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Ultrastructural Study on the Development of Lenticels of *Ficus microcarpa* L. f.

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ABSTRACT : The ultrastructural changes of lenticels on the branches of *Ficus microcarpa* L. f. during the developmental stages were studied. All the living precomplementary cells, complementary cells, and cells of the lenticel phellogen and the closing layer contained a large vacuole, nucleus, mitochondria, plastids, and dictyosome. During the differentiation and maturation of the lenticels, the cell walls of the closing layer, precomplementary cells, and complementary cells were progressively suberized. The electron microscopic examination of the suberized layers in the cell walls showed a lamellar appearance. The suberized outer tangential walls of the mature cells in the closing layer were found to be separated from their primary walls, and in the central vacuole fibrous fragements were observed. The subcellular localizations of acid phosphatase were mainly distributed in the cell walls and vacuoles near the tonoplast. In the closing layer, acid phosphatase was detected only in the plasmodesmata.

KEY WORDS : ***Ficus microcarpa*, Lenticel development, Ultrastructure, Suberin lamella, Acid phosphatase.**

INTRODUCTION

Lenticels usually form in the periderm of stems and roots of dicotyledons with pronounced secondary growth (Eames and MacDaniels, 1947; Esau, 1977; Fahn, 1990). The distribution of lenticels on the branches is environmentally influenced (Kuo-Huang and Hung, 1995). Nevertheless, the determination of the structural type of the lenticel is genetically controlled. The distribution and morphology of lenticels in the higher plants have been studied for taxonomical or functional purposes. But only a few reports (Wetmore, 1926; Clements, 1935; Jacob *et al*., 1989) were concerning with the developmental processes of lenticels. One of the reasons for this might be due to the hardness and complex composition of cells in the samples, so the sample preparation is difficult.

In our earlier paper (Kuo-Huang and Hung, 1995), we have described the distribution and gross anatomy of lenticels in the branches of *Ficus microcarpa*. In this study, the ultrastructural changes of lenticels during the differentiation and maturation processes were investigated. Besides, the subcellular localizations of acid phosphatase as revealed by cytochemical methods were also described for cells in the mature lenticels.

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MATERIALS AND METHODS

Branches of *Ficus microcarpa* L. f. were collected from the campus of National Taiwan University. Excised small stem samples with lenticels at different developmental stages were fixed in a 0.1 M phosphate buffer (pH 6.8) containing 2.5% glutaraldehyde for 2 –4 h, post-fixed in buffered 1% OsO_4 for 2h, dehydrated in an acetone series, and embedded in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate and then examined with a Hitachi H 600 TEM.

For the cytofluorescent test of suberized cell wall, some samples with lenticels at different developmental stages were fixed in FAA (formalin-acetic alcohol) for 24 h, dehydrated in a TBA (tert-butyl alcohol) series, and then infiltrated and embedded in paraffin. The 10 μm sections were deparaffinized with xylene, stained with 0.05% toluidine blue O for 45 min and with 0.1% neutral red for 1 min (Lulai and Morgan, 1992), and then photographed with a Leitz Diaplan microscope (excitation filter BP350-460; suppress filter LP5220; dichromatic mirror RKP510).

For the subcellular localization of acid phosphatase, some samples of mature lenticels were prefixed with 2.5% glutaraldehyde at 4°C for 4 h, washed with acetate buffer, and then reacted with Gomori's solution at 35°C for 90 min (Chen *et al.*, 1992). The material was postfixed with 1% OsO_4 for 2 h and then processed for TEM as described above.

RESULTS

The differentiation processes of lenticels on the tips of growing branches of *Ficus microcarpa* were investigated. The maturation of lenticels was noted to end within a short distance from the tip of the branches. The mature lenticels produce several strata of closing layer and complementary cells yearly (Kuo-Huang and Hung, 1995). Of the different sections examined, the following sequence of ultrastructural changes was deduced.

The initiation and formation of lenticel phellogen

In comparison with the angular collenchymatous cortical cells (beneath the ordinary epidermal cells), the loosely arranged precomplementary cells (beneath the stomata) were small and thin-walled parenchyma (Fig. 1). They divided in different planes (Fig. 2). However, the periclinal divisions of precomplementary cells progressed inwards and the lenticel phellogen was formed. The cytoplasm of precomplementary cells and cells of the lenticel phellogen appeared to be fairly active, containing large numbers of organelles (mitochondria, chloroplasts and dictyosome) as well as considerable areas of polysome-covered rough endoplasmic reticulum (Fig. 3).

The differentiation of closing layer and complementary cells

Centrifugally, the phellogen of the lenticel produced firstly 1 or 2 closing layers containing cells with suberized outer tangential walls (Figs. 4, 6) and then 1 –4 layers of unsuberized complementary cells. At this stage the lenticel phellogen also produced centripetally 1 or 2 layers of unsuberized phelloderm cells. Besides, soon after the formation of lenticel phellogen, the cortical cells beneath the ordinary epidermal cells formed the

periderm phellogen, which produced the suberized cells of phellem to the outside of the phellogen (Fig. 24). The cytoplasm of the cells in the lenticel phellogen was similar to previous stages. There were a considerable number of short dispersed fragments of rough endoplasmic reticulum, and some starch grains were found in the plastids (Fig. 5). The cytoplasm of the tannin cells in the lenticel phellogen was more densely stained.

The mature lenticel

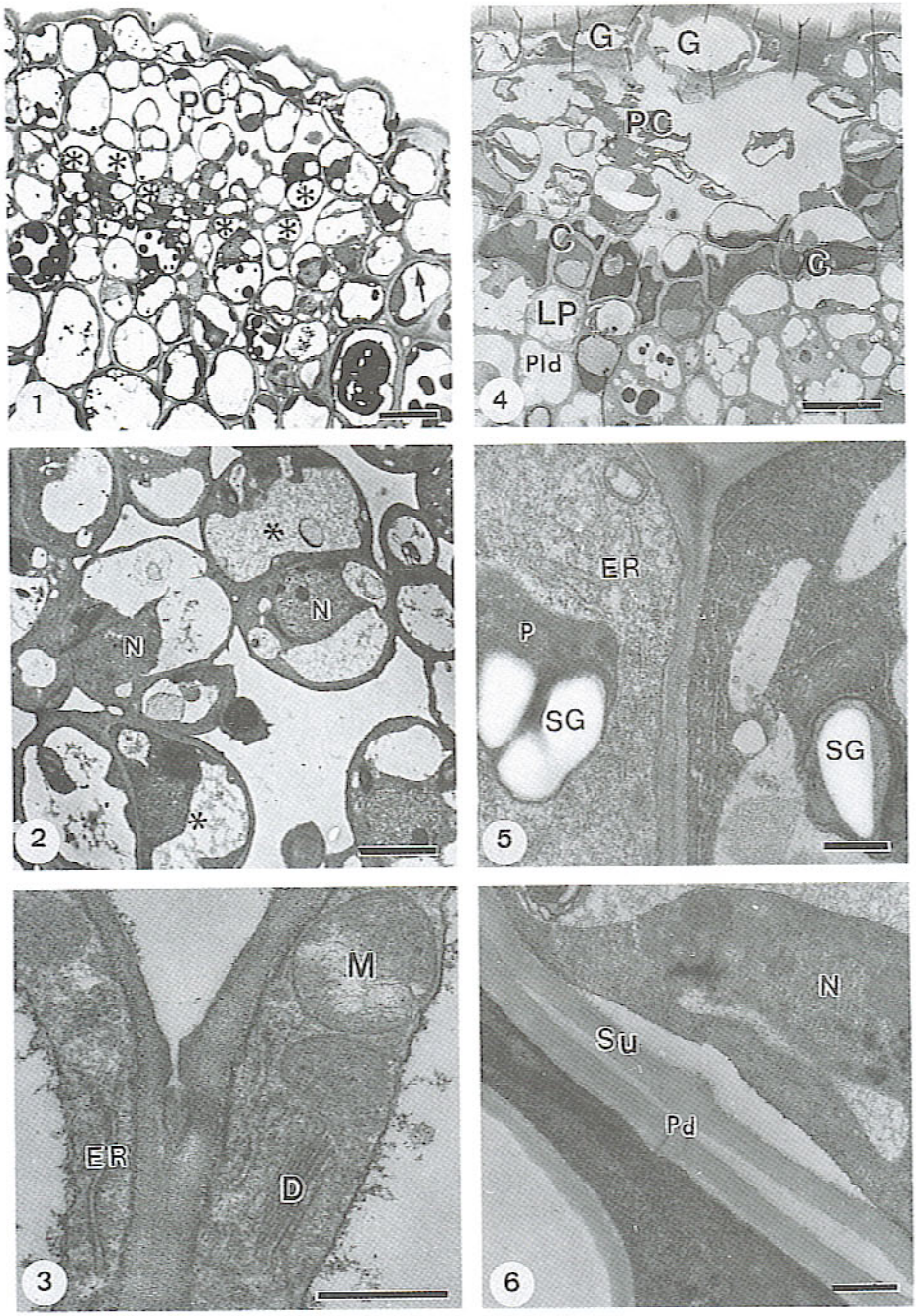
The phellogen of the lenticel alternately produced one compact closing layer and one to four layers of loosely arranged unsuberized complementary cells (Fig. 7). The whole lenticel was interspersed with intercellular spaces. Some cells of the phellogen were filled with tannin, and from them periclinally produced cells of the closing layer and complementary cells also contained tannin.

The cells of the phellogen in mature lenticels were compactly arranged, and between the cells plasmodesmata were found (Fig. 8). However, in the areas of complementary cells there were abundant intercellular air spaces (Fig. 9). The cytoplasm of the cells in the closing layer contained a nucleus, mitochondria, plastids with some starch grains, and a large central vacuole with membraneous fragments (Figs. 10, 12). In the cell walls of the closing layer, the lamellar suberin layers firstly appeared in the outer tangential walls (Fig. 13) and then in the radial walls between the cells of the closing layer (Fig. 15). The inner tangential walls were also lightly suberized (Fig. 14). The suberized outer tangential walls of the mature cells in the closing layer were thicker than the other cell walls and were found to be separated from their primary walls. These cells were concave in shape (Fig. 12). The cell walls of the precomplementary cells were also suberized (Fig. 11), and the cytoplasm was more densely stained. These precomplementary cells were collapsed. The increase in number of layers of the closing layer and complementary cells from the lenticel phellogen caused the rupture of the epidermis from the sites of stomata. The precomplementry cells and some outer strata of the closing layer and the complementary cells were then progressively pushed out (Figs. 22, 23). The outermost closing layer replaced the epidermis to become the protective layer.

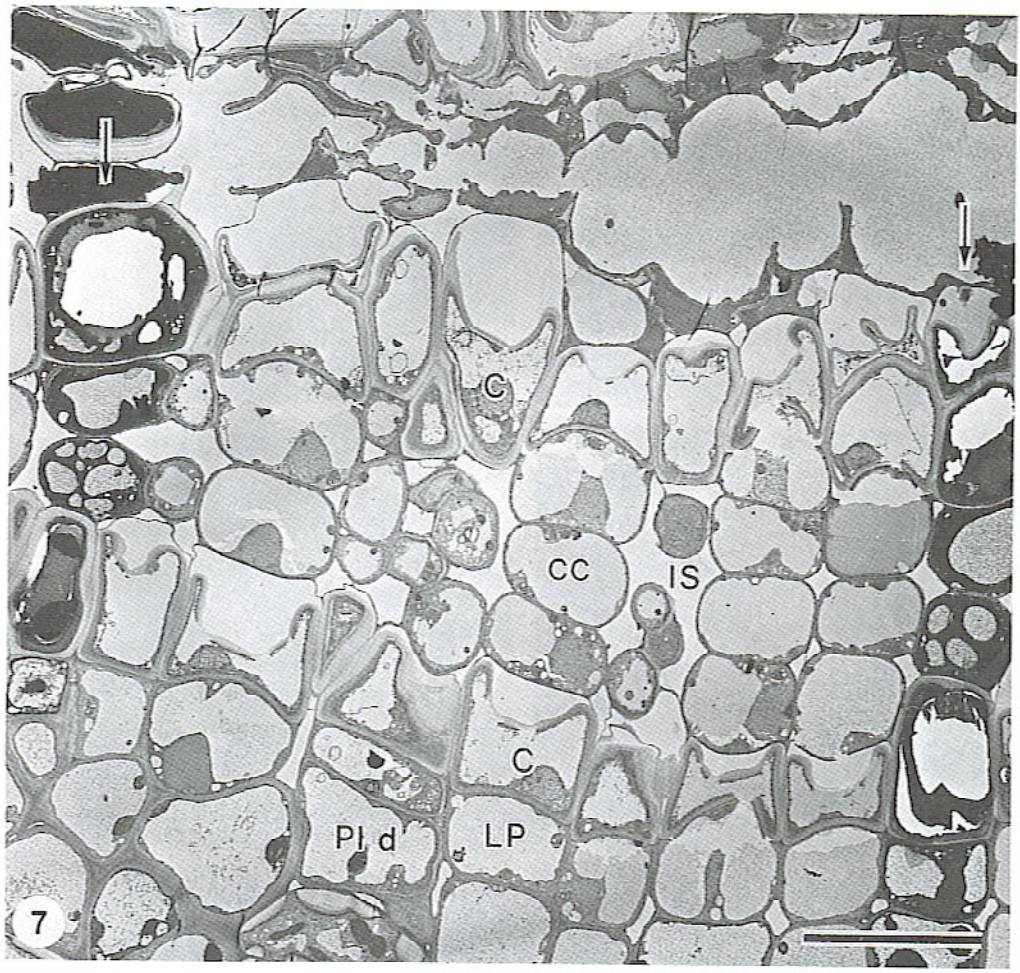
By the treatment with neutral red/toluidine blue O as a fluorescent probe for suberin, fluorescence appeared in the cells of the closing layers and the precomplementary cells (Figs. 16-21). In the cells of the closing layer, the presence of fluorescence for suberin was found in the outer tangential walls, radial walls, and also lightly in the inner tangential walls (Figs. 22, 23). Fluorescence of suberin was observed in the outer surfaces of the epidermal and guard cells and also in the cell walls of the sclereids and phellem (Figs. 24, 25). The inner tangential walls of the deepest layer of cells in the phellem produced light fluorescesce.

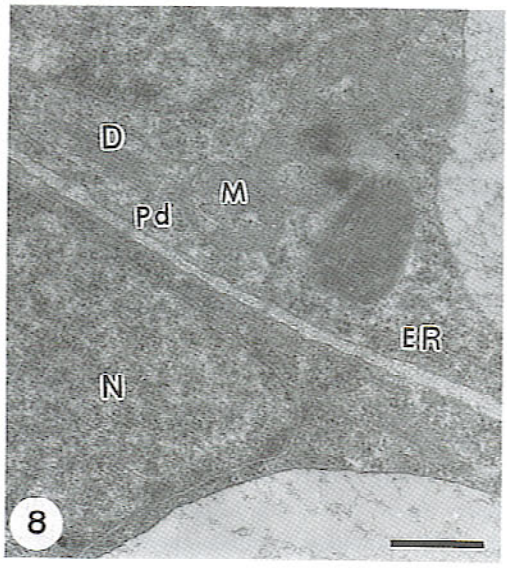
The localizations of acid phosphatase in the mature lenticel were found in the precomplementary cells, complementary cells, lenticel phellogen and in the phelloderm (Fig. 26). This enzyme was mainly distributed in the cell walls and vacuoles near the tonoplast. But in the cells of the closing layer, acid phosphatase was detected only in the plasmodesmata of the inner tangential wall (Figs. 27-29).

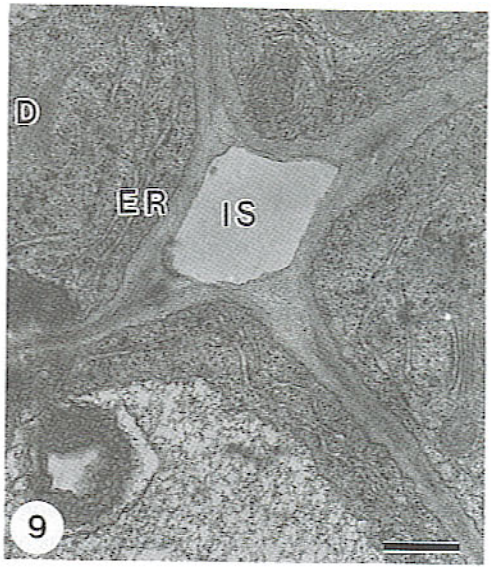
Abbreviations: C: Closing layer. CC: Complementary Cell. D: Dictyosome. ER: Endoplasmic Reticulum. G: Guard cell. IS: Intercellular Space. LP: Lenticel phellogen. M: Mitochondrium. N: Nucleus. P: Plastid. PC: Precomplementary Cell. Pd: Plamodesma. Pld: Phelloderm. PW: Primary Wall. SG: Starch Grain. Su: Suberin. SC: Sclereid.



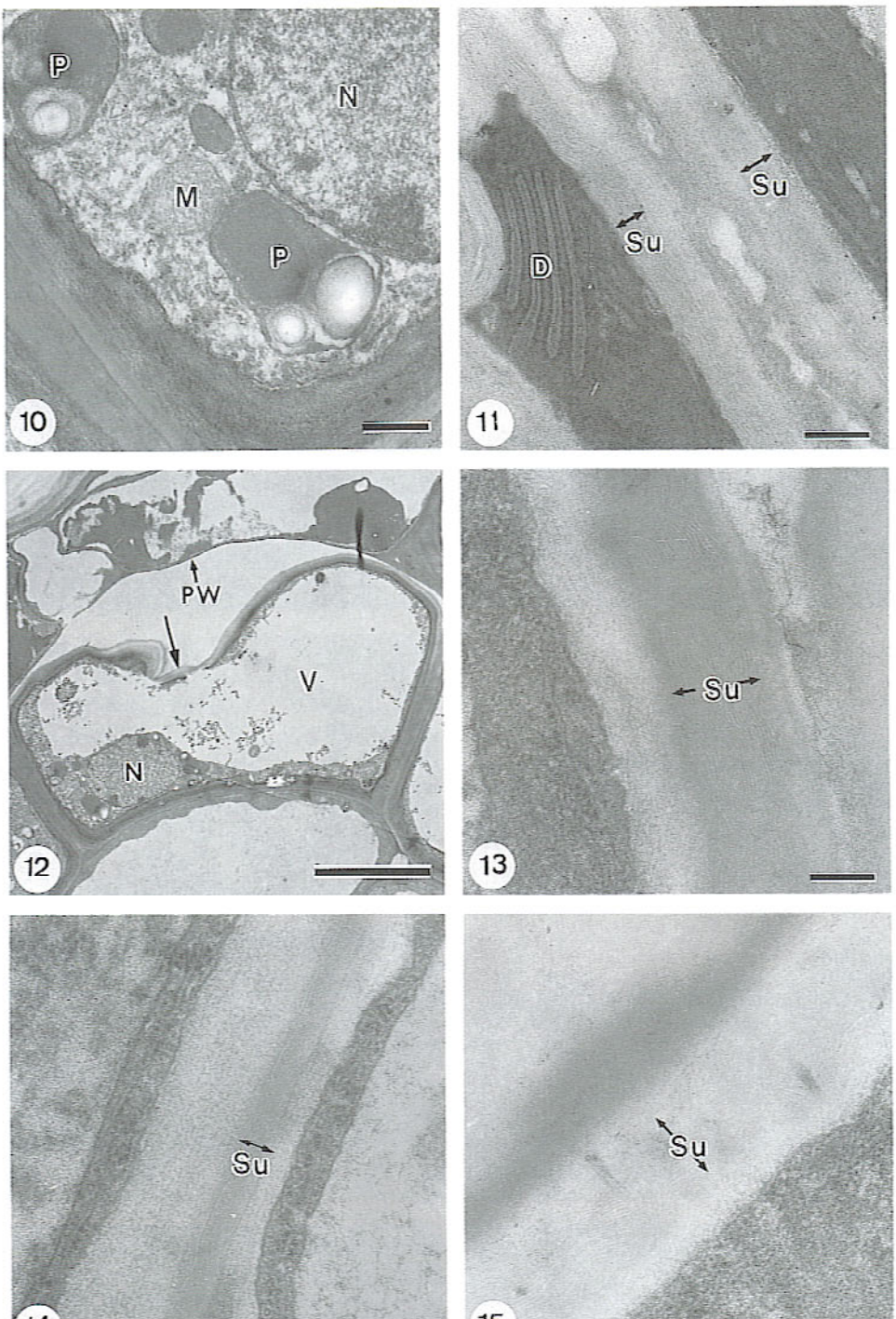
Figs. 1-3. Cross sections of lenticels at the stage of initiation and formation of lenticel phellogen. 1. Collenchymatous cortical cells (arrows) located beneath the ordinary epidermal cells, and the loosely arranged precomplementary cells being small and thin-walled parenchyma. Periclinal divisions of precomplementary cells progressed in the cortex inwards forming lenticel phellogen (*). Bar= 10μm. 2. Precomplementary cells and cells of lenticel phellogen. Bar= 10μm. 3. Portion of cytoplasm of cell in lenticel phellogen. Bar = 0.5μm. Figs. 4-6. Cross sections of lenticels at formation of closing layer and complementary cells. 4. Lenticel phellogen producing closing layer to the outside and one layer of phelloderm to the inside Precomplementary cells and outer surrounding epidermal cells including guard cells are collapsed. Bar= 10 μm 5. Portion of cells in lenticel phellogen. Bar= 0.5μm. 6. Portion of cells of closing layer showing the secondary wall with lamellar structure of suberin layers and plasmodesma between the cells of closing layer. Bar= 0.5μm.



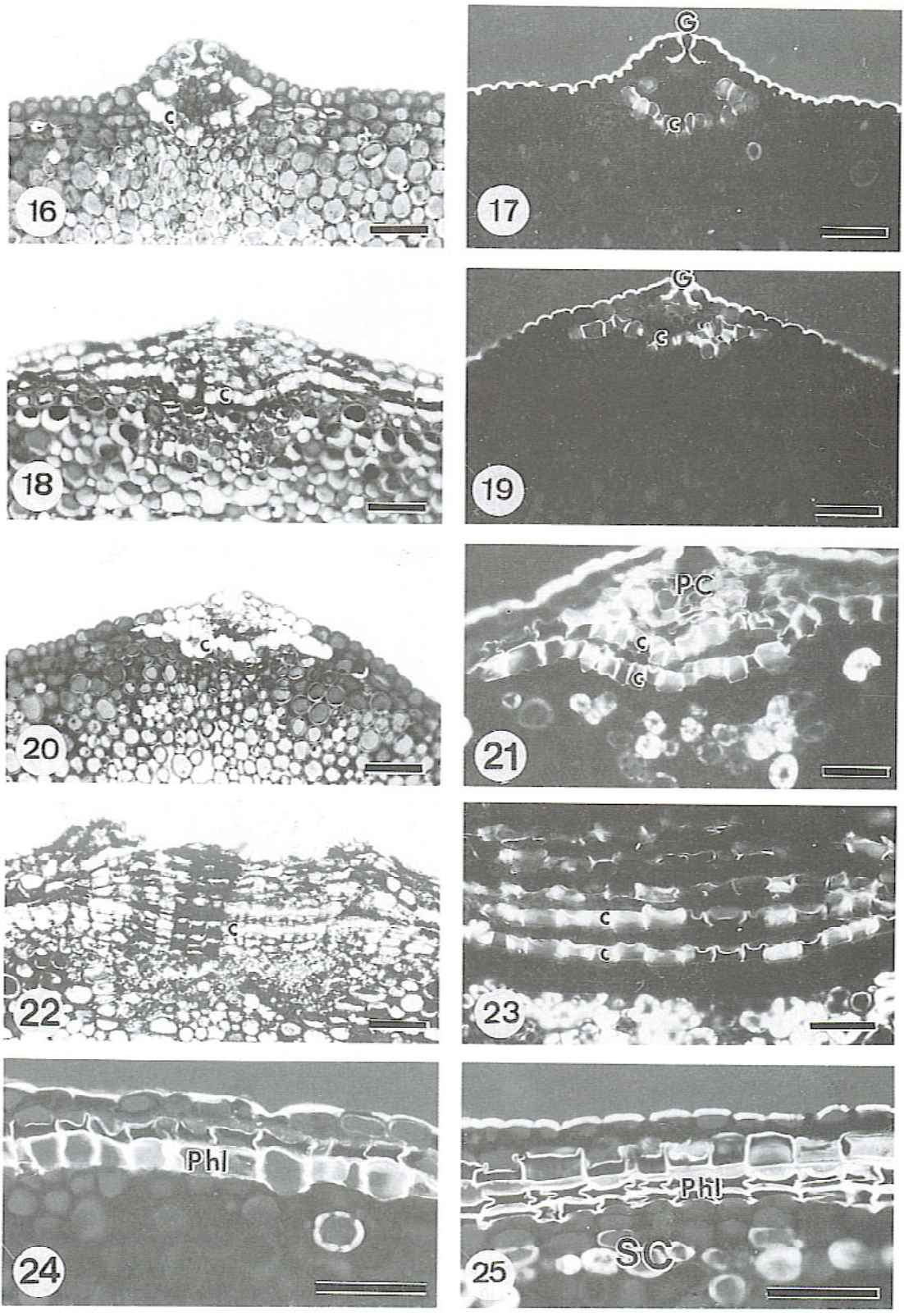




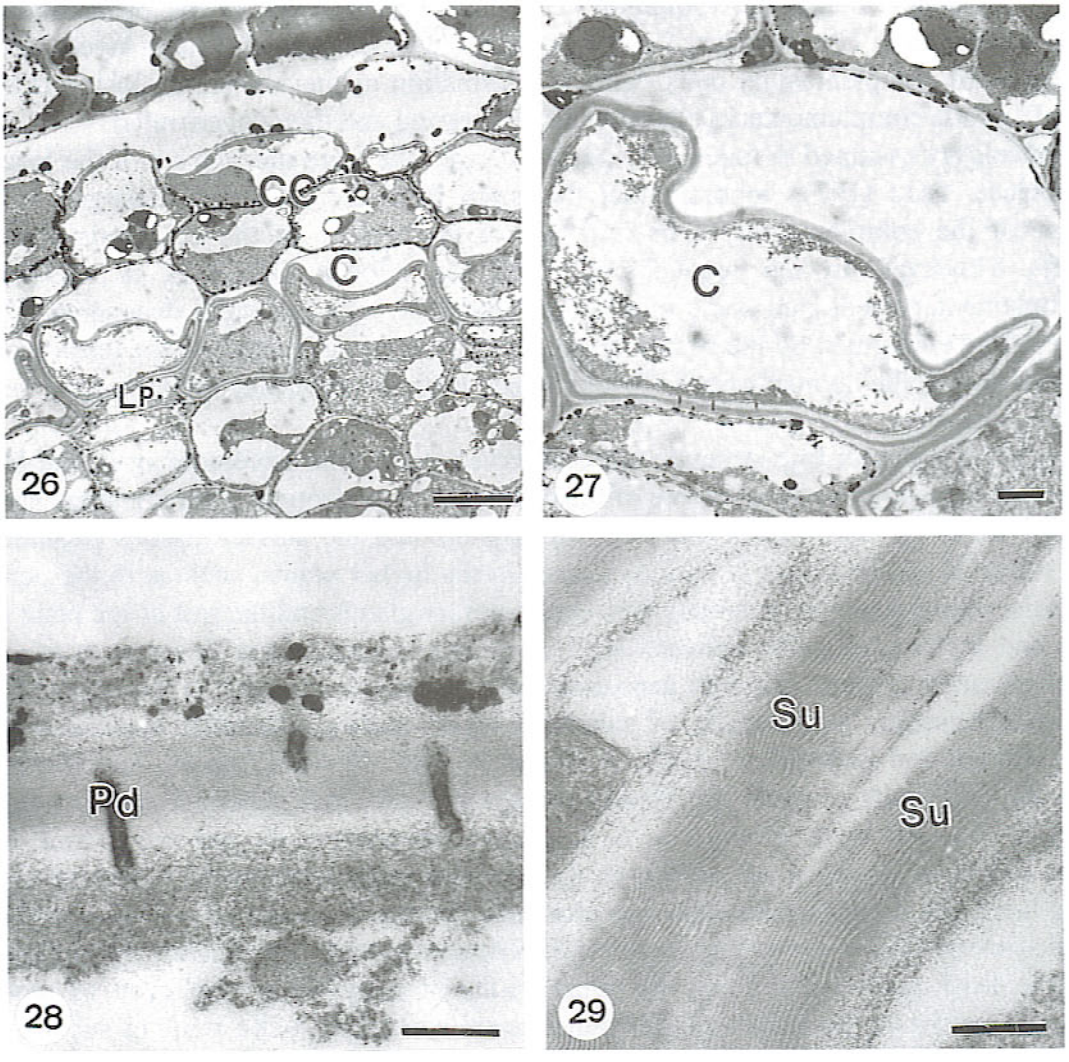
Figs. 7-9. Cross sections of mature lenticels. 7. Lenticel phellogen producing closing layers and layers of complementary cells alternately. Bar= 5μm. 8. Portion of cells in lenticel phellogen. Bar= 0.5μm. 9. Portion of complementary cells showing the intercellular space. Bar= 0.5μm.



Figs. 10-15. Cross sections of mature lenticels. 10. Portion of cell in the closing layer. Bar= 0.5μm. 11. Portion of precomplementary cell showing the lamellar suberin layers in the cell wall. Bar= 0.5μm. 12. Cell of the closing layer showing the suberized outer tangential wall separated from the primary wall. Bar = 5μm. 13. Portion of outer tangential wall of a cell in the closing layer. Bar=0.2μm. 14. Portion of inner tangential wall of a cell in the closing layer. Bar= 0.2μm. 15. Portion of radial wall of a cell in the closing layer. Bar= 0.2 μm.



Figs. 16-23. Cross sections of lenticels at different developmental stages by normal light or fluorescent light (17, 19, 21, 23) microscopy. 24, 25. Cross sections of periderm by fluorescent light microscopy. All bars= 50μm.



Figs. 26-29. Subcellular localizations of acid phosphatase in mature lenticel. 26. Cross section of mature lenticel showing the activity of acid phosphatase located in precomplementary cells, complementary cells, lenticel phellogen and phelloderm and the precipitates mainly distributed in the cell wall and vacuoles near the tonoplast. Bar= 1 μm. 27. Cross section of cell in closing layer showing precipitates in the plasmodesmata of inner tangential wall. Bar= 5 μm. 28. Portion of inner tangential wall of cell in closing layer. Bar= 0.2 μm. 29. Portion of radial wall of cells in closing layer. Bar= 0.2 μm.

DISCUSSION

According to the structural types of lenticels grouped by Esau (1977), the lenticels of *Ficus microcarpa* show the highest degree of specialization. The cells of the lenticel phellogen produce several strata of the closing layer and complementary cells yearly. In an ultrastructural study, the development of this kind of lenticel has found only in the branches of *Fagus sylvatica* (Jacob *et al.*, 1989). In general, our findings on the cellular ultrastructural changes agree with that study. However, in *Fagus*, the newly formed lenticel phellogen produces at first some layers of complementary cells. These cells are unsuberized, but they

begin to degenerate before the formation of the first closing layer. In our observations, the lenticel phellogen of *Ficus* produced at the beginning 1 or 2 layers of closing layers, and then 1 –4 layers of complementary cells. After the formation of the suberized closing layer, the cell walls of the complementary cells were also suberized and then degenerated.

Lenticels are assumed to function in gas exchange. The open mechanism of the lenticel is still unclear. Esau (1977) reported that the force from the osmoticly enlarged volumn-increase of the complementary cells may induce the rupture of the epidermis above the lenticels. However, in the lenticels of *Ficus* and *Fagus* (Jacob *et al.*, 1989), the precomplementary cells and some outer complementary cells gradually degenerated before rupture of the epidermis. So the rupture may primarily occur as a result of the tension caused by an increase in the number of cells inside, which leave crushed precomplementary cells on the exposed surface.

Distinct changes in fine structure were observed during the development of the lenticel, particularly in relation to the structure of the cell walls. Suberization occurs in some layers of cells that lie at the interface between the atmosphere and the surface tissues (Kolattukudy, 1981, 1984). It can be found in various tissues in the higher plants, such as in the periderm, epidermis, endodermis, the boundary between secretory glands and the rest of the plant (Fahn, 1990), and in the connection between seed coats and vascular tissue (Espelie *et al.*, 1980). The suberization process involves deposition of a polymeric material in the cell wall. The electron microscopic examination of suberized layers shows a lamellar appearance (Sitte, 1962; Schmidt and Schoenherr, 1982) as shown in Figs. 13 –15. Suberin lamellae appear within the cell walls towards the plasma membrane side, and suberization may progress throughout the cell walls giving rise to a lamellar appearance for the entire wall. In the lenticel of *Fagus* (Jacob *et al.*, 1989), thick suberized walls were found in the cells of the closing layer, but no suberin lamella was observed.The lamellae seen after OsO_4 fixation are generally interpreted as alternating lamellae differing in polarity, the electron-lucent lamellae being the least polar ones. Thus, the electron-lucent lamellae are believed to represent suberin waxes, while the electron-dense lamellae are considered to be made of suberin (Sitte, 1975).

A technique using the lipid fluorochrome neutral red as a cytochemical probe was employed to detect the hydrophobic/lipid domain of suberin in native and wound periderm of potato tuber (Lulai and Morgan, 1992). And toluidine blue O is used as a counterstain to quench autofluorescence. By this staining method, fluorescence for suberin clearly appears in all the suberized cell walls in the lenticels and the periderm of *Ficus*.

The localization of acid phosphatase has been studied in many different plant tissues (Chen *et al.*, 1992). The subcellular distributions of acid phosphatase in the plant cells may be in the cell walls, vacuole, cytoplasm and the organelles. In this study, under the electron microscope, acid phosphatase activity in the lenticel cells was found largely associated with the cell walls. Similar results had been observed in the cells of the separation zone in the leaf petioles of *Phaseolus* (Hall and Sexton, 1974). Although the role of acid phosphatase in metabolism is still not throughly understood, but it is certainly involved in the processes of differentiation and senescence of plant tissues (Baker and Tadakazu, 1973; Chen *et al.*, 1992). Based on the results of this study, we suggest that the acid phosphatase may be concerned with the function of autolysis of cytoplasm, digestion of primary wall, and formation of

suberized cell wall. The lack of acid phosphatase in cells of the closing layer may be the result of poor infiltration of substrates during the sample preparation.

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榕樹皮孔發育過程中微細構造的研究

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摘　　要

本文研究榕樹枝條上皮孔發育過程中微細構造的變化。皮孔內尚存活的前充塞細胞、填充細胞、皮孔形成層細胞與封閉層細胞均含有大液胞、細胞核、粒線體、色素體與高爾基體。皮孔分化與發育過程中封閉層細胞、前充塞細胞與填充細胞的細胞壁均會漸漸地木栓化。以電子顯微鏡觀察木栓化的細胞壁可看到層狀的構造。成熟封閉層細胞之木栓化的外切面細胞壁與初生細胞壁分離，而在其液胞內具有纖維狀片段。酸性磷酸酶的次細胞分布，主要是在細胞壁及液胞內近液胞膜處，而在封閉層細胞則只分布於胞間連絲。

關鍵詞：榕樹、皮孔發育、微細構造、木栓層狀構造、酸性磷酸酶。

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