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Study on laminar hydathodes of *Ficus formosana* (Moraceae) III. Salt injury of guttation on hydathodes

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ABSTRACT. The salt concentrations of gutted solution of laminar hydathodes on leaf usually increase after the repetition of guttation and eva-transpiration, and thus situation may lead to injure the hydathodes. The aim of this study is to investigate the salt injury of gutted solution on hydathodes of *Ficus formosana* Maxim. by using electron microscopy. Ultrastructural studies show that the hypertonic stress of gutted solution caused by evaporation could lead the injury of hydathodes. The major symptoms of salt injury caused by hypertonic stress are as the follows: many electron dense particles are spread in the nucleus and other organelles; the nucleolus is condensed and then disappeared; the endomembrane system is collapsed and then entirely become osmiophilic materials in the cytoplasm. Upon dehydration, the collapsed membranes become myelin-like structures are also observed. According to different degrees of salt injury within hydathodes, the abilities of tissue's salt-tolerance are diversified and tolerance ability of the epithem is better than other tissues. These results imply that epithem possesses some special mechanisms that have been evolved to adapt the damage stress. In addition to physiological regulation, we suggest that some morphological changes such as the sinuous cell wall, proliferation of peroxisomes and the abundant endomembrane systems, and the conspicuous fluid-phase endocytosis. Epithem promotes the tolerant efficiency of vacuoles by increasing the contact surface with environment to accelerate salt tolerance.

Keywords: Epithem; *Ficus formosana* Maxim.; Fluid-phase endocytosis; Hydathodes; Sheath layer; Salt injury; Water pore.

INTRODUCTION

Guttation, a process of water excretion from leaves in liquid form, occurs in a wide range of vascular plants. During the early stage of leaf development, guttation does not make any visible injury to plants, but in the later stages don't show a certainty. According the viewpoint of Curtis (1943), three things may happen to the guttation drop on a plant: 1) it may roll off; 2) it may evaporate; or 3) it may be sucked back into the leaf. So, the gutted solution will be condensed through many times of guttation and evaporation. Ivanoff (1963) proposed that the injuries of concentrated gutted solution are related to three kinds of casual bases. First, injuries are connected with loss and depletion of usual amounts of vital nutrient substances. Secondly, injuries are caused by the accumulation and concentration of guttation products on localized areas of the plants. Finally, the entrance of various foreign agents and pathogen causes injuries since they go through water pore into the hydathodes during active guttation periods. Chlorosis and necrosis, two guttation injury symptoms, are usually observed on leaves whose injuries are generally caused by direct action of concentrated guttation solution and microorganisms' infection (Yarwood, 1952; Carlton et al., 1998; French and Elder, 1999). Several previous studies have suggested that mineral salts of guttation solution may be exuded outside hydathodes and/or sucked back into leaves through water pores, and that the hypertonic solution can damage those cells in hydathodes (Curtis, 1943; Ivanoff, 1944, 1963). Moreover, icing water drops could enter plant through stomata and hydathodes, causing frozen damage to leaves (Pearce, 2001). Furthermore, there are reports to claim that epithem cells not only process the retrieval function of nutrients from guttation liquid, but also play an important role in removing salt from guttating plants (Broyer and Hoagland, 1943; Klepper and Kaufmann, 1966; Wilson et al., 1991). However, under such stresses the epithem and water pores of hydathodes play an important role in competence for the demand of nutrient retrieval function and they must have some unique mechanisms to adapt such hypertonic condition.

Our previous studies (Chen and Chen, 2005; 2006) on the ultrastructure and morphogenesis of the laminar hydathodes of *F. formosana* showed that: 1, epithem cells have sinuous cell wall to increase their absorption surface area of cells; 2, both vigorous membrane

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endocytosis and actively pumping endomembrane systems are induced by plasmolysis-deplasmolysis cycles that support the membrane surface changes of epithem cells; 3, proliferated peroxisomes in epithem cells may depress the free radicals, which are produced by high salt stress; 4, observed many salt-glandular trichomes occur in the vicinity of hydathodes' surface during the early stage of leaf development. Epithem cells couldn't endure the strict stress circumstance coming even possess above characteristics, and the salt damage of membrane systems still happen (Kuchitsu et al., 1992; Hernandez et al., 1993; Huang, 1996). It is interesting to see whether these salt-glandular trichomes have a function of removing and eliminating excess salt during leaf development.

In this study, we tried to investigate the symptoms of salt injury of hydathodes caused by concentrated gutted solution and focused on the cytological characteristics of water pore, epithem, and sheath layer by using electron microscopy. Besides, we also observed the ultrastructure of salt glandular trichomes and discussed the possible mechanisms of epithem cell using to adapt to salt stress.

MATERIALS AND METHODS

Plant Materials

The mature expansion leaves of *F. formosana* Maxim. f. *Shimadai* Hayata (15 day normal leaves and 30 day old leaves with conspicuous chlorotic symptom) were prepared for studying the salt injury in hydathodes. Meanwhile, salt glandular trichomes surrounding the hydathode on the adaxial surface of leaves at 3 and 7 day were collected also for further study.

Transmission Electron Microscopy (TEM)

Mature leaves containing hydathodes with or without conspicuous chlorotic symptoms were observed under a dissecting microscope, cut into 1×1 mm^2, fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0) at room temperature for 6 h, and washed in a rinse buffer (0.1 M sodium cacodylate buffer) three times. Washed samples were post fixed with 1% OsO_4 in 0.1 M sodium cacodylate buffer (pH 7.0) at room temperature for 8 h. After three-times rinsing with 0.1 M sodium cacodylate buffer of pH 7.0, samples were dehydrated with a gradient acetone series and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections in golden color were cut with a diamond knife and picked up on the formvar-coated 75 mesh grids. The section-mounted grids were stained with aqueous uranyl acetate for 25 min and lead citrate for 5 min. The stained sections were examined in a Hitachi H-600 transmission electron microscope at 75 kV.

Scanning Electron Microscopy (SEM)

Fixation of samples and buffer washers were done as the described above. Fixed samples were dehydrated through an ethanol series up to 100%, transferred to pure acetone, and critical point-dried in a Hitachi Critical Point Dryer HCP-2. Afterwards, specimens were mounted on aluminum stab with silver paste and coated with palladium-gold in an ion coater (Eiko Engineering, Ltd. IB-2 ion coater) and viewed in a Hitachi S-520 SEM.

RESULTS

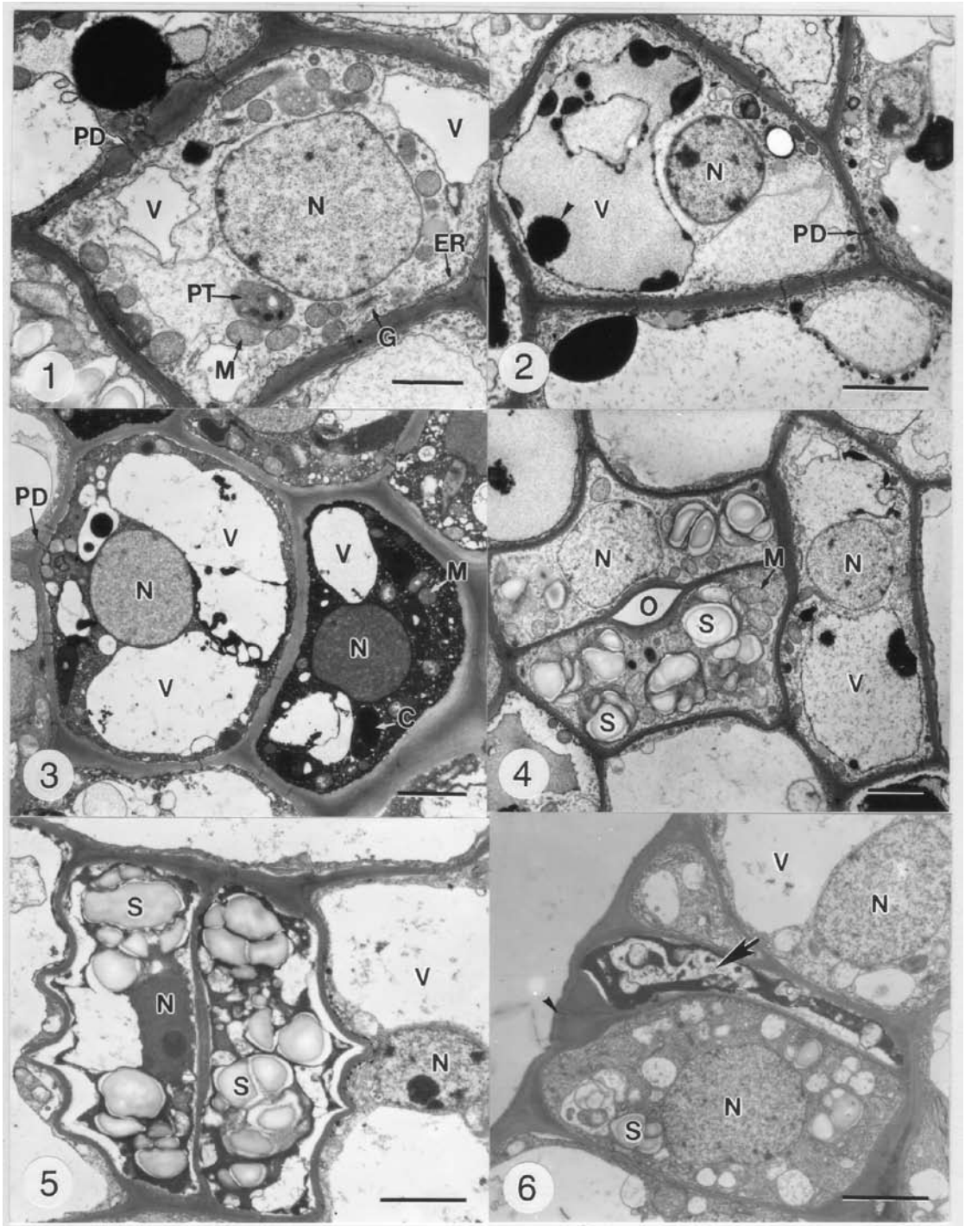
Salt effects on epidermis and water pores of hydathode

In normal mature hydathodes, the epidermal cells have a prominent nucleus, many mitochondria and plastids, endoplasmic reticulum, vacuoles, and Golgi apparatus (Figure 1). When mature leaves are getting old, electron dense tannin granules are accumulated in the vacuoles of epidermal cells (Figure 2). The salt injury on hydathodes would occur when the local salt concentration increased drastically by repeated evaporation after guttation. Several salt-injury symptoms of epidermis were observed: the nucleolus is condensed and disappeared; the nucleus becomes heterochromatinized; many electron-dense materials are accumulated in the cytoplasm; and membranous organelles are broken down and become osmiophilic (Figure 3). As the Figure 3 show: left cell is the front stage of salt injury there are electron-dense cytoplasm; right cell is more serious injury stage, membranous organelles are broken down and become osmiophilic and electron-dense.

The normal water pore consists of two guard cells, which are specialized cells containing many amyloplasts, mitochondria, and general endomembrane systems (Figure 4). Especially, their middle region of pore is permanently open then the outside region toward atmosphere overlapped by outer ridges. Salt stress arrests the differentiation of water pores during developmental process that damages the guard cells pair destroys one or both to cause malformation of water pore (Figures 5 and 6). Hypertonic stress causing guard cells plasmolysis and their salt-injury symptoms are similar epidermal cells as the described above.

Salt effects on epithem of hydathode

In the inner hydathode, the normal young epithem cells are lobed in shape and have general organelles in appearance (Figure 7). Under salt stress epithem cells are not only have condensed tannins granules spreading in the vacuoles, but also their cytoplasm becomes electron-dense (Figure 8). These phenomena are more serious and obviously relevant with maturation of hydathode (Figure 9). Drastic salt and osmotic stresses resulted in the failure of some epithem cells to regulate and adapt, and serious injuries were finally observed (Figure 10 middle cell). While the tolerant epithem cells with proliferated peroxisomes are obviously observed and peroxisomes number increases with aging and extreme stress period (Figures 9 and 10 left cell). Moreover, many vesicular structures, puffy endoplasmic reticulum and Golgi apparatus associated with electron-dense materials



Figures 1-6. TEM micrographs of salt injury on epidermis and water pore in hydathodes of *F. fomasana* Maxim. f. *Shimadai* Hayata. 1, Epidermis near the water pore; 2, Epidermis at initial stage under salt stress. Epidermal cells have a group of mitochondria, several large vacuoles containing tannin granules (arrowhead), and a few plasmodesmata on cell wall between two of them; 3, Two levels of salt injury in epidermis under sharp salt stress. Left cell is at early stage of salt injury showing nucleolus condensed and disappeared, cytoplasm containing electron-dense materials and small osmiophilic droplets. Right cell is at late stage of salt injury showing nucleolus and cytoplasm became more electron-dense, and organelles containing osmiophilic droplets; 4, Epidermis at paradermal view showing a normal water pore paired with one open pore; 5, Parademal view of epidermis showing water pore paired with two guard cells pair under ionic toxicity and plasmolysis caused by salt stress. The nucleoli are condensed and containing many electron-dense particles. Because of destroy of two guard cells let the pore can't perform; 6, Cross section of a malformed water pore showing one of the guard cells pair is destroyed (arrow) under salt stress that caused water pore cannot completely differentiate. Arrowhead indicates the pore site. (1, bar scale = 1.25 μm; 2-6, all bar scales = 2.5 μm). Figure abbreviations: C, chloroplast; CW, cell wall; ER, endoplasmic reticulum; G, Golgi body; IS, intercellular space; M, mitochondrion; N, nucleus; P, peroxisome; PD, plasmodesmata; PT, plastid; S, starch-containing plastid; T, trichome; TC, tracheid cell; V, Vacuole; WP, water pore.

and many fluid-phase endocytosis were observed in the cytoplasm of tolerant epithem (Figures 11, 12 and 13). Another character of tolerant epithem cells is the accumulation of electron-dense material in vacuole (Figure 14). Damaged epithem cells are easily observed nearby the tracheid cells or under the water pore (Figures 10 and 14). In addition to the electron dense materials accumulated in cytoplasm, the other major symptom of cell damage is the collapse of nuclear and organelle membranes. As shown in Figure 15, a heterochromatinized nucleus with many electron-dense granules, a condensed nucleolus, and collapsed chloroplasts were observed in a damaged epithem cell under hypertonic stress. The plasmodesmata between normal epithem cells are connected. However, in damaged epithem cells, callose materials are synthesized nearby the plasmodesmata to block their coupling with other healthy cells (Figure 16). Particularly, some structures of the fluid-phase endocytosis are observed in the early stage of a damaged epithem cell (Figure 17). When hypertonic stress is severe, epithem cells will confront with the threat of osmotic and ionic stresses and result as the collapsed membranes fragments and myelin-like structures in the cytoplasm (Figure 18).

Salt effects on sheath layer

The salt-injury symptoms of sheath layer cells are different from those described above tissues. Under hypertonic stress, the advanced plasmolysis occurs and the large central vacuole disappears. The peripheral remnant cytoplasm is mixed and dehydrated to become electron-dense materials that contain collapsed organelles and desiccated chloroplasts (Figure 19). Their stacked membranes of desiccated chloroplasts are electron-loose under dehydration (Figure 20).

Morphology and ultrastructure of salt-glandular trichomes by hydathodes

Many salt-glandular trichomes surrounding the hydathodes occur on the adaxial surface of leaves during leaf development (Figure 21). These salt glandular trichomes often appear in a large group of cells in early stages of hydathodes development and then are dropping off gradually during maturation. A normal salt glandular trichome consists of a basal cell, a stalk cell and eight-celled head cells (Figure 22). As shown in Figure 23, at the four-celled stage, the salt glandular trichome contains one basal cell, one stalk cell and two head cells that there are many plasmodesmata presented between them. In this stage, cells are characterized by a large nucleus and dense cytoplasm, which contains numerous ribosomes and organelles, such as mitochondria, Golgi bodies, ER and plastids. In the maturation stage, particularly head cells have many condensed tannins or granules precipitation accumulated in the large central vacuole and conspicuous salt injury symptoms such as membrane collapsed and electron-dense materials in the cytoplasm are also observed (Figure 24). Basal cell has a large vacuole and other organelles are pericytomatic distribution. A mature stalk cell connected the basal cell and head cells there are many puffy endoplasmic reticulum system, plastids, mitochondria, Golgi body and small vesicles presented in the cytoplasm (Figure 25).

DISCUSSION

Effects of repeating guttation and evaporation on hydathodes

The guttated solution contains a large quantity of solutes consisting of not only mineral salts but also organic materials (Goatley and Lewis, 1966; Mizuno et al., 2002). When evaporation occurs during daytimes, solutes in guttated solution can be concentrated on the margin or the inside region of the hydathodes (Wilson et al., 1991). The local solute condensation can inflict the salt stress on tissues of hydathodes (Ivanoff, 1963). Beside evaporation, cuticular transpiration also occurred on surface of hydathodes that could increase the solute concentration of xylem sap. In general, there are two kinds of deleterious

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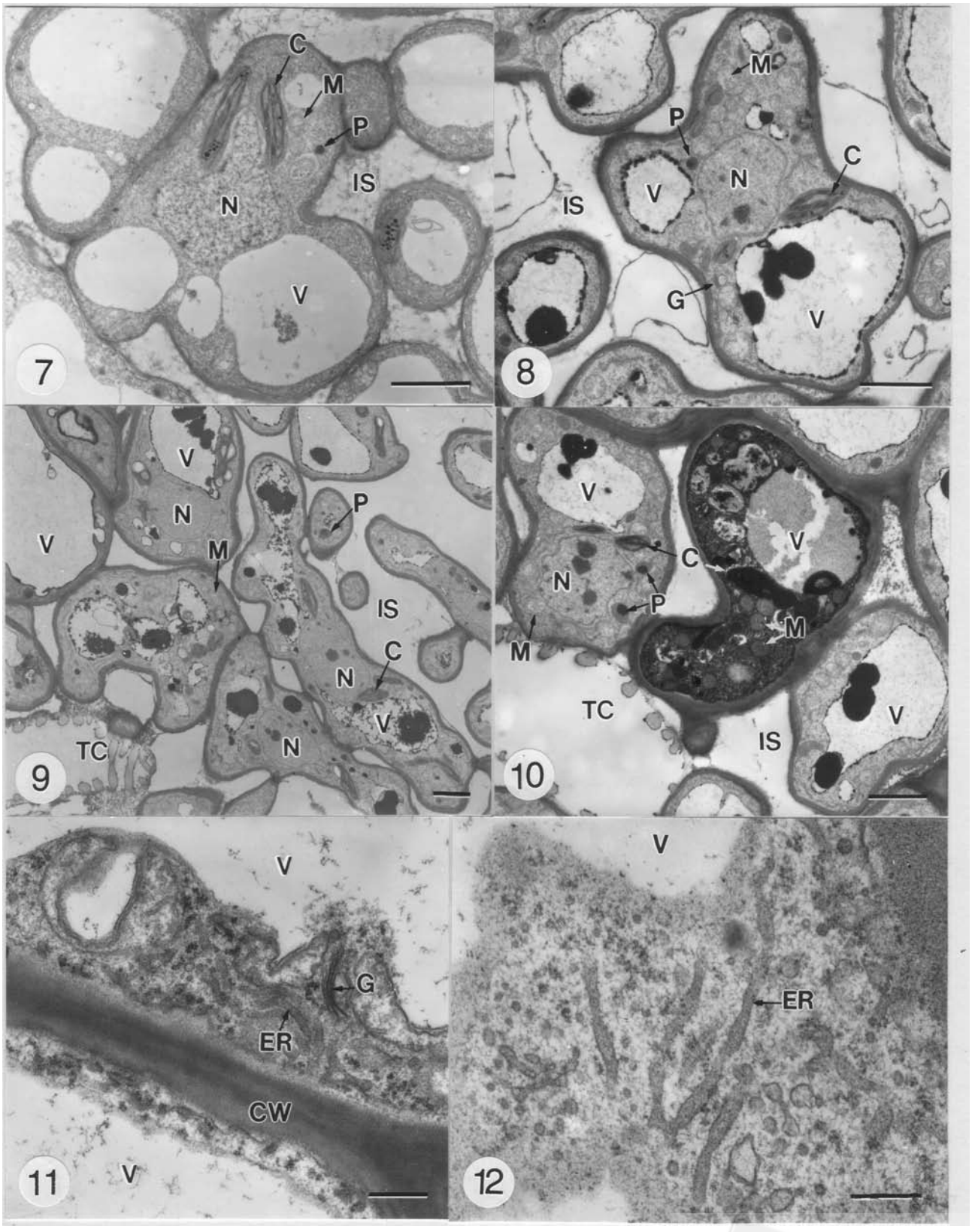
Figures 7-18. TEM micrographs showing salt injury on the epithem of hydathodes in *F. fomasana* Maxim. f. *Shimadai* Hayata. 7, Normal epithem cells; 8, Salt-susceptible epithem cells located beneath water pores under salt stress; 9, Salt-tolerant epithem cells above tracheid cells under salt stress showing their cytoplasm become electron-dense and a lot of electron dense materials accumulated in vacuoles; 10, Salt injury on epithem cell close to tracheids showing cytoplasm with electron-dense materials and many osmiophilic droplets and chloroplasts become electron-dense and osmiophilic; 11, Epithem cells with dilations of ER membrane and Golgi body stacking membrane caused by salt stress; 12, Paradermal section under cell wall showing the dilated endoplasmic reticular system; 13, Salt-tolerant epithem cells at stage later than that of Figure 8 showing cytoplasm with more electron-dense materials, many mitochondria and numerous osmiophilic droplets in vacuoles, and some fluid-phase endocytosis (arrowheads) were observed; 14, Collapsed epithem cell under water pore (star) caused by direct ionic toxicity under salt stress. Their nucleolus and cell organelles were collapsed and cytoplasm became osmiophilic; 15, Epithem cells under sharp salt stress showing nucleus with many osmiophilic particles (white arrowheads) and a condensed nucleolus (arrowhead), and their chloroplasts also collapsed and osmiophilic; 16, Ultrastructure of plasmodesmata between normal and salt-damage cells. Arrowhead indicates the callose synthesis presented on the side of damaged cell; 17, Fluid-phase endocytosis (arrowhead) occurred in early stage of salt damage epithem cell under salt stress; 18, Epithem cell with dehydrated organelle membranes and myelin-like structures (arrowheads) under desiccation. (7-10 and 13-14, all bar scales = 2.5 μm; 15-17, all bar scales = 0.5 μm; 11, 12 and 18, bar scales = 0.25 μm). Figure abbreviations: C, chloroplast; CW, cell wall; ER, endoplasmic reticulum; G, Golgi body; IS, intercellular space; M, mitochondrion; N, nucleus; P, peroxisome; PD, plasmodesmata; PT, plastid; S, starch-containing plastid; T, trichome; TC, tracheid cell; V, Vacuole; WP, water pore.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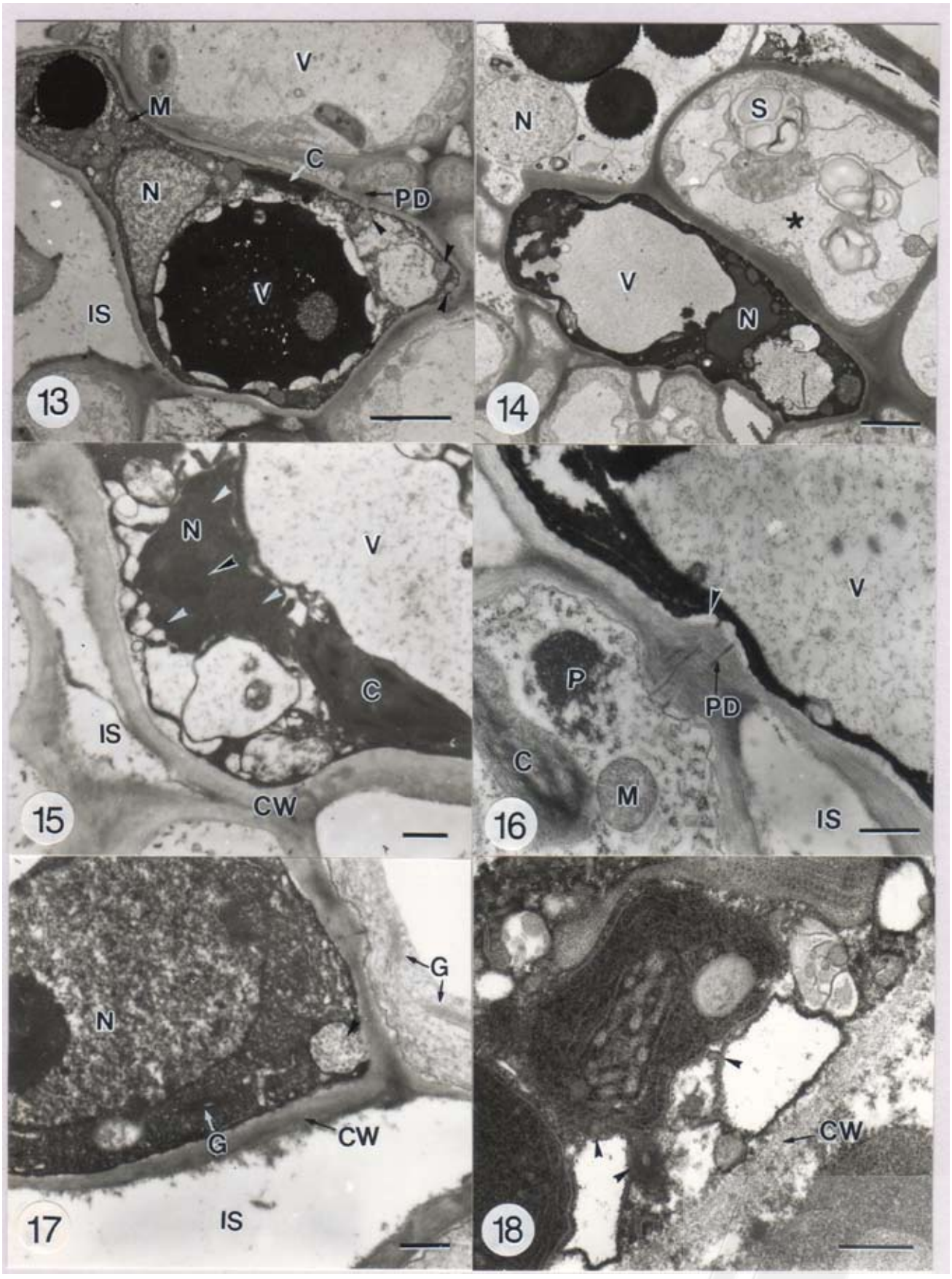
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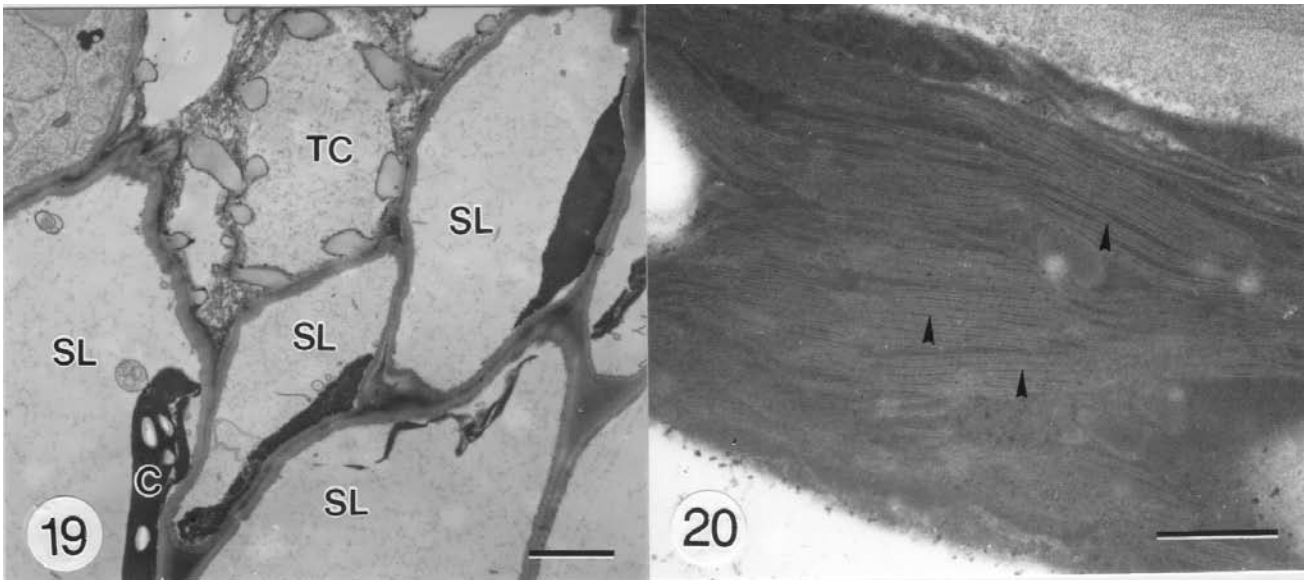


Figures 19-20. TEM micrographs showing salt injury on a sheath layer of hydathodes in *F. formosana* Maxim. f. *Shimadai* Hayata. 19, Chloroplasts of the bundle sheath cell become electron dense under dehydrated state; 20, Chloroplast enlargement from Figure 19 showing their thylakoid membrane becomes less stained during dehydration. (19, bar scale = 2.5 μm; 20, bar scale = 0.25 μm). Figure abbreviations: C, chloroplast; CW, cell wall; ER, endoplasmic reticulum; G, Golgi body; IS, intercellular space; M, mitochondrion; N, nucleus; P, peroxisome; PD, plasmodesmata; PT, plastid; S, starch-containing plastid; T, trichome; TC, tracheid cell; V, Vacuole; WP, water pore.

effects of high salts on the cells. In addition to osmotic stress, plants are suffered from the potential hazards of specific ion toxicities because of excessive accumulations of ions, such as Cl^-, SO_4^{2-}, Na^+ and Mg^{2+} (Zaitseva and Sudnitsyn, 2001). Of course, hydathodes also face the same problem of high salt condition caused by evaporated condensation. So both specific ion-toxic and osmotic-induced damages can be observed in hydathodes.

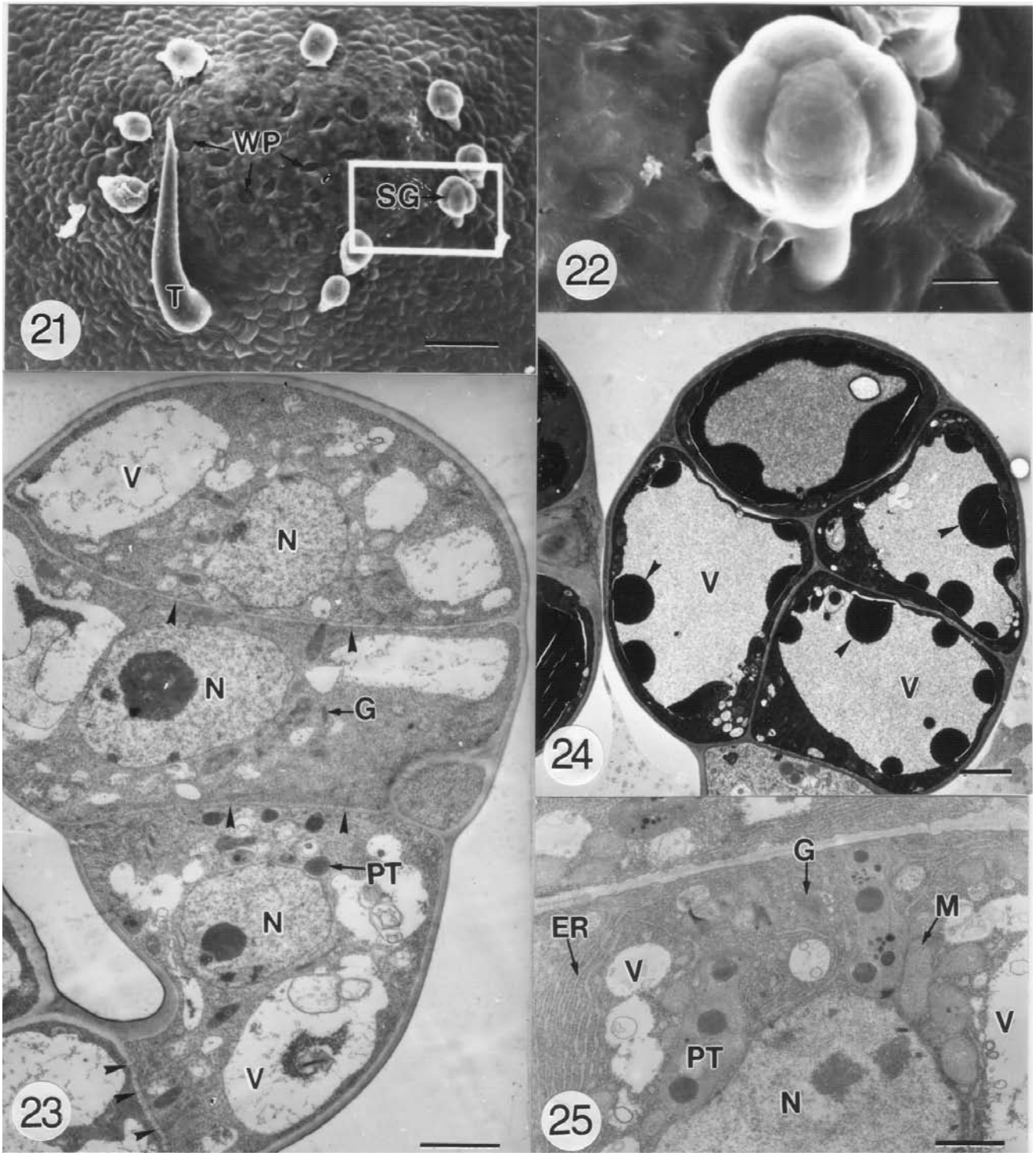
The evaporation triggers hypertonic conditions in the epithem cells within hydathodes. The plasma membrane of epithem cells is very unstable. In our previous observation on the structure of plasmalemmosome of epithem cell (Chen and Chen, 2005), we thought that the particular structures might be the derivatives of membrane-invagination were induced by hypertonic stress. There are two reasons to support our suggestions. First, the epithem cells immerge fully in guttated fluid that contains a large number of solutes consisting of mineral salts and organic materials during guttation (Choi et al., 1997; Mizuno et al., 2002). Evaporation follows the guttation will result in the sharp change of solute concentrations to cause high salt and high osmotic conditions in the hydathodes (Wilson et al., 1991), and this hypertonic situation induces the plasma membrane invagination (Gordon-Kamm and Steponkus, 1984). Second, the sinuous cell wall of the epithem cell has a potential ability to propose the larger membrane surface and to regulate a unique membrane area/cell volume ratio. This ability is important for epithem especially under the plasmolysis and deplasmolysis cycles be induced by repeating guttation and evaporation. During the transition between plasmolysis and deplasmolysis, osmotic stress induced the fluid-phase endocytosis is easy to take place (Oparka et al., 1990; Wartenberg et al., 1992; Bahaji et al., 2003).

Salt effects on water pores development

Curtis (1943) demonstrated that three things might happen to the guttation drop on a plant: (1) it may roll off, (2) it may evaporate, or (3) it may be sucked back into the leaf. However, even it may evaporate or be sucked back into the leaf, the water pore and epidermal cells of hydathodes' surface are the first to be affected. If normal water pores under high salt and osmotic changes it often results in damages of cells. As shown in Figures 5 and 6, one or two guard cells of water pore are necrotic and malformed due to high salt and high osmotic stresses. This salt injury can occur in any stages of water pores development that affects the pore formation and terminates their differentiation and abolishes their normal function.

Salt effects on epithem development and sheath layer

The guttation water is sucked back into hydathode where the hypertonic solution is harmful to the cells; another damage is going by new products or changes in the guttation fluid which was produced by bacteria, molds or enzymes, it may be toxic to the epithem cells when the fluid is sucked back (Curtis, 1943). Serial sections of salt-injured epithem cells show that epithem cells abutting



Figures 21-25. Electron micrographs showing non-glandular and glandular trichomes present near the hydathode in *F. formosana*. 21, Adaxial surface of laminar hydathodes consisting of a group of water pores, a non-glandular trichome and 9 salt-glangular trichomes surrounding the hydathode; 22, Larger magnification of the insert in Figure 21 showing matured salt glandular with a short stalk and eight-celled head cells; 23, Six cells stage of salt glandular showing one basal cell, one stalk cell and four-celled head cells. Several plasmodesmata are observed between a stalk cell and basal cell, and also between stalk cell and head cells (arrowheads); 24, A mature glandular trichome at ten cells stage showing: four head cells with a large central vacuole containing tannin droplets (arrowheads) and an electron-dense peripheral cytoplasm; 25, Magnification of a stalk cell at mature glandular trichome showing a puffy endoplasmic reticulum system associated with small vesicles, mitochondria, and plastids with osmiophilic droplets. (21, bar scale = 50 μm; 22, bar scale = 10 μm; 23-24, bar scales = 2.5 μm; 25, bar scale = 1 μm). Figure abbreviations: C, chloroplast; CW, cell wall; ER, endoplasmic reticulum; G, Golgi body; IS, intercellular space; M, mitochondrion; N, nucleus; P, peroxisome; PD, plasmodesmata; PT, plastid; S, starch-containing plastid; T, trichome; TC, tracheid cell; V, Vacuole; WP, water pore.

water pores are easily damaged and the symptoms of salt injury spread gradually from water pores to tracheid vein-ends. This phenomenon is corresponding to the condensation of guttated solution induced by evaporation that salt concentration near water pores is higher than that of tracheid-ends. Epithem has two types of cells that can be observed under salt stress. One type is a tolerant cell and their major characteristics are tannins and electron-dense material accumulated in several vacuoles and many proliferated peroxisomes in the cytoplasm. The other is a salt susceptible cell, whose cells can't effectively respond to the salt stress on time and become collapsed and dehydrated, and usually there is nothing in their vacuoles. It is interesting that susceptible cells under salt stress could first synthesize and accumulated the callose on the side of the plasmodesmata and to block the communication with the other normal cells. It seems to prevent the further enlargement of salt damage effects.

In contrast to epithem cells, the sheath layer cells do not have sinuous cell walls but have a big central vacuole. Although sheath layer cells have a central vacuole that still haven't the sufficient ability to survive under the restricted salt stress as that of epithem cells. Base on this observation, the sheath layer cells undergo harmful plasmolysis and, even, dehydration under high osmotic stress. In particular, the thylakoid membranes of chloroplast are less stained under dehydration (Figure 20); this phenomenon reflects the possibility of fast dehydration occurred in this case. However, the thylakoid membrane desiccation of chloroplast in epithem cells is less drastic. It might imply that some compatible solutes are biosynthesized and accumulated in vacuoles which result in a dehydration of epithem cells.

How do epithem cell adapt high salt and osmotic stresses?

We hypothesized that epithem cells have special regulated mechanisms for morphological and physiological adjustments to adapt high salt stress. Those changes have involved the polyamine biosynthesis that seems to function in osmotic adjustment, protection, and also in regulating ion uptake and compartmentation as well (Bohnert et al., 1995). The lobed shape epithem cells have prominent sinuous cell walls for increasing the contact surface of epithem cells with environment to enhance cell's absorption rate (Sattelmacher, 2001; Chen and Chen, 2005). Larger vacuolation increases the ratio of vacuole surface volume to cytoplasm and elevated the vacuole's efficiency substantially. These morphological adjustments increase the capability of cells to tolerate environment stresses (Chang et al., 1996; Rahman et al., 2002). In addition, mineral ions (Na^+, Cl^-, La^{3+} and NO_3^-) are also absorbed by membrane endocytosis through the multivesicular bodies into larger vacuoles that have a minimal effect on cytoplasm (Lazzaro and Thomson, 1992; Kurkova and Balnokin, 1994). This mechanism may let many electron-dense materials to accumulate in the vacuoles. Moreover, the endoplasmic reticulum is a unique type of endomembrane system of plant cells in the response to environmental stresses (Hayashi et al., 2001; Matsushima et al., 2002), so that we can observe the puffy ER structure in epithem (Figures 11 and 12). Furthermore, many plasmodesmata present among epithem cells that perform as supercellular-network structure having more ability to regulate the stress than ordinary one's (Lucas and Lee, 2004).

Particularly, there are plasmolemmasome structures forming in salt-tolerant epithem cells. It illustrates that fluid-phase endocytosis can alleviate salt stress for cells (Oparka et al., 1990; Wartenberg et al., 1992). Indeed, plasmalemma invaginated into the cytoplasm is the obligatory process that cells accommodating an osmotic drive decrease the membrane surface area (Kubitscheck et al., 2000). Epithem cells can regulate the salt stress through adjusting the rate of membrane trafficking into cytoplasm from the plasma membrane (Levine, 2002).

Epithem cells with function to retrieve nutrient from xylem sap were documented (Dieffenbach et al., 1980; Wilson et al., 1991). So, epithem having the ability of retrieval nutrient and salt-tolerance under such high salt stress should obtain it through the evolution. From the viewpoints of morphological data, we suggest that the epithem might have several adaptation mechanisms. First, epithem cells develop sinuous cell wall and many large vacuoles to increase their tolerance under stress conditions. The sinuous cell wall reduces the membrane tension that stimulates the endocytosis and increases the macromolecular uptake under osmotic stress (Apodaca, 2002; Bahaji et al., 2003). Moreover, many larger vacuoles also increase the vacuolar volume during salt stress and can serve as salt tolerance mechanisms in plant cells (Mink, 1993; Kinoshita et al., 1998; Marty, 1999; Mimura et al., 2003). Second, abundant and prosperous endomembrane systems, containing more stacking Golgi apparatus, ER membrane and a numerous small traffic vesicles in epithem cells, are used to support membrane equilibration between the processes of endocytosis and exocytosis that are caused by the plasmolysis and deplasmolysis cycles (Oparka et al., 1993; Reuzeau et al., 1997; Staehelin, 1997; Steer, 1988; Battey et al., 1999; Mazel et al., 2004). Third, epithem cells possess special antioxidant organelles, which particularly increase the number of peroxisomes and their activity. High salt stress not only increases the production of the superoxide free radicals, but also induces the synthesis of antioxidant isozymes and increases their activity in peroxisomes that can metabolize reactive oxygen species and reduce free radials (Palma et al., 1991; Hernandez et al., 1993; Lopez-Huertas et al., 2000; Corpas et al., 2001). Finally, abundant plasmodesmata existed between epithem cells contributes to improve the transport efficiency between cells (Crawford and Zambryski, 1999; Cantrill, et al., 1999; Lucas, 1995; Epel, 1994; Lucas and Lee, 2004).

The function of salt-glandular trichomes during the leaf development

Guttation happens in the later stage of leaf development of *F. formosana* and its running smoothly must be based on maturity of hydathodes, especially on water pores and tracheid-ends maturation, and the intercellular space formation (Chen and Chen, 2006). Having a problem prior to the hydathode function is how to remove excessive salt ions from leaves when the salt concentration increases in xylem sap following transpiration in the early stage of leaf development. So, the role of salt-glandular trichomes in the early stage of leaf development is very important for eliminating excess salt in xylem saps of hydathode. In guttation, epithem cells of hydathodes can reduce the salt concentration in xylem sap (Klepper and Kaufmann, 1966). As shown in Figure 21, salt-glandular trichomes obviously occur in the vicinity of hydathodes' surface in the early stage of leaf development. We thought that the role of these salt-glandular trichomes near hydathodes is to exclude excessive salt ions. At first, the basal and the stalk cells play an import role in collecting and transporting the excess of salt ions into head cells from xylem saps. Afterwards, salt ions are accumulated in head cells and they gradually fall off during the maturation stage of leaf development.

Hydathode, an ideal system for study in response of cells under high salt and high osmotic stresses in plant

Under salt stress, hydathodes procure both nutrient retrieval and surviving functions that they have more efficiently regulating and resisting mechanisms for salt stress. From this viewpoint, the epithemal cytological data of hydathodes may provide useful information for studying cell in response to salt and osmotic stresses.

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細葉天仙果葉部泌水器的研究：III. 泌溢作用對泌水器的鹽傷害

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由於泌溢溶液內含有機及無機鹽類，加上白天蒸散作用的濃縮效果，提高了泌水器內鹽類的濃度，高鹽逆境對泌水器造成組織的傷害。本研究以電子顯微鏡觀察高鹽逆境對細葉天仙果葉部泌水器所引起的傷害。從超微構造主要的鹽害病徵是許多濃電子密度粒子分佈於細胞核與各種胞器內，造成細胞核仁濃縮、消失；細胞質內部胞器與內膜系崩解變成親過氧化鐵的物質存在；隨著急速脫水作用使崩解的膜系呈透明化而形成類囊膜鞘構造。由不同的鹽害程度發現，不同組織呈現不同鹽害耐性，末梢組織耐高鹽能力較其他組織高，顯示泌水器內末梢組織細胞似乎已演化出一些有效的調節機制，用來適應高鹽及高滲透壓的逆境；除了生理代謝的調節適應外，包括了迂迴的細胞壁，大量增殖的過氧化小體、發達的內膜系統與內噬作用等形態構造上的改變，藉由增加與環境接觸面積來提高液胞功能的有效性以增加細胞對高鹽逆境的耐受性。

關鍵詞：泌水組織；天仙果；液相吞食；泌水器； 鞘層； 鹽害；水孔。