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Abaxial and adaxial stomatal density, stomatal conductances and water status of bean primary leaves as affected by paclobutrazol

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Abstract

The plant growth retardant, paclobutrazol at 8.5 or 17.0 µM concentrations effectively inhibited the stem elongation and primary leaf expansion of bean seedlings. Although the retardant reduced the relative water content in well-watered plants, the water and pressure potentials remained high in the primary leaves. K^+, Na^+, Mg^{2+} and Ca^{2+} contents in the primary leaves of the paclobutrazol-treated plants were not significantly different from those in the control. The stomatal density increased on both surfaces but the length of guard cells was not reduced significantly on the adaxial epidermes of the paclobutrazol-treated primary leaves. The inhibitory effect of paclobutrazol on the abaxial stomatal conductances became more pronounced with time during the light period but the adaxial surfaces displayed similar or slightly higher conductances than those of the control. The transpiration rate on a unit area basis did not change significantly or increased in the treated leaves thus the reduced water loss of paclobutrazol-treated plants was due to the reduced leaf area. Stomatal conductances of the adaxial surfaces responded more intensively to exogenous abscisic acid and the total leaf conductance decreased faster with increasing ABA concentration in the control than in the paclobutrazol-treated leaves. Paclobutrazol, an effective inhibitor of phytosterol biosynthesis, not only amplified the stomatal differentiation but increased the differences between the adaxial and abaxial stomatal conductances of the primary leaves.

Additional key words: abscisic acid, abaxial/adaxial surface, *Phaseolus vulgaris* L., primary leaf, stomatal index, stomatal size, water relations.

Introduction

The fitness of plants under water-limited conditions can be improved by the application of triazole growth retardants which are effective inhibitors of gibberellic acid and sterol biosynthesis (Grossmann 1990).

Paclobutrazol (PB) [(2RS, 3RS)-1(4-chlorophenyl)-4,4-dimethyl-2-(1, 2, 4-triazol-1-yl) pentan-3-ol)] decreased the water loss of apple seedlings under water-stressed (Asamoah and Atkinson 1985) or non-stressed conditions (Steffens *et al.* 1983) and induced drought resistance in jack pine (Marschall *et al.* 1991). White spruce seedlings maintained relatively high pressure potential in the tissues of needles and withstood prolonged drought after PB treatment by changing the elastic properties of the cell walls (Marschall and Dumbroff 1999).

The reduction of the leaf area by growth retardants often results in an increased stomatal density (Yim *et al.* 1997) and the size or density of the stomata may vary over the surface of the leaf. Stomatal heterogeneity is manifested in different average conductances in different regions of the leaf (Terashima *et al.* 1988, Pospíšilová and Šantrůček 1994, Lawson *et al.* 1998a). In combination with hydraulic mechanisms, spatial changes in the concentrations of different diffusible signals such as IAA, ABA, potassium ion or internal CO_2 concentration (c_i), determine the steady-state stomatal aperture (Lawson *et al.* 1998b, Mott and Buckley 1998). Although it is commonly accepted, that stomatal conductance (g_s) is largely linearly related to stomatal aperture, it has recently

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Abbreviations: ABA - abscisic acid; E - transpiration rate; g_s - stomatal conductance; IAA - indole-3-acetic acid; PB - paclobutrazol; ψ_s - pressure potential; ψ_s - osmotic potential; ψ_w - water potential.

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been demonstrated, that the steepest increase of g_s occurred at small degrees of stomatal opening in *Sambucus* leaves (Kaiser and Kappen 2001). Moreover, the stomatal conductances may be different for the two surfaces (Mott *et al.* 1993).

External factors and plant hormones generally affect not only the function, but also the differentiation of the stomata. The theory of Bünning (1956) concerning the stomatal differentiation of dicotyledonous plants postulates that the existing stomata inhibit the differentiation of new ones, but stomatal differentiation may also be determined by long-distance signals originating from the mature leaves (Brownlee 2001). There are stomatal density mutants in *Arabidopsis* which display a highly increased stomatal density and have a mutation in a gene of a putative subtilisin-like protease converting an inactive peptide signals into an active form (Berger and Altmann 2000). The differentiation of stomata depends on the external CO_2 concentration, the stomatal density being lower in plants that grow in high concentrations of CO_2 (Woodward and Kelly 1995). Exposure of *Vigna sinensis* L. plants from shade to bright light for 1 d also increased the stomatal indices on the upper epidermis of the third trifoliate leaves (Schoch *et al.* 1980).

In contrast, water stress and abscisic acid (ABA) reduced the stomatal index, increased the stomatal density and decreased the stomatal conductance on the adaxial surface of spring wheat leaves (Quarrie and Jones 1977). Similar results have been found for stomatal conductances after short-term ABA treatment in tobacco plantlets acclimatizing to *ex vitro* conditions (Pospíšilová 1996, Pospíšilová *et al.* 1998). Cytokinins have an opposite effect: natural and synthetic cytokinins have been shown to promote stomatal opening (Jewer and Incoll 1980). Indoleacetic acid (IAA) and synthetic auxins increased the stomatal apertures and acted antagonistically to ABA (Dunleavy and Ladley 1995). IAA was remarkably effective in producing wide adaxial apertures and eliminated the normal disparity in the stomatal opening between abaxial and adaxial stomata (Pemadasa 1982a), and the responses of adaxial and abaxial stomata to light, particularly in the blue region, also differed in the epidermal peels of *Commelina* (Pemadasa 1982b).

Plant growth retardants modify the concentrations of different hormones, including those which are effective in the regulation of stomatal movement. In short-term experiments, the ABA content exhibited a transient increase followed by a decline in oilseed rape after triazole treatment (Häuser *et al.* 1990). According to Asare-Boamah *et al.* (1986), triadimefon protected bean plants from water stress by increasing the concentration of ABA. In contrast, reduced ABA contents have been found after PB treatment in non-stressed or water stressed apple (Wang *et al.* 1987) and wheat seedlings (Buta and Spaudling 1991).

The concentration of IAA in the primary leaves of bean was elevated by PB (Nagy *et al.* 1991) and the cytokinin contents in different parts of soybean seedlings were also increased by triazole (LAB 150987) treatment (Grossmann *et al.* 1987). Thus, the changes in the endogenous hormone concentrations may favour the stomatal opening in PB-treated plants.

The present paper describes the effects of PB on the stomatal development of bean primary leaves with respect to the differences between the abaxial and adaxial surfaces, and examines the effects of changes in the stomatal frequency and water status on the stomatal conductance and transpiration rate of bean primary leaves. We were also interested in the sensitivity of the stomatal conductance to exogenous abscisic acid in PB-treated plants.

Material and methods

Seeds of *Phaseolus vulgaris* L. cv. Juliska were immersed in 8.5 or 17.0 μM PB solution for 2 h and then germinated in a thermostat at 25 °C. After 48 h the seeds were sown in garden mould and the plants were grown in a chamber (*Controlled Environments*, Winnipeg, Canada) under a 14-h photoperiod, 25/20 °C day/night temperatures, and 65 % relative humidity. The plants were watered daily by sub-irrigation. Measurements were made on the fully expanded primary leaves of 14-d-old seedlings. The experiments were repeated twice. The stomatal index was defined as the number of stomatal complexes per 100 epidermal cells.

The epidermal morphology of the leaves of 15-d-old plants was examined by using polystyrene replicas of both leaf sides (Quarrie and Jones 1977). The leaf surfaces were divided into three regions at equal distances on the main vein: the apical, middle and basal regions. The leaf imprints were examined under a light microscope, and the cell dimensions and stomatal indices were recorded for 9 different primary leaves. The peristomatal groove distance was defined and measured after Lawson *et al.* (1998b). One experiment was conducted with epidermal peels, which did not reveal significant differences from the data for the imprints.

Stomatal conductance was determined in the middle part of the abaxial and adaxial sides of fully expanded primary leaves at an irradiance of 180 μmol m^{-2} s^{-1} with a diffusion porometer (Model *VP-2000*, *Cayuga Development Research Instruments*, New York, USA) The water potential of primary leaves (ψ_w) was measured in a pressure chamber (*PMS Instrument Co.*, Corvallis, USA). The osmotic potential (ψ_s) of the expressed sap was determined with an osmometer via determination of the freezing point depression. The pressure potential (ψ_p) was calculated from the difference between ψ_w and ψ_s. The appropriate concentrations of (±)-ABA were dissolved in 0.05 % *Tween 20* solution and sprayed onto both surfaces of the primary leaves. The control leaves were treated with 0.05 % *Tween 20*. Stomatal conductances were measured 24 h after ABA treatment.

Whole leaf transpiration was determined gravimetrically (Schubert *et al.* 1991). The detached leaves were allowed to dry adaxial side up in the growth chamber in which the plants were growing and their mass was determined for 10 min at 1-min and for the next 3 h at 30-min intervals. The transpiration rate (E) was calculated from the initial slope of the water loss curves recorded immediately after leaf excision. Leaf areas were also measured at the harvest with a digital planimeter (*Tamaya Technics Inc.*, Tokyo, Japan).

Concentrations of K^+, Na^+, Ca^{2+} and Mg^{2+} were determined after wet digestion of 100 mg dried plant material in a mixture of nitric acid and 30 % H_2O_2 (5:4; v/v) at 200 °C for at least 3 h. The metal contents of the primary leaves were measured with a Hitachi Z-8200 Zeeman polarized atomic absorption spectrophotometer (*Hitachi Ltd.*, Tokyo, Japan).

Results and discussion

PB has proved to be very effective in the reduction of leaf expansion in a number of species, including bean (Tari and Nagy 1994). A significant reduction in the growth rate occurred in the linear phase of the expansion of primary leaves, the leaf area was 35 and 55 % lower in plants treated with 8.5 and 17.0 µM PB, respectively. Differentiation of the stomata took place mainly during the exponential phase of primary leaf growth and further

Table 1. Stomatal densities, abaxial/adaxial ratios and stomatal indices in different zones of the two surfaces of control and PB-treated primary leaves in 15-d-old bean plants. Means ± SE (3 × 3 leaves from 3 pots, 10 microscopic areas per leaf).

PB [µM]		Stomatal density [mm^{-2}]			Stomatal index [%]	
		adaxial	abaxial	abax/adax	adaxial	abaxial
0	apex	47.4 ± 6.6	207.1 ± 19.0	4.37	9.4 ± 0.8	14.9 ± 1.4
	middle	40.4 ± 8.8	172.5 ± 20.7	4.26	7.2 ± 0.7	14.5 ± 0.3
	base	39.5 ± 6.6	169.4 ± 22.4	4.28	14.9 ± 1.4	15.6 ± 1.1
8.5	apex	166.7 ± 36.6	568.5 ± 17.8	3.41	12.3 ± 0.7	25.9 ± 0.4
	middle	83.7 ± 9.1	433.9 ± 13.7	5.20	10.6 ± 0.8	24.5 ± 1.2
	base	101.8 ± 9.1	392.2 ± 34.3	3.85	11.4 ± 0.6	23.3 ± 0.7
17.0	apex	165.8 ± 9.2	737.0 ± 19.9	4.45	10.3 ± 0.1	19.1 ± 0.2
	middle	88.6 ± 5.8	449.2 ± 53.2	5.05	10.4 ± 0.4	15.7 ± 0.8
	base	98.3 ± 14.1	315.8 ± 16.1	3.21	10.9 ± 0.7	16.3 ± 1.8

Table 2. Effect of PB on the length of guard cells and on the peristomatal groove distance in different zones of the adaxial and abaxial epidermes of primary leaves in 15-d-old bean plants. Stomatal characteristics were determined on the imprints prepared 2 h after the beginning of the light period. Means ± SE (3 × 3 leaves from 3 pots, 10 microscopic areas per leaf).

PB [µM]		Guard cell length [µm]		Peristomatal groove distance [µm]	
		adaxial	abaxial	adaxial	abaxial
0	apex	26.0 ± 1.2	24.6 ± 0.7	12.6 ± 0.4	12.2 ± 0.1
	middle	28.4 ± 1.4	25.2 ± 0.8	13.8 ± 0.8	12.1 ± 0.3
	base	29.5 ± 0.3	25.8 ± 0.5	14.8 ± 0.1	14.0 ± 0.2
8.5	apex	26.7 ± 1.1	23.3 ± 0.2	13.5 ± 0.8	12.6 ± 0.2
	middle	29.5 ± 1.6	24.3 ± 0.5	13.9 ± 0.5	12.2 ± 0.4
	base	29.0 ± 0.8	25.2 ± 1.0	14.0 ± 0.1	12.2 ± 0.2
17.0	apex	26.0 ± 0.3	22.7 ± 0.2	12.1 ± 0.2	11.7 ± 0.2
	middle	28.1 ± 1.6	23.9 ± 0.4	13.6 ± 0.3	11.0 ± 0.4
	base	29.0 ± 0.6	25.1 ± 0.5	13.2 ± 0.5	11.4 ± 0.5

expansion decreased the stomatal density (unpublished results).

In control plants stomatal density of primary leaves decreased from the apical to the basal region of both the adaxial and the abaxial surfaces (Table 1). The stomatal frequency was highly increased by PB and the differences between the leaf regions were more significant than in the control. The differentiation of the stomatal complexes was also modified. PB increased the stomatal indices, the percentage of guard cell pairs among the epidermal cells on the apical and middle parts of both surfaces, which suggested, that in accordance with Bünning's theory, the size of the inhibition field around the existing stomatal complex might have been reduced. The length of the stomata on the adaxial surface did not change significantly after PB treatment (Table 2), although other stress factors, such as water stress or ABA (Quarrie and Jones 1977), which reduced the expansion of the leaves, were also known to reduce the stomatal size. The decrease in the peristomatal groove distance, which had been introduced as a surrogate measure for stomatal aperture in bean primary leaves (Lawson *et al.* 1998b), was obvious only on the abaxial surface after 17.0 μM PB treatment. This suggests that the control of g_s by pore area reduction is substantially higher in abaxial than in adaxial stomata in retardant-treated leaves .

High concentrations of triazole retardants such as PB inhibit not only the biosynthesis of gibberellins, but also that of plant sterols, *e.g.*, campesterol (Grossmann 1990). Theoretically, these inhibitors should reduce the concentrations of brassinosteroids but the decrease in the accumulation of common phytosterols also plays an important role in the regulation of membrane fluidity, permeability and other membrane-coupled processes. It was found, that the *cpd* (constitutive photomorphogenesis and dwarfism) mutant of *Arabidopsis* deficient in the biosynthesis of brassinosteroids, exhibited an enhanced stomatal differentiation on the hypocotyl, cotyledon and leaf epidermes (Molnár 2001). PB induced similar changes in stomatal differentiation to those of brassinosteroid-deficient mutant plants in the bean primary leaves. Moreover, PB induced similar changes in the distribution of IAA, ethylene production, ethylene precursor 1-aminocyclopropane-1-carboxylic acid between apical and basal parts of bean hypocotyls, as light and increased the IAA content in the leaf blades in our system (Nagy *et al.* 1991, Tari and Mihalik 1998). These hormonal changes contribute to the control of cell cycle and differentiation of epidermal cells complementing the primary effects of the retardant.

Table 3. The effects of PB on the relative water content (RWC), water potential (ψ_w), osmotic potential (ψ_s), calculated leaf pressure potential (ψ_p) and transpiration rate (E) in primary leaves of bean. The measurements were made 2 h after the beginning of the light period. Values are means ± SE. *n* = 18. Means denoted by * or *** are significantly different from the control at $P \leq 0.05$ or 0.001 levels, respectively, as determined by Student's *t*-test.

PB [μM]	RWC [%]	ψ_w [MPa]	ψ_s [MPa]	ψ_p [MPa]	E [mmol leaf^{1} min^{-1}]	E [mmol m^{-2} s^{-1}]
0	90.0 ± 1.1	-0.55 ± 0.10	-0.67 ± 0.04	0.122	0.24 ± 0.01	0.68 ± 0.05
8.5	87.9 ± 1.5	-0.40 ± 0.06	-0.56 ± 0.03*	0.156	0.11 ± 0.02***	0.85 ± 0.50
17.0	84.1 ± 0.6***	-0.36 ± 0.05	-0.58 ± 0.05	0.224	0.14 ± 0.02***	1.05 ± 0.05***

Table 4. Ion contents [μmol g^{-1} (d.m.)] in primary leaves of 15-d-old bean plants after PB treatment. Means ± SE, *n* = 3. Means were not significantly different from the control as determined by Student's *t*-test.

PB [μM]	K^+	Ca^{2+}	Mg^{2+}	Na^+
0	593.0 ± 38.7	607.7 ± 16.2	312.3 ± 62.0	36.1 ± 2.0
8.5	689.7 ± 41.5	611.0 ± 20.0	275.7 ± 96.7	31.2 ± 0.4
17.0	651.7 ± 19.5	588.0 ± 50.6	184.7 ± 7.2	30.9 ± 1.0

The water status of tissues may determine the stomatal conductance and transpiration rate of the leaf. In well-watered 15-d-old plants, PB treatment resulted in constant but not necessarily significant increases in water, osmotic and pressure potential (Table 3). The tissue concentrations of those macroelements which play a role in some way or another in the regulation of stomatal movement (K^+, Ca^{2+}, Mg^{2+} and Na^+) did not change significantly in the treated leaves (Table 4), although the Mg^{2+} content was lower in the treated tisssues.

The stomatal conductance of bean primary leaves exhibits a daily pattern (Davies and Van Volkenburg 1983): the stomata open at the beginning and close gradually during the last third of the light period. PB treatments affected g_s on adaxial and abaxial surfaces differently. Depending on the phase of the light period, the retardant reduced g_s on the abaxial surface, but on the adaxial surface g_s remained similar to that of the control or increased slightly as a function of time (Fig. 1). The observed changes in g_s were not directly related with the changes in stomatal frequency. The two surfaces responded also differently to exogenous ABA. The adaxial g_s was less sensitive than the abaxial one to exogenous ABA in treated plants, and as a consequence the total g_s was not affected or was even slightly higher in the leaves treated with 17.0 μM PB at a wide ABA concentration range (Fig. 2).

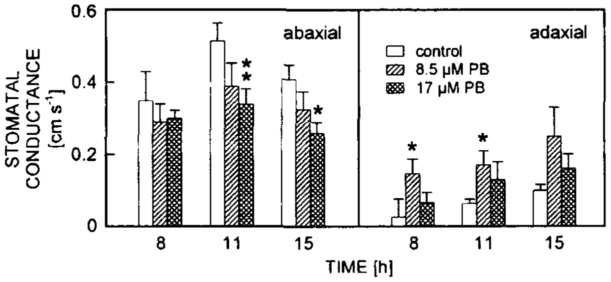

Fig. 1. Effects of 8.5 or 17.0 μM paclobutrazol treatment on adaxial and abaxial stomatal conductances of 15-d-old bean primary leaves in the light period (Means ± SE for 3 pots with 10 plants in each). Means denoted by * and ** are significantly different from the appropriate control at $P \leq 0.05$ and 0.01 as determined by Student's *t*-test.

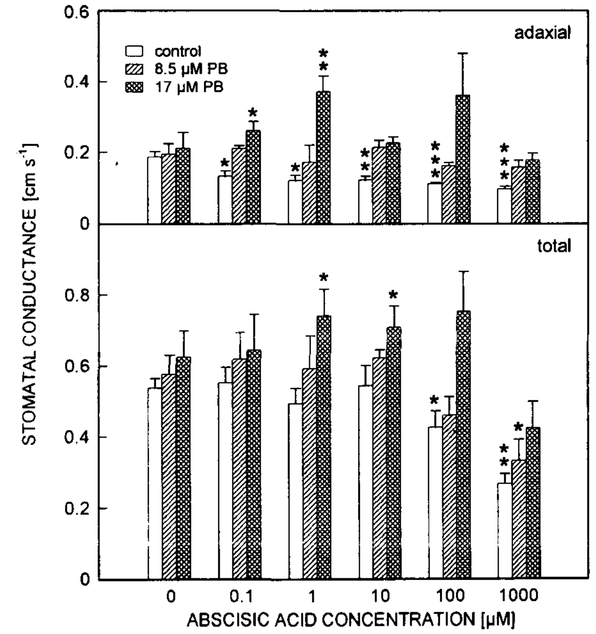

Fig. 2. Effects of 0.1 - 1000 μM abscisic acid on adaxial and total stomatal conductances in bean primary leaves treated with 8.5 or 17.0 μM paclobutrazol Stomatal conductances were measured two hours after the beginning of the light period Means ± SE (for 3 pots with 10 plants in each). Means denoted by *, ** and *** are significantly different from the untreated control at $P \leq 0.05$, 0.01 and 0.001 as determined by Student's *t*-test.

It is known that a highly sensitive relationship exists between guard cells and their environment. The sensitivity of stomata on the abaxial and adaxial surfaces are different to auxin (Pemadasa 1982a). The guard cells responde to IAA by opening in the presence of appropriate K^+ concentration which may vary in different parts of the leaves. ABA counteracts the effect of IAA on stomatal opening in a concentration dependent manner (Dunlavy and Ladley 1995). We found higher IAA contents in the blades of PB-treated leaves which may interfere with the effects of exogenously applied ABA. The guard cells responde to the concentration changes of ABA in the apoplast, but depending on the apoplastic pH, ABA can be readily trapped in the cytoplasm or in the chloroplasts of the surrounding cells (Hartung and Slovik 1991) which decreases the effect of ABA on stomatal closure. This process is highly enhanced by light when the light reactions of photosynthesis increase the pH of the stroma. The sequestration of the hormone among cell compartments may be changed by the retardant and the capacity of mesophyll or epidermal cells to metabolize ABA can also modify the amount of the hormone arriving to guard cell plasma membranes in PB-treated plants.

As calculated from the slope of the water loss curves immediately after excision, there was a significant increase in the transpiration rate on a unit area basis at 17.0 μM PB concentration. The effect could be attributed to the extremely high stomatal frequencies, but the water loss of whole primary leaves decreased significantly in PB-treated plants (Table 3). This suggests that, under well-watered conditions, the effect of PB on the transpiration rate is a very complex phenomenon comprising the actual hormonal status, water potential and stomatal density changes of leaves. The retardant enhanced the difference between the stomatal frequencies and between conductances of the two leaf surfaces but the higher stomatal density was not necessarily coupled with higher g_s on the abaxial surface. Moreover, the heterogeneity of various stomatal characteristics on the same leaf surface results in fluctuations in g_s, thus the amount of transpired water is primarily regulated by the reduction of leaf area in PB-treated leaves, as a result plants able to limit water loss.

References

Asamoah, T.E.O., Atkinson, D.: The effects of (2RS, 3RS)-1-(4-chlorophenyl) 4, 4-dimethyl-2-(1H-1, 2, 4 triazol-1-yl) pentan-3-ol (paclobutrazol:PP333) and root pruning on the growth, water use and response to drought of Colt Cherry rootstocks. - Plant Growth Regul. 3: 37-45, 1985.

Asare-Boamah, N.K., Hofstra, G., Fletcher, R.A., Dumbroff, E.B.: Triadimefon protects bean plants from water stress through its effects on abscisic acid. - Plant Cell Physiol.

27: 383-390, 1986.

Berger, D., Altmann, T.: A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis*. - Genes Dev. **14**: 1119-1131, 2000.

Brownlee, C.: The long and the short of stomatal density signals. - Trends Plant Sci. **6**: 441-442, 2001.

Buta, J.G., Spaulding, D.W.: Effect of paclobutrazol on abscisic acid levels in wheat seedlings. - J. Plant Growth Regul. **10**: 59-61, 1991.

Bünning, E.: General processes of differentiation. - In: Milthorpe, F.L. (ed.): The Growth of Leaves. Pp. 18-30. Butterworths, London 1956.

Davies, W.J., Van Volkenburgh, E.: The influence of water deficit on the factors controlling the daily pattern of growth of *Phaseolus* trifoliates. - J. exp. Bot. **34**: 87-999, 1983.

Dunleavy, P.J., Ladley, P.D.: Stomatal responses of *Vicia faba* L. to indole acetic acid and abscisic acid. - J. exp. Bot. **46**: 95-100, 1995.

Grossmann, K., Kwiatkowski, J., Siebecker, H., Jung, J.: Regulation of plant morphology by growth retardants. Effects on phytohormone levels in soybean seedlings determined by immunoassays. - Plant Physiol. **84**: 1018-1021, 1987.

Grossmann, K.: Plant growth retardants as tools in physiological research. - Physiol. Plant. **78**: 640-648, 1990.

Hartung, W., Slovik, S.: Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: stomatal regulation by abscisic acid in leaves. - New Phytol. **119**: 361-382, 1991.

Häuser, C., Kwiatkowski, J., Rademacher, W., Grossmann, K.: Regulation of endogenous abscisic acid levels and transpiration in oilseed rape by plant growth retardants. - J. Plant Physiol. **137**: 201-207, 1990.

Jewer, P.C., Incoll, L.D.: Promotion of stomatal opening in the grass *Anthephora pubescens* Nees. by a range of natural and synthetic cytokinins.- Planta **150**: 218-221, 1980.

Kaiser, H., Kappen, L.: Stomatal oscillations at small apertures: indications for a fundamental insufficiency of stomatal feedback-control inherent in the stomatal turgor mechanism. - J. exp. Bot. **52**: 1303-1313, 2001.

Lawson, T., Weyers, J., A'Brook, R.: The nature of heterogeneity in the stomatal behaviour of *Phaseolus vulgaris* L. primary leaves. - J. exp. Bot. **49**: 1387-1395, 1998a.

Lawson, T., James, W., Weyers, J.: A surrogate measure of stomatal aperture. - J. exp. Bot. **49**: 1397-1403, 1998b.

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.

Marschall, J.G., Dumbroff, E.B.: Turgor regulation via cell wall adjustment in white spruce. - Plant Physiol. **119**: 313-320, 1999.

Molnár, G.: [Investigation of brassinosteroid-regulated gene expression in *Arabidopsis thaliana*.] - Ph.D. Thesis, University of Szeged, Szeged 2001. [In Hungarian.]

Mott, K.A., Buckley, T.N. Stomatal heterogeneity. - J. exp. Bot. **49**: 407-417, 1998.

Mott, K.A., Cardon, Z.G., Berry, J.A.: Asymmetric patchy stomatal closure for the two surfaces of *Xanthium strumarium* L. leaves at low humidity. - Plant Cell Environ. **20**: 25-34, 1993.

Nagy, M., Tari, I., Bubán, T.: IAA distribution in the hypocotyls and primary leaves of *Phaseolus vulgaris* L. treated with paclobutrazol in relation to their rooting capacity. - Biochem. Physiol. Pflanz. **187**: 447-451, 1991.

Pemadasa M.A.: Differential abaxial and adaxial stomatal responses to indole-3-acetic acid in *Commelina communis* L. - New Phytol. **90**: 209-219, 1982a.

Pemadasa, M.A.: Abaxial and adaxial stomatal responses to light of different wavelengths and to phenylacetic acid on isolated epidermes of *Commelina communis* L. - J. exp. Bot. **33**: 92-99, 1982b.

Pospíšilová, J.: Hardening by abscisic acid of tobacco plantlets grown *in vitro*. - Biol. Plant. **38**: 605-609, 1996.

Pospíšilová, J., Šantrůček, J.: Stomatal patchiness. - Biol. Plant. **36**: 481-510, 1994.

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.

Quarrie, S.A., Jones, H.G.: Effects of abscisic acid and water stress on development and morphology of wheat. - J. exp. Bot. **28**: 192-203, 1977.

Schoch, P.-G., Zinsou, C., Sibi, M.: Dependence of the stomatal index on environmental factors during stomatal differentiation in leaves of *Vigna sinensis* L. - J. exp. Bot. **31**: 1211-1216, 1980.

Schubert, J., Röser, K., Grossmann, K., Sauter, H., Jung, J.: Transpiration-inhibiting abscisic acid analogs. - J. Plant Growth Regul. **10**: 27-32, 1991.

Steffens, G.L., Wang, S.Y., Steffens, C.L., Brennan, T.: Influence of paclobutrazol (PP333) on apple seedling growth and physiology. - Proc. Plant Growth Regul. Soc. Amer. **10**: 195-205, 1983.

Tari, I., Nagy, M.: Enhancement of extractable ethylene at light/dark transition in primary leaves of paclobutrazol-treated *Phaseolus vulgaris* seedlings. - Physiol. Plant. **90**: 353-357, 1994.

Tari, I., Mihalik, E.: Comparison of the effects of white light and the growth retardant paclobutrazol on the ethylene production in bean hypocotyls. - Plant Growth Regul. **24**: 67-72, 1998.

Terashima, I., Wong, S-C., Osmond, C.B., Farquhar, G.D.: Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. - Plant Cell Physiol. **29**: 385-394, 1988.

Wang, S.Y., Sun, T., Ji, Z.L., Faust, M.: Effect of paclobutrazol on water stress-induced abscisic acid in apple seedling leaves. - Plant Physiol. **84**: 1051-1054, 1987.

Woodward, F.I., Kelly, C.K.: The influence of CO_2 concentration on stomatal density. - New Phytol. **131**: 311-327, 1995.

Yim, K-O., Kwon, Y.W., Bayer, D.E.: Growth responses and allocation of assimilates of rice seedlings by paclobutrazol and gibberellin treatment. - J. Plant Growth Regul. **16**: 35-41, 1997.