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Microstructural Characteristics of the Ordered and Disordered Leaves in *Citrus junos* Sieb.

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ABSTRACT

We compared microstructural features of the ordered cell and disordered leaves in *Citrus junos* Sieb. by electron microscopy. In the cell of the ordered leaves, many chloroplasts and large vacuoles were particularly observed. Also a lot of vessel, companion cell and big nucleus were presented in vascular bundle regions. The mitochondria and the other organelles were interspersed among the chloroplasts in a thin, peripheral layer of cytoplasm. The chloroplast possessed typical grana and intergranal lamellae, numerous starch grains and a few small osmophilic globules. Besides, microbodies were closely associated with the mitochondria and the chloroplast. The process of the formation of the secondary cell wall from primary cell wall was observed the vessel elements, the tonoplast wall and the secondary cell wall. It was observed that the oil sac with the unique perfume distributed the adjacent cell wall. In the cell of disordered leaves, the all of the organelles were thrust toward the cell wall due to the fusion of vacuoles in the cells. It was observed that a lot of the very small particles spreaded in the cytoplasm. The loss of unique perfume of the leaves was resulted in the destruction of the oil sac. Also, there was not observed grana, lamellae, starch and osmophillic globules in the chloroplast. The small distributed organelles was not observed but the elongation of the cell wall was proceed no longer. Therefore, the plasma membrane diverged from the cell wall. All of organelles in the cell had poor function and deformation. A massive vacuole was fulfilled in single cell and the vacuole contains a lot of large and small particles. The organelles were presented on the side of the cell wall according to the enlargement of vacuole and they were observed to be breakdown.

Key Words : *Citrus junos* Sieb., Ordered leaf, Disordered leaf, Microstructure, Organelles,

INTRODUCTION

Yooja(*Citrus junos* Sieb.) is the old fruit tree and it' s cultivation is increased on the suddenly to economic fruit tree in the islands of Namhea at the present. The cultivation is excellent in the weather of hot and high humidity. The yooja is the cold-resistant of an indeciduous latifoliate tree. If it fall down the many leaves of yooja in the winter, a little fruit yield in the next year because flower buds doesn' t division nearly. The generation of new leaf in the yooja of a ever-green

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tall tree is three times in the young tree yearly. The average life of the leaf is 16-20 months but some leaves are more than 3 years. The old leaf of *Citrus* fall down after turned into yellow leaf between November and December. The leaf of yooja is covered cutin and wax in the shruds plant and has nine chromosomes of two gametophyte. But it happens three to four gametophyte sometimes. The seeds of yooja are an egg shape and color is green or milk white color. The sarcocarp of yooja is strong sour and have a little seeds(Gavish *et al.*,1989). The cultivation of yooja is increased but wasn' t systematically studied. The research is studied about the method of cultivation, morphological and the damages by blight and harmful insects up to date(Kim, Han *et al.*,1991). In particular, a characteristic researches of yooja was studied about shape, classification, physiological property and the secondary production, specialized synthesis in addition to mechanism of flavonone glucoside to the inside and outside of the country.

Therefore, this paper was observed to the microstructural properties of yooja leaf and affinity relationship of small organelles. Hence this paper will use to the fundamental data of morphological research in citrus plant.

MATERIALS AND METHODS

The ordered and disordered leaves of the spring' s and the summer' s stems of the collected yooja at Chunnam Yando of the southern sea in Korea were used in this study.

The freshed mature leaves were sampled and processed immediately for the experiment. Small pieces of tissue, 1㎜, were dissected from the middle portion of leaves avoiding midrib. The tissues were fixed in a mixture of 3% paraformaldehyde in 0.1M phosphate buffer(pH 6.8 - 7.2) at room temperature.

After fixation for 3 hours followed by a short rinse in buffer, the material was postfixed with 2% OsO_4in the same buffer for 2 hours to overnight at 4℃. After postfixation followed by 4 times of 15 min rinses, specimens were dehydrated in a graded alcohol series, substituted with acetone, and embeded in Spurr' s low viscosity resin(Spurr,1969) ca.1㎛ thick sections were made and stained in 5% toluidine blue. Thin section(60 - 90 ㎚) were cut on a LKB-V ultramicrotome with diamond knife. The sections were staining with lead citrate. Ultrathin sections were viewed in a JEM 100 CX-II transmission electron microscope at 100kv.

RESULTS AND DISCUSSION

The microstructrural characteristics of ordered leaf cell

The external shape of the leaf is long elliptical and have sharp top. The structure of chlorenchyma is composed to the epidermis system of the exterior protected envelope in the inner part. The fundamental tissue system to achived photosynthesis and the important physiological function, also the vascular system to achived the pathway function. The arrangement of stomata at hypostamatic leaf were distributed generally the reverse side than the surface. The mesosophyll as the main body of metabolism in the leaf was composed palisade parenchyma distributed a lot of chloroplast and spongy parenchyma(Gong Shic *et al.*,1991). The ordered leaf of yooja was developed vacuole with large scale than the other plant' s cell. The inside of vacuole was included an organic substance so it was played an important role for the secretion of yooja. Different from the other plants, the number of oil sacks with unique perfume in yooja were widely distributed close to the cell wall. The chloroplast generally contained quite reduced starch grains and osmophilic granules, large granulic microbodies around the chloroplast were observed. The shapes of mitochondria with cristae were detected circle form or rod form. The mitochondria and microbodies were very closely associated with chloroplasts.

All organelles in the cell and the substances were located close to the cell wall according to the developmental vacuole. The turgor pressure was promoted and the regular shape and the size of the cell were generally observed. The inside of cell to arranged the cell of palisade tissue of regular size was distributed organelles of nucleus, chloroplast, endoplasmic

Fig. 1. Electron micrograph of palisade tissue, showing a lot of organelles in the odered leaf cell; Endoplasmic reticulum(ER), nucleus(N), nucleolus(no), chloroplast(C) and vacuole(V).(× 9,400)

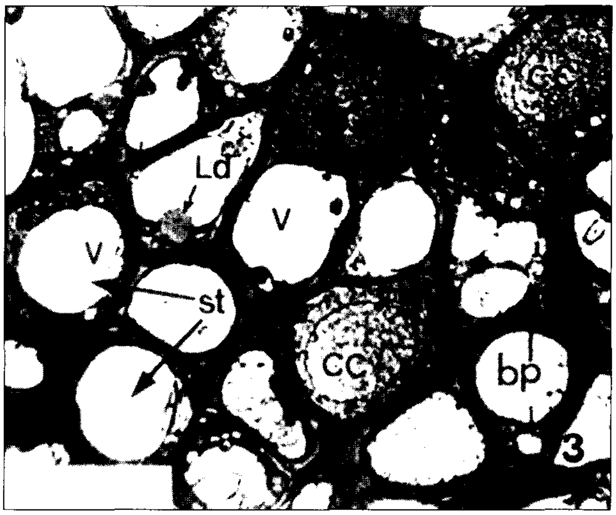

Fig. 3. Electron micrograph of the ordered leaf tissue; companion cells(CC),sievetubes(st), a bag of perfume(bp), lipid droplet(Ld).(× 6,700)

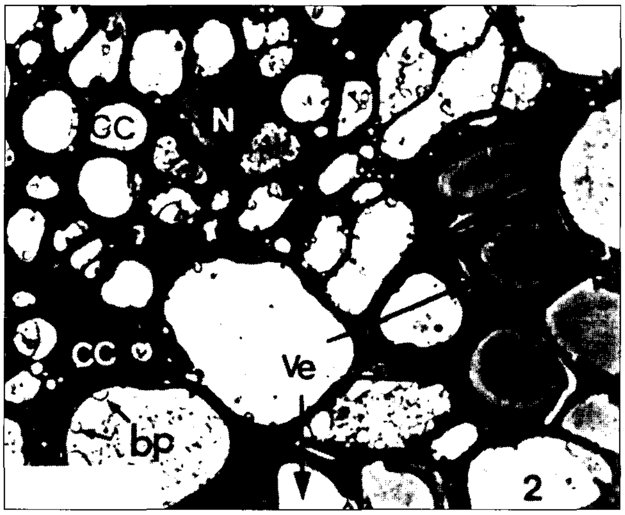

Fig. 2. Electron micrograph of vascular bundle region in the ordered leaf cell ; A lot of vessels(Ve),companion cell(CC),abagofperfume(bp) and nucleus(N).(× 3,500)

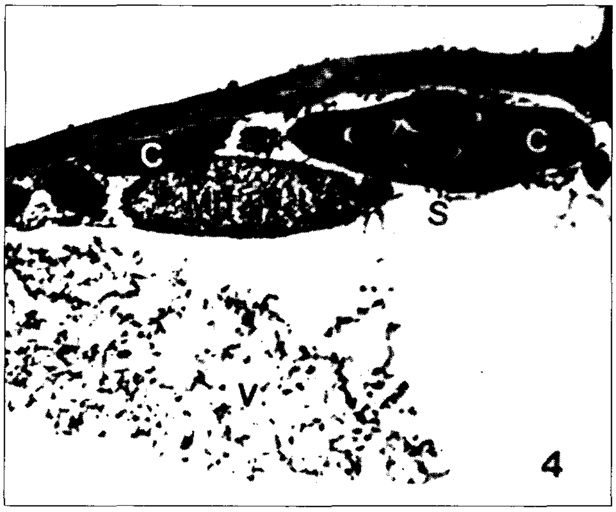

Fig. 4. Electron micrograph of a single cell in ordered leaf. Microbody(Mb) and many starch grains(S) are observed. All of organelles are closed by the cell wall.(× 9,000)

reticulum, vacuole and so on. The cytoplasm was highly vacuolate, so cells contain numerous organelles in a centrifugal position. In some cells the vacuole could not identified since organelles appeared to fill the entire cell(Fig. 1).The distintive protrusions of plasmodesmata into the cytoplasm were typical cell wall abnormalities found only in the leaf tissues(Fig. 1). Paramural bodies and large vesicles were often present together at the same site along the cell wall(Fig.1,4,8). The oil sacks having unique the perfume similar to vacuole morphologically were adjacent to cell wall different from the other plants(Fig. 2,3,5). Companion cell having sieve element and nucleus, unmatured cell and so on were detected in the observation of vein position. The companion cell was specially observed highly large nucleus and highly vacuolate in some position. The cells consist of vascular bundle were unregulared in the shape and the size(Fig.2,3,5). The huge intercellular spaces occupied a large portion of spongy tissue and the cytoplasm were highly vacuolated since most of all substances in cytoplasm were located close to the cell wall. The chloroplast with extremely numerous starch grain and osmophilic granule and the microbodys were observed(Fig.4). Sun(1962), Newcomb(1967) and Parks(1984) examined about the crystal in prochloroplastid. Newcomb(1967) reported that the

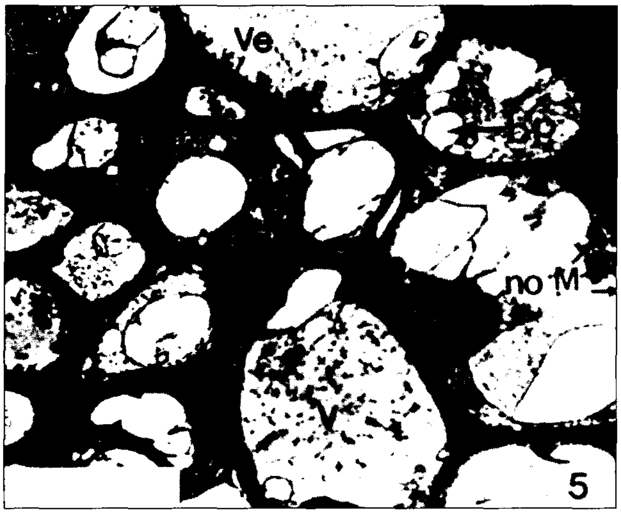

Fig. 5. Electron micrograph of a lot of large and small vacuoles in the odered cell. Nucleus(N) and mitochondria(M) arecontiguous to the inside of the cell wall due to the fusion ofvacuolesin the cells; A bag of perfume(bp), mitochondria(M), vessel(Ve), vacuole (V), nucleolus(no).(× 6,700)

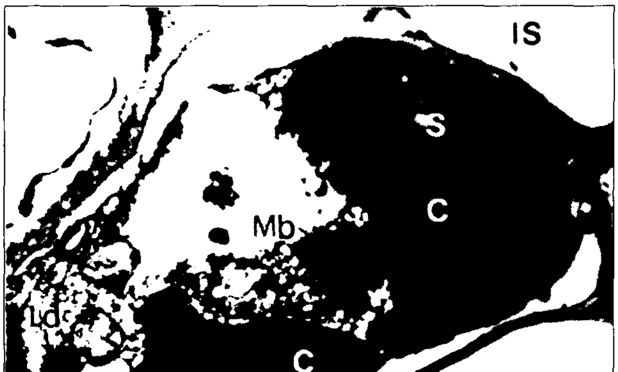

Fig. 6. Electron micrograph of the immature cell contains chloroplastwithstarchgrainsintheorderdcell; chloroplast microbody(Mb), starch(S), osmophilic grains(Og), lipid droplets (Ld), intercellular space(IS). (× 13,800)

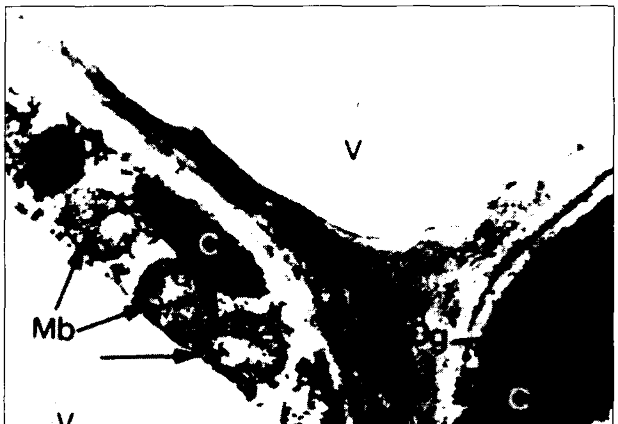

Fig. 7. Manymicrobodiesin ordered leaf cell areclose bythechloroplast withosmophilicgrains(Og), microbody (Mb),mitochondria (M) and large vacuoles(V). (× 17,000)

crystal is constantly composed to the boundary of membrane. Subsequently Ricardo(1967), Ross et al(1984) reported about crystal in the structure of unit membrane.During the developmental process from unmatured leaf to matured leaf of yooja, the cell wall was promoted and the intercellular space was highly developed. In addition to large chloroplast with osmophilic granules and starch grains and microbodies were frequently observed (Fig. 6) (Lilley et al., 1975).

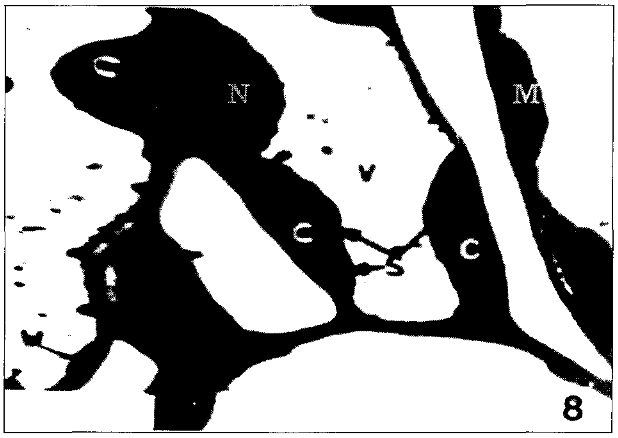

Fig. 8. Electron micrograph of the various form of ordered cell; chloroplast(C) with starch grains(S), mitochondria(M) and nucleus(N) in the side of the cell wall.(× 9,200)

Fig. 9. Electron micrograph of the xylem parenchyma(Xp) of the ordered leaf cell; vessel(Ve), a bag of perfume(bp). (× 6,500)

The development of vacuole consist of ergastic substance with the unique alkaloid materials in yooja was distinguished according to the kind of cells. The shape of vacuole formed by the fusion of a very small vacuoles was appeared the form of large circle and free form (Fig. 3,7,8). In the cross section of developed vascular bundle, it was observed xylem parenchyma consist of the cell of irregular small and large circular and free form (Fig. 9).

The mirostructure of cell in the disordered leaf

In the cell of disordered leaf , very big vacuole, tonoplast and a little mitochondria were observed, addition to a lot of abnormal particles in very big vacuole of center were detected different from the cell of ordered leaf. All organelles according to the growing of vacuoles was the most early deformed (Fig. 10) (Douce et al., 1985;Lilley et al.,1975).

Also some of cells due to undeveloped cell wall were observed large intercellular space and rod or spherical mitochondria. In the vessel and the cell having the developed secondary cell wall, a lot of small particles

Fig. 10. Electron micrograph of the cell in disordered leaf tissue ; small particles(Sp), vacuoles(V), endoplasmic reticulum(ER), tonoplast(T), mitochondria(M), abagof perfume(bp), plasma membrane(PM) andcell wall(CW). Vacuole has many particles and endoplasmic reticulum has ribosomes(R). Free ribosomes are presented in cytoplasm. (× 23,000)

was detected at chloroplastid with lipid droplets and a variety of microstructural abnormalities have been found in the destructed cell. One of these defective chlroplasts was the most common type obseved which contained a less degenerated grana and a electron-dense stroma with a number of osmophilic globules but seems regular in shape (Fig. 11, 12) (Klotal et al.,1967; Kunisada et al.,1983). The other disordered leaf tissues revealed the abnormalities of cell wall, wall thickenings and protrusions of the cell wall into the cytoplasm. The cell wall thickenings due to the deposition of additional materials occurred, particularly in proximity to plasmodesmata.

The oil sacks closed to cell wall due to the destruction of vacuole were destructed. The special perfume was gradually lost. At last the functions and shapes of the organelles were generally lost. The distinctive protrusions of plasmodesmata into the cytoplasm were typical cell wall abnormalities found only in the disordered leaf tissues. The separation of plasma membrane was appeared at the parenchyma of xylem and pholem. One of these detective chloroplasts was the special type observed which contained a less

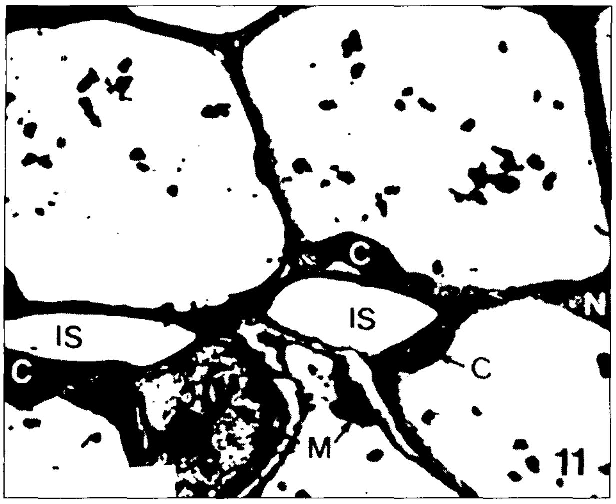

Fig. 11. Electron micrograph of the disordered cell in early stage of immatured leaf tissue. All of organelles are observed on the inside of the cell wall; intercelluar space(Is), smallnparticle(Sp), chloroplast(C), nucleus(N), nucleolus(no), itochondria(M).(× 9,000)

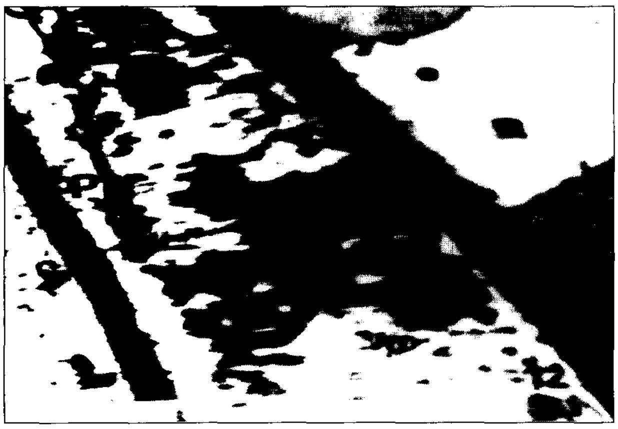

Fig. 12. Electron micrograph of the disordered plastid of the destructed leaf cell. Many particles are observed in the cell, showing plastid.Lipid droplets presented in the plastid. Nucleus has lipid droplets(Ld) and in the plastid. Nucleus has lipid droplets(Ld) and small particles(Sp), abag of perfume(bp),showing secondary wall(SW).(× 26,000)

Fig. 13. Electron micrograph of the disordered cell with the destructed chloroplast. A lot of vacuoles(V) and the fusion of vacuoles appeared in the disordered cell. Chloroplast contain starch grains(S) and a lot of chloroplast(C) containing osmophilic granules(Og) and a bag of perfume(bp) are well observed in the cell.(× 9,000)

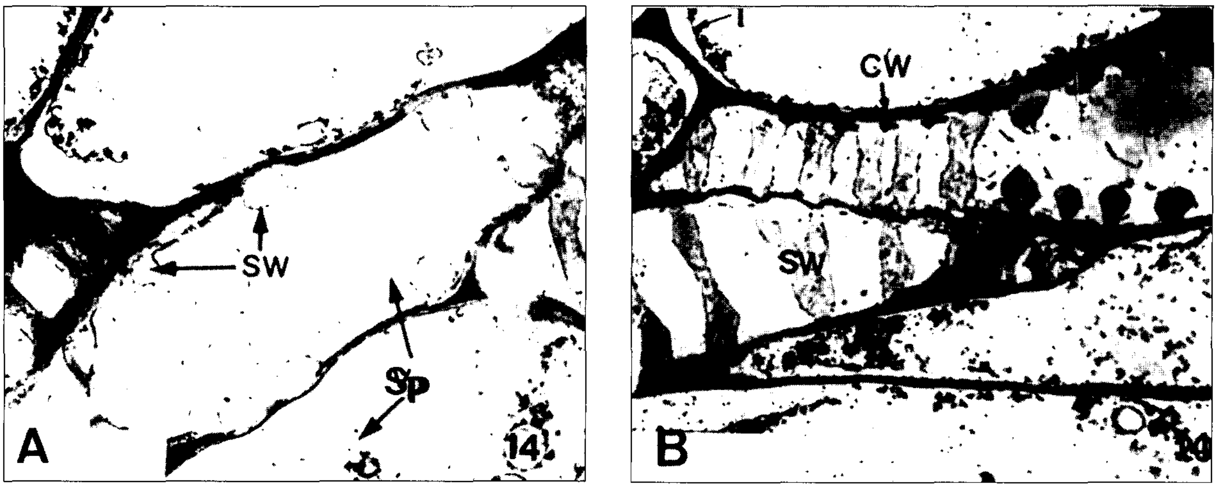

Fig. 14. A, B. Electron micrograph of the disordered cell during of the formatiom of the secondary cell wall.
A. The early formation stage of the secondary cell wall from primary cell wall.(× 9,000)
B. The formation secondary cell wall; secondary cell wall(SW), tonoplast(T), cell wall(CW).(× 6,000)

degenerated grana and an electron-dense stroma with a number of osmophilic globules. The centrifugal arrangement of the chloroplasts appears to be ideal for the exchange of the photosynthetic intermediates between these chloroplasts. The normal chloroplasts are known to contain few osmophilic globules, an increase in size and number of osmophilic globules is closely related with the breakdown of thylakoids in chloroplast affected by the physical or abiotic treatments(He et al., 1994; Ahn et al., 1995). Also mitochondria and microbodies cells were very closely associated with chloroplasts (Fig. 9,10,11,12,13). Mitochondria were very small and aparse. Almost the other of peripheral organelles were not observed in general. Some of the cells had the large vacuole considered typical of fully differentiated cells. Vacuole was appeared to fill the entire cell(Fig. 11, 12, 14).

The separation of plasma membrane at the parenchyma of xylem and pholem was appeared. Vessel elements and tonoplast presented in during the formation of secondary cell wall from primary cell wall was showed (Fig. 15).

In the disordered leaf tissue, the shape of vacuole was deformed and the shape of the cell was deformed to the very various forms. The oil sacks were gradually lost the unique perfume together with structural destruction due to the destruction of vacuole. (Fig. 12, 13).

The segregation of plasma membrane was gradually appeared the most organelles in the cell deformed. The weeked cell wall was not maintained the cell no longer (Fig. 15). Also the nuclear membrane was destructed

Fig. 15. Electron micrograph of the destructed palisade tissueinthe disordered leaf; small particles(Sp); chloroplast(C).(× 9,000)

and abnormal expanded (Fig. 16, 17). The materials of cytoplasm came out by little from inside of cell according to the partial destruction of plasma membrane together with the its segregation (Fig.16).

Also Tomson *et al.*(1980) announced chloroplastid have not relation with secreted reaction in the leaf of *Citrus junos*. But Wooding (1965), Amelunexe (1967) and Fahn (1974) contended chloroplastid played the role of secernent synthesis site in the cell.

The cell wall didn' t maintained basic cellular

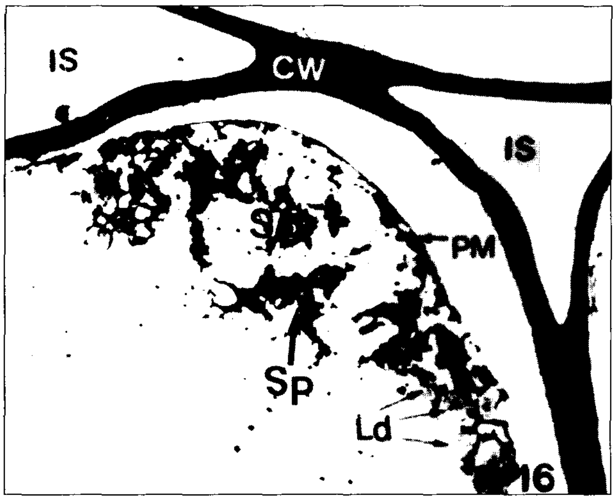

Fig. 16. Electron micrograph of the plasmolysis from the disordered immature cell wall; Intercellular space(Is), plasma membrane(PM) and cell wall(CW), Lipid droplet((Ld), small particles(Sp).(× 18,000)

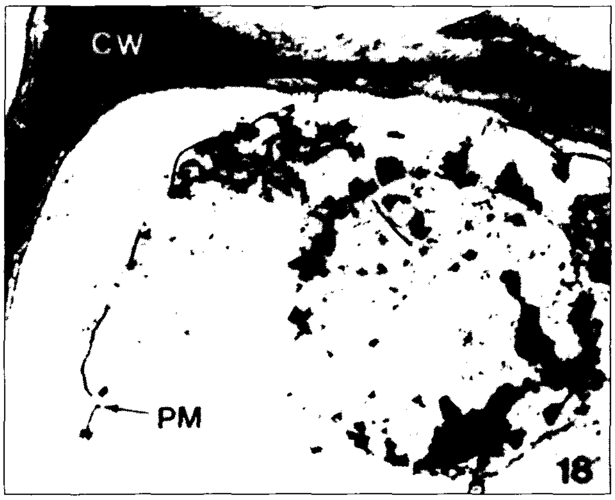

Fig. 18. Electron micrograph of the extremely destructed all of organelles in the disordered cell; plasma membran (PM), cell wall(CW).(× 30,000)

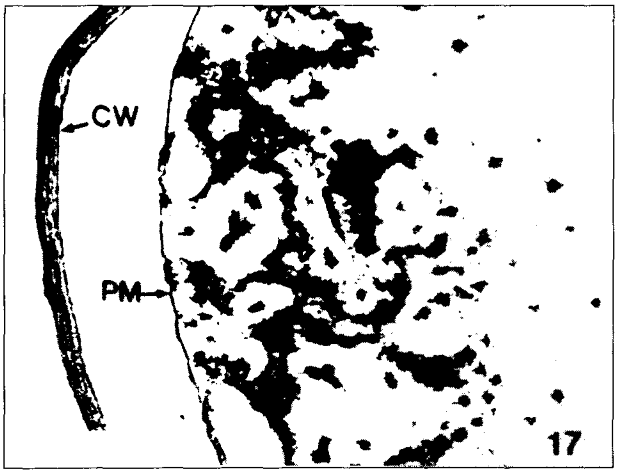

Fig. 17. Electron micrograph of the single disordered cell. Plasma membrane are diverged to the disordered cell wall; cell wall(CW), plasma membrane(PM), small particle. (× 36,800)

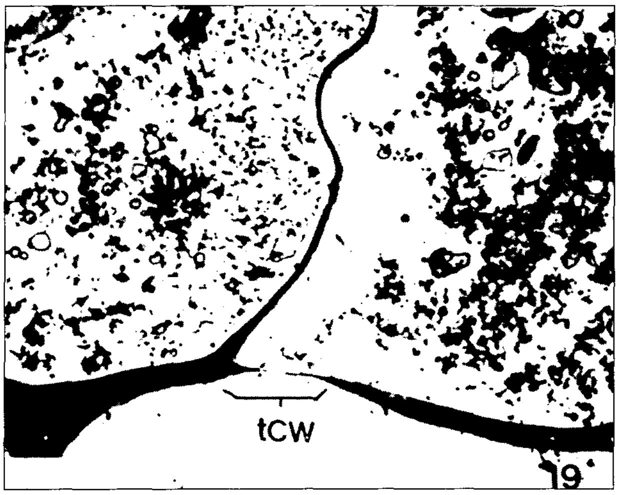

Fig. 19. Electron micrograph of the extremely destructedprotoplasm and thined cell wallin the disordered leaf tissure; thined cell wall (tCW). (× 12,000)

form(Fig. 18.). W.e observed the transformation of partialy thined cell wall as a results of leaf disease. And the biger vessel cell than the cell of xylem parenchyma tissue and parenchyma tissue was observed (Fig. 19).

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