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REVERSAL OF ABSCISIC ACID INDUCED STOMATAL CLOSURE BY BENZYL ADENINE

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SUMMARY

Benzyl adenine enhanced stomatal opening in isolated epidermal strips of *Commelina benghalensis* and *Tridax procumbens*. The stimulation was maximum at a concentration of 5×10^{-5} M BA. But kinetin had no remarkable effect on stomatal opening. The activity of benzyl adenine was observed over a narrow range of concentrations, from 10^{-5} M to 10^{-4} M. The increase in stomatal aperture was more pronounced in *Commelina* than in *Tridax*. Presence of benzyl adenine in the medium prevented the stomatal closure expected from abscisic acid and could reverse considerably the stomatal closure induced earlier by abscisic acid. It is felt that the balance between abscisic acid and cytokinins can possibly control the stomatal aperture effectively.

INTRODUCTION

The control of stomatal aperture and transpiration through the exogenous application of chemicals has been receiving considerable attention. Abscisic acid (ABA) is a powerful inhibitor of stomatal movement. It significantly reduced the stomatal aperture when sprayed on to leaves (Mittleheuser and Vansteveninck, 1969, 1971; Jones and Mansfield, 1970; Talha and Larsen, 1975) and was considered to be as an ideal antitranspirant. On the other hand, transpiration and stomatal opening were increased by the application of cytokinins (Livne and Vaadia, 1965; Meidner, 1967; Luke and Freeman, 1967).

Abscisic acid was effective in decreasing the stomatal aperture even in isolated epidermal strips. But stomata did not respond to kinetin in epidermal strips (Tucker and Mansfield, 1971; Horton, 1971). A recent study indicated the significant interaction between ABA and kinetin on stomatal opening in barley (Cooper, Digby and Cooper, 1972). In view of the conflicting evidence, we examined in detail the influence of cytokinins, kinetin and benzyl adenine (BA) and their interaction with ABA.

MATERIALS AND METHODS

Lower epidermal strips of about 1.0×0.5 cm size were prepared from the leaves of *Commelina benghalensis* L. and *Tridax procumbens* L. (Raghavendra and Das, 1972). The incubation medium for stomatal opening in light was 0.067 M phosphate buffer at pH 7.0 with 10 mM KCl, the various additional compounds were included when

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required at the given final concentrations and the pH of the incubation medium was checked and adjusted to 7.0.

The epidermal strips were floated on 20 ml of incubation medium in Petri dishes of 5 cm diameter either illuminated or kept in the dark. The light source was a bank of incandescent bulbs and the light intensity after passing through the water filter was 12 Klux at the surface of the experimental material. The temperature was maintained at $27 \pm 2°C$. At regular intervals one of the epidermal strips was removed out and examined under the microscope. The size of stomatal aperture was measured with the help of a precalibrated occular micrometer. Each time an average of thirty stomata selected at random was calculated. The experiment was repeated at least thrice on different days. The average of these is reported.

Abscisic acid was dissolved in a drop of saturated Na_2CO_3 solution, whereas kinetin and BA were dissolved in 1 N NaOH. They were diluted with a few millilitres of distilled water, pH adjusted to 7.0 and made up to required volume with 0.067 M phosphate buffer pH 7.0.

Results

Only benzyl adenine enhanced stomatal opening in epidermal strips of *Commelina benghalensis* L. and *Tridax procumbens* (Table 1). Kinetin exerted no remarkable effect.

Table 1. *Effect of kinetin and benzyl adenine on stomatal opening in* Commelina benghalensis *and* Tridax procumbens

Treatment	Stomatal aperture (μm)	
	C. benghalensis	*T. procumbens*
Initial*	4.2	2.4
After illumination†		
Control	12.7	8.5
+ Kinetin 10^{-5} M	11.8	8.6
+ Kinetin 5×10^{-5} M	12.9	8.8
+ Kinetin 10^{-4} M	12.5	8.4
+ BA 10^{-5} M	13.2	8.8
+ BA 5×10^{-5} M	17.6	9.8
+ BA 10^{-4} M	12.8	9.0

* At the start of the experiment.
† At an intensity of 12 Klux for 5 h.

The stimulatory effect was more pronounced in case of *Commelina*, but it was also quite significant in *Tridax*. Fig. 1 shows the critical importance of the concentration of BA in stimulation of stomatal opening. Since the increase of aperture was maximum in 5×10^{-5} M BA. This concentration was used in the following experiments.

Stomata of *Commelina benghalensis* opened wider in 5×10^{-5} M BA but the stimulation was partially suppressed by the presence of ABA in the medium (Fig. 2). The stomata closed when ABA was alone present in the medium, but it was evident that BA maintained the stomatal opening to a remarkable level even in the presence of ABA. Addition of BA rapidly reversed the closure induced earlier by ABA. Both the action of ABA as well as the reversal of it by BA was rapid.

Discussion

The relative insensitivity of stomatal aperture to kinetin and the narrow range of effective concentrations of BA, clarify the controversies which arise from previous observations.

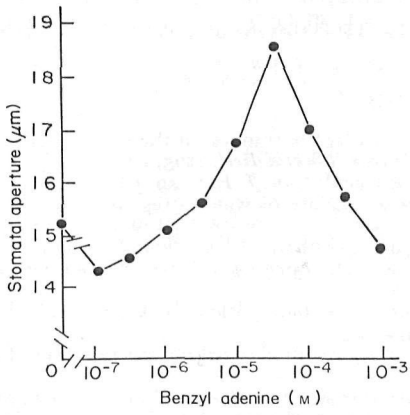

Fig. 1

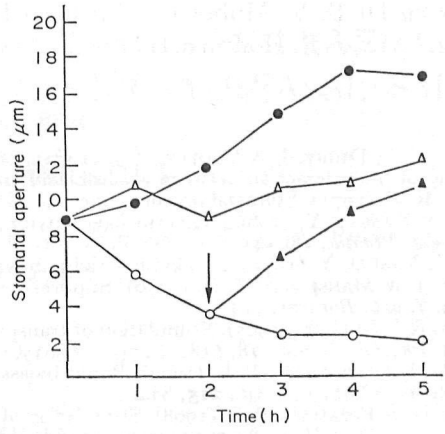

Fig. 2

Fig. 1. Stimulation of stomatal opening by benzyl adenine in isolated epidermal strips of *Commelina benghalensis*. The epidermal strips were incubated in a light of 12 Klux.

Fig. 2. Reversal of abscisic acid induced stomatal closure by benzyl adenine. The epidermal strips of *Commelina benghalensis* were incubated in a light of 12 Klux. Stomata opened as usual in control sets (●) whereas the inclusion of ABA in the medium (○) closed them. However ABA induced stomatal closure was rapidly reversed when BA was added after two hours (▲). The arrow indicates the addition of BA. The course of stomatal opening in presence of both BA and ABA (△) is also shown.

The failure of stomata in epidermal strips to respond to kinetin is in agreement with the findings of Tucker and Mansfield (1971). Horton (1971) also could not find any effect of either BA or kinetin on the stomatal movement in *Vicia*. The present findings make it clear that BA can significantly enhance stomatal opening, particularly within a limited range of concentration. Previous reports have indicated a stimulatory effect of kinetin on stomatal opening and transpiration in leaves (Livne and Vaadia, 1965; Meidner, 1967; Luke and Freeman, 1967, 1968; Cooper *et al.*, 1972).

The stomata of *Commelina benghalensis* responded more than those of *Tridax procumbens* to the presence of BA in the medium. Such a differential response strikes a similarity to the earlier findings of Luke and Freeman (1968) and Cooper *et al.* (1972) who suggested that the stimulatory effects of kinetin might be confined to graminaceous genera. The reasons for such differences can be explained only after further detailed investigations.

There is convincing evidence for the possible control of transpiration in leaves by a balance between cytokinins and abscisic acid. It is now well established that leaves of water-stressed plants contain more ABA (Wright, 1969; Milborrow and Noddle, 1970; Zeevart, 1971; Most, 1971) and less cytokinins (Itai and Vaadia, 1965, 1971). Detailed investigations with the wilty mutant of tomato also indicated that wilting of plants was associated with the increase in cytokinin content and decrease in ABA (Tal and Imber, 1970, 1971; Tal, Imber and Itai, 1970).

It seems clear that ABA and BA can cause stomatal opening or closure depending upon their relative contents. This fact reflects the key importance of their level in the leaf in controlling the water loss from the plants.

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