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Effects of pre-treatments with abscisic acid and/or benzyladenine on gas exchange of French bean, sugar beet, and maize leaves during water stress and after rehydration

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Abstract

Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) during water stress and after rehydration were measured in *Phaseolus vulgaris*, *Beta vulgaris*, and *Zea mays*. Immediately before imposition of water stress by cessation of watering, plants were irrigated with water (control), 100 μM abscisic acid (ABA), and/or 10 μM N^6-benzyladenine (BA). In all three species, application of ABA decreased g_s, E, and P_N already 1 h after application. However, during water stress g_s, E, and P_N in plants pre-treated with ABA remained higher than in plants pre-treated with water. Positive effects of ABA application were observed also after rehydration. In contrast, the effects of pre-treatment with BA were species-specific. While in bean plants BA application ameliorated negative effect of water stress, only very slight effects were observed in maize, and in sugar beet BA even aggravated the effects of water stress.

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

Introduction

Different mechanisms were developed during plant evolution to prevent severe, dangerous water deficit, and to tolerate mild water deficit. Not only successful survival during stress but also the recovery after stress is crucial. Shoot desiccation is avoided mainly by stomatal regulation of transpiration rate (E). Simultaneously with reduction of water efflux, stomatal closure decreases the CO_2 influx which limits photosynthesis under mild water stress and supports photoinhibition under high irradiance. Decreased stomatal conductance (g_s) leads to a decrease in substomatal CO_2 concentration. Under mild water stress also diffusion of CO_2 *via* mesophyll cell walls, membranes, cytoplasm, and chloroplast envelope is decreased which leads to decreased chloroplastic CO_2 concentration (Flexas *et al*. 2002, Terashima and Ono 2002). The severe water stress directly affects photosynthetic capacity of the mesophyll causing decrease in carboxylation as well as electron transport chain activities, and/or induces ultrastructural changes in chloroplasts (for review see, *e.g*., Cornic and Massacci 1996). Differences among species and in the rates of imposition of water deficits, as well as the interactions with other environmental stresses, play a role in the relative importance of stomatal and non-stomatal limitations of photosynthesis under drought (*e.g*. Maroco *et al*. 2002).

Abscisic acid (ABA) might be root-to-shoot chemical signal of water stress. It is generated in the roots and transported to the leaves or directly in the leaves (*e.g.* Davies and Jeffcoat 1990, Liang *et al*. 1997). ABA can regulate stomatal aperture by changes in *1*) osmotic potential of guard cells, *2*) mechanical properties of guard cells, and *3*) gene expression (for recent reviews, see *e.g*., Schroeder *et al*. 2001, Pospíšilová 2003a).

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Abbreviations: ABA - abscisic acid; BA - N^6-benzyladenine; E - transpiration rate; g_s - stomatal conductance; P_N - net photosynthetic rate; RWC - relative water content; WUE - water use efficiency (P_N/E); WUE_i - intrinsic water use efficiency (P_N/g_s).
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To simulate effects of water stress, feeding shoots with ABA has often been used. ABA applied to plants can be readily absorbed (Trouverie *et al.* 2003). While stomatal closure immediately after ABA application was proved many times, long-term effects of ABA are much less known. For example treatment with ABA not only reduced transpiration, but also improved water use efficiency (WUE), prolonged development of water stress, retention of chlorophyll and survival of water-deprived barley and wheat seedlings (Mizrahi *et al.* 1974). In addition to lowering stomatal conductance, the application of ABA resulted in the accumulation of proline and total saccharides (Hose *et al.* 2000), or in promotion of wax deposition on the leaf surface (Xu *et al.* 1994).

In contrast to ABA, the stress-induced changes in contents of cytokinins and their possible effects on stomata are not sufficiently known (for recent review see Pospíšilová *et al.* 2000, Pospíšilová 2003a, Pospíšilová and Dodd 2004). Cytokinins can also delay stomatal closure induced by ABA (Blackman and Davies 1984, Radin and Hendrix 1988, Drüge and Schönbeck 1992).

In previous experiments with French bean we found that benzyladenine (BA) simultaneously applied with ABA partially reversed effects of ABA on g_s, E, and net photosynthetic rate (P_N) in plants sufficiently supplied with water (Pospíšilová 2003b). In addition, both substances ameliorated negative effects of severe water stress on gas exchange parameters. To determine if these effects are only species specific or more general we did similar experiments also with one C_3 species (sugar beet) and one C_4 species (maize). The aim of this contribution is comparison of these three species.

Materials and methods

Seedlings of French bean (*Phaseolus vulgaris* L. cv. Jantar), sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll, cv. Elán), and maize (*Zea mays* L. cv. Anjou 245) were grown in pots with coarse sand or fine *Perlite* sufficiently moistened with Hewitt nutrient solution. Plant were cultivated in growth chambers at 16-h photoperiod, irradiance of 250 μmol(photon) m^{-2} s^{-1} (400 - 700 nm), 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). Water stress was induced by cessation of watering. Before imposition of water stress, the plants were pre-treated with 50 cm^3 of water (control), 100 μM ABA, and/or 10 μM BA, and gas exchange parameters were measured 1 h after application, during water stress when visible wilting occurred, and 2 d after rehydration. Simultaneous application of 100 μM ABA + 10 μM BA was used only before water stress because the accumulation of endogenous ABA is induced due to water stress (for review see *e.g.* Pospíšilová 2003a).

P_N, E, and g_s were measured on attached leaves using the 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 m^{-2} s^{-1}, CO_2 concentration of 350 μmol mol^{-1}, and relative humidity of about 30 %. Water use efficiency (WUE) was calculated as P_N/E ratio and intrinsic water use efficiency (WUE_i) was calculated as P_N/g_s. Mature leaves with P_N, E, and g_s at near maximum values were usually used. Relative water content (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).

For each parameter a mean and a standard error of mean were calculated from two independent experiments.

Results and discussion

In plants sufficiently supplied with water, the highest values of g_s and E were found in bean and the highest values of P_N in maize (Fig. 1). WUE and WUE_i were relatively low in bean in comparison with sugar beet and especially maize (Table 1). After cessation of watering g_s, E and P_N decreased earlier than the decrease in relative water content (RWC) was observed. After 5 - 7 d visible wilting was observed, RWC decreased to about 65 - 75 %, and P_N, E, and especially g_s decreased significantly. The least decrease was observed in sugar beet. In bean, gas exchange parameters did not recover completely 2 d after rehydration even if RWC reached the pre-stress level. Much better recovery was observed in maize and especially in sugar beet (*cf.* Figs. 1 and 2). Under water stress, WUE did not change markedly in bean leaves, it decreased slightly in maize and considerably in sugar beet. WUE_i decreased under water stress in sugar beet and maize while it increased in bean (Table 1). Therefore in bean plants stomatal limitations to photosynthesis were dominant under water stress, however, in sugar beet and maize, non-stomatal limitations were also important. After rehydration, WUE still remained lower that before imposition of water stress in sugar beet, slightly increased in bean and was high in maize. WUE_i increased in all species which suggested slower recovery of g_s than P_N after rehydration.

Already 1 h after application, ABA decreased g_s, E, and P_N in all three species and the most marked effects were observed in bean. Only slight changes in WUE or WUE_i were observed after ABA application, therefore the ABA-induced decrease in P_N was probably mostly due to restricted CO_2 supply.

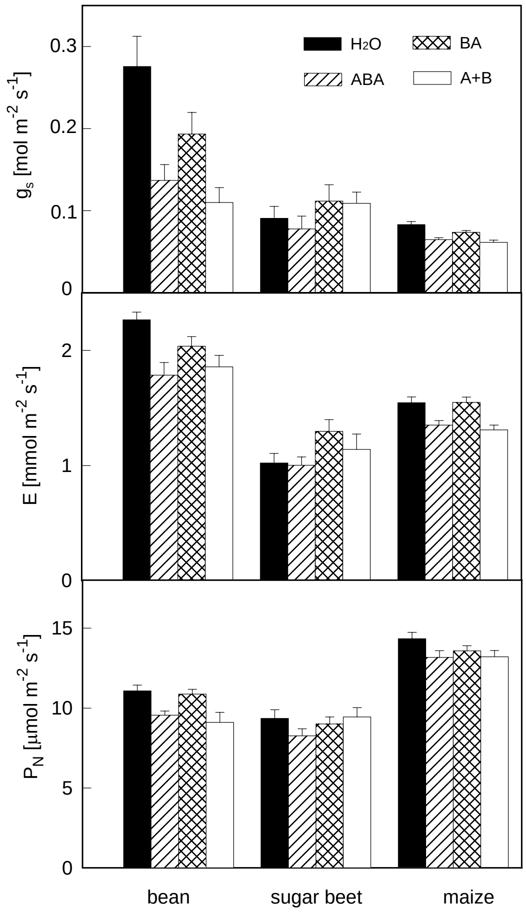

Fig. 1. Stomatal conductance (g_s), transpiration rate (E), and net photosynthetic rate (P_N) of attached French bean, sugar beet and maize leaves measured 1 h after application of 50 cm^3 H_2O (control), 100 µM ABA, 10 µM BA, or combination of 100 µM ABA + 10 µM BA (A+B) into the substrate. Means ± SE, *n* = 19 (bean), 18 (sugar beet), and 35 (maize).

On the contrary to non-stressed plants, the values of g_s, E, and P_N in all three species remained higher during water stress in plants pre-treated with ABA than in plants pre-treated with water. ABA pre-treatment also stimulated recovery of gas exchange paramenters after rehydration (Fig. 2). These results are in agreement with previous results with bean plants (Pospíšilová 2003b) and results of those authors who showed alleviation of negative effects of water stress by ABA application, *e.g.* in barley and wheat (Mizrahi *et al.* 1974), pine (Marshall *et al.* 1991), wheat (Nayyar and Kaushal 2002), and *in vitro* grown tobacco plants during *ex vitro* transfer (Pospíšilová *et al.* 1998).

In bean plants, application of BA decreased g_s and E (Fig. 1). The results obtained were similar to those in previous experiments where BA was applied to the substrate (Pospíšilová 2003b) but different from those where g_s was increased after immersion of bean roots into 5, 10 or 20 µM BA solution (Pospíšilová *et al.* 2001). After simultaneous application of ABA+BA, the values of E and P_N were similar to those when ABA alone was applied and g_s was even lower (Fig. 1). However, when ABA+BA were sprayed on bean leaves, BA ameliorated the negative effect of ABA on gas exchange parameters (Pospíšilová 2003b). In sugar beet plants, application of BA slightly increased g_s and E. In previous experiments (Pospíšilová *et al.* 2001), similar increase in E was observed in leaves sprayed with BA but not in leaves of plants with roots immersed in BA solution. Application of ABA+BA increased g_s similarly as BA. All gas exchange parameters were higher after ABA+BA application than when ABA was applied alone. Thus under simultaneous application, BA partially reversed the effect of ABA. In maize plants, application of BA did not significantly influence gas exchange parameters and combination ABA+BA decreased them similarly as application of ABA (Fig. 1). These results proved again that CK effects on stomatal opening, E, and P_N are strongly dependent on the plant species, CK concentration used, and way of application (Pospíšilová *et al.* 2001, Rulcová and Pospíšilová 2001, Vomáčka and Pospíšilová 2003), and the same holds for their interactions with ABA.

In bean plants, pre-treatment with BA ameliorated effects of water stress on gas exchange parameters in the same direction but less markedly than ABA did (Fig. 2). 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).

In contrast to bean plants, pre-treatment with BA accelerated development of water stress in sugar beet and maize. Lower values of RWC were reached in BA pre-treated plants than in plants pre-treated with water at the same time after cessation of watering (Fig. 2). Also g_s, E, and P_N decreased more during water stress in plants pre-treated with BA than in those pre-treated with water or ABA (Fig. 2). In sugar beet plants, the negative effect of BA on E and P_N, but not g_s, remained visible also after rehydration. In maize plants, the values of gas exchange parameters after rehydration did not differ significantly between plants pre-treated with BA and water (Fig. 2).

Table 1. Effects of ABA and/or BA pre-treatment on WUE (P_N/E) and WUE_i (P_N/g_s) determined 1 h after application, under water stress (RWC 65 - 75 %) and 2 d after rehydration. Comparison of French bean, sugar beet and maize plants.

		WUE			WUE_i		
		bean	beet	maize	bean	beet	maize
Application	H_2O	4.88	9.18	9.25	39.57	104.00	179.10
	ABA	5.36	8.27	9.76	50.26	103.40	219.50
	BA	5.33	6.93	8.76	77.71	81.90	194.00
	ABA+BA	4.90	8.28	10.08	82.82	85.81	220.20
Stress	H_2O	4.39	3.77	7.43	92.77	87.45	81.42
	ABA	5.36	4.40	11.00	120.62	74.64	90.14
	BA	8.17	4.29	4.87	149.20	100.00	184.10
Rehydration	H_2O	5.38	6.91	13.27	105.93	134.53	271.04
	ABA	5.84	6.85	11.60	121.35	108.30	247.03
	BA	5.95	5.60	14.62	118.95	85.75	235.96

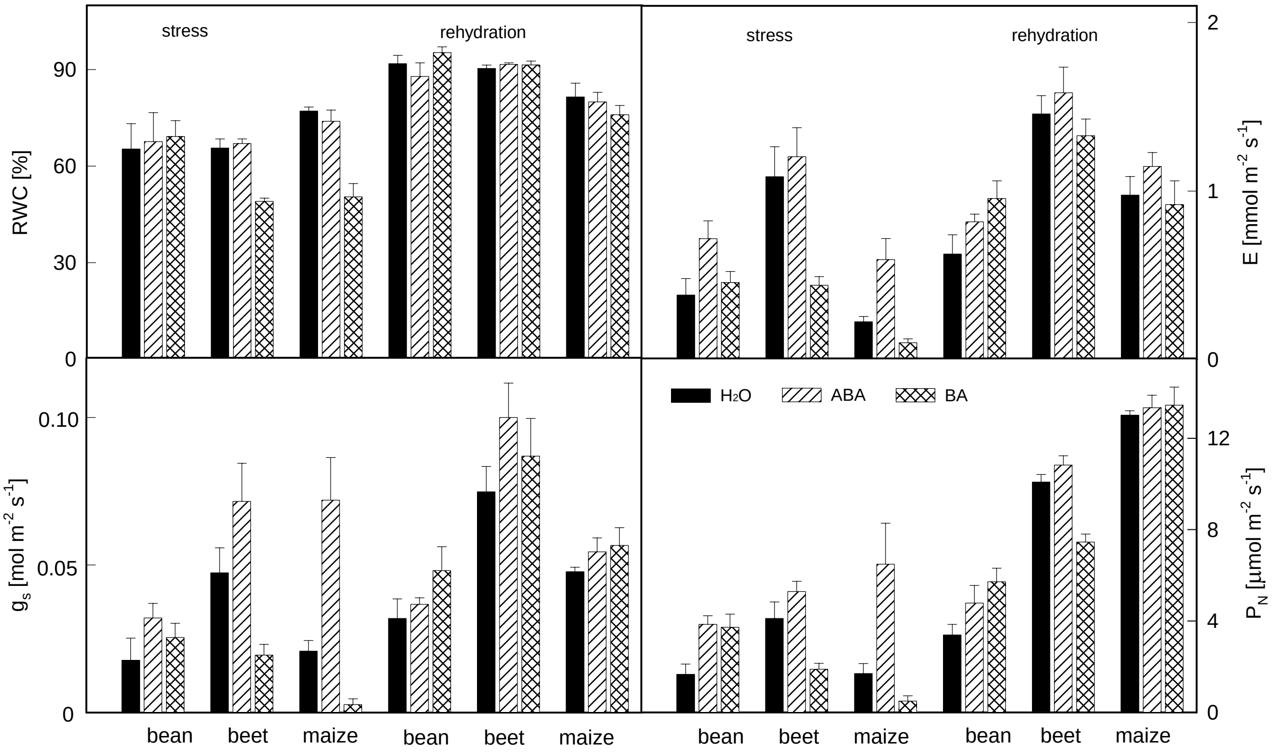

Fig. 2. Effect of pre-treatment with H_2O (control), 100 µM ABA, or 10 µM BA added to the substrate on relative water content (RWC), stomatal conductance (g_s), transpiration rate (E), and net photosynthetic rate (P_N) in French bean, sugar beet and maize under water stress and recovery after rehydration (2 d). Means ± SE, *n* = 33 (bean), 24 (sugar beet), 18 (maize).

In summary, these results proved positive effects of ABA application on gas exchange parameters during water stress and subsequent recovery after rehydration in all studied species. Thus amelioration of negative effect of water stress by ABA might be of general character. However, effects of BA-pretreatments were species-specific and ranged from positive to negative.

References

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

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

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.

Cornic, G., Massacci, A.: Leaf photosynthesis under drought stress. - In: Baker, N.R. (ed.): Photosynthesis and the Environment. Pp. 347-366. Kluwer Academic Publishers, Dordrecht 1996.

Davies, W.J., Jeffcoat, B. (ed.): Importance of Root to Shoot Communication in the Responses to Environmental Stress. - British Society for Plant Growth Regulation, Bristol 1990.

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.

Flexas, J., Bota, J., Escalona, J.M., Sampol, B., Medrano, H.: Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. - Funct. Plant Biol. **29**: 461-471, 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.

Liang, J., Zhang, J., Wong, M.H.: Can stomatal closure caused by xylem ABA explain the inhibition of leaf photosynthesis under soil drying? - Phosynth. Res. **51**: 149-159, 1997.

Maroco, J.P., Rodrigues, M.L., Lopes, C., Chaves, M.M.: Limitations to leaf photosynthesis in field-grown grapevine under drought-metabolic and modelling approaches. - Funct. Plant Biol. **29**: 451-459, 2002.

Marshall, 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.

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.

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.

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.

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

Pospíšilová, J.: Interaction of cytokinins and abscisic acid during regulation of stomatal opening in bean leaves. - Photosynthetica **41**: 49-56, 2003b.

Pospíšilová, J., Dodd, I.C.: Role of plant growth regulators in stomatal limitation to photosynthesis during water stress. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis, 2nd Ed., in press. Marcel Dekker, New York 2004.

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., Wilhelmová, N., Synková, H., Čatský, J., Krebs, D., Tichá, I., Hanáčková, B., Snopek, J.: Acclimation of tobacco plantlets to *ex vitro* conditions as affected by application of abscisic acid. - J. exp. Bot. **49**: 863-869, 1998.

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.

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

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.

Terashima, I., Ono, K.: Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. - Plant Cell Physiol **43**: 70-78, 2002.

Trouverie, J., Thévenot, C., Rocher, J.-P., Sotta, B., Prioul, J.-L.: The role of abscisic acid in the response of a specific vacuolar invertase to water stress in adult maize leaf. - J. exp. Bot. **54**: 2177-2186, 2003.

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

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.