Stomatal Morphology and Effect of Potassium Chloride, Sodium Chloride, Calcium Chloride and Abscisic Acid in the Stomatal Regulation of two Species of *Commelina* L.

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SUMMARY

The structure of mature epidermis of *Commelina benghalensis* L. and *C. erecta* L. is described. The leaves of both taxa are hypostomatic, mature hexacytic stomata having been found only on the abaxial surface. Stomatal abnormalities recorded include superposed contiguous stomata in *C. benghalensis*, aborted stomata in *C. erecta* and single guard celled stomata in both taxa.

Different concentrations of potassium chloride (KCl), sodium chloride (NaCl) and calcium chloride ($CaCl_2$) influenced the stomatal opening. A concentration of 0.75M KCl was most effective in causing stomatal opening in *C. erecta* while 0.25M $CaCl_2$ induced the widest opening in *C. benghalensis*. Abscisic acid (ABA) caused closure of KCl, NaCl and $CaCl_2$ induced open stomata of the two taxa. However, these chemicals were able to reverse to some extent stomatal closure induced by ABA.

INTRODUCTION

According to Chawan (1978) epidermal surfaces of most land plants present major barriers to the escape of water vapour and to the exchange of gases with the atmosphere. Stomatal pores which penetrate the epidermis are major ports of exit and entry to the interior of the plant. Since these pores can be opened and closed by the very specialised cells, the guard cells, which surround them, regulate vapour and gas exchanges between the plant and its environment. Thus stomata exert a profound influence on the physiology of the plant.

Stomatal movement is known to be influenced by a number of factors. Glinka (1971), has shown that changes in the turgor of the guard cells and the neighbouring epidermal cells cause stomatal movement. According to Bhandari and Sen (1974) plasmolysis of the epidermal cells is the cause of stomatal movement. The importance of starch sugar conversion in the guard cells in regulating stomatal movement has also been stressed by various authors (Raschke and Fellows 1971 and Bhandari and Sen 1972).

Monovalent cations such as K^+, Li^+, and K^+ are known to stimulate stomatal opening whereas divalent cations such as Ca^{2+} and Mg^{2+} can depress opening (Wilmer and Mansfield 1970; Sen 1973; Bhandari and Sen 1974 and Chawan 1978). The role of abscisic acid (ABA) in stomatal

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movement has also been reported; (Jones and Mansfield, 1972; Horton 1971; Sen and Chawan 1975 and Chawan 1978).

The epidermal structure and the organisation of the stomatal complex of some taxa of the family Commelinaceae have previously been described (Stebbins and Khush 1961; Tomlinson, 1969, and Nayawuame and Gill 1990). However, available literature indicates that apart from the work of Wilmer and Mansfield (1970) stomatal movement in the genus *Commelina* has not preciously been investigated. The present study has therefore been undertaken to provide information on (1) the epidermal structure and variation in the organisation of the stomatal complex and (2) to put on record the role of K^+, Na^+ and Ca^{2+} cations as well as ABA on stomatal movement in *C. benghalensis* L. and *C. erecta* L., the two common weeds of moist waste places.

MATERIALS AND METHODS

Foliar materials used during the present study have been collected fresh from plants growing in natural conditions in the environs of Benin City (Lat. 6.5°N, Long. 6.0°W). Epidermal peelings were obtained following the method of Gill and Karatela (1983). The peels were stained with 1% safranin solution in 50% aqueous ethanol and mounted in pure glycerine on a glass slide. Ten preparations were made for *C. benghalensis* and *C. erecta*. Stomatal measurements are the averages of 100 readings. Line drawings of the epidermal structure have been made at a uniform magnification of x260 using the light microscope.

Different sets of epidermal peelings were used to study stomatal its movement in the two taxa. The peelings were floated in respective concentrations (0.10, 0.25, 0.50, 0.75 and 1.0 M) of KCl, NaCl, and $CaCl_2$ solutions. Distilled water (DW) served as control. The peelings were incubated for three hours in these solutions under diffused light conditions at room temperature (30 ± 2°C). In another experiment the peelings were incubated for three hours in optimum concentrations of KCl, NaCl and $CaCl_2$ solutions before transforming to 10^{-3} M ABA solution for one hour incubation period. The peels were transferred back to their respective solutions of KCl, NaCl and $CaCl_2$ for three hours in cubation period. The width of stomatal aperture was measured at the end of each incubation period. The measurements are the means of at least 50 stomata at each concentration.

RESULTS AND DISCUSSION

The structure of mature epidermis along with stomatal features of *C. benghalensis* and *C. erecta* have been summarized in table 1. Stomatal abnormalities recorded are presented in Fig. 1a - d. The effects of the various concentrations of KCl, NaCl, and $CaCl_2$ solutions on stomatal aperture are summarized in tables 2a and 2b. The effect of interaction of ABA with these solutions on stomatal aperture is given in Tables 3a and 3b.

Earlier contributions to the phytodermology of the genus *Commelina* are by Stebbins and Khush (1961) and Tomlinson (1969, 1974). Tomlinson (1969) states that both the upper and lower epidermis of members of the family Commelinaceae are composed of polygonal cells with straight anticlinal and periclinal walls. He further states that trichomes on the epidermal surface may be unicellular or multicellular. During the present study, epidermal cells in both *C. benghalensis* and *C. erecta* have been found to be regular with straight anticlinal and periclinal walls. Both unicellular and bicellular eglandular trichomes have been recorded in the two taxa.

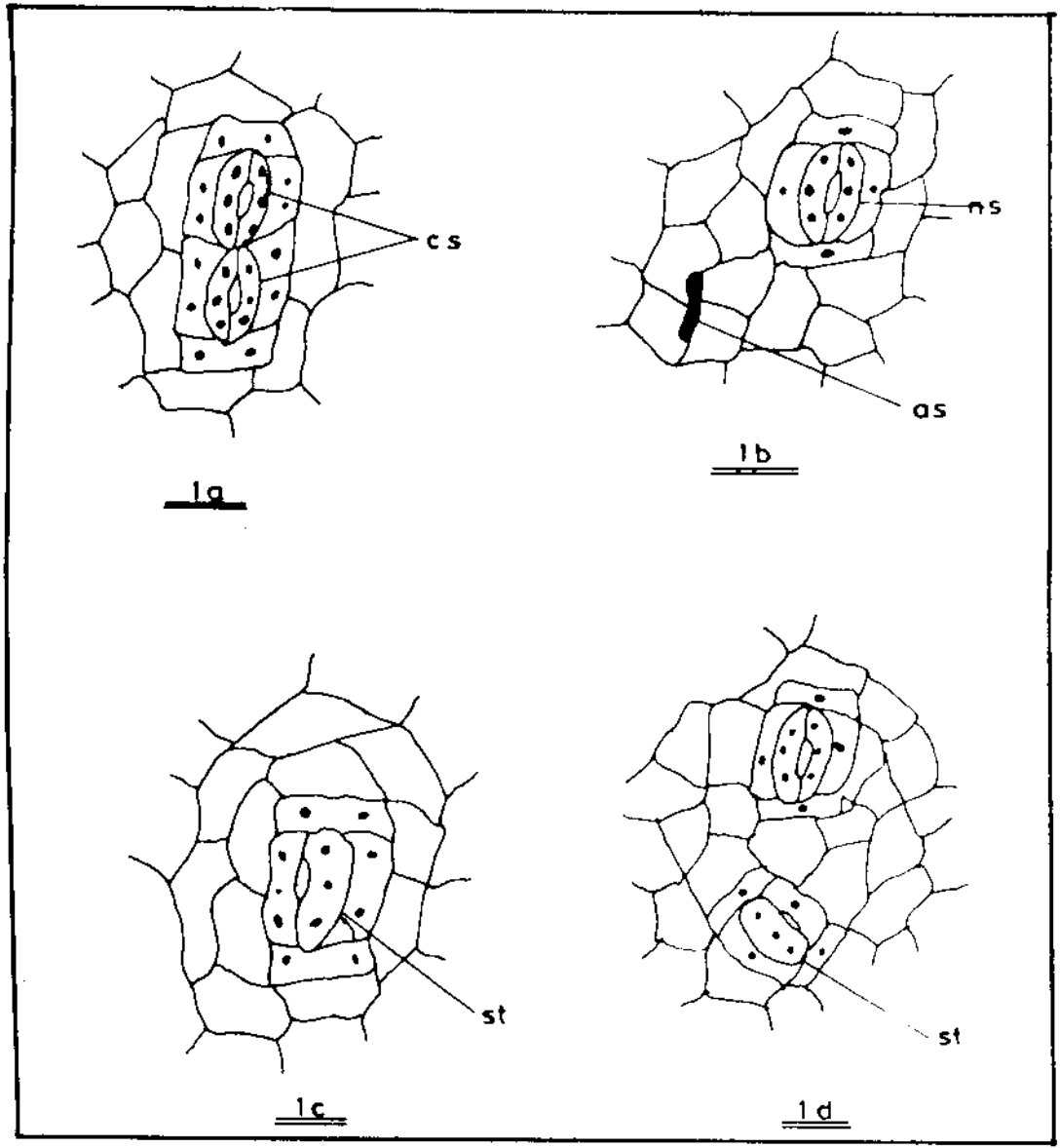
Tomlinson (1969), and Nyawuame and Gill (1990) have reported hypostomatic leaves in the family Commelinaceae. Hypostomatic leaves observed in these two taxa confirm the previous reports of these authors. According to Stebbins and Khush (1961), stomata in most monocotyledonous taxa are arranged in files and the two guard cells of each stoma are surrounded by 4-6 subsidiary cells alligned in all the four directions. Hexacytic stomata arranged in files have been observed in both *C. benghalensis* and *C. erecta* which confirm the earlier report of Stebbins and Khush (1961). (Table 1)

The stomata of *C. benghalensis* are of smaller dimension (36.4 x 174 x 5.8 um) than those of *C. erecta* (39.4 x 20.5 x 6.2 um). However, stomatal frequency is of almost the same order in both taxa but a little lower in *C. benghalensis* ($18.0 \pm 1.4/mm^2$) than *C. erecta* ($19.0 \pm 1.8/mm^2$). Nyawuame and Gill (1990) reported various stomatal abnormalities in some monocotyledonous taxa. During the present study single guard celled stomata have been observed in both taxa. However, aborted stomata have been observed only in *C. erecta* while superposed contiguous stomata have been recorded in *C. benghalensis*. These abnormalities are reported here for the first time. (Fig. 1a-d)

The favourable effect of monovalent and bivalent cations such as K^+, Na^+, Ca^{2+} and Mg^{2+} on stomatal opening has been reported by various authors (Raschke and Fellows 1971; Bhandari and Sen 1972; Sen 1973; and Harsh and Sen 1974). During the present study, stomata were observed to be initially closed in the epidermal peelings of *C. benghalensis* and *C. erecta*. Water incubation of the peelings caused only turgidity of the guard cells. Solutions

Table 1 : Summary of mature epidermis and stomatal features of *Commelina benghalensis* and *C. erecta*.

Taxon	Leaf surface	Wall pattern	Distribution of stomata	Morphological type of stomata	Stomatal measurements			
					Length (um)	Breadth (um)	Pore width (um)	Frequency (no. x mm^{-2})
C. benghalensis L.	U	Straight	Hypostomatich	Hexacytic	36.3± 1.7	17.4± 3.3	5.8± 0.2	18.1± 1.4
	L	Straight						
C. erecta L.	U	Straight	Hypostomatic	Hexacytic				
	L	Straingt			39.9± 0.6	20.5± 0.4	6.2± 0.8	19.0± 0.7



Explanation to figures

Figure 1a: *Mature epidermal peeling of Commelina benghalensis showing superposed contiguous stomata (cs).*

Figure 1b: *Mature epidermal peeling of C. erecta showing a normal stomata (ns) and aborted stomata (as).*

Figure 1c: *Mature epidermal peeling of C. benghalensis showing a single guard-celled stomata (st).*

Figure 1d: *Mature epidermal peeling of C. erecta showing a normal stomata (ns) and a single guard-celled stomata (st).*

Note: All peelings are from the abaxial surface.

Table 2a : Effect of different concentrations of KCl, NaCl and $CaCl_2$ stomatal pore width of C. benghalensis after 3 hrs incubation period.

Conc. (M)	KCl	NaCl	$CaCl_2$
		Stomatal width (um)	
0.0 (DW)	0.0	0.0	0.0
0.25	$5.4^{\pm}2.3$	$5.8^{\pm}0.8$	$11.1^{\pm}0.8$
0.50	$9.0^{\pm}1.5$	$6.3^{\pm}1.1$	$7.9^{\pm}2.4$
0.75	$9.7^{\pm}1.8$	$7.9^{\pm}2.0$	$9.5^{\pm}2.4$
1.00	$7.7^{\pm}1.6$	$8.5^{\pm}2.5$	0.0

Table 2b: Effect of different concentrations of KCl, NaCl and $CaCl_2$ on stomatal pore width of *C. erecta* after 3 hrs incubation period.

Conc.(M)	KCl	Stomatal pore width(um) NaCl	$CaCl_2$
0.0 (DW)	0.00	0.0	0.0
0.25	$3.7^{\pm}1.0$	0.0	$5.4^{\pm}0.6$
0.50	$11.1^{\pm}2.3$	$8.2^{\pm}2.7$	$10.0^{\pm}1.2$
0.75	$14.4^{\pm}1.8$	$4.4^{\pm}1.1$	$9.2^{\pm}1.1$
1.00	$13.7^{\pm}3.4$	0.0	0.0

of KCl, NaCl and $CaCl_2$ induced stomatal opening in the epidermal strips of the two taxa. $CaCl_2$ (0.25M) induced the widest stomatal opening (11.1 ± 0.8 um) in *C. benghalensis* while KCl (0.75M) was the most effective in *C. erecta*, bringing about an opening of 14.9 ± 1.8 um (Table 2a, 2b). The stomatal aperture in KCl/ NaCl/ $CaCl_2$ induced open stomata of the two taxa was reduced to a great extent when transferred to ABA (10^{-3}M)for one hour incubation period (Table 3a 3b). It is interesting to note that the ABA incubated stomata of both *C. benghalensis* and *C. erecta* never fully reopened when transferred back to the respective optimal concentrations of KCl, NaCl, and $CaCl_2$ solutions. Horton (1971), Jones and Mansfield (1972) and Chawan (1978) have reported similar findings in *Vicia faba*, *Xanthium strumarium*, and *Barleria* and *Convolvulus* species respectively. So far, the mechanism whereby ABA is able to regulate stomatal aperture remains unknown.

Table 3a : Effect of optimum concentrations of KCl, NaCl and $CaCl_2$ on stomatal aperture of epidermal strips of *C. benghalensis* incubated for 1 hr in ABA.

Solution	Stomatal pore wisth (um) before treatment with ABA	Stomatal pore width (um) after treatment with ABA
0.75M KCl	9.7± 1.8	
1.00M NaCl	8.8± 1.9	6.8± 1.7
0.25M $CaCl_2$	11.7± 2.3	5.6± 1.5

Table 3b : Effect of optimum concentrations of KCl, NaCl, and $CaCl_2$ on stomatal aperture of epidermal strips of C.erecta incubated for 1 hr. in ABA.

Solution	Stomatal pore width (um) before incubation in ABA	Stomatal pore width (um) after incubation in ABA
0.75M KCl	12.5± 1.8	5.6± 2.0
0.50M NaCl	7.5± 2.1	4.6± 1.5
0.50M $CaCl_2$	10.2± 1.1	4.4± 1.5

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