BIOLOGIA PLANTARUM 34(3-4): 259-265, 1992

Stomatal responses to ABA and IAA in isolated epidermal strips of *Vicia faba* L.

M. ŘIČÁNEK and M. VICHERKOVÁ

Department of Plant Physiology and Anatomy, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czechoslovakia

Abstract

Epidermal strips from well-watered faba-bean plants were subjected to a range of abscisic acid (ABA) and indolyl-3-acetic acid (IAA) concentrations (10^{-5} to 1 mM) in the presence or absence of CO_2 in light or dark. ABA had inhibitory effect on abaxial stomatal apertures in all the concentrations studied and retained them closed even after addition of KCl (50 and 100 mM) to the incubation medium. It also influenced stomatal responses to CO_2. In the presence of CO_2 apertures were greater than in its absence in light as well as in darkness. This relationship remained unchanged also after addition of KCl. The action of ABA inhibited accumulation of potassium in the guard cells. IAA stimulated stomatal opening and its effect was quite opposite to ABA; in the presence of CO_2 the apertures were smaller than in its absence. IAA, however, was able to inhibit the closing effect of darkness, CO_2, and ABA, and stimulated potassium accumulation in the guard cells. Simultaneous action of ABA+IAA manifested effects of both substances.

Introduction

It is well known that factors such as light, temperature, humidity and K^+ concentration induce changes in stomatal apertures. However, there still exists contradictory views on the effects of phytohormones and their interactions, especially under different environmental conditions. It is clear that ABA is an inhibitor of stomatal opening which plays an important physiological role particularly in water-stressed plants. However, the possible intraction between ABA and CO_2 still remains an open question. Independence of action was suggested by Orton and Mansfield (1974) and Mansfield (1976) who found that the response of the stomata of *Xanthium strumarium* to ABA was virtually the same in CO_2-free and normal air. Raschke (1975a,b) found an above mentioned interaction in stomatal responses and noted the necessity, at least in some plant species, of the presence of ABA for the regulation of apertures by CO_2.

Received 5 November 1990, *accepted* 14 February 1991.

He reported that the sensitivity of stomata to CO_2 in well-watered plants is variable, but these plants can be made sensitive by the external application of ABA. On the other hand, Mansfield and Wilson (1981) showed no evidence of increased sensitivity to CO_2 when the epidermis was taken from well-watered plants of *Commelina communis* and was incubated in different ABA concentrations. However, Wilson (1981) showed that the sensitivity of the stomata of *C. communis* to CO_2 was increased if the plants had been subjected to water stress. The attempts to show statistically significant interaction between CO_2 and ABA in well-watered plants of *X. strumarium* (Mansfield 1976) were not successful, even though there was evidence for some interpendence between these two regulators.

Certain interrelations were also found between the effects of CO_2, ABA and naturally occuring or synthetic cytokinins (Blackman and Davies 1984). Some reports suggest (Pemadasa 1982, Snaith and Mansfield 1982, Eamus and Wilson 1984) that a similar interactions exist also between CO_2, ABA and IAA. It is questionable whether or not the effects of IAA, like the effects of CO_2 and ABA, can result from a direct or indirect modulation of ion fluxes in the guard cells.

The following experiments dealing with the effects of ABA, IAA, CO_2 and light on stomatal aperture on the epidermis detached from leaves of *Vicia faba*try to contribute to solution of the above mentioned problems.

Material and methods

Vicia faba L., cv. Chlumecký were grown on combination of Richter's (macroelements) and Hoagland's (microelements) nutrient solutions. Plants were maintained in an air-conditioned room at the temperature of 25 to 27 °C with 200 μmol m^{-2} s^{-1} photon flux density during a 16 h photoperiod. 15 to 30-d-old plants were used throughout the experiments. The abaxial epidermis was peeled from fully expanded leaves of the 3rd leaf storey, being cut into *ca.* 3 × 10 mm strips. The epidermal strips were incubated in dishes containing 2 cm^3 of medium during 3 h at 25 ± 1 °C in dark or light (photon flux density of 120 μmol m^{-2} s^{-1}) in the presence (350 μmol mol^{-1}) or absence of CO_2. The standard incubation medium consisted of 10 mM MES buffer (2[*N*-morpholino]ethanesulfonic acid) adjusted to pH 6.15 with KOH; ABA (mixed isomers of 90% purity from *Sigma*, St. Louis, USA) 10-5 to 1 mM, IAA (*L. Light*, Colnbrook, Bucks, England) 10^{-5} to 1 mM and KCl (50 or 100 mM) singly or in combination were incorporated into buffer.

Stomatal apertures were measured under the microscope fitted with a calibrated ocular micrometer. Stomatal potassium was stained histochemically as cobaltous sulfide (Willmer and Mansfield 1970). Histochemical reactions were carried out with epidermal strips after incubation in buffered solutions of ABA (10^{-5} to 1 mM) and IAA (10^{-5} to 1 mM), in the absence of CO_2 in light or in dark (standard incubation medium: MES 10 mM + 50 mM KCl). Dark manipulations and measurements were carried out under green light and a green filter was placed on the condensor of the microscope. The measured values were further evaluated using the two-sample analysis.

Results and discussion

ABA acts as an inhibitor, while IAA as a stimulator, on stomatal apertures (Fig. 1). Both effects were observed in the whole range of concentrations used in the light and in the dark in the presence and absence of CO_2.

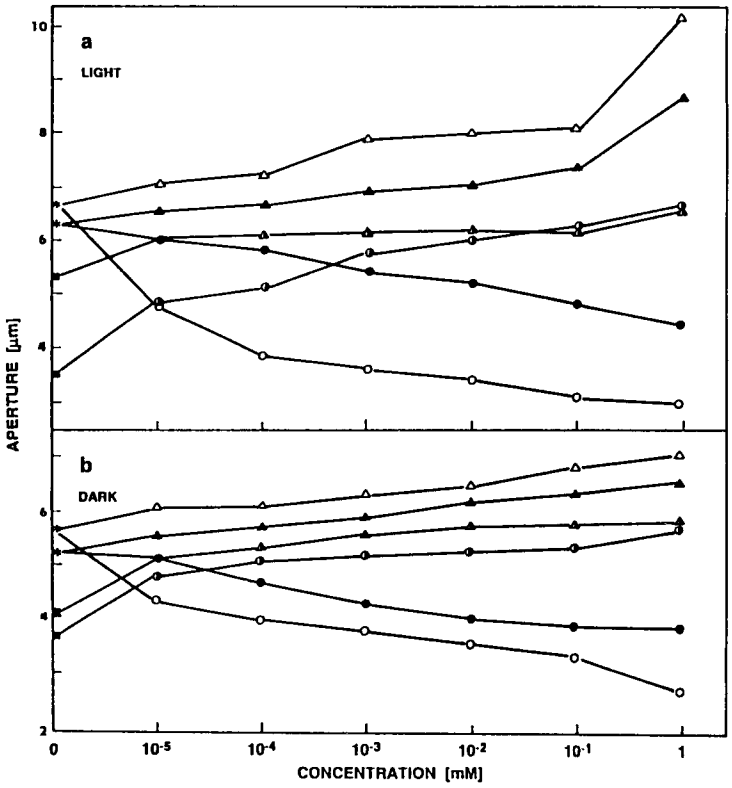

Fig. 1. Stomatal apertures on detached abaxial epidermal strips of *Vicia faba* after incubation in a range of buffered ABA (10^{-5} to 1 mM), IAA (10^{-5} to 1 mM) or IAA+ABA (IAA: 10^{-5} to 1 mM + ABA: 10^{-2} mM) media in the presence (+CO_2) or absence (-CO_2) of CO_2, under irradiance (*a*) or in darkness (*b*). IAA -CO_2 (*open triagles*), IAA +CO_2 (*closed triangles*), IAA + ABA +CO_2 (*semiclosed triangles*), IAA + ABA -CO_2 (*semiclosed circles*), ABA - CO_2 (*open circles*), ABA + CO_2 (*closed circles*), 10 mM MES (*asterisks*), 10 mM MES + 10^{-2} mM ABA (*closed squares*). Each point is the mean of 60 measurements. Error bars are omitted, but all standard errors were < ± 0.12 µm.

The inhibiting influence of ABA on stomatal apertures has also been found by other authors in various plants, for example, on epidermal strips of *Commelina communis* (Tucker and Mansfield 1971, Snaith and Mansfield 1982), *Vicia faba* (Horton 1971, Vicherková 1988, 1989), or on intact leaves of *Xanthium strumarium* (Jones and Mansfield 1970).

Epidermal strips being incubated in the buffer alone showed the stomatal apertures to be significantly smaller in the presence than in the absence of CO_2 in light and in dark. This also agrees with the hitherto published results of certain authors (Wilson 1981) and this dependence apparently applies to the other plant species as well, though there is a considerable variability of the results obtained by different authors. In the incubation media with ABA the ascertained dependence on CO_2 was quite contrary. In the presence of CO_2, stomatal apertures were in this case greater than in the CO_2-free air both in light and in dark. The differences were highly significant in the whole range of concentrations. It is possible that stomata can restrict their closing under water-stress by means of these contrary effects of ABA and CO_2 on the stomatal apertures.

Table 1. Histochemical reactions of potassium in *Vicia faba* guard cells after 3 h incubation under absence of CO_2 in light and in dark. MES - basic incubation medium (10 mM MES + 50 mM KCl), -- CoS is absent, - CoS in 10 % of cell areas, + CoS in 11 to 30 % of cell areas, ++ CoS in 31 to 50 % of cell areas, +++ CoS in more than 51 % of cell areas.

Incubation medium	Histochemical reaction of K^+	
	light	dark
MES	++	
MES + ABA 10^{-5} mM	+	-
MES + ABA 10^{-4} mM	+	-
MES + ABA 10^{-3} mM	-	--
MES + ABA 10^{-2} mM	--	--
MES + ABA 10^{-1} mM	--	--
MES + ABA 10^{-0} mM	--	--
MES + IAA 10^{-5} mM	++	-
MES + IAA 10^{-4} mM	++	-
MES + IAA 10^{-3} mM	++	-
MES + IAA 10^{-2} mM	+++	+
MES + IAA 10^{-1} mM	+++	+
MES + IAA 10^{-0} mM	+++	+

It is beyond dispute that the ion concentration of the incubation medium has a fundamental influence on the results obtained at a given irradiance and CO_2 relations (Willmer and Mansfield 1970, Vicherková and Kostřica 1977, Jarvis and Mansfield 1980, Vicherková 1989), and our experiments fully confirm this fact, too (Fig. 2). When the buffered incubation medium with ABA was enriched with KCl (50 or 100 mM), the aperture sizes were markedly increased. However, ABA was able even in the presence of KCl, to diminish apertures the more, the greater its concentration was (*cf.* Vicherková 1988). The correlation between the content of K^+ and the increase in aperture size was high, and ABA was unable, even in the highest concentration used, to abolish the differences in aperture sizes due to different KCl concentrations.

In the incubation media ABA + KCl the same effect of CO_2 was seen as in the absence of KCl. Stomatal apertures were greater in the presence of CO_2 than in its absence. The same relationship remained unchanged also in the standard buffered

incubation medium with 50 or 100 mM KCl (without ABA). This effect of CO_2 on stomata of the isolated epidermis of *Vicia faba* was also observed in the absence of ABA in the non-buffered media with 10 mM KCl (Spence *et al.* 1984 a, b).

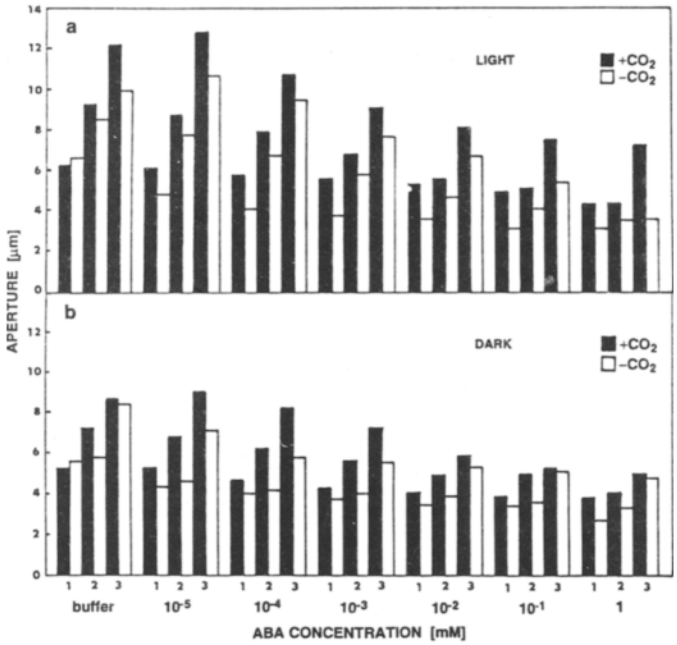

Fig. 2. Effect of different concentrations of KCl (1 = without KCl, 2 = 50 mM KCl, 3 = 100 mM KCl) on stomatal apertures of *Vicia faba* after incubation in a range of ABA concentrations in the presence (+CO_2) or absence (-CO_2) of CO_2, in the light (*a*) or in dark (*b*). Each point is the mean of 60 measurements. Error bars are omitted, but standard errors were < ± 0.18 µm.

ABA is evidently able to influence the permeability of plasma membranes (Lea and Collins 1979). Instead of the earlier view that ABA inhibits ion uptake by guard cells, the recent findings indicate that ABA affects stomatal aperture by stimulating the K^+ efflux rather than by inhibiting their influx (Weyers and Hillman 1980). Histochemical reactions to K^+ in the guard cells (Table 1) confirmed an obvious decrease of K^+ content with the increasing concentrations of ABA in the incubation solutions of our experiments both in light and dark.

An effect, quite opposite to that of ABA, was determined by histochemical reactions to K^+ for IAA (Table 1) which stimulated K^+ accumulation in the guard cells. The influence of IAA was strongly dependent on concentration levels: the higher was the IAA concentration, the more K^+ was contained in the guard cells. This finding is in full agreement with the results of Pemadasa (1982), who firstly demonstrated the stimulating activity of IAA in conjunction with an increase of K^+ content in the stomatal apparatus of *C. communis*.

It follows from our experiments that IAA also enhances stomatal opening of the abaxial epidermis of *Vicia faba*, that can be seen under all incubation conditions and in the whole range of IAA concentrations. This also agrees with the results of Pemadasa (1982) and Snaith and Mansfield (1982) for *C. communis*, and of Levitt *et al.* (1987) for *V. faba*. In the presence of CO_2 (+ IAA), stomatal apertures were significantly lower than in the absence of CO_2, and this dependence was seen, without exception, in the whole range of IAA concentrations in light as well as in dark. Also, the greatest apertures were achieved in light in the absence of CO_2, while the smallest were obtained in the presence of CO_2 in dark. CO_2 then was clearly seen to preserve in this case its inhibitory effect, although, due to IAA, the apertures were markedly increased.

The incubation of *V. faba* epidermis in the mixture of IAA (10^{-5} to 1 mM) and ABA (10^{-2}) lead to the greater apertures than when the incubation tooks place in the media with ABA alone. In the presence of CO_2 stomatal apertures are not smaller (as in the case with IAA) but, on the contrary, they are greater, both in light and dark. The sizes of apertures increase with increasing IAA concentration. Thus it seems that IAA may regenerate the ABA-inhibited opening. Snaith and Mansfield (1982), who tested the the effect of IAA on the stomata of *C. communis*, also showed that IAA in the incubation medium markedly influenced stomatal responses. These authors, though not having found the effect of IAA in the absence of CO_2, did demonstrate the opening of stomata to be clearly dependent on the concentration of IAA in the presence of CO_2 (700 μmol mol^{-1}). It is then probable, and our results support such assumption, that there exists an interaction between CO_2, ABA and IAA.

References

Blackman, P. G., Davies, W. J.: Modification of the CO_2 responses of maize stomata by abscisic acid and by naturally occuring synthetic cytokinins. - J. exp. Bot. **35** : 174-179, 1984.

Eamus, D., Wilson, J. M.: A model for the interaction of low temperature, ABA, IAA and CO_2 in the control of stomatal behaviour. - J. exp. Bot. **35** : 91-98, 1984.

Horton, R.F.: Stomatal opening: the role of abscisic acid. - Can. J. Bot. **49** : 583-585, 1971.

Jarvis, R. G., Mansfield, T. A.: Reduced stomatal responses to light, carbon dioxide and abscisic acid in the presence of sodium ions. - Plant Cell Environ. **3** : 279-283, 1980.

Jones, R. J., Mansfield, T. A.: Suppression of stomatal opening in leaves treated with abscisic acid. - J. exp. Bot. **21** : 714-719, 1970.

Lea, E. J. A., Collins, J. C.: The effect of the plant hormone abscisic acid on lipid bilayer membranes. - New Phytol. **82** : 11-18, 1979.

Levitt, L. K., Stein, D. B., Rubinstein, B.: Promotion of stomatal opening by indoleacetic acid and ethrel in epidermal strips of *Vicia faba* L. - Plant Physiol. 85 : 318-321, 1987.

Mansfield, T. A.: Delay in the response of stomata to abscisic acid in CO_2-free air. - J. exp. Bot. **27**: 559-564, 1976.

Mansfield, T. A., Wilson, J. A.: Regulation of gas exchange in water-stressed plants. - In: Johnson, C. B. (ed.) : Physiological Processes Limiting Plant Productivity. Pp. 237-251. Butterworths, London 1981.

Orton, P. J., Mansfield, T. A.: The activity of abscisic acid analogues as inhibitors of stomatal opening. - Planta **121** : 263-272, 1974.

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

Raschke, K.: Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium strumarium* L. - Planta **125** : 243-259, 1975a.

Raschke, K.: Stomatal action. - Annu. Rev. Plant Physiol. **26** : 309-340, 1975b.

Snaith, P. J., Mansfield, T. A.: Control of the CO_2 responses of stomata by indolyl-3-acetic acid and abscisic acid. - J. exp. Bot. **33** : 360-365, 1982.

Spence, R. D., Sharpe, P. J. H., Powell, R. D., Wu, H.: Responses of guard cells to temperature at different concentrations of carbon dioxide in *Vicia faba* L. - New Phytol. **97** : 129-144, 1984a.

Spence, R. D., Sharpe, P. J. H., Powell, R. D.: The role of the epidermal cells in the carbon dioxide response of stomata of *Vicia faba* L. - New Phytol. **97** : 145-154, 1984b.

Tucker, D. J., Mansfield, T.A.: A simple bioassay for detecting "antitranspirant" activity of naturally occuring compounds such as abscisic acid. - Planta **98** : 157-163, 1971.

Vicherková, M.: Interaction of calcium, ABA and potassium influencing stomatal movement regulation in *Vicia faba* L. - In: 6th Congress of FESPP. Plant Water Relations. Pp. 9-33. FESPP, Split 1988.

Vicherková, M.: Effect of abscisic acid on stomatal closing at presence of potassium and sodium ions. [In Czech.]. - In : Dny Rostlinné Fyziologie V. P. 90. ČSBS, Brno 1989.

Vicherková, M., Kostřica, P.: The influence of potassium on stomatal apertures in *Vicia faba* L. cultivated under sufficient and deficient potassium nutrition. - [In Czech]. - In: Dni Rastlinnej Fyziológie I. Pp. 86-89. ČSBS, Bratislava 1977.

Weyers, J. D. B., Hillman, J. R.: Effect of abscisic acid on $^{86}Rb^+$ fluxes in *Commelina communis* L. leaf epidermis. - J. exp. Bot. **31** : 711-720, 1980.

Willmer, C. M., Mansfield, T. A.: Further observations on cation-stimulated stomatal opening in isolated epidermis. - New Phytol. **69** : 639-645, 1970.

Wilson, J. A.: Stomatal responses to applied ABA and CO_2 in epidermis detached from well-watered and water-stressed plants of *Commelina communis* L. - J. exp. Bot. **32** : 261-269, 1981.