

## Stomatal response of chlorocholine chloride and indole-3-acetic acid in *Commelina communis* L.

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**Abstract.** The apertures of stomata on isolated epidermal peels from both the leaf surfaces of *Commelina communis* were reduced in response to chlorocholine chloride as the concentrations increased from 0.01–10.0 mol m<sup>-3</sup>. When chlorocholine chloride was applied in combination with different concentrations of indoleacetic acid, stomatal closure occurred only when a high concentration of chlorocholine chloride was applied with a low concentration of indoleacetic acid, while low concentrations of chlorocholine chloride did not affect the stimulation of aperture caused by indoleacetic acid treatments. Chlorocholine chloride treatments caused depletion of K<sup>+</sup> from the guard cells while indoleacetic acid resulted in accumulation of K<sup>+</sup>.

**Keywords.** Stomatal response; chlorocholine chloride; indoleacetic acid; *Commelina communis* L.

### 1. Introduction

Control of stomatal behaviour by application of exogenous chemicals which induce their partial closure, in order to check excessive transpirational rates and improve water economy without causing any damaging side effects on the metabolism of plants, would be of great practical importance. It could, for example, be used on field crops to overcome temporary water deficits. A large number of compounds have been screened and tested on different plant species but they are unsatisfactory because they have some side effects or their effects are short lived (Mansfield and Davies 1981). Chlorocholine chloride (ccc) and its allied compounds have been found to increase tolerance to salinity (El Domaty *et al* 1964) and to improve drought resistance (Halevy and Kessler 1963). The present experiments have been performed to investigate the impact of ccc on the opening and closing mechanisms of stomata on isolated epidermal peels from leaves of *Commelina communis*, and its interactions with indoleacetic acid (IAA).

### 2. Materials and methods

Plants of *C. communis* L. grown in a greenhouse of the Department of Biological Sciences, Lancaster University, Lancaster were used and the techniques for studying stomatal opening and closing were similar to those described by Pemadasa (1981). The plants were first transferred to a growth chamber maintained at 25°C ± 1°C at 95% RH and provided with 140–150 μmol m<sup>-2</sup> s<sup>-1</sup> photon flux density, 2–3 days prior to experimentation, so as to acclimatise them to experimental conditions. Two young fully expanded leaves from plants 5–6 weeks old were removed and floated on distilled water with their dorsal surfaces facing upward. From these leaves 5 mm wide strips were cut

from the lamina parallel to the mid-vein avoiding the midrib, and epidermal peels from adaxial and abaxial surfaces of the same strips were removed. The detached epidermal peels were then floated over distilled water with their cuticular surfaces facing upward. These epidermal strips were cut into pieces  $10 \times 5$  mm in size, and 3 pieces were selected randomly for each treatment. The stomatal apertures of 10 stomata taken at random from each epidermal piece were measured after the incubation period, with a Watson Hilux 70 microscope fitted with a projection eye piece.

The epidermal pieces were incubated in 10 ml solution in 5 mm diameter petridishes containing  $10 \text{ mol m}^{-3}$  morpholino ethane sulphonic acid (MES) buffer at pH 6.15 together with  $50 \text{ mol m}^{-3}$  KCl, supplemented with CCC and IAA separately or in their various combinations as stated in table 1. The petridishes were placed in a glass water bath at a temperature of  $25^\circ\text{C} \pm 1^\circ\text{C}$  and illuminated from below by fluorescent tubes which provided white light of  $140\text{--}150 \mu\text{mol m}^{-2} \text{ s}^{-2}$  photon flux density.  $\text{CO}_2$  free air was bubbled into the medium at  $100 \text{ cm}^3 \text{ m}^{-1}$  through hypodermic needles. The duration of incubation was 3 hr and the experiments were performed between 9 AM and 1 PM (GMT).

Histochemical localization of  $\text{K}^+$  in the guard cells was achieved using the techniques of Willmer and Mansfield (1970) and the amount of  $\text{K}^+$  was assessed subjectively by arbitrary scoring. Six main categories were recognised (0–5) depending upon the amount of black precipitate of cobalt sulphide present in the guard cells (Pemadasa 1979a,b) and the results were evaluated statistically.

### 3. Results

Two types of experiments were performed to investigate the mechanism of opening and closing of stomata of *C. communis* in response to the CCC treatments. The responses of stomata to adaxial and abaxial surfaces were studied with different concentrations of CCC (0.01, 0.1, 1 and  $10 \text{ mol m}^{-3}$ ) on an optimal incubation medium ( $10 \text{ mol m}^{-3}$  MES +  $50 \text{ mol m}^{-3}$  KCl at 6.15 pH) in light and  $\text{CO}_2$ -free air. The stomata of both the surfaces responded to CCC by closing, the magnitude of response increasing with the CCC concentration from 0.01– $10 \text{ mol m}^{-3}$ . On the ventral surface the stomata were,

**Table 1.** Showing the presence of  $\text{K}^+$  in guard cells as revealed by black precipitate.

ccc $\text{mol m}^{-3}$	IAA $\text{mol m}^{-3}$			
	–IAA	0.01 IAA	0.1 IAA	1 IAA
–ccc	2	<2	2	> 3
0.01	2	<2	2	3
0.1	<2	<2	<2	3
1.0	<1	<2	<2	<2
10	0	<1	<1	<1

(0), Absent; (1), black grains present; (2), 1/4 guard cells covered with black precipitate; (3), 1/2 guard cells covered with black precipitate; (4), 3/4 guard cells covered with black precipitate; (5), whole guard cell covered with black precipitate.

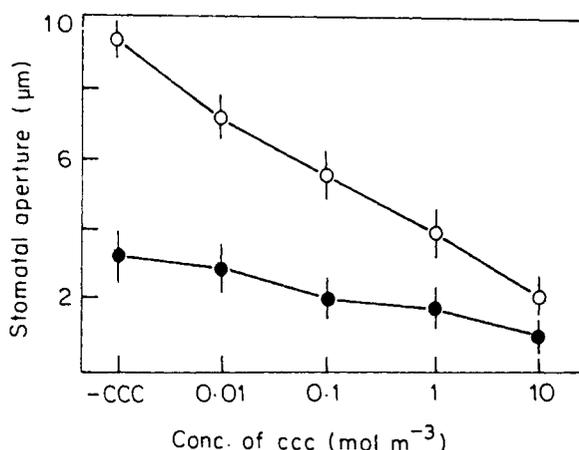
however, almost closed in  $10 \text{ mol m}^{-3}$  CCC while on the dorsal surface they were partially open in this concentration (figure 1).

In the second set of experiments the incubation medium was supplemented with CCC (0.1, 1 and  $10 \text{ mol m}^{-3}$ ) and different regimes of IAA (0.01, 0.1 and  $1 \text{ mol m}^{-3}$ ). The experiments were performed under similar conditions of light, temperature and  $\text{CO}_2$ -free air as in those described previously. It was observed that IAA alone in the absence of CCC removed the differential opening of stomata of adaxial and abaxial surfaces (figures 2 and 3). The apertures of stomata on both the surfaces was almost the same, and this effect was most pronounced in IAA  $1 \text{ mol m}^{-3}$ , while in the other two concentrations it was less evident. With CCC and varying concentrations of IAA it was observed that in the highest concentration of CCC ( $10 \text{ mol m}^{-3}$ ) and the lowest concentration of IAA ( $0.01 \text{ mol m}^{-3}$ ) the stomata closed on both the surfaces, but higher concentrations of IAA (1 and  $0.1 \text{ mol m}^{-3}$ ) and lower concentrations of CCC (1,  $0.1 \text{ mol m}^{-3}$ ) produced no significant change in the apertures obtained (figures 2 and 3).

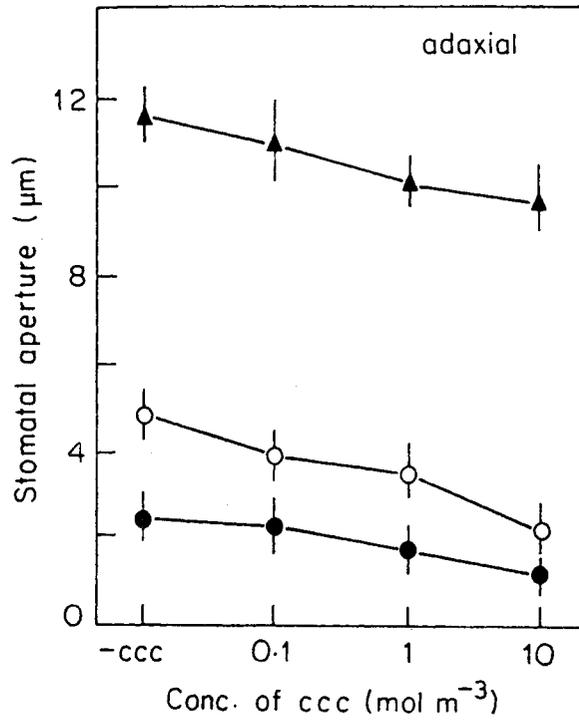
To study the effect of CCC on  $\text{K}^+$  influx and efflux in the guard cells, the histochemical localization of  $\text{K}^+$  was carried out in the epidermal peels incubated with different concentrations of CCC and IAA, alone or in combinations. It was observed that CCC ( $10 \text{ mol m}^{-3}$ ) reduced the influx of  $\text{K}^+$  from the incubating medium, as there were only a few dark grains of cobalt sulphide dispersed in the guard cells while more  $\text{K}^+$  was observed in the epidermal cells. On the other hand in a medium without CCC, and in a medium with IAA alone, large amounts of  $\text{K}^+$  were found the arbitrary scores being 2 and 3 respectively (table 1).

#### 4. Discussion

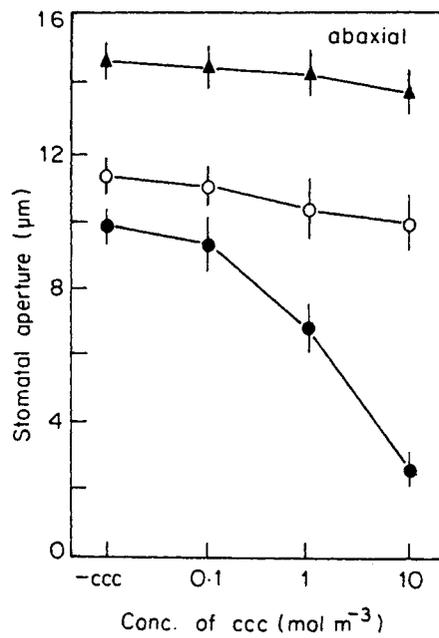
Plant cell membranes are greatly influenced by the application of exogenous growth substances. Scalla and Gauvrit (1983) proposed that compounds with herbicidal properties can interact with plant membranes either by binding themselves on to the membranous site, thus altering the permeability or fluidity of the membrane, or by



**Figure 1.** Mean stomatal aperture of adaxial and abaxial surfaces in response to CCC concentrations (○, adaxial; ●, abaxial). Vertical bars represent SD.



**Figure 2.** Mean stomatal aperture of adaxial surface in response to different regime of CCC and IAA (○, ●, ▲ represent 0.01, 0.1 and 1 mol m<sup>-3</sup> IAA). Vertical bars represent SD.



**Figure 3.** Mean stomatal aperture of abaxial surface in response to different regime of CCC and IAA, (○, ●, ▲ represent 0.01, 0.1 and 1 mol m<sup>-3</sup> IAA). Vertical bars represent SD.

exerting indirect effects on membranes which may lead to cellular death. Blein and Scalla (1983) reported that the herbicide lanacil stimulates  $K^+$  uptake in *Acer* cells. The role of  $K^+$  influx and efflux in changing the solute potential of guard cells is well established (Raschke 1975). Influx of  $K^+$  depends on the membrane potential generated by proton extrusion and the permeability of the membrane to  $K^+$ , while efflux may occur along the concentration gradient (Travis and Mansfield 1979a,b). Thus a net gain of  $K^+$  by the guard cells results in stomatal opening and a net loss causes closure. The present experiments reveal that CCC results in the closure of stomata of both adaxial and abaxial surfaces and the extent of closure depends upon the concentration of CCC. CCC also negates the effect of IAA, which stimulates stomatal opening on adaxial epidermis, removing or reducing the difference in opening between the adaxial and abaxial surfaces. A high concentration of CCC ( $10 \text{ mol m}^{-3}$ ) caused a considerable reduction in stomatal aperture even in the presence of IAA (0.01, 0.1 and  $1 \text{ mol m}^{-3}$ ).

Histochemical localization of  $K^+$  in the guard cells gave results that correspond with the stomatal apertures recorded. In CCC-treated epidermal peels, the  $K^+$  accumulation was less in the guard cells and more in the epidermal cells, but in IAA-treated peels more  $K^+$  accumulated in guard cells than in epidermal cells. The combination of a high concentration CCC and a low concentration of IAA resulted in the closure of stomata, but in a low concentration of CCC and a high concentration of IAA the stomata remained open though their apertures were reduced. Thus it appears that CCC either binds itself on to the membrane sites or causes a change in permeability or fluidity of the membrane, resulting in the inhibition of influx of  $K^+$ , or an accelerated efflux of  $K^+$ , from the guard cells. Its antagonistic effect with IAA reveals that it may be either binding itself on to the site of IAA action on the membrane acting competitively with IAA.

Another important known property of CCC is that it increases the salt tolerance (El Damaty *et al* 1964) and drought resistance (Halevy and Kessler 1963; Larter *et al* 1965) in plants. Singh *et al* (1973) observed that after the application of CCC, there is a retardation in growth of the wheat plant, but there is no change in water potential of the leaves when the plants were subjected to water stress; simultaneously, however, there is a high accumulation of proteins in the leaves. Although there is no record of the effect of CCC on guard cell membranes or on the proline content of these cells, it appears from the present experiments that CCC either acts directly on guard cell membranes or it acts through the synthesis of proline or similar compounds like betaine which may affect the membrane leading to reduced  $K^+$  in guard cells, and causing stomatal closure. Finally, it may be suggested that the external application of CCC could to some extent help in overcoming a period of drought, but trials are needed before undertaking such practice on a larger scale.

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