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Effect of benzyladenine and hydroxybenzyladenosine on gas exchange of bean and sugar beet leaves

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Abstract

Using bean seedlings, the effects of benzyladenine (BA) on stomatal conductance (g_s), transpiration rate (E), and net photosynthetic rate (P_N) were examined in order to find out dose and time responses. In bean seedlings, BA applied to roots in concentrations of 1, 5, 10, and 20 μM increased g_s and P_N of leaves already 1 h after application. E was not markedly affected and water use efficiency (WUE) was increased. However, the effects were mostly transient and after 24 h P_N only at 1 and 5 μM BA was increased, and other parameters were not affected or even decreased. In sugar beet seedlings, the effects of hydroxybenzyladenosine (HBA) in addition to those of BA on the same parameters were determined. The both cytokinins were applied in 1, 5, 10, and 20 μM concentrations either to roots or sprayed on leaves. However, the effects were inconsistent and the positive effect was observed only after 24 h on P_N in plants with roots immersed in 5 and 10 μM BA, or 10 μM HBA, and on E in plants sprayed with 5 μM BA or 10 μM HBA. Thus the stimulation of gas exchange by exogenously applied cytokinins is rather exceptional than general.

Additional key words: *Beta vulgaris*, cytokinins, net photosynthetic rate, *Phaseolus vulgaris*, stomatal conductance, transpiration rate, water use efficiency.

Introduction

Cytokinins (CKs), acting both in synergy and antagonism with other plant hormones, influence a wide range of events during plant growth. Plant hormones including CKs are also main signals in root-to-shoot communication and *vice versa* (for reviews see, *e.g.*, Schulze 1986, Davies and Zhang 1991, Tardieu and Davies 1993, Davies 1995, Naqvi 1995). The major portion of CKs is produced in meristematic regions of the roots and transported via xylem to the shoot. These CKs, along with the locally synthesized CKs, control development and senescence of the whole plant. CKs promote leaf expansion, accumulation of chlorophyll, conversion of etioplasts into chloroplasts, and delay leaf senescence (for reviews see Synková *et al.* 1997, Naqvi 1999). In addition, they can alleviate negative effect of water stress on chlorophyll and carotenoid contents, photochemical activities of photosystems 1 and 2, and content and activity of Rubisco (Chernyad'ev 1997, Metwally *et al.* 1997, Chernyad'ev and Monakhova 1998, Singh *et al.* 2001) and on chloroplast ultrastructure (Stoyanova and Yordanov 1999).

Both synthetic and naturally occurring CKs, when added exogenously, increased transpiration rate (E) and stomatal aperture, and/or delayed stomatal closure induced by abscisic acid, *e.g.* in *Anthephora*, *Commelina*, *Kalanchoe*, *Tradescantia*, *Tridax*, *Vicia*, *Vitis*, and *Zea* (Incoll and Jewer 1987, Incoll *et al.* 1990, Morsucci *et al.* 1991, Pharmawati *et al.* 1998, Stoll *et al.* 2000). Thus the reduction in CK concentration and activity in xylem sap (Bano *et al.* 1993, Naqvi 1995, 1999, Shashidhar *et al.*

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Abbreviations: ABA - abscisic acid; BA - N^6-benzyladenine; CK - cytokinin; E - transpiration rate, g_s - stomatal conductance; HBA - N^6-(*m*-hydroxybenzyl)adenosine; P_N - net photosynthetic rate, WUE - water use efficiency.
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1996) in leaves of water stressed plants might amplify leaf responses to an increasing concentration of ABA (Davies and Zhang 1991). However, in water stressed cotton and sunflower, the ABA/CKs ratio increased in apoplastic solution but the CK concentration was not significantly changed (Hartung *et al.* 1992, Masia *et al.* 1994). The mechanism of CK action on guard cell might involve direct induction of membrane hyperpolarization by stimulation of electrogenic H^+-pump, stimulation of adenylate cyclase activity which could lead to an increase in intracellular adenosine 3′,5′-cyclic monophosphate content, stimulation of guanylate cyclase activity, or interaction with calcium-calmodulin system, and with ABA regulation of ion channel permeabilities (Incoll *et al.* 1990, Morsucci *et al.* 1991, Pharmawati *et al.* 1998). The antagonism between CKs and ABA may be the result of metabolic interactions: CKs share, at least in part, a common biosynthetic origin with ABA (Cowan *et al.* 1999).

However, positive effect of CKs on stomatal opening and E was not observed in all cases (for review see Pospíšilová *et al.* 2000). In cotton, flax, maize, and sugar beet CKs 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), but in maize and flax decreased stomatal response to ABA. In *Commelina* epidermal strips or leaf fragments, zeatin or kinetin even decreased stomatal opening and had no effect on ABA-induced stomatal closure (Blackman and Davies 1983). In the root hemiparasite *Melampyrum arvense,* application of CKs increased stomatal opening only in preparasitic stage (Lechowski 1997). E of tobacco plants and stomatal aperture of *Digitalis* were increased when grown *in vitro* on medium with CKs but the effect was concentration dependent (Diettrich *et al.* 1992, Pospíšilová *et al.* 1993). Similarly in our previous experiments, slight positive effect of 1 μM benzyladenine (BA) on g_s and P_N of water stressed and rehydrated bean plants was found but 10 μM BA had negative effect (Rulcová 2000, Rulcová and Pospíšilová 2001).

The above mentioned contradictory results provoke a question whether the stimulation of stomatal opening, and in consequence of E and P_N by CKs, is of general or exceptional character. Therefore, the aim of these experiments was to determine whether the effects of CKs on gas exchange parameters depend on CK type and concentration, way of application, and plant species used.

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* sufficiently moistened with Hewitt nutrient solution. Both plant species were cultivated in growth chambers 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 %. Air temperature and humidity were measured with the *JUMO Humitherm TDAc-70* (*M.K. Juchheim,* Fulda, Germany). Irradiance was measured with the *LI 185B* radiometer with a quantum sensor (*Li-COR*, Lincoln, USA).

P_N, E, and g_s were measured on attached leaves using commercial gas exchange system *LCA-4* (*ADC Bio Scientific*, Hoddesdon, UK) with leaf chamber *LC4/PLC4BT-1/E* at a temperature of 25 °C, irradiance of 750 μmol(photon) m^{-2} s^{-1}, relative humidity of 50 %, and CO_2 concentration of 350 μmol mol^{-1}. WUE was calculated as P_N/E ratio. Mature leaves with P_N, E and g_s at or near maximum were usually used.

In previous experiments N^6-benzyladenine (BA) in two concentrations (1 and 10 μM) was used, and it was applied either added to the substrate (sand + nutrient solution) or sprayed on primary leaves of plants sufficiently supplied with water, or of water-stressed plants. Photosynthetic characteristics were measured 72 h after application. In contrast, in these experiments BA was used in a wider range of concentrations (1, 5, 10, and 20 μM), under different way of application (immersion of roots directly into BA solution), and measurements were done already 1 and 24 h after application. The first pair of secondary leaves was also used as these leaves in bean plants morphologically differ from primary leaves.

In sugar beet plants two synthetic CKs, BA and N^6-(*m*-hydroxybenzyl)adenosine (HBA), were applied in concentrations 0, 1, 5, 10, and 20 μM. Two ways of application were used: *1*) the roots of intact plants were immersed in the CK solutions, and *2*) the CK solutions were sprayed on the leaves. P_N, E, and g_s were measured 1, 4, and 24 h after application. The immersion of petioles of detached sugar beet leaves in the CK solutions was also tested but the values of measured parameters in these leaves were always (even when immersed in water) lower than those of intact plants; therefore this application was not used in further experiments.

For each parameter a mean and standard error of mean were calculated and the statistical significance of differences between control and treated plants were evaluated by Student's *t*-test.

Results and discussion

The effects of BA in different concentrations (0, 1, 5, 10, and 20 μM) on P_N, E and g_s of primary leaves of 12-d-old bean plants sufficiently supplied with water were determined 1 and 24 h after application. P_N and g_s of plants with roots immersed in 1, 5, 10, or 20 μM BA solutions were higher that those of control plants with roots immersed in water when measured 1 h after application. At the same time E was not markedly affected. Therefore WUE was increased by BA treatments. When measured 24 h after application, values of P_N were slightly increased only at lower BA concentrations (1 and 5 μM). 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 results obtained are mostly in agreement with previous experiments where 1 or 10 μM BA solutions either added to the substrate or sprayed on primary leaves, and photosynthetic parameters were measured 72 h after application (Rulcová 2000, Rulcová and Pospíšilová 2001). In these plants 1 μM BA slightly increased P_N, but g_s was not significantly affected. However, 10 μM BA had negative effects on the parameters measured.

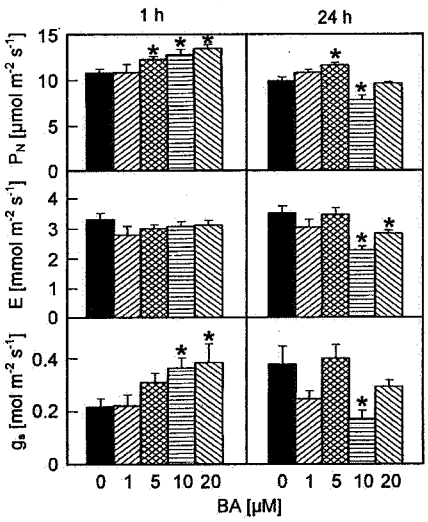

Fig. 1. Effects of root immersion in BA solutions of different concentrations (0, 1, 5, 10, and 20 μM) on P_N, E, and g_s of bean leaves. All parameters were measured 1 and 24 h after application. Means ± SE, $n = 9$, * - values significantly different at $P < 0.05$ from corresponding ones measured on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

The results showed, that the effect of BA on bean plants was dependent on concentration and time: 1 h after application the positive effect on P_N was observed at all concentrations used, but later (24 and 72 h after application) only at lower concentrations. The way of application was 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. Similarly, the effect of plant age was also not decisive: the effects of BA on secondary leaves (the first pair) of 28-d-old bean plants which are morphologically different from primary leaves were not significantly different from the effects of BA on primary leaves (data not shown).

In previous experiments, BA in concentrations was 0, 1, and 10 μM was also added to the substrate or sprayed on leaves of water-stressed bean plants and photosynthetic parameters were measured after complete rehydrations (after 72 h; relative water content higher than 90 %). Application of 1 μM BA slightly improved recovery of plants in terms of increased P_N and g_s (parameters which were markedly decreased by mild water stress). Higher concentration of BA (10 μM BA) had again negative effects on the parameters measured (Rulcová 2000, Rulcová and Pospíšilová 2001). However, immersion of bean roots into 1 or 5 μM BA did not stimulate recovery of primary leaves during the first day of rehydration. The values of P_N, E, and g_s measured 4 or 24 h after application were similar or even slightly lower in plants with roots immersed in BA solution than those in plants with roots immersed in water (unpublished data).

Promotion of stomatal opening in bean leaves induced by 1 μM BA was in accordance with the reviews of Incoll and Jewer (1987) and Incoll *et al.* (1990), and results of Meinzer *et al.* (1991), Morsucci *et al.* (1991), and Pharmawati *et al.* (1998). In addition, spraying of potted

Table 1. Effects of root immersion in H_2O or BA solutions of different concentrations on P_N [μmol m^{-2} s^{-1}], E [mmol m^{-2} s^{-1}] and g_s [mol m^{-2} s^{-1}] of sugar beet leaves. All parameters were measured 1, 4, and 24 h after application. Means ± SE, $n = 10$; [a] - statistically significant ($P < 0.05$) decrease in respective parameter in comparison with corresponding one in leaves treated with H_2O (P_N, E and g_s in intact plants in pots before experiment were 10.61 μmol m^{-2} s^{-1}, 3.01 mmol m^{-2} s^{-1}, and 0.26 mol m^{-2} s^{-1}, respectively).

Time [h]	BA [μM]	P_N	E	g_s
1	0	9.80 ± 0.47	3.20 ± 0.34	0.18 ± 0.03
	10	9.82 ± 0.81	2.60 ± 0.32	0.14 ± 0.03
	20	7.98 ± 0.26[a]	2.39 ± 0.37	0.21 ± 0.04
4	0	10.80 ± 0.40	3.02 ± 0.18	0.24 ± 0.03
	10	10.35 ± 0.59	2.84 ± 0.21	0.17 ± 0.02
	20	7.75 ± 0.16[a]	2.41 ± 0.19[a]	0.24 ± 0.02
24	0	11.17 ± 0.40	2.93 ± 0.15	0.23 ± 0.02
	10	9.25 ± 0.22[a]	1.67 ± 0.22[a]	0.08 ± 0.02[a]
	20	8.68 ± 0.51[a]	3.47 ± 0.33	0.27 ± 0.04

grapevines by BA alleviate negative effect of water stress on stomatal conductance (Stoll *et al.* 2000). Increased g_s already 1 h after BAP application is in agreement with the results of Vogelmann *et al.* (1984) who found that BA was readily taken up by plants.

While in bean plants E was usually not significantly increased after BA application, CKs supplied to leaves *via* xylem increased transpiration rate in wheat and oak (Badenoch-Jones *et al.* 1996). Increased transpiration rate in rehydrated plants treated by CK before water stress was observed by Todorov *et al.* (1998).

As mentioned above, P_N was usually more stimulated by BA than E and therefore WUE was increased. It might be, in agreement with literature, due to the influence of BA on photosynthetic apparatus in addition to its effect on g_s. Slightly stimulated contents of chlorophyll (Chl) *a*, and Chl *b*, and parameters of Chl *a* fluorescence kinetic in primary bean leaves by 1 μM BA were found in previous experiments (Rulcová 2000, Rulcová and Pospíšilová 2001).

Quite different effects of CK applications were observed in sugar beet plants. The effects of 10 and 20 μM BA on P_N, E and g_s of mature leaves of 3-month-old sugar beet plants were determined 1, 4, and 24 h after immersion of roots into BA solutions. Immersion in water served as control. On the contrary to bean plants, no positive effect of BA application on P_N, E, and g_s was observed (Table 1), and 20 μM BA negatively affected P_N. WUE was slightly increased after application of 10 μM BA.

Therefore, in further experiments treatments with lower BA concentrations (1, 5, and 10 μM) were tested. In addition to root immersion in the respective solutions, solutions of BA were used for spraying of leaves. In these experiments the effects of BA application were inconsistent and 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, and on E after 24 h in plants sprayed with 5 μM BA (Table 2). All the P_N, E, and g_s values were lower than those presented in Table 1 because younger seedlings (6- to 8-week-old) were used in these experiments.

Table 2. Effects of BA and HBA in different concentrations on P_N [μmol m^{-2} s^{-1}], E [mmol m^{-2} s^{-1}] and g_s [× 10^{-1} mol m^{-2} s^{-1}] of sugar beet leaves. All parameters were 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 respective parameter in comparison with corresponding one in leaves treated with H_2O; [a] - statistically significant ($P < 0.05$) decrease in respective parameter in comparison with corresponding one in leaves treated with H_2O.

			H_2O	BA			HBA		
			0	1	5	10	1	5	10
P_N	roots	1	3.91 ± 0.36	4.13 ± 0.39	4.93 ± 0.41	3.39 ± 0.21	3.31 ± 0.32	4.54 ± 0.27	4.74 ± 0.47
		24	3.94 ± 0.36	3.62 ± 0.48	5.47 ± 0.28*	5.57 ± 0.23*	4.27 ± 0.42	4.17 ± 0.52	5.53 ± 0.31*
	leaves	1	4.08 ± 0.45	3.77 ± 0.53	4.84 ± 0.30	3.95 ± 0.55	3.87 ± 0.28	5.22 ± 0.58	4.72 ± 0.33
		24	4.84 ± 0.49	4.77 ± 0.67	4.41 ± 0.45	4.05 ± 0.40	4.93 ± 0.50	5.29 ± 0.49	4.99 ± 0.36
E	roots	1	1.16 ± 0.06	0.84 ± 0.08[a]	0.95 ± 0.17	1.57 ± 0.29	0.67 ± 0.05[a]	1.00 ± 0.08	1.12 ± 0.09
		24	1.45 ± 0.13	0.61 ± 0.07[a]	1.17 ± 0.15	1.59 ± 0.20	0.84 ± 0.07[a]	1.25 ± 0.09	1.20 ± 0.10
	leaves	1	1.11 ± 0.18	1.36 ± 0.19	1.27 ± 0.12	0.94 ± 0.11	0.84 ± 0.06	1.11 ± 0.14	1.35 ± 0.08
		24	1.08 ± 0.05	0.93 ± 0.07	1.35 ± 0.12*	1.27 ± 0.12	1.16 ± 0.14	1.29 ± 0.12	1.44 ± 0.10*
g_s	roots	1	0.66 ± 0.08	0.53 ± 0.07	0.75 ± 0.16	0.47 ± 0.07[a]	0.27 ± 0.02[a]	0.61 ± 0.11	0.70 ± 0.08
		24	0.77 ± 0.07	0.46 ± 0.07	0.53 ± 0.07	0.68 ± 0.08	0.37 ± 0.06[a]	0.59 ± 0.08	0.73 ± 0.09
	leaves	1	0.57 ± 0.09	0.73 ± 0.12	0.40 ± 0.06	0.65 ± 0.11	0.48 ± 0.05	0.77 ± 0.10	0.61 ± 0.07
		24	0.67 ± 0.09	0.44 ± 0.05[a]	0.74 ± 0.08	0.75 ± 0.09	0.80 ± 0.10	0.83 ± 0.11	0.77 ± 0.10

In addition to BA, also HBA in the same concentrations and ways of application was used. Neither HBA, which more efficiently delayed leaf senescence than BA in sugar beet plants in field experiments (Čatský *et al.* 1996), significantly stimulated gas exchange of sugar beet leaves. Positive effect on gas exchange parameters was only exceptional: 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 solution (Table 2). WUE was increased in many cases after BA or HBA application, but not in every case.

The results with sugar beet are in agreement with the results of Radin *et al.* (1982), Blackman and Davies (1983), Radin and Hendrix (1988), and Drüge and Schönbeck (1992).

We conclude that 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. Rapid response to CK application may suggest some possibility of involvement of CKs in regulation of gas exchange probably in interaction with ABA. Therefore, further experiments focused on interactions between CKs and ABA are in progress.

References

Badenoch-Jones, J., Parker, C.W., Letham, D.S., Singh, S.: Effect of cytokinins supplied via the xylem at multiplies of endogenous concentrations on transpiration and senescence in derooted seedlings of oat and wheat. - Plant Cell Environ. **19**: 504-516, 1996.

Bano, A., Dörfling, K., Bettin, D., Hahn, H.: Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. - Aust. J. Plant Physiol. **20**: 109-115, 1993.

Blackman, P.G., Davies, W.J.: The effect of cytokinins and ABA on stomatal behaviour of maize and *Commelina*. - J. exp. Bot. **34**: 1619-1626, 1983.

Čatský, J., Pospíšilová, J., Kamínek, M., Gaudinová, A., Pulkrábek, J., Zahradníček, J.: Seasonal changes in sugar beet photosynthesis as affected by exogenous cytokinin N^6-(*m*-hydroxybenzyl)adenosine. - Biol. Plant. **38**: 511-518, 1996.

Chernyad'ev, I.I.: [Plant photosynthesis under conditions of water stress and the protective effect of cytokinins.] - Priklad. Biokhim. Mikrobiol. **33**: 5-17, 1997. [In Russ.]

Chernyad'ev, I.I., Monakhova, O.F.: The activity and content of ribulose-1,5-bisphosphate carboxylase/oxygenase in wheat plants as affected by water stress and kartolin-4. - Photosynthetica **35**: 603-610, 1998.

Cowan, A.K., Cairns, A.L.P., Bartels-Rahm, B.: Regulation of abscisic acid metabolism: towards a metabolic basis for abscisic acid-cytokinin antagonism. - J. exp. Bot. **50**: 595-603, 1999.

Davies, P.J.: The plant hormone concept: concentration, sensitivity and transport. - In: Davies, P.J. (ed.): Plant Hormones. Pp. 13-38. Kluwer Academic Publishers, Dordrecht - Boston - London 1995.

Davies, W.J., Zhang, J.: Root signals and the regulation of growth and development of plants in drying soil. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 55-76, 1991.

Diettrich, B., Mertinat, H., Luckner, M.: Reduction of water loss during *ex vitro* acclimatization of micropropagated *Digitalis lanata* clone plants. - Biochem. Physiol. Pflanz. **188**: 23-31, 1992.

Drüge, U., Schönbeck, F.: Effect of vesicular-arbuscular mycorrhizal infection on transpiration, photosynthesis and growth of flax (*Linum usitatissimum* L.) in relation to cytokinin levels. - J. Plant Physiol. **141**: 40-48, 1992.

Hartung, W., Weiler, E.W., Radin, J.W.: Auxin and cytokinins in the apoplastic solution of dehydrated cotton leaves. - J. Plant Physiol. **140**: 324-327, 1992.

Incoll, L.D., Jewer, P.C.: Cytokinins and stomata. - In: Zeiger, E., Farquhar, G.D., Cowan, I.R. (ed.): Stomatal Function. Pp. 281-292. Stanford University Press, Stanford 1987.

Incoll, L.D., Ray, J.P., Jewer, P.C.: Do cytokinins act as root to shoot signals? - In: Davies, W.J., Jeffcoat, B. (ed.): Importance of Root to Shoot Communication in the Responses to Environmental Stress. Pp. 185-197. British Society for Plant Growth Regulation, Bristol 1990.

Lechowski, Z.: Stomatal response to exogenous cytokinin treatment of the hemiparasite *Melampyrum arvense* L. before and after attachment to the host. - Biol. Plant. **39**: 13-21, 1997.

Masia, A., Pitacco, A., Braggio, L., Giulivo, C.: Hormonal responses to partial drying of the root system of *Helianthus annuus*. - J. exp. Bot. **45**: 69-76, 1994.

Meinzer, F.C., Grantz, D.A., Smit, B.: Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. - Aust. J. Plant Physiol. **18**: 329-338, 1991.

Metwally, A., Tsonev, T., Zeinalov, Y.: Effect of cytokinins on the photosynthetic apparatus in water-stressed and rehydrated bean plants. - Photosynthetica **34**: 563-567, 1997.

Morsucci, R., Curvetto, N., Delmastro, S.: Involvement of cytokinins and adenosine 3',5'-cyclic monophosphate in stomatal movement in *Vicia faba*. - Plant Physiol. Biochem. **29**: 537-547, 1991.

Naqvi, S.S.M.: Plant/crop hormones under stressful conditions. - In: Pessarakli, M. (ed.): Handbook of Plant and Crop Physiology. Pp. 645-660. Marcel Dekker, New York - Basel - Hong Kong 1995.

Naqvi, S.S.M.: Plant hormones and stress phenomena. - In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. Pp. 709-730. Marcel Dekker, New York - Basel 1999.

Pharmawati, M., Billington, T., Gehring, C.A.: Stomatal guard cell responses to kinetin and natriuretic peptides are cGMP-dependent. - Cell. mol. Life Sci. **54**: 272-276, 1998.

Pospíšilová, J., Čatský, J., Synková, H., Macháčková, I., Solárová, J.: Gas exchange and *in vivo* chlorophyll fluorescence in potato and tobacco plantlets *in vitro* as affected by various concentrations of 6-benzylaminopurine. - Photosynthetica **29**: 1-12, 1993.

Pospíšilová, J., Synková, H., Rulcová, J.: Cytokinins and water stress. - Biol. Plant. **43**: 321-328, 2000.

Radin, J.W., Hendrix, D.L.: The apoplastic pool of abscisic acid in cotton leaves in relation to stomatal closure. - Planta **174**: 180-186, 1988.

Radin, J.W., Parker, L.L., Guinn, G.: Water relation of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. - Plant Physiol. **70**. 1066-1070, 1982.

Rulcová, J.: [Effect of Cytokinins on Plant Recovery after Water Stress.] - Thesis, Faculty of Natural Sciences, Charles University, Prague 2000. [In Czech.]

Rulcová, J., Pospíšilová, J.: Effect of benzylaminopurine on rehydration of bean plants after water stress. - Biol. Plant. **44**: 75-81, 2001.

Schulze, E.-D.: Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. - Annu. Rev. Plant Physiol. **37**: 247-274, 1986.

Shashidhar, V.R., Prasad, T.G., Sudharshan, L.: Hormone signals from roots to shoots of sunflower (*Helianthus annuus* L.). Moderate soil drying increases delivery of

abscisic acid and depresses delivery of cytokinins in xylem sap. - Ann. Bot. **78**: 151-155, 1996.

Singh, D.V., Srivastava, G.C., Abdin, M.Z.: Amelioration of negative effect of water stress in *Cassia angustifolia* by benzyladenine and/or ascorbic acid. - Biol. Plant. **44**: 141-143, 2001.

Stoll, M., Loveys, B., Dry, P.: Hormonal changes induced by partial rootzone drying of irrigated grapevine. - J. exp. Bot. **51**: 1627-1634, 2000.

Stoyanova, D., Yordanov, I.: Influence of drought, high temperature, and carbamide cytokinin 4-PU-30 on photosynthetic activity of plants. 2. Chloroplast ultrastructure of primary bean leaves. - Photosynthetica **37**: 621-625, 1999.

Synková, H., Wilhelmová, N., Šesták, Z., Pospíšilová, J.: Photosynthesis in transgenic plants with elevated cytokinin contents. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 541-552. Marcel Dekker, New York - Basel - Hong Kong 1997.

Tardieu, F., Davies, W.J.: Root-shoot communication and whole-plant regulation of water flux. - In: Smith, J.A.C., Griffiths, H.: Water Deficits. Plant Responses from Cell to Community. Pp. 147-162. Bios Scientific Publishers, Oxford 1993.

Todorov, D., Alexieva, V., Karanov, E.: Effect of putrescine, 4-PU-30, and abscisic acid on maize plants grown under normal, drought, and rewatering conditions. - J. Plant Growth Regul. **17**: 197-203, 1998.

Vogelmann, T.C., Bornman, C.H., Nissen, P.: Uptake of benzyladenine in explants of *Picea abies* and *Pinus sylvestris*. - Physiol. Plant. **61**: 513-517, 1984.