Yun et al. 1996

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J. Plant. Res. 109: 339-342.

REFNO:

KEYWORDS:

Palisade cell, Saintpaulia

J. Plant Res. 109: 339-342, 1996

Journal of Plant Research

Short Communication

Acute Morphological Changes of Palisade Cells of *Saintpaulia* Leaves Induced by a Rapid Temperature Drop

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Early changes in fine structures of intracellular organelles of *Saintpaulia* leaves exhibiting chilling-induced degeneration of photosynthetic activities were investigated by electron microscopy. As early as 3 min after exposure to 10C water, thylakoid lamellae in the palisade cells showed extensive disarrangement, especially in intergrana lamellae. Simultaneously, other organelles showed drastic morphological changes, including the condensation of chromatin in the nucleus.

Key words: Chilling injury — Electron microscope — Leaf spot — Palisaide cell — *Saintpaulia*

A rapid drop in temperature of *Saintpaulia* leaves causes a chilling injury called leaf spot and only palisade cells are injured, resulting in cell death (Elliot 1946, Maekawa *et al.* 1987). A few seconds after the rapid temperature drop, the intensity of chlorophyll fluorescence reduces and photosynthetic activities are simultaneously damaged (Yun *et al.* 1995). However, no morphological studies have been conducted on the developement of leaf spot of *Saintpaulia*.

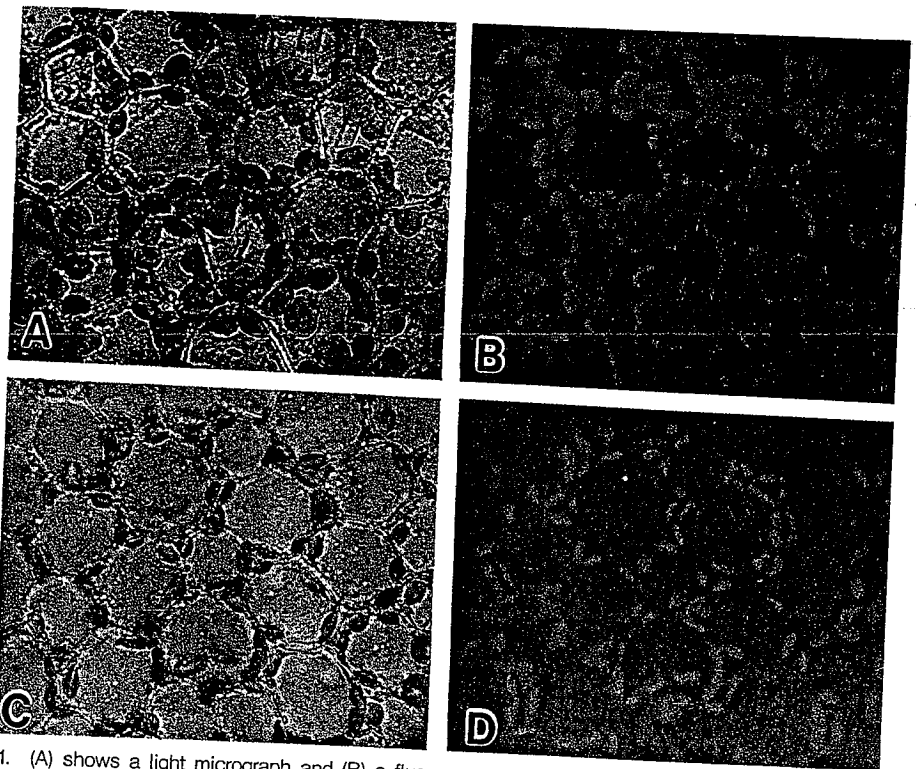

Fig. 1. (A) shows a light micrograph and (B) a fluorescence micrograph (EX450-490) of the same intact palisade cells. (C) shows a light micrograph and (D) a fluorescence micrograph of the same palisade cells 1 hr after the chilling treatment.

In this study, morphological changes in the structure of palisade cells of *Saintpaulia* leaves were investigated with special attention paid to the initial stages of this injury when reduction of chlorophyll fluorescence and degeneration of photosynthetic activities become prominent.

Saintpaulia ionantha cv. Ritali was cultivated in a greenhouse under the temperature range of 20-30C. After being kept at 35C for 10 min, detached mature leaves were exposed to 10C water for 3 sec. This treatment will be called chilling treatment hereafter. The chilling-treated leaves were observed for morphological changes of the palisade cells after being left in the ambient air for indicated periods. For the electron microscopy, small pieces of the treated or untreated leaves were fixed in Karnovsky's fixative (Yasuda *et al.* 1981) supplemented with 0.1% Tween 20 at room temperature for 2 hr and then fixed in 2% OsO_4 for 10 hr, and stained with 2% uranyl acetate. After dehydration through ethanol series, samples were embedded in epoxy resin. Ultrathin sections were stained with lead citrate and 2% uranyl acetate and observed under an electron microscope (JEM-1010, JEOL, Tokyo, Japan).

Figure 1 shows the light and fluorescence micrographs of the intact (Fig. 1A, B) and treated palisade cells (Fig. 1C, D). The chloroplasts in the intact palisade cells were green and round, and emitted fluorescence strongly (Fig. 1A, B). The chilling of the leaves reduced the intensities of chlorophyll

Fig. 2. Electron-micrographs of vertical sections of an intact cell (A) and a cell 3 min after the chilling treatment (B). n shows nucleus; nc, nucleolus; v, vacuole; ch, chloroplast; cw, cell wall. Arrow indicates invagination of tonoplast; arrow head, electron dense granule; double arrow, separation of cytoplasm from the cell wall. Bar shows 1 μm.

fluorescence and the chloroplasts were flattened in shape (Fig. 1C, D). The chloroplast in the treated cells appeared to have been dehydrated and shrunk.

Figure 2 shows the electron micrographs of the intact cell (Fig. 2A) and the treated cell (Fig. 2B) 3 min after the chilling treatment. The ultrastructure of all the organelles in the treated cells (Fig. 2B) have changed drastically. In the treated cell, the electron density of cytoplasm have increased and the cytoplasm has separated from the cell wall in some places (double arrow, Fig. 2B). This result shows that cytoplasm shrank and plasmolysis occurred after the chilling treatment. Electron dense particles (arrow heads, Fig. 2B) have extruded from the cytoplasm toward the cell walls. These electron dense particles are similar to the lipid extrusions which appeared in chilling injured cells of grapefruit rind (Platt-Aloin and Thomson 1976). Some invaginations of tonoplast filled with cytosol into the vacuole were observed (arrows, Fig. 2B). Electron dense aggregates have increased in the vacuole (Fig. 2B). This increase might reflect an environmental change in the vacuole caused by the chilling treatment but the composition of these aggregates has not yet been determined.

In the chloroplast of the intact cells, the electron density of the grana thylakoid space was high (Fig. 3A). On the other hand, the lumen spaces in the grana thylakoid in the chilling-treated cells were more transparent (Fig. 3B), indicating that some electron dense material in the lumen spaces had disappeared after the chilling treatment. However, the width of the grana thylakoids was almost the same as that in the intact cells and the membrane structure of the grana thylakoids appeared to remain intact. By contrast, the arrangement of the intergrana lamellae had been disordered and the lamella structure had become less clear (Fig. 3B). These results indicated that the structure of the intergrana thylakoids was more susceptible to the chilling than that of the grana thylakoids. Another line of our studies on the chilling-treated leaves revealed that photosystem I (PS I) activity was much more sensitive to chilling than photosynthesis II (PS II) activity (Yun *et al.* unpublished data). Since PS I activity is concentrated in the intergrana lamellae rather than the grana lamellae (Anderson and Melis 1983), the instability of intergrana lamellae structure to chilling observed in the present study may have reflected the faster degradation of PS I activity.

In the nucleus of the treated cells, the chromatin was condensed (Fig. 2B). In the microbodies, numerous unknown electron dense irregularly-shaped particles were observed over the microbody matrix (Fig. 3D). In the mitochondria, the cristae became narrower and the electron density of the mitochondrial matrix was lower than that in the

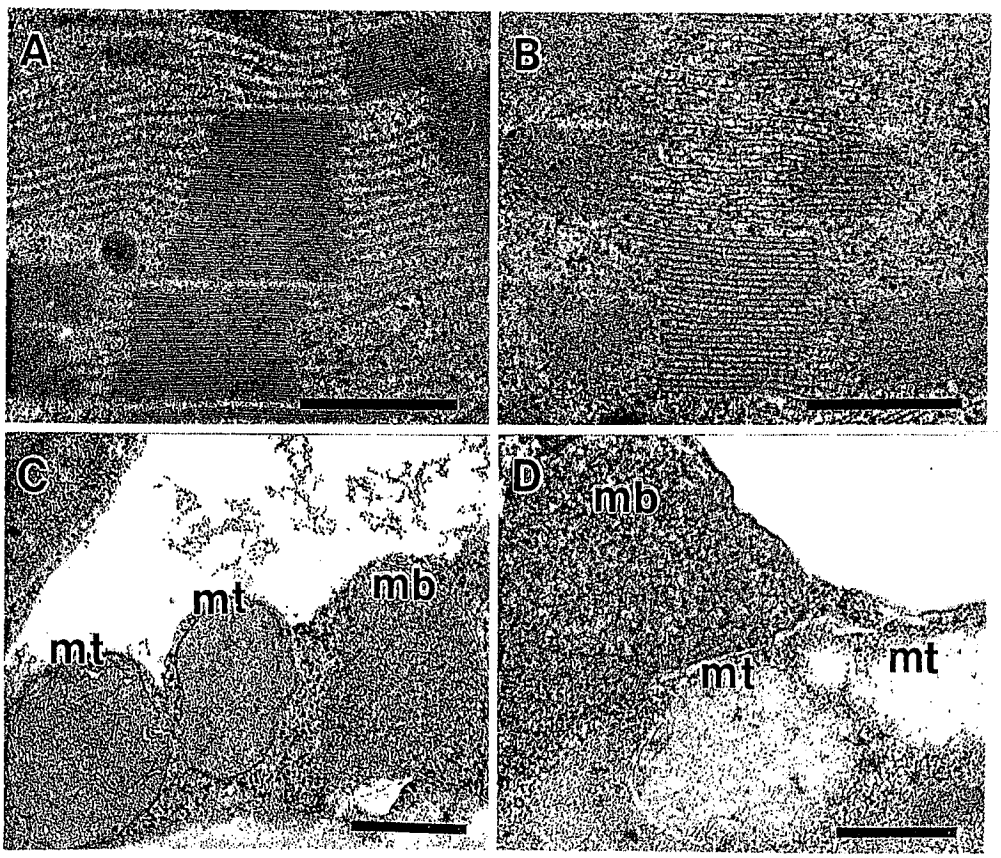

Fig. 3. Electron micrographs of thylakoids (A, B), and mitochondria and microbodies (C, D) in an intact cell (right column) and a cell 3 min after the chilling treatment (left column). mt shows mitochondria; mb, microbody. Bar shows 0.5 μm.

intact cells. We observed that these morphological changes had already occurred in all the cell organelles in the palisade cells 3 sec after chilling treatment (Yasuda unpublished data). These observations suggested that the drastic changes occurred simultaneously in all the cell organelles of palisade cells as a result of chilling.

In the injured cells of *Saintpaulia*, the ultrastructural damage became visible a few seconds after chilling. In the injured cells of other cold sensitive plants, the onset of visible ultrastructural damages takes several hours or more (Lyons 1973, Saltveit and Morris 1990). In the chilling injured areas of other cold sensitive plants, necrosis occurs following morphological changes such as swelling of both the thylakoids in the chloroplasts and the cristae in the mitochondria, and breakage of the tonoplast (Taylor and Crang 1971, Kimball and Salisbury 1973, Lyons 1973, Ilker *et al.* 1976, Moline 1976, Niki *et al.* 1978, Wise *et al.* 1983, Wise and Naylor 1987, Saltveit and Morris 1990). In the injured palisade cells of *Saintpaulia*, swelling was not seen in chloroplast thylakoids nor in the mitochondrial cristae, nor was the breakage of the tonoplast observed. The morphological changes including shrinkage of cytoplasm and condensation of chromatin, observed in the palisade cells of *Saintpaulia* after chilling, were not necrotic, but very similar to that of apoptosis observed in animal cells (Kerr *et al.* 1972).

We would like to thank Dr. K. Maruyama in Kyoto University and Dr. T. Ehara in Tokyo Medical College for their helpful discussions. Our thanks are also due to Miss. A. Soda for her technical assistance.

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(Received March 15, 1996: Accepted July 11, 1996)