Sci. Bull. Fac. Educ., Nagasaki Univ., No. 36, pp. 7～15 (1984)

Possible Role of Subsidiary Cells on Stomatal Movement in *Commelina benghalensis* subsp.

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(Received Oct. 31, 1984)

Abstract

Stomatal movement was investigated after distilled water treatment in *Commelina benghalensis* subsp.. Distilled water treatmen+ induced the cytoplasmic shrinkage of surroudning subsidiary cells on guard cells, resulting stomatal opening up to about 11 μm even in dark.

Distilled water-induced stomatal opening was not accompanied with accumulation of potassium. The open stomata did not close in response to 0.1 mM ABA alone in dark but opened further in 10 mM MES-KOH buffer (pH 6.5) containing 100 mM KCl under light.

There was an inverse relationship between the aperture and potassium content in guard cells with shrunk subsidiary cells induced by subsequent distilled water treatment. That is, the aperture did not reduce strongly in spite of a rapid reduction of potassium in guard cells. On the other hand, when subsidiary cells were intact the aperture was paralleled with potassium content in guard cells on both normal stomatal opening and closing.

From these results, it seems possible that subsidiary cells have an important role on the stomatal closure.

Key words : *Commelina benghalensis* subsp. —— Role —— Stomatal movement —— Subsidiary cells.

Introduction

Stomatal movements have been generally investigated, taking focus on guard cells. There have been a few study concerning effect of surrounding cells of guard cells on stomatal movements.

Abbreviation : ABA, abscisic acid.

Distilled water treatment of epidermis induced the cytoplasmic shrinkage of surrounding subsidiary cells, and resulted in stomatal opening.

Squire and Mansfield (1972) and MacRobbie and Lettau (1980) showed that stomatal opening was enhanced by the destruction of the epidermal and subsidiary cells at low pH. More recently, Jinno (1984) showed that stomatal opening was also induced by the destruction of surrounding subsidiary cells using a disrupter.

In this present study, the role of subsidiary cells on the stomatal movement will be discuss in *Commelina benghalensis* subsp.

Material and Methods

Plant of *Commelina benghalensis* subsp. were grown in a greenhouse under natural light conditions. The first and second fully expanded leaves from the top were used for the experiments. They were cut with a razor at their petiole and transferred to the experimental room, and kept in dark for about 15 hr at 30℃ to ensure closed stomata, dipping the petiole in distilled water in an open petri dish.

Lower epidermal strips of about 3×3 mm at the center part of leaf blade were peeled off with a forceps.

For the experiment of distilled water-induced stomatal opening, immediately after peeling the epidermal strips were immersed in 5 ml of cold distilled water at about 5℃ for 15-30 min in a refrigerator in order to remove air in stomatal cavity.

After the cold distilled water treatment, epidermis were incubated subsequently for 3 hr in distilled water at 30℃ in dark and were divided into two portions. One was followed by the incubation for 2 hr in 10 mM MES-KOH buffer at pH 6.5 containing 10 mM KCl in light of about 10 Klux using a bank of three 20 W white fluorescent tubes. The other was transferred to 0.1 mM ABA alone in dark. The aperture and potassium content were measured at appropriate intervals.

For the experiments of normal opening, leaves were floated on distilled water upper surface up under the light condition as described above. At appropriate intervals, lower epidermis were peeled from the light-treated leaf with a razor and forceps and aperture and K^+content were measured during 3 hr. After the measurement of the epidermis of light-treated whole leaf for 3 hr, strips were transferred to distilled water and 0.1 mM ABA alone. Thereafter, aperture and K^+content were measured at appropriate intervals.

The cytoplasmic shrinkage of subsidiary cells could be recognized from the microscopic appearance.

The plasmolytic method was employed for subsidiary cell viability, allowing the epidermis to immerse in 0.5 M mannitol for about 10 min. The viability was recognized from the ability to plasmolyze under high magnification.

Measurements of the stomatal aperture and potassium content were carried out as described previously (Jinno and Kuraishi 1982). Ten to 15 stomata were observed in each of at least three trips and the average was calculated. Each data was obtained from the same leaf.

After histochemical determination of starch staining with I_2/IK, the same procedure as described previously (Jinno and Kuraishi 1982) was employed for the measurement of its content.

The osmotic pressure of the guard cells and subsidiary cells was determined by the plasmolytic method using a graded series of mannitol solution.

Results

As showned in Fig. 1, guard cells of *Commelina benghalensis* subsp. were surrounded by six subsidiary cells. The epidermis before distilled water treatment had intact subsidiary cells (Fig. 1a). Almost all epidermal cells were destroyed by the peeling (data not shown). After distilled water treatment, a cytoplasmic shrinkage was induced at only subsidiary cells and resulted in open stomata of about 11 μm (Fig. 1 and 2). The microscopic appearance was similar to that of the plasmolyzed cell. However, the distilled water-induced open stomata had no potassium in guard cells, and they did not close in response to 0.1 mM ABA alone (Fig. 2).

The distilled water-induced could open further in 10 mM MES-KOH buffer (pH 6.5) containing 100 mM KCl (Fig. 2). During 2 hr incubation in light, the stomata opened further to about 19 μm, and the opening was accompanied with accumulation of potassium (Fig. 2 and Table 1). Thus the stomata found to have a ability to open.

When the open stomata in MES buffer was subsequently transferred to distilled water, the stomatal closure was more slower (Fig. 2) compared with normal closure (Fig. 3). The aperture reduced from 19 to 14 μm during 2 hr. However, potassium content in guard cells reduced to nearly zero state (Fig. 2). Thus, the leakage or excretion of potassium from guard cells did not induced rapid stomatal closure.

When whole leaf was floated on distilled water in light condition described above at 30℃, the stomatal opening was considerably fast (Fig. 3). During 3 hr, stomata opened up to about 21 μm. At the end of light treatment of whole leaves, epidermis were peeled off and when the strips was transferred to distillted water and 0.1 mM ABA alone, rapid stomatal closure occurred on intact subsid-

iary cells. In ABA alone, the open stomata closed completely at least within 30 min. However, they opened again to about 10 μm, followed by the shrinkage of subsidiary cells during 2 hr. No poatssium accumulation occurred in this opening.

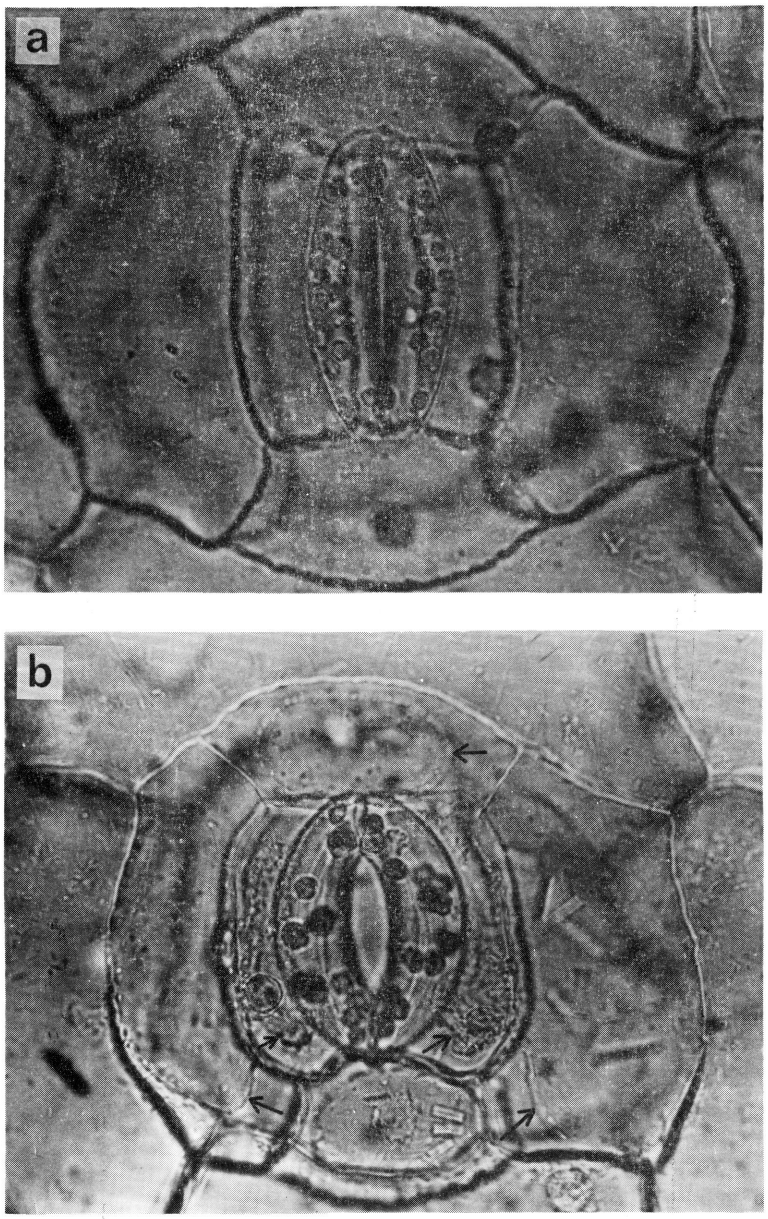

Fig. 1 Appearance of the stomatal complex of *Commelina benghalensis* subsp. Before (a) and after (b) distilled water treatment. Note open stomata at stomatal complex with shrunk subsidiary cells (arrows). × 160.

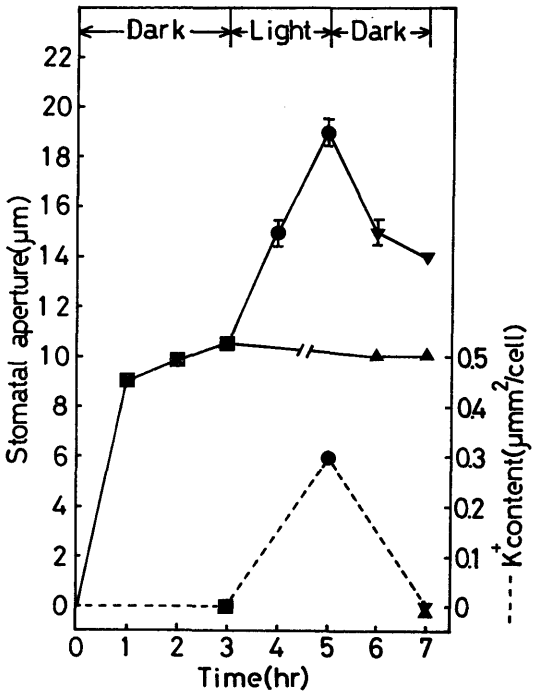

Fig. 2 Changes in stomatal aperture (solid line) and K^+ content in guard cells (broken line). After cold distilled water treatment, strips were incubated further in distilled water at 30℃ in dark (-■-), and these were divided into two groups. One was transferred to 10 mM MES-KOH buffer (pH 6.5) containing 100 mM KCl (-●-) in light and followed by the distilled water incubation (-▼-). The other was transferred to 0.1 mM ABA alone (-▲-).

In distilled water, the open stomata closed completely within 1 hr with intact subsidiary cells, and after 3 hr incubation shrinkage of subsidiary cells was occurred and resulted in open stomata of about 10 μm (Fig. 3.). This opening was not also accompanied with potassium accumulation in guard cells. On the contrary, the closure of stomota with shrunk subsidiary cells was more slower. The aperture reduced from about 21 to 15 μm during the first 1 hr, and thereafter a slight closure occurred during the period of 2 to 3 hr remaining the aperture about 13 μm. However, potassium content of guard cells reduced rapidly to nearly zero state within 1 hr. Thus the aperture did not paralleled with a reduction of potassium content.

The changes in stomatal aperture, contents of cellular compoment and osmotic pressure were investigated under the appropriate treatment. As shown in Table 1, initially closed stomata had no potassium in guard cells but had in subsidiary cells. Although distilled water-induced open stomata had no potassium in both guard and subsidiary cells, stomatal opening was induced in MES buffer + K^+. Potassium content in guard cells increased with increasing aperture, but that of subsidiary cells decreased inversely. Thus, subsidiary cells were found to be functional, changing their potassium content.

There was an inverse relationship between starch content of the guard cell chloroplast and the stomatal aperture (Table 1).

Osmotic pressure of guard cells was slightly higher than that of subsidiary cells at initially closed stomata, although potassium content of guard cells was less than that of subsidiary cells. At distilled water-induced open stomata, the

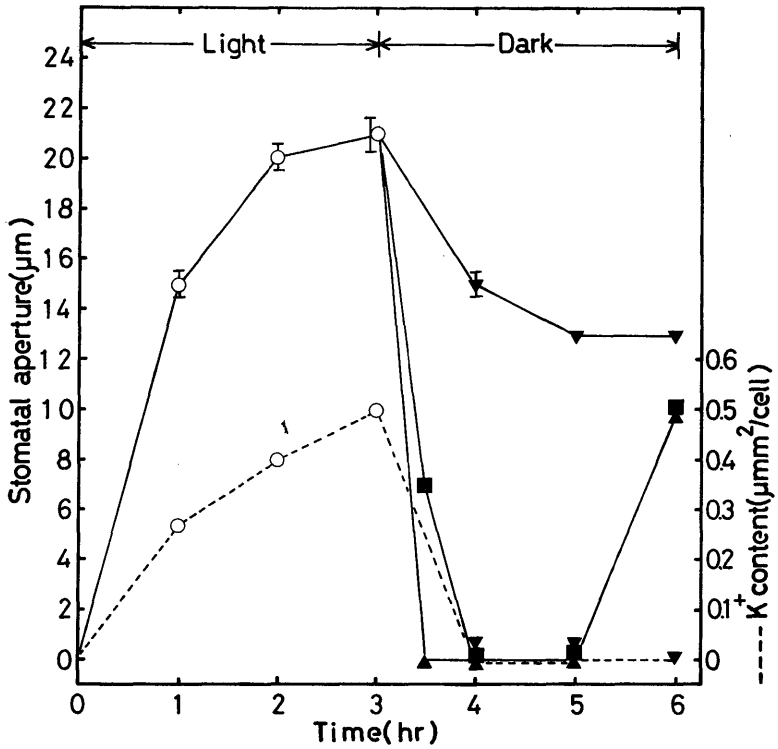

Fig. 3 Changes in stomatal aperture (solid line) and K^+ content of guard cells (broken line). The epidermis of whole leaves was peeled at hourly intervals followed by the measurement of the aperture (-○-). After 3 hr light treatment of leaves, strips were transferred to the treatment solution to observe stomatal closure in dark. The closure at intact (-■-), shrunk (-▼-) subsidiary cells in the distilled water and at intact subsidiary cells in 0.1 mM ABA alone (-▲-). Note the stomatal opening unaccompanied by the potassium accumulation after 3 hr incubation in both ABA and distilled water.

osmotic pressure of guard cells was slightly higher than that of subsidiary cells. On the other hand, guard cells of open stomata in distilled water under light had a considerably higher pressure than that of subsidiary cells due to the accumulation potassium into guard cells (Table 1.).

On opening at MES buffer + K^+, the osmotic pressure of guard cells increased with increasing aperture, while that of subsidlary cells did not change.

Thus, there was a close relationship between potassium content and osmotic pressure in guard cells. After 3 hr incubation in MES buffer, initial osmotic pressure increased by 6 and 2 times at guard and subsidiary cells, respectively.

Table 1 Changes in the cellular components.

Measurement	Whole leaf	Epidermal strips			
	Initially closed stomata	Open stomata after 3 hr treatment			
		In distilled water		In MES buffer+100 mM K^+	
		Dark	Light	Light[d]	Light
Stomatal aperture (μm)	0	12.50±0.62 (10)	18.37±0.85 (15)	11.32±0.58 (15)	21.02±0.75 (13)
Starch content of guard cell chloroplast (μmm^2)	326.55±17.80 (15)	331.11±36.58 (15)	198.66±23.99 (14)	230.15±23.15 (13)	177.85±21.35 (11)
K^+ content (μmm^2/cell)					
Guard cells[a]	NM	NM	0.283±0.025 (17)	0.182±0.015 (23)	0.324±0.021 (12)
Subsidiary cells[b]	0.047±0.005 (10)	NM	NM	0.056±0.005 (23)	0.031±0.005 (12)
Osmotic pressure (bars)					
Guard cells	4.28	4.89	21.99	13.44	26.88
Subsidiary cells	3.05	4.28[c]	4.28[c]	6.11	6.11

Data given are the means ± S. E. Values in parenthesis indicate the number of cells measured. NM, not measureable.
[a]Values per area of a pair of guard cells.
[b]Values per area of six subsidiary cells.
[c]Value measured in intact subsidiary cells.
[d]After stomata opened to the nearly same degree as that of distilled water-induced open stomata, epidermis were treated for this measurement of cellular components.

Discussion

It is less clear that the cytoplasmic shrinkage occur at only subsidiary cells in distilled water. As shown in Fig. 1 b, the shrunk appearance is very similar to that of plasmolyzed cells. However, if the shrinkage occurred once, the strunk state of protoplast did not restore to the initial state even in MES buffer solution. From this point, the shrunk subsidiary cells seems to be unfunctional and similar to that of the destroyed subsidiary cells by the treatment at low pH (Squire and Mansfield 1972; MacRobbie and Lettau 1980).

The cytoplasmic shrinkage of subsidiary cells was always accompanied with stomatal opening up to 11 μm in distilled water under darkness. This opening may be mainly caused by the release of the back pressure of surrounding cells (Squire and Mansfield 1972). By comparison, maximum opening in *Vicia faba* (Glinka 1971) and *Zantedeschia aethiopica* (Maier-Maercker 1983) was always accompanied with incipient plasmolysis of surrounding cells.

However, judging from the results that subsidiary cells had a little lower osmotic pressure than that of guard cells, it seems subsidiary cells have no turgor to compress guard cells. By the way, the stomatal closure in distilled water can be explained by the 'mechanical advantage' that the subsidiary cells in the stomatal complex have over the guard cells (DeMichelle and Sharp 1973). The subsidiary cells have a larger facing area than the guard cell, then the pressure of subsidiary cells may be able to balance or overcome against the higher pressure of guard cells. Thus, functional subsidiary cells seem necessary for the complete stomatal closure.

Distilled water-induced open stomata had no ability to close in responce to ABA alone but to open further in MES buffer + K^+. With *Vicia faba*, the stomata on epidermal strips are often due to epidermal cell destruction and do not close completely after ABA treatment (Glinka 1971). Moreover, fully open stomata with shrunk subsidiary cells of whole leaves did not close completely remaining turgid, although a rapid reduction of potassium content in guard cells was induced. Thus, intact subsidiary cells may be also necessary for the normal closure of normally open stomata. Furthermore, it appears that the elasticity of guard cell wall may be lost during shrinking in distilled water.

From these results, it is suggested that if only guard cells alone are alive, active stomatal opening can be induced. However, normal stomatal closing can not be induced by survival of guard cells alone. When guard cells are surrounded by the living subsidiary or epidermal cells and they function as potassium reservoir, the normal closure may occur. Therefore, it is conceivable that the stomatal closure is not caused by the action of guard cells alone. Subsidiary cells may have a main role on the stomatal closure. It seems possible that the subsidiary cell is capable to take up potassium from the guard cells resulting closed stomata.

I wish to express my gratitude to Dr. Masayoshi Fujino, not only for criticism, but also for his interest and encouragement.

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