the leaves (measured with ICP-MS).

*Correspondence to: Erika Kovács-Bogdán; Email: k-erika@elte.hu

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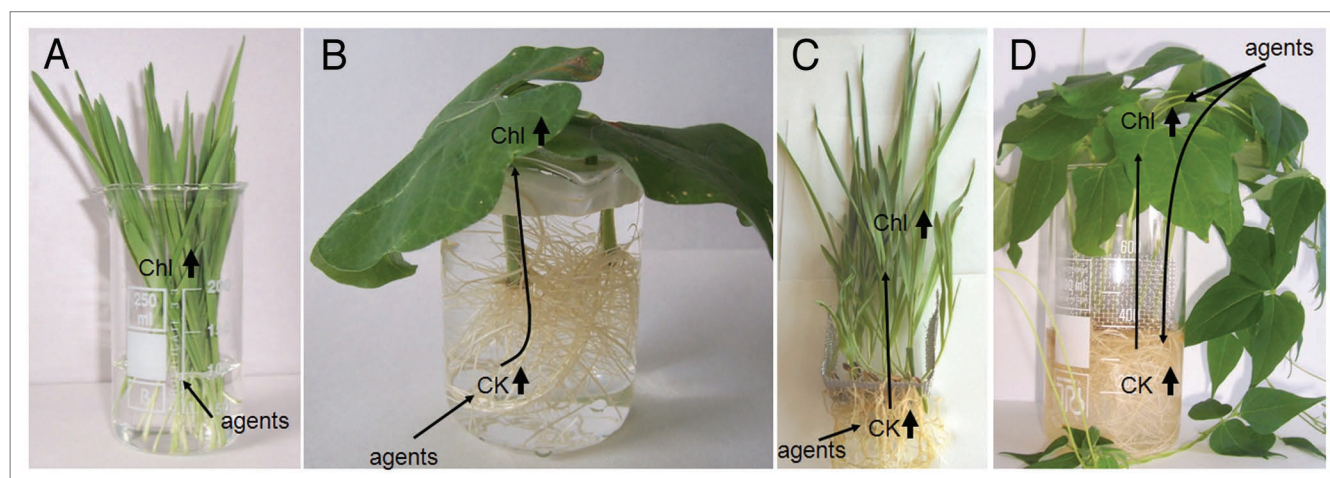


Figure 1. Effect of low-concentration stressors on the organs of the different model systems: (A) detached non-rooting leaves (barley); (B) detached rooting leaves (bean); (C) hydroponically treated seedlings (barley); (D) sprayed seedlings (bean). Chl, chlorophyll; CK, cytokinin.

Inhibition of the phosphoinositide pathway with LiCl,¹⁴ and blocking of protein kinase C (PKC) activity with stearylcarbitine chloride^{15,16} in agent-treated leaves, kept the parameters at the control level, suggesting that the phosphoinositide pathway together with PKC transferred the signal from the agents into the cells and led to slower ageing¹³ (Fig. 2a–c).

Detached, Rooting Leaves

Some plant species (e.g., bean and cucumber) are able to develop roots after leaf detachment and thereby return the initial senescence to rejuvenation. Using this more complex detached leaf system (getting closer to the whole plant models) Nyitrai et al.¹⁷ investigated the effect of low-concentration stressors [10^{-8} M $\text{Cd}(\text{NO}_3)_2$, 10^{-7} M $\text{Pb}(\text{NO}_3)_2$, NiSO_4 , DCMU or 10^{-6} M TiCl_3] in hydroponically treated, detached bean leaves (Fig. 1B). After one week of detachment root development was observed, and rejuvenation was intensively facilitated by the stressors: a strongly increased Chl amount (as the best sign for the stimulation) and a higher CO_2 -fixation activity was measured in the treated groups in contrast to the control.

According to data in the literature, treating plants with CKs increases the amount of Chl and different Chl-protein complexes, and the photosynthetic activity.^{18,19} CKs inhibit the loss of Chl and nucleic acids in yellowing leaves, consequently delaying senescence.^{20,21} Treatment with CKs also facilitates the rejuvenation of old leaves of decapitated plants.^{22,23} Moreover, Zatykó²⁴ found that pinto bean seedlings treated with a low concentration (20 ppm) of colchicin showed no negative effect, rather an increase in endogenous CKs.

These studies, among others, led Nyitrai et al.²⁵ to measure the amount of active CKs in detached and treated [5×10^{-8} M $\text{Cd}(\text{NO}_3)_2$, 10^{-7} M $\text{Pb}(\text{NO}_3)_2$ or DCMU] bean leaves, searching for a possible reason of stimulation. As expected, they observed a stronger increase of active CK level in the treated roots where CKs are synthesized,²⁶ but to a lesser extent also in the leaves. HPLC analysis revealed an increase in two kinds of CKs,

isopentenyl-adenine and trans-zeatin. The stimulation could be inhibited by LiCl, the inhibitor of the phosphoinositide pathway. The transport of the metal ions to the leaves—and there a direct effect—was excluded with ICP-MS analysis.

In conclusion, the agents have a direct effect in the roots: via the activation of the phosphoinositide pathway they induce CK synthesis, which hormone is transported to the leaves and stimulates Chl synthesis causing rejuvenation (Fig. 2d–i and c).

Interestingly, detached cucumber leaves showed the same facilitated rejuvenation, without any treatment.²⁷ After detachment, senescence was observed in the leaves in the first week, as shown by the larger plastoglobuli in chloroplasts, and the slightly decreased amount of Chl. After root development strong rejuvenation occurred: leaf area and thickness, Chl amount, chloroplast size, and the photosynthetic activity highly increased. Most of these changes could be imitated separately by the addition of different CKs to leaf discs, demonstrating the possible CK effect.

Hydroponically Treated Seedlings

Effects of low-concentration stressors were also investigated in whole plants: either by hydroponical treatments via the roots, or by spraying onto the leaves. Hydroponical treatment had been described to have a positive effect on the roots and on the leaves as well: 10^{-6} – 10^{-7} M CdSO_4 stimulated root development of pine seedlings.¹ Lemna species treated with low-concentration atrazine (1.15×10^{-6} M or 0.25 ppm) or CuSO_4 (10^{-6} M) in the nutrient solution showed an increased Chl amount, more developed grana system, smaller plastoglobuli and higher photosynthetic activity.^{28–30} Additionally, 10^{-3} M CdCl_2 pretreatment could protect temporarily the PSII activity of *Pisum sativum* and *Vicia faba* from heat stress via the increased D1 protein turnover.³¹ Karavaev et al.³² investigated the effect of 10^{-6} – 10^{-7} M CdCl_2 , SnCl_2 or CuCl_2 on *Vicia faba* and found an increased photosynthetic activity and oxygen evolution independently of the used agent. Different low-concentration stressors [5×10^{-8} M $\text{Cd}(\text{NO}_3)_2$, 10^{-7} M $\text{Pb}(\text{NO}_3)_2$, NiSO_4 , DCMU or 10^{-6} M TiCl_3] also have a

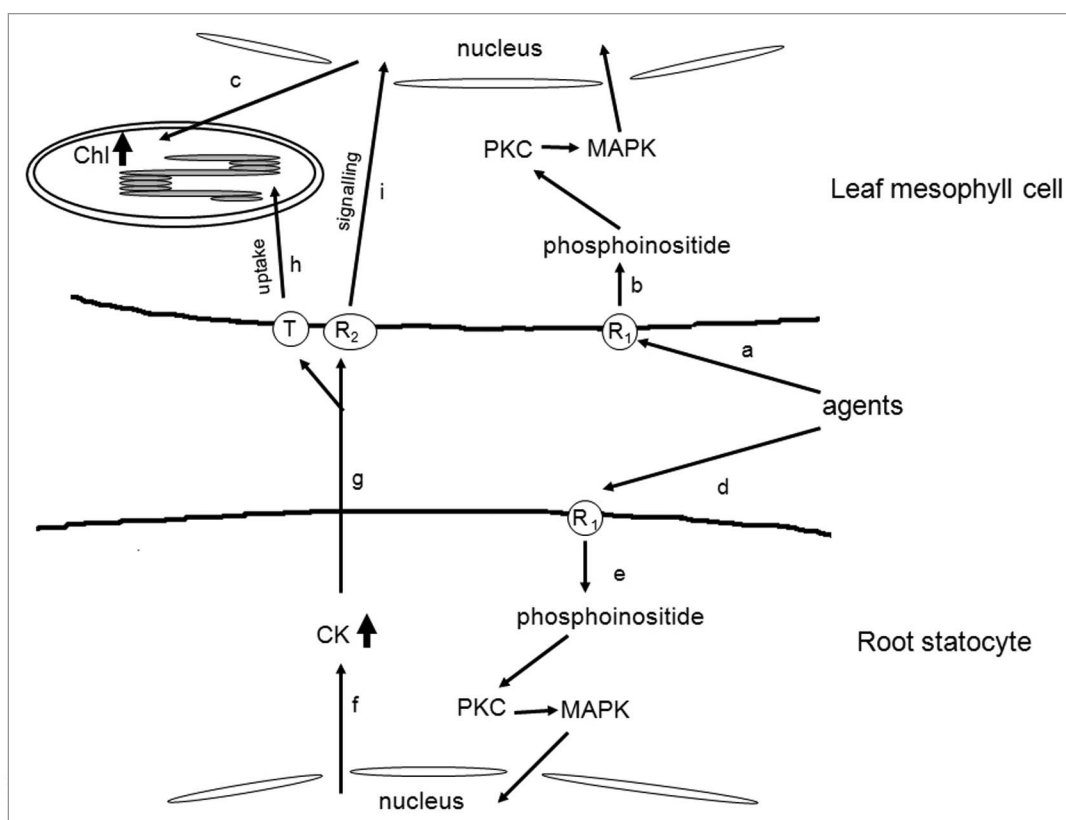


Figure 2. Mechanism behind the effect of low-concentration stressors. The four model systems (detached non-rooting leaves: a–c; detached rooting leaves: d–i, c; hydroponically treated seedlings: d–i, c; sprayed seedlings: a–c and d–i, c) are summarized. (a and d) activation of the receptor(s) (R_1) by the agents; (b and e) activation of the signalling pathways; (c) stimulation of chlorophyll (Chl) synthesis; (f) stimulation of cytokinin (CK) synthesis; (g) transport of CKs to the leaves; (h) uptake of CKs, (i) signalization activated by CKs. MAPK, mitogen activated protein kinase pathway; PKC, protein kinase C; R_2 , CK receptor; T, CK transferase.

non-specific stimulating effect on maize seedlings: Nyitrai et al.³³ measured an increased Chl amount and photosynthetic activity at all leaf storeys and developmental stages. Another study³⁴ showed similar stimulating effect of 5×10^{-8} M $\text{Cd}(\text{NO}_3)_2$ or 10^{-7} M DCMU in barley seedlings (Fig. 1C).

Kovács et al.³⁴ excluded the direct effect of the agents in the leaves after hydroponic treatment, on the basis of the negligible amount of Cd transported there. Increased CK amount was observed in roots and leaves, such as in rooting detached leaves (see above). Searching for the signal transduction pathway involved in the stimulation, they blocked not only the phosphoinositide pathway with LiCl, but also the MAPK pathway with PD 98059, a specific inhibitor of MEK1 and 2.³⁵ The MAPK pathway is activated in plants by high concentration of heavy metals (1–1,000 μM CdCl_2 or CuCl_2),³⁶ which raised the question if the same could also be experienced by low concentrations. Both of the inhibitors seemed to be effective, they abolished the stimulating effect in barley seedlings.³⁴

The increased amount of Cd in the roots required to test a possible oxidative stress in the seedlings. The amount of superoxide dismutase (the antioxidative enzyme) and malondialdehyde (the indicator of lipid peroxidation) was measured in the same study, and the authors concluded that oxidative stress did not play a role in the stimulation.

In summary, the stressors have a non-specific, direct effect on the roots, their signal is transmitted through the phosphoinositide pathway. This activates the MAPK pathway (possibly via DAG and PKC), without the involvement of reactive oxygen species. It leads to CK synthesis and transport to the leaves, which causes there stimulation (Fig. 2d–i and c).

In contrast to these studies, De Filippis et al.³⁷ found the inhibition of cell division and the decrease of Chl amount in *Euglena* after the treatment with 5×10^{-5} M ZnCl_2 , 10^{-7} M CdCl_2 or 10^{-8} M HgCl_2 , but the dry weight and the protein amount were increased. Gonen-Zurgil et al.³⁸ described the inhibition of photosynthetic activity and growth already with 10^{-7} M DCMU in different algal species. These findings indicate that algae are more sensitive to these stressors, a concentration that is stimulative in vascular plants can already be inhibitory in algae. Therefore they are good indicators of the change in water quality.

Sprayed Seedlings

The stimulating effect of low-concentration stressors was not only observed by hydroponical treatment, but also by spraying the agents onto the leaves. Nyitrai et al.³³ characterized it in details by measuring Chl amount, CO_2 -fixation, the increment of Chl-protein complexes, and fluorescence induction parameters

of bean seedlings after the treatment with 10^{-7} M $\text{Cd}(\text{NO}_3)_2$, 10^{-6} M $\text{Pb}(\text{NO}_3)_2$, NiSO_4 , DCMU or 10^{-5} M TiCl_3 (Fig. 1D). According to the authors, the Chl content increased in the first week, and remained above the control level during the second week. A change in the active CK levels was also observed, it increased in the first week in the roots and only after the second week in the leaves.⁴⁰ The signal transduction pathways involved in the stimulation showed to be the same as after hydroponical treatment with the stressors (phosphoinositide and MAPK, see above). Moreover, the content of the used heavy metals increased in the leaves and in the roots, as well.

These findings together let the authors to conclude, that the agents have a direct primary effect on the Chl synthesis in the leaves (such as in the detached non-rooting leaves) (Fig. 2a–c), then a secondary effect in the roots on the synthesis of CKs (such as in the root-possessing models), which are transported to the leaves and stimulate again Chl synthesis (Fig. 2d–i and c).

Conclusions

It has been demonstrated in several studies, that low-concentration stressors (10^{-5} – 10^{-8} M) have a positive effect on different plant species in contrast to high concentrations. The effects proved to be the same independently of the used agents (metals, including heavy metals, or herbicides): increased growth, Chl content, Chl-protein complexes and photosynthetic activity, accompanied with ultrastructural changes referring to stimulation. The exact concentration of course is dependent on the toxicity of the agent, the experimental system, and the treated organ. The general effect already suggests a general mechanism for stimulation.

After several studies, describing these stimulative effects, but not focusing on the background, the mechanism of the stimulation has been characterized in terms of the signalling pathways activated, distinguishing between primary (direct) and secondary

(indirect) effects. In non-rooting detached leaves, inhibition of aging is caused directly by the agents in leaves¹³ (Fig. 1A). In hydroponically treated rooting leaves or seedlings, stimulation is caused directly in roots via increased CK synthesis^{17,25,33,34} (Fig. 1B and C). Sprayed seedlings show both mechanisms: direct stimulation in leaves and induction of CK synthesis by accumulated agents in roots which leads again to a stimulation in leaves⁴⁰ (Fig. 1D). Accordingly, the level of stimulation (in Chl content) was somewhat higher after spraying, than that found in the hydroponic model systems, on the same time course.

Interestingly, the pathways are the same for all of the models: the phosphoinositide pathway together with PKC (via DAG) is activated both in roots and leaves, and lead to CK or Chl synthesis, respectively.^{13,25,34,40} In those models, where it was probed, the activation of MAPK pathway was also experienced showing that both pathways are necessary for stimulation, independently of the treated organ^{25,34,40} (Fig. 2).

What remains still unclear, it is the receptors which can sense the signal of the stressors in the leaves and in the roots. Are they the same in the different organs? Can the same receptor sense low-concentration metals and DCMU? According to the studies summarized here, we would expect the same receptors in the roots and in the leaves, which may be different for metal ions and DCMU. Regarding the identity of the receptor for metal ions, Ouelhadj et al.⁴¹ found the activation of a lysine motif receptor-like protein kinase in barley, after treatments with 50 μM or 1 mM heavy metal salts. Although the stimulative concentration applied in the reviewed studies was below the lower value in this study, this receptor worked at different (damaging and non-damaging) concentrations, so it may be considered also for the submicromolar range.

Another problem is the genes activated by stimulation. These are obviously different from those playing a role in the defensive reactions elicited by high-concentration stressors, but their identification remains for future studies.

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