PHOTOSYNTHETICA 41 (1): 49-56, 2003

Interaction of cytokinins and abscisic acid during regulation of stomatal opening in bean leaves

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Abstract

Effects of benzyladenine (BA) and abscisic acid (ABA) applied separately or simultaneously on parameters of gas exchange of *Phaseolus vulgaris* L. leaves were studied. In the first two experimental sets, 100 µM ABA and 10 µM BA were applied to plants sufficiently supplied with water. Spraying of leaves with ABA decreased stomatal conductance (g_s) and in consequence transpiration rate (E) and net photosynthetic rate (P_N) already 1 h after application, but 24 h after application the effect almost disappeared. 10 µM BA slightly decreased gas exchange parameters, but in simultaneous application with ABA reversed the effect of ABA. Immersion of roots into the same solutions markedly decreased gas exchange parameters and 24 h after ABA application the stomata were completely closed. The effect of ABA was ameliorated by simultaneous BA application, particularly after 1-h treatment. In the third experimental set, plants were pre-treated by immersing roots into water, 1 µM BA, or 100 µM ABA for 24 h and then the halves of split root system were dipped into different combinations of 1 µM BA, 100 µM ABA, and water. In plants pre-treated with ABA all gas exchange parameters were small and they did not differ in plants treated with H_2O+H_2O, H_2O+BA, or BA+BA. In plants pre-treated with BA or H_2O, markedly lower values of P_N were found when both halves of roots were immersed in ABA. Further, the effects of pre-treatment of plants with water, 1 µM BA, 100 µM ABA, or ABA+BA on the development of water stress induced by cessation of watering and on the recovery after rehydration were followed. ABA markedly decreased gas exchange parameters at the beginning of the experiment, but in its later phase the effect was compensated by delay in development of water stress. BA also delayed development of water stress and increased P_N in water-stressed leaves. BA reversed the effect of ABA at mild water stress. Positive effects of BA and ABA pre-treatments were observed also after rehydration.

Additional key words: benzyladenine; net photosynthetic rate; *Phaseolus vulgaris*; stomatal conductance; transpiration rate; water stress.

Introduction

Regulation of stomatal conductance (g_s) is the main mechanism by which plants control gas exchange and leaf temperature. During water stress stomata respond to hydraulic and chemical signals (*e.g.* Tardieu and Davies 1993). Abscisic acid (ABA) synthesised in the roots under water stress and transported to the leaves may act as a root-to-shoot chemical signal of water stress conditions and, together with ABA synthesised in the leaves themselves and released from physiologically inactive ABA-glucose conjugates, induce stomatal closure (for recent reviews see, *e.g.*, Assmann and Armstrong 1999, Blatt 2000, Rock 2000, Ng *et al.* 2001, Schroeder *et al.* 2001, Wilkinson and Davies 2002). ABA compartmentation in different tissues is largely caused by changes in pH (*e.g.* Sauter *et al.* 2001). Decisive for stomatal closure is ABA content in the vicinity of guard cells which depends not only on ABA supply, but also on changes in water content (Zhang *et al.* 2001, Zhang and Outlaw 2001a,b).

However, it is not reasonable to deny participation of other phytohormones in chemical signalling of water stress conditions (for recent reviews, see Naqvi 1999, Pospíšilová *et al.* 2000, Pospíšilová 2003). In contrast to

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Received 9 December 2002, *accepted* 26 February 2003.

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Abbreviations: ABA – abscisic acid; BA – N^6-benzyladenine; CK – cytokinin; E – transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate; RWC – relative water content.

Acknowledgements: The author acknowledges critical reading of manuscript by Dr. J. Čatský and Dr. Z. Šesták, skilful technical assistance of Mrs. L. Hávová, and the financial support of the Grant Agency of the Czech Republic (grant No. 522/02/1099).

ABA, our knowledge of the stress-induced changes in contents of other phytohormones and their possible direct effects on stomata and interactions with ABA is only fragmentary. Cytokinins (CKs) are often considered ABA antagonists in many processes including the regulation of stomatal opening (*e.g.* Thimann 1992).

In water stressed plants, a decreased content of CKs was found in rice (Bano *et al.* 1993), alfalfa (Goicoechea *et al.* 1995, 1997), wheat (Teplova *et al.* 1999), grapevine (Stoll *et al.* 2000), and severely water-stressed sunflower (Shashidhar *et al.* 1996). CK content was not significantly changed in apoplastic solution of water-stressed cotton and sunflower (Hartung *et al.* 1992, Masia *et al.* 1994), and in the xylem sap of *Prunus dulcis* (Fusseder *et al.* 1992).

CKs may induce stomatal opening but the effects are species specific and depend on CK type, concentration, and method of application. The increase in transpiration rate (*E*) or stomatal opening by CKs was mentioned in leaves of *Anthephora*, *Avena*, *Brassica*, *Commelina*, *Helianthus*, *Hordeum*, *Kalanchoë*, *Melampyrum*, *Saccharum*, *Tradescantia*, *Triticum*, and *Vigna* (Incoll and Jewer 1987, Santakumari and Fletcher 1987, Meinzer *et al.* 1991, Badenoch-Jones *et al.* 1996, Lechowski 1997, Pharmawati *et al.* 1998, Gupta *et al.* 1999) but the consistent stimulation of g_s or *E* was not observed in *Beta*, *Commelina*, *Gossypium*, *Linum*, *Pisum*, *Tridax*, *Triticum*, *Vicia*, and *Zea* (Radin *et al.* 1982, Incoll and Jewer 1987, Radin and Hendrix 1988, Drüge and Schönbeck 1992, Badenoch-Jones *et al.* 1996, Čatský *et al.* 1996, Pospíšilová *et al.* 2001, Vomáčka and Pospíšilová 2003). In *Digitalis*, *Nicotiana*, *Phaseolus*, and *Vicia* low CK concentrations slightly stimulated g_s and *E* but higher concentration mostly inhibited them (Morsucci *et al.* 1991, 1992, Diettrich *et al.* 1992, Pospíšilová *et al.* 1993, 2001, Rulcová and Pospíšilová 2001). In epidermal strips or leaf fragments of *Commelina*, CKs decreased stomatal opening (Blackman and Davies 1983). CKs can also delay stomatal closure induced by ABA, which was observed in *Gossypium*, *Linum*, and *Zea* (Radin *et al.* 1982, Blackman and Davies 1984, Radin and Hendrix 1988, Drüge and Schönbeck 1992).

ABA can regulate stomatal opening by changing various parameters: (*1*) Osmotic potential of guard cells which involves both an inhibition of the channels allowing K^+ entry and an activation of the channels determining release of K^+ and those determining release of anions. Ca^{2+} is a second messenger in some, but not all, of the ABA-induced changes in guard cell ion channels. ABA-induced inactivation of the plasmalemma inward K^+ channels is usually Ca^{2+} mediated, whereas ABA-induced activation of the plasmalemma outward K^+ channels is Ca^{2+} independent (*e.g.* MacRobbie 1997, Allen *et al.* 1998, Leung and Giraudat 1998, Assmann and Shimazaki 1999, Lemtiri-Chlieh *et al.* 2000). In the latter case, pH changes seem to be important (Allen *et al.* 1998, Leung and Giraudat 1998, Blatt 2000). (*2*) Mechanical properties of guard cells may be induced by reversible reorganisation of actin filaments and cortical microtubules (Jiang *et al.* 1996, Eun and Lee 1997). (*3*) Gene expression may lead to changes in content of water-transport proteins, ion-transport proteins, or proteins involved in carbon metabolism (Webb *et al.* 2001).

The mechanism of CK action on guard cell might involve direct induction of membrane hyperpolarization by stimulation of electrogenic H^+-pump, stimulation of adenylate or guanylate cyclase activity, or interaction with calcium-calmodulin system (Incoll *et al.* 1990, Morsucci *et al.* 1991, Pharmawati *et al.* 1998). The internal calcium concentration might be a candidate for mediating interactions between CKs and ABA (Hare *et al.* 1997). The antagonism between CKs and ABA may also be the result of metabolic interactions: CKs share, at least in part, a common biosynthetic origin with ABA (Cowan *et al.* 1999).

The above survey of literature shows that it is not possible yet to confirm or deny the hypothesis that antagonistic effects of ABA and CKs can participate in precise regulation of stomata opening. Therefore, the central question to be examined in this paper is the relative effect of ABA and CKs alone and in different combinations on g_s, and rates of transpiration and photosynthesis of plants sufficiently supplied with water or during development of water stress and subsequent rehydration.

Materials and methods

Plants and cultivation: Seedlings of French bean (*Phaseolus vulgaris* L. cv. Jantar) were grown in pots with coarse sand and nutrient solution at a 16-h photoperiod, irradiance of 150 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).

Treatments: To distinguish between direct effects and interactions, benzyladenine (BA) and ABA were applied separately or simultaneously. In the first two experimental sets, 100 μM ABA and 10 μM BA were applied to plants sufficiently supplied with water. Different types of application were used: in the first experimental set H_2O, BA, ABA, or BA+ABA were sprayed on leaves, while in the second experimental set roots were immersed into solutions of the same concentrations. Parameters of gas exchange were measured 1 and 24 h after application.

In further experimental sets 1 μM BA was used because 10 μM concentration of BA seemed to be unnecessarily high for application to roots. In the third experimental set, plants were pre-treated by immersing roots into water, 1 μM BA, or 100 μM ABA for 24 h. Then the root system was split and halves of roots were immediately dipped in small vessels and treated by different combinations of 1 μM BA, 100 μM ABA, and H_2O (Table 1). Then both vessels together with a part of root system overlapping the surface of solutions used were wrapped in aluminium foil to be in darkness and to avoid desiccation. Parameters of gas exchange were measured 2 h after splitting the roots.

Further, the effects of pre-treatment of plants with water, 1 μM BA, 100 μM ABA, or combination of 100 μM ABA + 1 μM BA on the development of water stress induced by cessation of watering and on the recovery after rehydration was followed. Relative water content (RWC) and gas exchange parameters were measured immediately after application (0 d), during mild water stress (3 and 4 d), during severe water stress (7 and 8 d), and after rehydration (2 d).

Methods determining water relations and photosynthetic parameters: Net photosynthetic rate (P_N), transpiration rate (E), and g_s were measured on attached leaves using a gas exchange system (*LCA-4*, *ADC Bio Scientific*, Hoddesdon, UK) with leaf chamber (*LC4/PLC4BT-1/E*) at temperature of 25 °C, irradiance (400-700 nm) of 750 μmol m^{-2} s^{-1} provided by four *Argaphoto-BM* (*Philips*, Eindhoven, The Netherlands) lamps cooled by a water filter, relative humidity of 50 %, and CO_2 concentration of 350 μmol mol^{-1}. Primary or secondary leaves were used in the stage of maximum or near maximum P_N. RWC was measured gravimetrically in leaf discs (0.5 cm^2) water-saturated by immersing into holes of fully moistened polyurethane foam under dark according to Čatský (1960).

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

Results

Effects of ABA, BA, and ABA+BA on plants sufficiently supplied with water: Gas exchange parameters of leaves of plants sufficiently supplied with water were differently affected by growth regulators according to the method of application. Spraying of leaves with water slightly increased gas exchange parameters. 100 μM ABA markedly decreased g_s and in consequence E and P_N 1 h after application, but 24 h after application the effect almost disappeared. 10 μM BA slightly decreased gas exchange parameters, but in simultaneous application with ABA it reversed the effect of ABA (Fig. 1).

Dipping of roots into the same solutions markedly

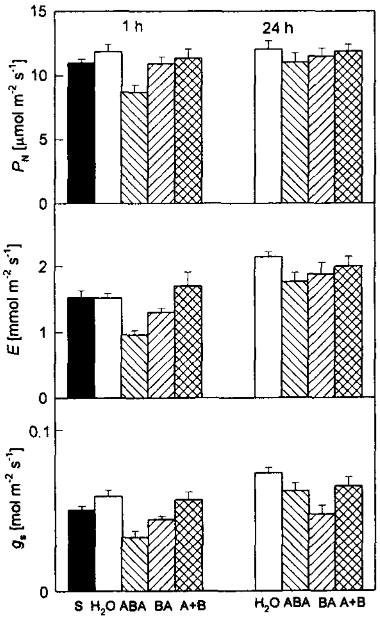

Fig. 1. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) in primary bean leaves 1 and 24 h after spraying with H_2O (control), 100 μM ABA, 10 μM BA, or combination of 100 μM ABA + 10 μM BA (A+B). Means ± SE, $n = 18$ (S – initial values before treatments).

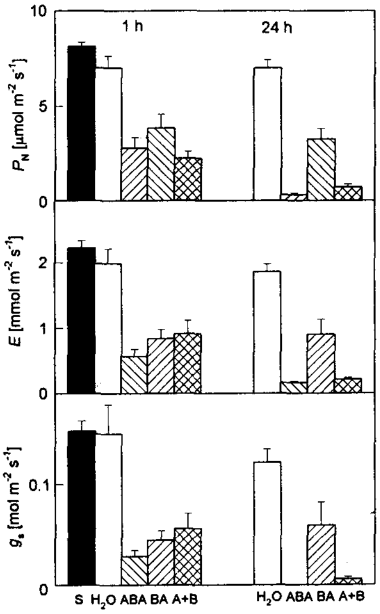

Fig. 2. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) in primary bean leaves 1 and 24 h after immersion of roots into H_2O (control), 100 μM ABA, 10 μM BA, or combination of 100 μM ABA + 10 μM BA (A+B). Means ± SE, $n = 12$ (S – initial values of plants in substrate).

decreased gas exchange. In this application the concentrations of ABA and BA seemed to be unnecessarily high. 24 h after ABA application the stomata were completely closed. The effect of ABA was ameliorated by simultaneous BA application, particularly after 1 h of treatment (Fig. 2).

Effects of different combinations of ABA, BA, and H_2O applied to halves of split root system: When plants were pre-treated by immersion of roots for 24 h in water and then the root system was split and both halves dipped in water, values of gas exchange parameters were nearly the same as before pre-treatment (Table 1). In all other combinations, P_N, E, and g_s were slightly decreased. Markedly lower values of P_N, E, and g_s were found when both halves of the roots were dipped in ABA (combination ABA+ABA), and values of E and g_s were also low when dipped into combination BA+BA. Values of gas exchange parameter of plants pre-treated with BA were mostly slightly lower than those of plants pre-treated with water. Markedly decreased P_N was observed in combinations ABA+ABA and ABA+BA. However, E and g_s were similar in all combinations used. In plants pre-treated with ABA, P_N, E, and g_s were low and they did not differ in plants treated with H_2O+H_2O, H_2O+BA, or BA+BA (Table 1).

Effect of pre-treatment of plants with ABA, BA, or ABA+BA on development of water stress and subsequent recovery: Pre-treatment of plants with 1 μM BA, 100 μM ABA, or combination of both affected the development of water stress and subsequent rehydration. RWC in control plants markedly decreased from day 7 after cessation of watering, when also visible wilting occurred. On day 2 after rehydration RWC did not reach the initial value. Application of ABA and BA delayed development of water stress and in consequence recovery after rehydration was improved. Simultaneous application (ABA+BA) was less effective in amelioration of water stress but also improved rehydration (Fig. 3).

Stomatal conductance markedly decreased during water stress in all plants and after eight days stomata were completely closed. The recovery was incomplete, which indicated occurrence of relatively severe water stress. ABA decreased g_s immediately after application, but changes from day 3 to day 7 were small. The decreased g_s was the reason for delay in water stress development. Positive effects of BA were found on day 4 and after rehydration. BA reversed the effect of ABA on day 3 (Fig. 3).

Transpiration rate decreased during water stress and slightly increased after rehydration. On day 8 transpiration was cuticular because the stomata were closed. ABA markedly decreased E on days 3 and 4. Further, the effect was compensated by a delay in development of water stress. Positive effects of BA were observed on days 3, 4, and 7 and after rehydration. BA reversed the effect of ABA on day 3 (Fig. 3).

P_N was less affected by water stress-induced decrease in g_s than E. Water stress was not so severe to induce changes in biochemistry of photosynthesis. This was shown by parallel measurements of chlorophyll fluorescence (results not shown). ABA decreased P_N on days 3 and 4. However, because of the delay in the development of water stress, P_N on day 8 and after rehydration was higher in ABA-pre-treated plants than in control plants. Positive effects of BA were observed on days 4, 7, and 8 and after rehydration. BA reversed the effect of ABA on day 3 (Fig. 3).

As a result of pre-treatment-induced changes in development of water stress, the relationships between RWC and g_s, E, or P_N were not markedly affected by pre-treatment of plants (values not shown).

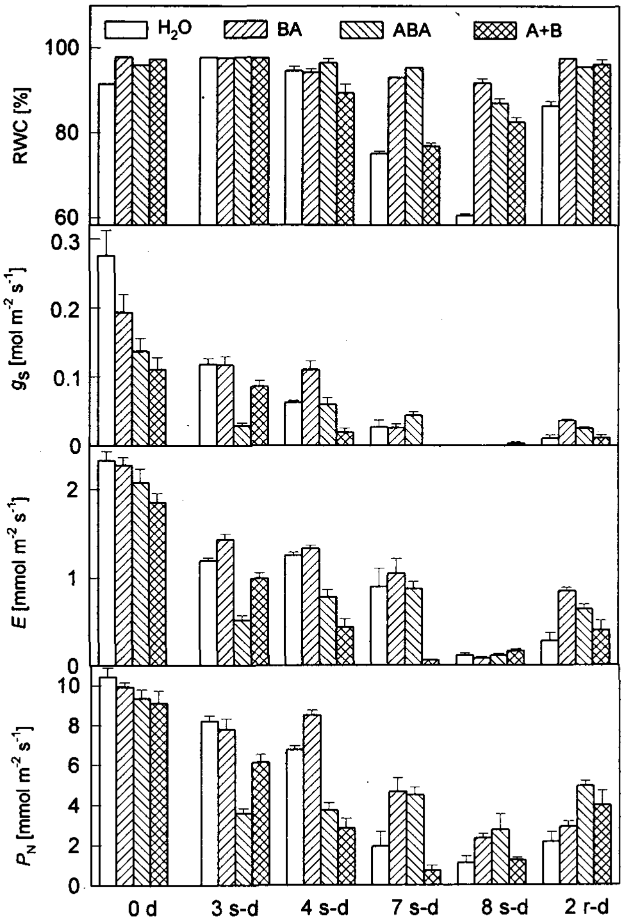

Fig. 3. Effect of pre-treatment with 1 μM BA, 100 μM ABA, or combination of 100 μM ABA + 1 μM BA (A+B) added to the substrate on development of water stress in primary bean leaves (3-8 d after cessation of watering) and subsequent rehydration (2 d) characterised by changes in relative water content (RWC), stomatal conductance (g_s), transpiration rate (E), and net photosynthetic rate (P_N). Means ± SE, n = 12. s-d = days during stress; r-d = days during rehydration.

Table 1. Bean plants were pre-treated by immersion of roots either in water or 1 μM BA or 100 μM ABA. After 24 h the root system was split into two halves which were immersed in different combinations of H_2O, BA, and ABA and gas exchange parameters were measured after 2 h. Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) of plants before pre-treatment were 8.40 μmol m^{-2} s^{-1}, 1.29 mmol m^{-2} s^{-1}, and 0.09 mol m^{-2} s^{-1}, respectively (in plants pre-treated with ABA only combinations which could re-open the stomata were used). Means ± SE, $n = 18$, * differences significant at $p < 0.05$ according to t-test.

Pretreatment – 24 h	Treatment – 2 h	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]
H_2O	H_2O+H_2O	8.36 ± 0.34	1.31 ± 0.08	0.101 ± 0.013
	H_2O+BA	6.20 ± 0.43*	1.19 ± 0.14	0.092 ± 0.019
	H_2O+ABA	7.45 ± 0.42	1.12 ± 0.12	0.063 ± 0.011*
	BA+BA	7.07 ± 0.40*	0.92 ± 0.12*	0.043 ± 0.006*
	BA+ABA	6.97 ± 0.36*	1.09 ± 0.10	0.062 ± 0.010*
	ABA+ABA	4.52 ± 0.47*	0.87 ± 0.12*	0.042 ± 0.007*
BA	H_2O+H_2O	6.99 ± 0.54*	0.93 ± 0.11*	0.043 ± 0.005*
	H_2O+BA	5.90 ± 0.40*	0.74 ± 0.11*	0.038 ± 0.006*
	H_2O+ABA	5.34 ± 0.16*	1.12 ± 0.07	0.051 ± 0.005*
	BA+BA	6.00 ± 0.14*	1.11 ± 0.12	0.054 ± 0.010*
	BA+ABA	4.82 ± 0.42*	1.07 ± 0.20	0.052 ± 0.011*
	ABA+ABA	4.54 ± 0.42*	0.88 ± 0.15*	0.044 ± 0.010*
ABA	H_2O+H_2O	3.49 ± 0.55*	0.61 ± 0.14*	0.028 ± 0.008*
	H_2O+BA	3.65 ± 0.52*	0.55 ± 0.13*	0.024 ± 0.007*
	BA+BA	3.46 ± 0.54*	0.63 ± 0.10*	0.027 ± 0.006*

Discussion

Effects of ABA on gas exchange parameters: In agreement with the literature, 100 μM ABA in leaves sufficiently supplied with water markedly decreased g_s and in consequence E and P_N. However, the effect on plants by spraying on leaves was only short-term and less efficient than dipping of roots into the solutions of the same concentrations. This was probably because of restricted ABA uptake in the former case as a result of low permeability of the cuticle and is probably one of the reasons why application of ABA into the transpiration stream has usually been used for simulation of effects of water stress (*e.g.* Correia and Pereira 1995, Heckenberger *et al.* 1996). However, spraying of ABA on sorghum leaves effectively reduced E (Xu *et al.* 1994).

In agreement with the above-mentioned experiments, pre-treatment of plants by immersing roots into 100 μM ABA for 24 h significantly decreased gas exchange parameters of plants with split-root systems and during the subsequent two hours stomata in plants with roots treated with water, BA, or combination of both did not fully reopen.

Leaf contents of endogenous ABA increased during development of water stress induced by cessation of watering and pre-treatment of plants with 100 μM ABA further increased endogenous ABA content (Pospíšilová and Vágner 2002). This resulted in immediate decrease in g_s and E and thus in delay of development of water stress (Fig. 3). In addition, increased content of endogenous ABA may increase root hydraulic conductivity (*e.g.* Zhang *et al.* 1995, Hose *et al.* 2000, 2002) which may contribute to delay in water stress development. Retardation of development of water stress under drought by ABA application has also been observed, *e.g.* in *Hordeum*, *Pinus*, *Solanum*, and *Triticum* (Mizrahi *et al.* 1974, Sukumaran *et al.* 1975, Marshall *et al.* 1991, Nayyar and Kaushal 2002). Consequently, g_s in ABA-treated plants was from day 4 to day 8 similar to that in the control plants and P_N on day 8 was even higher in ABA-treated plants than in the controls (Fig. 3). Similarly in wheat plants, ABA application during development of water stress induced by polyethylene glycol 6 000 increased g_s and P_N on days 4 and 6 after application (Nayyar and Kaushal 2002). Amelioration of water stress improved rehydration, and g_s, E, and P_N of ABA-treated plants were two days after rehydration higher than those of the control plants.

Effect of BA on gas exchange parameters: Spraying of leaves of plants sufficiently supplied with water with 10 μM BA did not markedly change gas exchange parameters, but dipping of roots into 10 μM BA decreased them. Thus dipping of roots in BA solution was more effective than spraying of leaves; this difference was similar to that found with ABA. The 10 μM concentration of BA was most likely unnecessarily high for application to roots and later a lower concentration (1 μM) was used. The results are in agreement with previous experiments with water-stressed and re-hydrated bean plants (Rulcová and Pospíšilová 2001) where 1 μM BA applied to substrate stimulated stomatal opening 72 h after application but 10 μM BA inhibited it. However, in previous experiments with bean plants sufficiently supplied with water (Pospíšilová *et al.* 2001), 10 μM BA slightly stimulated gas exchange parameters 1 h after application and decreased them only 24 h after application. Thus the effect of BA on gas exchange parameters of bean plants depends on application mode, time of observation of effects, plant age, and probably other factors, as was observed, *e.g.*, in *Beta*, *Commelina*, *Gossypium*, *Linum*, *Pisum*, *Tridax*, *Triticum*, *Vicia*, and *Zea* (Radin *et al.* 1982, Incoll and Jewer 1987, Radin and Hendrix 1988, Drüge and Schönbeck 1992, Badenoch-Jones *et al.* 1996, Čatský *et al.* 1996, Pospíšilová *et al.* 2001, Vomáčka and Pospíšilová 2003).

Interactions of BA and ABA during simultaneous application of BA+ABA or during water stress: CKs are often considered ABA antagonists. In bean plants sufficiently supplied with water, 10 μM BA effectively reversed the closing effect of ABA in both (leaf or root) ways of application. In maize, CKs reversed ABA mediated stomatal closure in both young and old leaves (Blackman and Davies 1984). In flax, zeatin decreased stomatal response to ABA (Drüge and Schönbeck 1992). In cotton, kinetin had little effect on stomatal response to ABA in control plants, but it decreased stomatal response to ABA in plants grown under nitrogen deficiency (Radin *et al.* 1982, Radin and Hendrix 1988).

In plants pre-treated with water or BA, P_N, E, and g_s were lower when the both halves of root system were treated with ABA than if combinations H_2O+ABA or BA+ABA were used. However, the explanation may be an interaction between BA and ABA, as well as the lower ABA concentration in the vicinity of guard cells (dilution of ABA solution delivered from the half of the roots immersed in ABA by water or BA solution delivered from the second half of the roots). The reduced values of gas exchange parameters after pre-treatment of roots in ABA solution for 24 h were not reversed by the subsequent immersion of one or both halves of the split root system in BA solution. Thus the comparison of the three experimental sets suggests that BA simultaneously applied with ABA may inhibit the closing effect of ABA, but BA applied after ABA is not able to induce re-opening of stomata previously closed by ABA.

Pre-treatment of plants with 1 μM BA also delayed development of water stress induced by cessation of watering. In contrast to ABA, the mechanism is not clear. One possibility is stimulation of osmotic adjustment that was observed in *Cicer* after BA application (Yadav *et al.* 1997) or in *Vigna* after kinetin application (Agarwal and Gupta 1995). Also in tobacco and *Mesembryanthemum* the addition of CKs induced accumulation of proline and osmotin (Thomas *et al.* 1992). During development of water stress, a positive effect of BA pre-treatment on g_s was found only on day 4 but on day 3 BA partially inhibited the closing effect of ABA. More evident was the effect of BA on E and P_N. Positive effects of BA on E were found on days 3, 4, and 7 and on P_N on days 4, 7, and 8. The results agree with observations showing amelioration of water stress-induced inhibition of photosynthesis by application of CKs (Metwally *et al.* 1997, Chernyad'ev and Monakhova 1998, Pandey *et al.* 2000, Singh *et al.* 2001). The explanation may be that endogenous ABA content increased during development of water stress much less in BA pre-treated bean plants than in the control plants (Pospíšilová and Vágner 2002). Pretreatment with BA (as with ABA) improved rehydration of plants and thus two days after re-watering g_s, E, and P_N of treated plants were larger than in control plants.

Hence application of CKs may slightly increase g_s, E, and P_N, especially in unstressed plants treated with ABA or during water stress and subsequent rehydration, when increased content of endogenous ABA is probable. Therefore, some interactions between ABA and CKs seem likely.

References

Agarwal, R.K., Gupta, S.C.: Plant growth substances as osmoregulants under salt stress in callus cultures of cowpea. – Indian J. Plant Physiol. **38**: 325-327, 1995.

Allen, G.J., Amtmann, A., Sanders, D.: Calcium-dependent and calcium-independent K^+ mobilization channels in *Vicia faba* guard cell vacuoles. – J. exp. Bot. **49** (Spec. Issue): 305-318, 1998.

Assmann, S.M., Armstrong, F.: Hormonal regulation of ion transporters: the guard cell system. – In: Hooykaas, P.J.J., Hall, M.A., Libbenga, K.R. (ed.): Biochemistry and Molecular Biology of Plant Hormones. Pp. 337-361. Elsevier, Amsterdam 1999.

Assmann, S.M., Shimazaki, K.-I.: The multisensory guard cell. Stomatal responses to blue light and abscisic acid. – Plant Physiol. **119**: 337-361, 1999.

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.

Blackman, P.G., Davies, W.J.: Age-related changes in stomatal response to cytokinins and abscisic acid. – Ann. Bot. **54**: 121-125, 1984.

Blatt, M.: Cellular signaling and volume control in stomatal movements in plants. – Annu. Rev. cell. dev. Biol. **16**: 221-241, 2000.

Čatský, J.: Determination of water deficit in disks cut out from leaf blades. – Biol. Plant. **2**: 76-78, 1960.

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., 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.

Correia, M.J., Pereira, J.S.: The control of leaf conductance of white lupin by xylem ABA concentration decreases with the severity of water deficits. – J. exp. Bot. **46**: 101-110, 1995.

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.

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.

Eun, S.O., Lee, Y.: Actin filaments of guard cells are reorganized in response to light and abscisic acid. – Plant Physiol. **115**: 1491-1498, 1997.

Fusseder, A., Wartinger, A., Hartung, W., Schulze, E.-D., Heilmeier, H.: Cytokinins in the xylem sap of desert grown almond (*Prunus dulcis*) trees: Daily courses and their possible interactions with abscisic acid and leaf conductance. – New Phytol. **122**: 45-52, 1992.

Goicoechea, N., Antolín, M.C., Sánchez-Díaz, M.: Gas exchange is related to the hormonal balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. – Physiol. Plant. **100**: 989-997, 1997.

Goicoechea, N., Doležal, K., Antolín, M.C., Strnad, M., Sánchez-Díaz, M.: Influence of mycorrhizae and *Rhizobium* on cytokinin content in drought-stressed alfalfa. – J. exp. Bot. **46**: 1543-1549, 1995.

Gupta, S., Gupta, N.K., Kumar, A.: Effect of abscisic acid (ABA) and kinetin (Kn) on water loss from cowpea (*Vigna unguiculata* L.) seedlings. – Ann. Biol. **15**: 77-79, 1999.

Hare, P.D., Cress, W.A., Van Staden, J.: The involvement of cytokinins in plant responses to environmental stress. – Plant Growth Regul. **23**: 79-103, 1997.

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.

Heckenberger, U., Schurr, U., Schulze, E.-D.: Stomatal response to abscisic acid fed into the xylem of intact *Helianthus annuus* (L.) plants. – J. exp. Bot. **47**: 1405-1412, 1996.

Hose, E., Sauter, A., Hartung, W.: Abscisic acid in roots – biochemistry and physiology. – In: Waisel, Y., Eshel, A., Kafkafi, U. (ed.): Plant Roots. The Hidden Half. 3rd Ed. Pp. 435-448. Marcel Dekker, New York – Basel 2002.

Hose, E., Steudle, E., Hartung, W.: Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes. – Planta **211**: 874-882, 2000.

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.

Jiang, C.-J., Nakajima, N., Kondo, N.: Disruption of microtubules by abscisic acid in guard cells of *Vicia faba* L. – Plant Cell Physiol. **37**: 697-701, 1996.

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.

Lemtiri-Chlieh, F., MacRobbie, E.A.C., Brearley, C.A.: Inositol hexabisphosphate is a physiological signal regulating the K^+-inward rectifying conductance in guard cells. – Proc. nat. Acad. Sci. USA **97**: 8687-8692, 2000.

Leung, J., Giraudat, J.: Abscisic acid signal transduction. – Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 199-222, 1998.

MacRobbie, E.A.C.: Signalling in guard cells and regulation of ion channel activity. – J. exp. Bot. **48**: 515-528, 1997.

Marschall, J.G., Scarratt, J.B., Dumbroff, E.B.: Induction of drought resistance by abscisic acid and paclobutrazol in jack pine. – Tree Physiol. **8**: 415-421, 1991.

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.

Mizrahi, Y., Scherings, S.G., Malis Arad, S., Richmond, A.E.: Aspects of the effect of ABA on the water status of barley and wheat seedlings. – Physiol. Plant. **31**: 44-50, 1974.

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.

Morsucci, R., Curvetto, N., Delmastro, S.: High concentration of adenosine or kinetin riboside induces stomatal closure in *Vicia faba*, probably through inhibition of adenylate cyclase. – Plant Physiol. Biochem. **30**: 383-388, 1992.

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.

Nayyar, H., Kaushal, S.K.: Alleviation of negative effects of water stress in two contrasting wheat genotypes by calcium and abscisic acid. – Biol. Plant. **45**: 65-75, 2002.

Ng, C.K.-Y., McAinsh, M.R., Gray, J.E., Hunt, L., Leckie, C.P., Mills, L., Hetherington, A.M.: Calcium-based signalling systems in guard cells. – New Phytol. **151**: 109-120, 2001.

Pandey, D.M., Goswami, C.L., Kumar, B., Jain, S.: Hormonal regulation of photosynthetic enzymes in cotton under water stress. – Photosynthetica **38**: 403-407, 2000.

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.: Participation of phytohormones in the stomatal regulation of gas exchange during water stress. – Biol. Plant. **46**: 491-506, 2003.

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., Rulcová, J., Vomáčka, L.: Effect of benzyladenine and hydroxybenzyladenosine on gas exchange of bean and sugar beet leaves. – Biol. Plant. **44**: 523-528, 2001.

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

Pospíšilová, J., Vágner, M.: [Effect of abscisic acid and benzyladenine on photosynthetic and transpiration rates and stomatal conductance during water stress.] – In: Proceedings of Conference "Effects of Abiotic and Biotic Stresses on Properties of Plants". Czech Agriculture University, Prague 2002. [In Czech.]

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.

Rock, C.D.: Pathway to abscisic acid-regulated gene expression. – New Phytol. **148**: 357-396, 2000.

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

Santakumari, M., Fletcher, R.A.: Reversal of triazole-induced stomatal closure by gibberellic acid and cytokinins in *Commelina benghalensis*. – Physiol. Plant. **71**: 95-99, 1987.

Sauter, A., Davies, W.J., Hartung, W.: The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot. – J. exp. Bot. **52**: 1991-1997, 2001.

Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M., Waner, D.: Guard cell signal transduction. – Annu. Rev. Plant Physiol. Plant mol. Biol. **52**: 627-658, 2001.

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.

Sukumaran, N.P., Sharda, R.T., Saha, S.N.: Effect of abscisic acid treatment on water deficits in potato. – Potato Res. **18**: 433-437, 1975.

Tardieu, F., Davies, W.J.: Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. – Plant Cell Environ. **16**: 341-349, 1993.

Teplova, I., Farkhutdinov, R., Mitrichenko, A., Kudoyarova, G.: Changes in zeatin and ABA content and water relations in wheat seedlings as influenced by elevated temperature. – Biol. Plant. **42** (Suppl.): S78, 1999.

Thimann, K.V.: Antagonisms and similarities between cytokinins, abscisic acid and auxin (mini review). – In: Kamínek, M., Mok, D.W.S., Zažímalová, E. (ed.): Physiology and Biochemistry of Cytokinins in Plants. Pp. 395-400. SPB Academic Publishing, The Hague 1992.

Thomas, J.C., McElwain, E.F., Bohnert, H.J.: Convergent induction of osmotic stress-response. Abscisic acid, cytokinin, and the effects of NaCl. – Plant Physiol. **100**: 416-423, 1992.

Vomáčka, L., Pospíšilová, J.: Rehydration of sugar beet plants after water stress: effects of cytokinins. – Biol. Plant. **46**: 57-62, 2003.

Webb, A.A.R., Larman, M.G., Montgomery, L.T., Taylor, J.E., Hetherington, A.M.: The role of calcium in ABA-induced gene expression and stomatal movements. – Plant J. **26**: 351-362, 2001.

Wilkinson, S., Davies, W.J.: ABA-based chemical signalling: the co-ordination of responses to stress in plants. – Plant Cell Environ. **25**: 195-210, 2002.

Xu, H.-L., Shida, A., Futatsuya, F., Kumura, A.: Effects of epibrassinolide and abscisic acid on sorghum plants growing under soil-water deficit. II. Physiological basis for drought resistance induced by exogenous epibrassinolide and abscisic acid. – Jap. J. Crop Sci. **63**: 676-681, 1994.

Yadav, N., Gupta, V., Yadav, V.K.: Role of benzyladenine and gibberellic acid in alleviating water-stress effect in gram (*Cicer arietinum*). – Indian J. agr. Sci. **67**: 381-387, 1997.

Zhang, J., Zhang, X., Liang, J.: Exudation rate and hydraulic conductivity of maize roots are enhanced by soil drying and abscisic acid treatment. – New Phytol. **131**: 329-336, 1995.

Zhang, S.Q., Outlaw, W.H., Jr.: The guard-cell apoplast as a site of abscisic acid accumulation in *Vicia faba* L. – Plant Cell Environ. **24**: 347-355, 2001a.

Zhang, S.Q., Outlaw, W.H., Jr.: Abscisic acid introduced into the transpiration stream accumulates in the guard-cell apoplast and causes stomatal closure. – Plant Cell Environ. **24**: 1045-1054, 2001b.

Zhang, S.Q., Outlaw, W.H., Jr., Aghoram, K.: Relationship between changes in the guard cell abscisic-acid content and other stress-related physiological parameters in intact plants. – J. exp. Bot. **52**: 301-308, 2001.