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Gas exchange and cytokinins

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Introduction

Cytokinins (CK) are believed to antagonize many physiological processes induced by water stress, mainly those mediated by abscisic acid (ABA), *e.g.*, to delay plant senescence, increase growth of shoots more than that of roots, and induce stomatal opening. The increase in transpiration rate (E) and stomatal aperture, and/or delay in stomatal closure induced by ABA was observed, *e.g.*, in *Anthephora*, *Commelina*, *Kalanchoe*, *Tradescantia*, *Tridax*, *Vicia, Vitis,* and *Zea* (Incoll *et al*. 1990, Morsucci *et al*. 1991, Pharmawati *et al*. 1998, Stoll *et al*. 2000). However, in *Beta*, *Gossypium*, *Linum*, and *Zea* CK did not significantly affect stomatal opening, E, or P_N (Radin *et al*. 1982, Blackman and Davies 1983, Radin and Hendrix 1988, Drüge and Schönbeck 1992, Čatský *et al*. 1996), and in *Commelina* CK even decreased stomatal opening (Blackman and Davies 1983). Exogenously applied CK alleviated negative effect of water stress on chlorophyll and carotenoid contents, photochemical activities of photosystems 1 and 2, and content and activity of Rubisco (Metwally *et al*. 1997, Chernyad′ev and Monakhova 1998, Singh *et al*. 2001).

The aim of these experiments was to answer a question whether the stimulation of stomatal opening and in consequence of E and net photosynthetic rate (P_N)by CK is of general or exceptional character. The changes in gas exchange parameters in dependence on CK type and concentration, way of application, and plant species used was followed.

Materials and methods

Seedlings of French bean (*Phaseolus vulgaris* L. cv. Jantar) and sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll, cv. Elán) were grown in pots with coarse sand or fine *Perlite* with nutrient solution at 16-h photoperiod, irradiance (400 - 700 nm) of 350 μmol m^{-2} s^{-1}, day/night temperature of 25/20 °C, and relative humidity of about 50 %. Two synthetic CK, benzyladenine (BA) and hydroxybenzyladenosine (HBA) in concentrations 0, 1, 5, 10, and 20 μM, were used. They were added to the substrate, or the roots of intact plants were immersed in the CK solutions, or the CK solutions were sprayed on the leaves. In addition to plants sufficiently supplied with water, effects of CK during rehydration of water-stressed plants was also followed.

P_N, E, and stomatal conductance (g_s) were measured 1, 24, or 72 h after application on attached mature leaves using the gas exchange system *LCA-4* (*ADC Bio Scientific*) at a temperature of 25°C, irradiance of 750 μmol m^{-2} s^{-1}, relative humidity of 50 %, and CO_2 concentration of 350 μmol mol^{-1}. Water use efficiency (WUE) was calculated as P_N/E ratio. Chlorophyll content was determined in 80 % acetone extract of leaves with the spectrophotometer *Hitachi U-3300*. Chlorophyll *a* fluorescence characteristics of attached leaves were measured with a PAM Chlorophyll Fluorometer (*Walz*).

Results and discussion

The responses of bean plants to BA application were dependent on concentration and time: P_N and g_s of primary leaves of 12-d-old bean plants with roots immersed in 1, 5, 10, or 20 µM BA solutions were higher that those of control plants when measured 1 h after application. This rapid increase in g_s was in agreement with the results of Vogelmann *et al.* (1984) who found that BA was readily taken up by plants. At the same time E was not markedly affected. Therefore WUE was increased by BA treatments. When measured 24 h after application, P_N was slightly increased only at lower BA concentrations. E and g_s of BA treated plants were not affected or were lower than those in control plants (Fig. 1). WUE was increased at 1 µM BA. The way of application and plant age were not decisive: similar effects were found when BA solutions were sprayed on leaves, added to the substrate, or when roots were directly immersed in BA solutions, as well as when primary or secondary leaves which are morphologically different were used (data not shown).

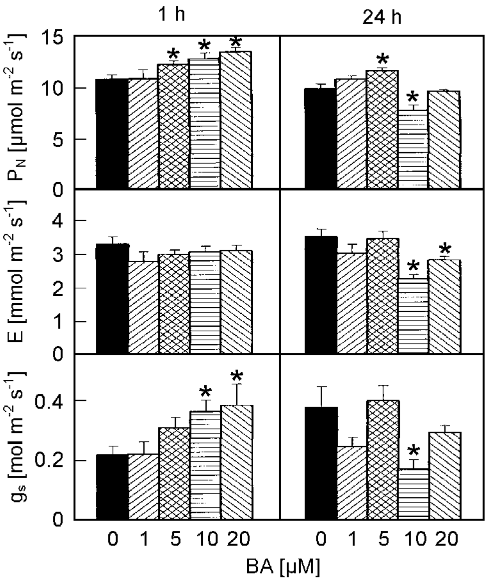

Fig. 1. Effects of root immersion in BA solutions of different concentrations (0, 1, 5, 10, and 20 µM) on P_N, E, and g_s of bean leaves. All parameters were measured 1 and 24 h after application. Means ± SE, $n = 9$, * - values significantly different at $P < 0.05$ from corresponding ones measured on plants with roots immersed in water.

In contrast to bean plants, P_N, E, and g_s of sugar beet plants were not consistently affected by immersion of roots in 1, 5, and 10 µM as well as by spraying the leaves with corresponding BA solutions. The only statistically significant positive effects on P_N were observed after 24 h in plants with roots immersed in 5 or 10 µM BA solution (Fig. 2), and on E after 24 h in plants sprayed with 5 µM BA. In addition to BA, also HBA in the same concentrations and ways of application was used. Neither HBA, consistently stimulated gas exchange of sugar beet leaves. 24 h after application, P_N was stimulated in plants with roots immersed in 10 µM HBA solution and E in plants sprayed with 10 µM HBA. WUE was increased in many cases after BA or HBA application, but not in every case. Thus, in agreement with data in review (Pospíšilová *et al.* 2000) effects of CK on gas exchange were dependent on plant species.

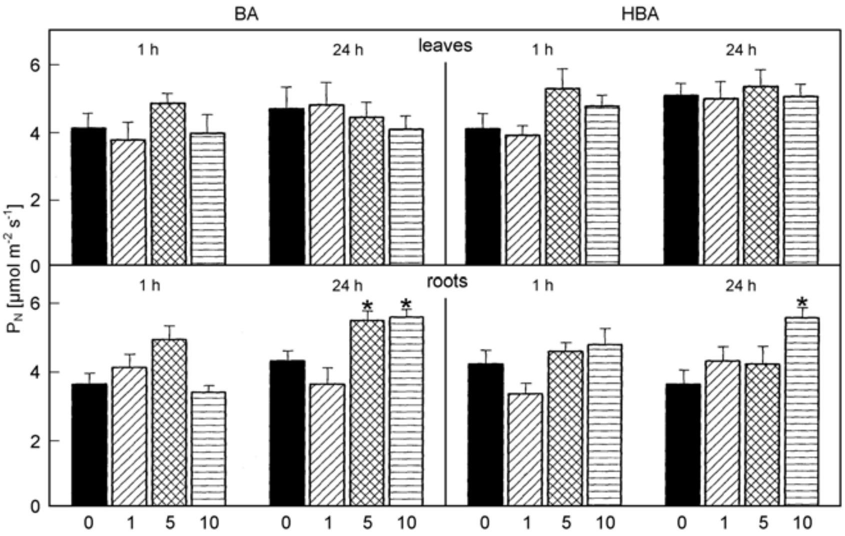

Fig. 2. Comparison of effects of BA and HBA on P_N of sugar beet leaves measured 1 and 24 h after application. Roots of plants were immersed in corresponding solutions (roots) or solutions were sprayed on leaves (leaves). Means ± SE, *n* = 15, * - statistically significant ($P < 0.05$) increase in the respective parameter in comparison with corresponding one in leaves treated with water.

In addition to plants sufficiently supplied with water, 1 and 10 μM BA was also applied to the substrate of water-stressed bean plants (relative water content about 70 %) or sprayed on their leaves. Application of 1 μM BA slightly delayed leaf senescence: in 17-d-old control plants, P_N and chlorophyll (Chl) content, and when sprayed on leaves also some of Chl *a* fluorescence kinetic parameters of BA-treated leaves were slightly higher than those of untreated leaves. Both types of application of 1 μM BA slightly improved recovery of plants during rehydration after water stress in terms of slightly increased g_s and P_N, *i.e.*, parameters which were markedly decreased by mild water stress. However, contents of Chl *a* and Chl *b*, and parameters of Chl *a* fluorescence kinetic were not markedly affected by mild water stress and after rehydration were usually not stimulated by 1 μM BA. 10 μM BA had mostly negative effects on the parameters measured (Fig. 3). Stimulation of P_N by BA or HBA was also observed during rehydration of sugar beet plants, but only after severe water stress.

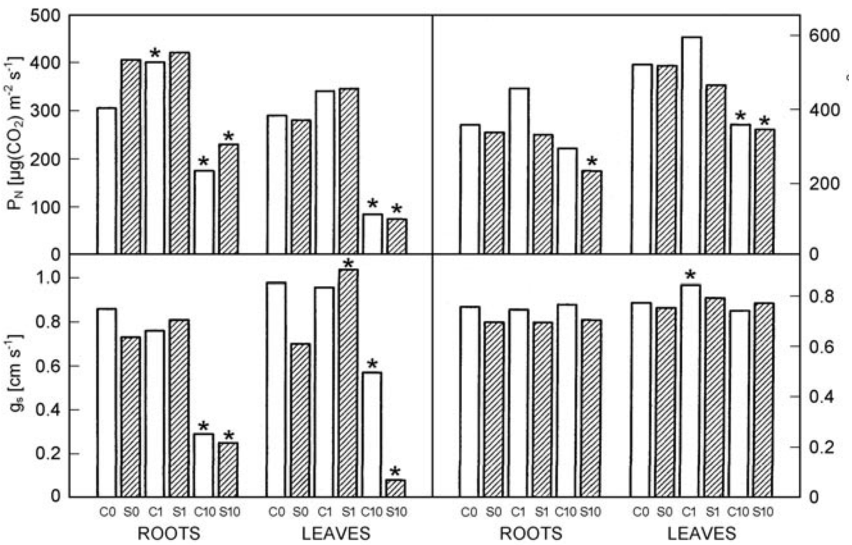

Fig. 3. P_N, g_s, Chl *a+b* content, and photochemical efficiency (Fv/Fm) in control 17-d-old bean plants (C0), water-stressed and rehydrated plants (S0), and in control, and water-stressed and rehydrated plants treated by 1 μM BA (C1, S1) or 10 μM BA (C10, S10). BA was applied either to the substrate (roots) or sprayed on the leaves (leaves). Means of 10 repetitions. * - statistically significant ($P < 0.05$) differences in the respective parameters between BA-treated and control plants.

Conclusions

The stimulation of stomatal opening, E and P_N by exogenously applied CKs is rather exceptional than general. The effects strongly depend on plant species and concentration but only weakly on way of application. Nevertheless, these results cannot definitively reject any role of CKs in regulation of gas exchange and further experiments are focused on interactions between CKs and ABA.

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