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The Fine Structure of the Guard Cell
of *Commelina communis* L.

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Abstract

The guard cell and subsidiary cell of *Commelina communis* L. were observed with electronmicroscope.

There were many organellas in the guard cell different from the epidermal cell. In cross section, the cell wall of guard cell was fibrous, and the wall of upper and lower side of guard cell was very thick, but the wall facing on the stoma and that between guard cell and inner subsidiary cell was very thin. Many plasmodesmatas were found in the wall between these two cells.

There were many densely staining vacuoles in the guard cell.

Introduction

Many investigations on the mechanism of stomatal movement have been carried out. Since Williams (1954), the idea of active movement using energy have been proposed.

Recently, Fujino (1967, 1969), Fischer (1968a, b, 1971), Fischer and Hsiao (1968), Humble and Hsiao (1969) reported that the transport of potassium in and from the guard cell was carried out at stomatal movement. Fujino (1967, 1969) reported that ATP and ATPase in the guard cell were concerned with the transport of potassium. To know the mechanism of stomatal movement, it is necessary to investigate from the view of ultrastructure of the guard cell and subsidiary cell.

Brown and Johnson (1962), Kaufman, Petering, Yocum and Baic (1970), Thomson and Journett (1970) made the reports of the ultrastructure of guard cell of grass, *Avena, Opuntia*, respectively. But the stomatal movement of these plant is not sensitive. Milthorpe (1969) also reported on *Commelina cyanea*,...
but did not describe in detail.

To know the mechanism of stomatal movement, plant having large and sensitive guard cell is good material. Thus, in present experiment, the fine structure of guard cell and subsidiary cell of *Commelina communis* L. that have large and sensitive guard cell were observed.

**Material and Methods**

Fully grown and fresh leaf of *Commelina communis* L. was cut into small pieces $1 \times 2$ mm$^2$.

The material was fixed in 2.5% KMnO$_4$ solution at room temperature for 2 hours and was followed by brief washing in distilled water 2 to 3 times, dehydration in a graded series of ethanol, and 3 changes of propylene oxide, and embedded in Epon mixture. Ultrathin sections were cut with Reichert OmU 2 ultramicrotome, and stained with uranyl acetate and lead nitrate. After carbon vacuum evaporation, all preparations were examined with HU-125 DS electronmicroscope.

**Observation**

The guard cells of *Commelina communis* L. are surrounded by six subsidiary cells, that is, inner, outer and polar ones which are adjacent to epidermal cell. First authors will wish to describe the fine structure of the guard cell.

A. (a) **Nucleus** The nucleus varied considerably in size and form probably due to the plane of the section. At center cross section of the cell, the nucleus occupied 70 to 80% of the cell, and presented the triangular form. The chromatin was recognizable as dense, granular area, and presented a slight contrast against the nuclear matrix of relatively low density. The nuclear envelope was double-layered membrane and had small nuclear pores. The outer membrane was rarely associated with poor endoplasmic reticulum.

(b) **Mitochondria** The majority of the mitochondria occurred in the cytoplasm, although occasionally a few could be seen around the nucleus. The form of mitochondria was spherical or oval. The mitochondria was bounded by double-layered membrane. The cristae usually had a random orientation, and was short and obscure. The matrix is fine granular, but intramitochondrial granules were not present.

(c) **Chloroplast** Chloroplast contained several well-developed grana which consisted of stacks of electron-dense thylakoid. Connections between the granas could be seen. Usually one or more starch grains were contained in each chloroplast. In the chloroplast containing starch grains, grana was pressed and became thin.
The matrix of chloroplast was fine granular and it’s structure and density were similar to that of cytoplasm.

(d) **Endoplasmic reticulum** The small, smooth and tubular endoplasmic reticulum were observed in the periphery of the cytoplasm. These endoplasmic reticulum were not lamellar form, but were consisted of individual cisternae or tubules. A few endoplasmic reticulum were seen, and did not connect to a plasmodesmata.

(e) **Golgi apparatus** Golgi apparatus was observed in this cell. It was composed of a small stack of plate-like cisternae, but was not associate with vesicles. It was poor and simpler than the Golgi apparatus of animal cell. The association of the Golgi apparatus with other membrane system did not occur.

(f) **Vacuole** Vacuoles were one of characteristic feature, they consisted of various sizes and types, and occupied the majority of the guard cell. In the center of the cell there was a large vacuole, and in the periphery of it there were some small ones which frequently fuse into a single large one. The tonoplast was not clear. They were filled with highly electron-dense and homogenous substance.

(g) **Other cell inclusion** There were single-membraned spherical bodies and multivesicular bodies containing less electron-dense and fine granular substance.

(h) **Cell wall** Another characteristic feature of the cell was the presence of the cell wall, composed of a internal fine fibrous layer and a external fine granular layer. This fibrous structure was well-developed. In cross section, the wall of upper and lower side of the cell was very thick, but the wall facing on the stoma and that between guard cell and inner subsidiary cell was very thin. When stoma was under the closing, the folds like wave were seen at the parts of these thin wall.

(i) **Plasmodesmata** Many plasmodesmatas were found in the wall between the guard cell and the inner subsidiary cell.

B. **Subsidiary cell** In subsidiary cell, vacuole occupied the large part of the cell. Small cytoplasm was seen along the cell wall. There were a little organella such as mitochondria in inner subsidiary cell, and more little in outer subsidiary cell. Vacuole was lower in electron-dense compared with that of the guard cell and became coarse.

The cell wall was not so fibrous and thick as that of the guard cell. There were many plasmodesmatas in the wall between inner and outer subsidiary cell, and a little between in the wall between outer subsidiary cell and epidermal cell.
The fine structure of the guard cell is very elaborate compared with the epidermal cell. It is thought then, that the guard cell have functions different from the epidermal cell.

The cell wall of the guard cell is fibrous, and the wall of upper and lower side was very thick, but the wall facing on the stoma and that between the guard cell and inner subsidiary cell is very thin. Thus, the guard cell must be permitted to change the form by folding or expansion of the thin part of the wall at the movement.

Fujino (1967, 1969), Fischer (1968b, 1971), Fischer and Hsiao (1968), Humble and Hsiao (1969) reported that the transport of potassium in and from the guard cell is carried out at stomatal movement, and they consider that the transport of potassium is the cause of turgor-variation of the guard cell.

As the wall between the guard cell and inner subsidiary cell is very thin, diffusion must certainly occur rapidly across such a thin wall. Brown and Johnson (1962), Thomson and Journett (1970), Milthorpe (1969) do not find the plasmodesmata between the guard cell and the subsidiary cell of grass, Opuntia and Commelina respectively, Kaufman, Petering, Yocum and Baic (1970) find the plasmodesmata between these two cells of Avena.

In Commelina communis L. used in present observation, many plasmodesmata are found between the guard cell and inner subsidiary cell and a little plasmodesmata are also found between inner subsidiary cell and outer subsidiary cell.

Thus, the diffusion of water and solute must more rapidly occur between the guard cell and subsidiary cell.

Fujino (1967, 1969) reported that ATP and ATPase are involved in the transport of potassium in and from the guard cell. In fact, there are many mitochondrias in the guard cell, especially in the cytoplasm facing on inner subsidiary cell. Thus, the apparently high concentration of mitochondria in the guard cell may be relation to ATP production as a source of energy for rapid transport of solute such as potassium through plasmodesmata.

Milthorpe (1969) found the densely staining vacuole in the guard cell of Commelina cyanea. In Commelina communis L., there are many vacuoles which are more deeply stained. In most case, small vacuole fuse into large one. Whaly, et el (1960) reported that the vacuole of meristematic cell was deeply stained only when the sample was fixed with KMnO₄.

It is not clear whether densely staining of vacuole of Commelina communis L. is due to fixation reagent or due to particular substance in vacuoles. Ribosomes were not observed in the guard cell of Commelina communis. It is not clear whether the absence of ribosomes are due to fixation reagent or due to
the poorness of protein synthesis.

Literature Cited

The Fine Structure of the Guard Cell of *Commelina communis* L.

Fig. 1. Cross section of the guard cell of *Commelina communis*. ×7,500.

Fig. 2. Magnification of a part of fig. 1. ×21,000. N, Nucleus; V, Vacuole; C, Chloroplast; M, Mitochondria; CW, Cell wall.
Fig. 3. Magnification of a part of fig. 1. × 7,800.

Fig. 4. Cross section of the guard cell of Commelina communis. × 8,500. ISC, Inner subsidiary cell; S, Stoma; SER, Smooth endoplasmic reticulum; OCI, Other cell inclusion.
Fig. 9. Cross section of inner and outer subsidiary cell of *Commelina communis*. ×18,400.

Fig. 10. Cross section of outer subsidiary cell and epidermal cell of *Commelina communis*. ×21,100.
Fig. 5–6. Cross section of the guard cell and inner subsidiary cell of *Commelina communis.* ×21,000. GA, Golgi apparatus; P, Plasmodesmata.
Fig. 7. Cross section of inner and outer subsidiary cell of Commelina communis. ×27,000.

Fig. 8. Cross section of inner and outer subsidiary cell of Commelina communis. ×18,500. OSC, Outer subsidiary cell.