



# HOKKAIDO UNIVERSITY

Title	A SEM Observation of Organelles in the Cambial and Living Xylem Cells in Todomatsu ( <i>Abies sachalinensis</i> )
Author(s)	HIRAKARA, Yasuhiko; 平川, 泰彦; ISHIDA, Shigeo et al.
Citation	北海道大學農學部 演習林研究報告, 36(2), 459-468
Issue Date	1979-07
Doc URL	<a href="https://hdl.handle.net/2115/21010">https://hdl.handle.net/2115/21010</a>
Type	departmental bulletin paper
File Information	36(2)_P459-468.pdf



**A SEM Observation of Organelles in the Cambial  
and Living Xylem Cells in Todomatsu  
(*Abies sachalinensis*)\***

By

**Yasuhiko HIRAKAWA\*\*, Shigeo ISHIDA\*\*  
and Jun OHTANI\*\***

走査電子顕微鏡によるトドマツの形成層並びに  
木部細胞の細胞器官の観察

平川泰彦\*\* 石田茂雄\*\* 大谷 諄\*\*

**CONTENTS**

Introduction . . . . .	459
Materials and methods . . . . .	460
Results . . . . .	461
1. Ray parenchyma cells . . . . .	461
2. Tracheids . . . . .	462
Discussion and conclusion . . . . .	463
References . . . . .	465
要    約 . . . . .	466
Explanation of photographs . . . . .	467
Photographs (6 plates)	

**Introduction**

Secondary tissues of woody plants, originated from vascular cambium, have been studied by many researchers, at the macroscopic, microscopic and ultrastructural levels. Recent advances at the latter level of study of wood have been accelerated by the employment of modern electron microscopies. In the study of wood cell wall formation, where living cells are main subject to consider, electron microscopes, especially transmission electron microscope (TEM) have exhibited their high ability for the purpose. Scanning electron microscope (SEM) which is highly evaluated for studying such subjects as sculpturings of matured cell walls (OHTANI and ISHIDA, 1976) is also expected to be a characteristic and useful tool for the study on this kind of subjects, soft tissues. Only a few works, however, have

---

\* Received July 31, 1978. This report was presented at the 28th Annual Meeting of the Japan Wood Research Society, 1978, Nagoya.

\*\* Laboratory of Wood Physics, Dept. of Forest Products, Faculty of Agriculture, Hokkaido University.

北海道大学農学部林産学科木材理学教室

been reported (WODZICKI and HUMPHREYS, 1972, 1973; WODZICKI and BROWN, 1973), because of its later employment than TEM and of some difficulties in the specimen preparation for such soft tissues as the cambium.

Aiming at contribution to clarify the cell wall formation or the aging of cell, in relation to the behavior of some organelles there, the present authors carried out an experiment to observe the organelles in the cambium and living xylem cells neighbouring it in Todomatsu (*Abies sachalinensis* MAST.), with regard to the season. Principally SEM was used for the observation and the results obtained were compared with that using TEM by the authors themselves and by those who have already reported valuable results (TIMELL, 1973). In this paper the results of observation of micromorphological aspects and seasonal changes of the living cells are briefly described.

The most important problems lie on the specimen preparation and thus not a few works which are helpful for us have been reported (KEITH and GODKIN, 1976). The present authors also tried to find out the way which makes possible to carry out this work, and used the procedures obtained for it.

### Materials and methods

Materials for this study were obtained from a twenty five-year old Todomatsu tree (*Abies sachalinensis*), grown in a plantation in Tomakomai Experiment Forest, Hokkaido University. They were collected at two-week intervals during the active period of the cambium and one-month intervals during the remainder of the annual cycle in 1976 and 1977.

Small cubes of fresh material ( $L: 2\text{ mm}$ ,  $R: 3\text{ mm}$ ,  $T: 4\text{ mm}$ ) containing xylem, cambial zone and phloem were fixed for three hours at room temperature or  $4^{\circ}\text{C}$  in 3% or 6% glutaraldehyde buffered to pH 7.2 with 0.05 M sodium cacodylate and they were washed with buffer for two hours. In addition to this fixative, a mixed fixative (3% glutaraldehyde · 1% acrolein · 1% formaldehyde) was also employed in winter samples. The aldehyde fixed material was then post fixed in buffered 2% osmium tetroxide for two hours, subsequently washed for one hour in several changes of glassredistilled water, followed by the dehydration in graded series of ethanol or acetone.

After the dehydration, two different kinds of specimen preparation were employed for SEM: (1) materials were dried by critical point drying, then fractured in radial direction with a razor blade, and (2) small pieces saturated with 100% ethanol were quenchfrozen in liquid nitrogen and then cryofractured in radial or tangential direction, and dried by critical point drying. All the specimens were coated with gold using Ion Coater. SEM observations were made with JSM-2 at 25 kV, and JSM-F7, a field emission SEM. For TEM observation, the specimens embedded in spurr were sectioned with a diamond knife on LKB Ultratome 8800. Sections were stained on the grid with uranyl acetate and lead citrate. Observations were made with HITACHI HS-8.

## Results

The resumption of the cambial activity began early in May. Late in June cell divisions reached to maximum and about 10~15 layers of differentiating cells were present. The active differentiating cells as well as the cambial were rich in all types of cellular organelles in them; mitochondria, plastids, rough endoplasmic reticulum (ER), golgi bodies, ribosomes and so on. Fusiform cells were found highly vacuolated.

In November cambial activity was almost quiescent and immature fusiform cells were rich in small vacuoles. Cytoplasm of ray parenchyma cells was almost covered with a number of lipid droplets and starch grains in amyloplasts in the cells were larger than that in summer. Cytoplasmic ground substances were denser than those in summer.

### 1. Ray parenchyma cells

Parenchyma cells in growing season showed a characteristic aspect in them. Photo 1 illustrates inside of a differentiating ray parenchyma cell, showing a large nucleus placed at the left, many small global lipid droplets (0.5~2  $\mu\text{m}$  in diameter) scattered within the cell, a few disc-like plastids and many rod-like mitochondria. As shown in photo 2, mitochondria were mostly rod-like, definitely not much more than 1  $\mu\text{m}$  in diameter and 2~4  $\mu\text{m}$  in length. But it was occasionally found that they were branched as shown in photos 3 and 4 and elongated up to several  $\mu\text{m}$  or more in the same photographs. Slight inside view of a mitochondrion of a ray parenchyma cell is visible in photo 5 which was taken at the freeze-fractured specimen.

Plastids contained a few osmiophilic bodies and/or small starch grains in them and varied considerably in shape and size in a TEM photograph as shown in photo 6, but in SEM images they were nearly disc-like or occasionally oval-sphere-like, not more than about 5  $\mu\text{m}$  in diameter as in photo 7. Rough ER is shown in a SEM photograph (photo 8), being represented as a large area of membrane with granular surface. It was difficult to decide whether all particles on the membrane were ribosomes or not.

In active ray parenchyma cells there were some small vacuoles, the tonoplast of which was found smooth in its surface and pouch-like, and it appeared to continue with the plasma membrane as shown in photo 9. Plasma membrane was able to distinguish from the other membranes because of its placement adjacent to the cell wall as in photo 10.

Nucleus was slightly elongated except global only quite near the initial and the pattern of the pore distribution was clearly seen. In photo 11, the pores appear to be arranged circularly, each approximately 500~2000 Å in size. Cytoplasmic ground substances were net-like and they appear to attach to the organelles in photo 4. In SEM no golgi bodies were discerned in all cells examined.

In winter, almost ray parenchyma cells were packed with a number of lipid droplets, amyloplasts containing large starch grains and cytoplasmic ground sub-

stances denser than in summer as shown in photo 12 which corresponds well to that in photo 13 taken by TEM. Amyloplasts were oval-sphere-like not more than  $5\ \mu\text{m}$  in diameter, and it was seen that the membranes of them were broken and thus the starch grains were exposed, as shown in photos 14 and 15. In photo 16 taken from a slightly plasmolized ray parenchyma cell, it is clearly seen that the lipid droplets, even in its size and packed densely, cover the interior cytoplasmic components of the cell. Mitochondria were similar to ones in activity and they were rod-like and/or branched.

## 2. Tracheids

In the growing season differentiating fusiform cells were highly vacuolated. They had a large central vacuole separated by a tonoplast from the peripheral cytoplasm in which there were many organelles as shown in photo 19. Wherever these cells were observed by SEM, the stages of differentiation were judged by the position and internal features of each cell. In the case of this study, differentiating cells of later stage were selected to examine because cells near the initials were more complicated by their shape and organelles within them.

In photo 17, the cell has a large area of vacuolar membrane and a few organelles between vacuolar and plasma membranes. The cell in photo 18, on the other hand, has not such a vacuolar membrane. There are a number of vesicles of  $1\sim 5\ \mu\text{m}$  in diameter and the tube-like membrane on or connected with the plasma membrane. It must be noticed that the former is less mature than the latter.

The cell in photo 20 appears to be slightly more mature than that in photo 17, and less mature than the case of photo 18. There are mitochondria between plasma membrane and vacuolar membrane which is probably broken by artifacts in photo 21. It is clearly seen in photo 22 that there are a number of fine fibrillar structures ( $100\sim 300\ \text{\AA}$  in diameter) which lie on the plasma membrane in Z-helix at about 45 degrees in respect to the longitudinal axis of the cell. In photo 23, vacuolar membranes seem to connect with plasma membrane where the fibrillar structures located. These fibrillar structures changed their orientation into about 90 degrees to the cell axis on the plasma membrane at the much later stages of differentiation as shown in photo 24. Many small vesicles are also visible in the same photograph. A vesicle attached to the plasma membrane is shown in photo 25. Morphological differences in mitochondria, plastids and lipid droplets were not found in fusiform from ray parenchyma cells.

Dormant fusiform cells were characterized by the presence of many small vacuoles as in photo 26. Though both features obtained by SEM and TEM correspond well about organelles in winter, it was rather difficult to observe the organelles except vacuoles because of the denser cytoplasmic ground substances compared with that in summer. The connection of tonoplast is clearly seen as shown in photos 26 and 27. A particular structure, i. e., small tube-like structure was found connected mutually and with plasma membrane. Diameter of the tube was ca.  $0.05\sim 0.1\ \mu\text{m}$  as shown in photos 28 and 29.

### Discussion and conclusion

There are particular chances to occur artifacts during the drying, the coating and the fracturing for preparation of SEM specimens, even though the employment of fixation is same as in TEM. Accordingly when we observe the delicate tissues such as cambial tissues using SEM, we must be deliberate to interpret the photographs obtained, since we need to discern between the natural features and the products of artifacts if any. And it is needed to compare SEM observation with that using TEM by which many works have been reported already (BARNETT, 1973; CRONSHAW, 1965-a, b; ITOH, 1971; MURMANIS, 1969; ROBARDS and KIDWAI, 1968; SRIVASTAVA, 1966). The freeze fracture method is considered specially effective to clear the internal structure of organelles. However, it also appears that the artifacts might occur when the samples freezed and melted (ECHLIN and BURGESS, 1977). For example, the features of cytoplasmic ground substances obtained by freeze fracture showed slight difference from that fractured after the critical point drying. So, in this study freeze fracture method was not often employed.

The results obtained are briefly summarized as follows: lipid droplets were always found global, mitochondria generally rod-like, but occasionally elongated and/or branched, plastids disc-like. They all were obviously distinguished each other based upon the shape and size.

The lipid droplets were quite different in their distribution within the cell between both growing and dormant seasons. They were increased in number and arranged as if covered the interior cytoplasm in dormancy. This seems to be similar to that of spherosomes of *Pinus strobus* in winter (MURMANIS, 1971), and to imply the frost hardiness. Their diameter discerned exactly by SEM is found varied by TEM depending on the location of a droplet sectioned. There was not a marked tendency to increase the size of the droplets in dormancy.

It has been said that diameter of mitochondria rarely exceeds  $1\ \mu\text{m}$  and the length extends to several  $\mu\text{m}$ . The observation by the authors gave  $0.5\sim 1\ \mu\text{m}$  diameter and that the length was various as below. The majority of them were elongated about  $2\sim 4\ \mu\text{m}$  long, occasionally  $5\sim 7\ \mu\text{m}$  or more. The branched one which was occasionally found appears to be similar to that of *Acer* (CATESSON, 1974), and the nodular one that also occasionally appeared has been reported in *Pinus strobus* (MURMANIS, 1971). Seasonal changes of mitochondria in the shape and size were not found in this observation, but the detail of this must be searched using SEM which is useful for that. In freeze-fractured samples the inner parts of a mitochondrion were seen. However, it was difficult to discern the double membranes and cristae because of the resolving power of SEM used.

The majority of plastids were disc-like in activity and oval-sphere-like in dormancy. In the case of SEM, the inner parts of plastids are not observable except in freeze-fractured samples. So it was difficult to discern exactly various kinds of plastids when the freeze fracture method was not used, except amyloplasts the membrane of which was often found broken and thus the starch grain exposed.

It seems that lipid droplets and mitochondria are almost definite shape and size, but plastids are rather indefinite except amyloplasts. The features of plastids are obviously distinct from that of lipid droplets and mitochondria. The large starch grain in amyloplasts seems to be connected with storage in dormancy.

The pores were present on the surface of nuclei in active ray parenchyma cells, but not in dormancy. They must be nuclear pores. The diameter of pores was irregular (500~2000 Å), and they seem to be more or less affected by the ion sputter coating. Therefore, although the actual shape and size are obscure, the distribution of them is clearly shown through the photographs. The pores were arranged circularly, similar to those of peas (NORTHCOTE, 1968). That the pores were not found in dormancy, might be caused by fixation or dense cytoplasmic ground substances attached.

Rough ER has been said to consist of a system of plate-like or sheet-like, flattened cisternae with ribosomes. Present SEM observation shows that rough ER is the large area of membrane extending the interior of a ray parenchyma cell. It is evidently different from tonoplast of small vacuoles in appearance, because the surface is granular in rough ER, but pouch-like and smooth in tonoplast. It was difficult to discern in fusiform cells. In dormancy it was not found in both ray parenchyma and fusiform cells, in agreement with that some workers have reported already (MURMANIS, 1971).

The large vacuolar membranes were easily seen in active fusiform cells and they were slightly ruptured in places, thus peripheral cytoplasm and plasma membrane were able to see by SEM. The membranes observed by SEM were obviously situated in the lumen side in comparison with the plasma membrane and they were absent in nearly matured cells, showing that they seem tonoplast of large vacuole, and in addition, many small vacuoles almost remained intact in dormancy, so they seem to be preserved in activity.

There were small vesicles on the plasma membrane or attached to it. They were similar to the paramural bodies (MARCHANT and ROBARDS, 1968; MURMANIS, 1973). Although they should be discussed after the inner parts of them are observed in detail, these vesicles may be concerned with the cell wall formation.

In photo 23, there were particular structures of vacuolar membranes connected with the plasma membrane, not found by TEM, and it seemed to be no artifacts. They look similar to the structures of small tubular membranes in dormant fusiform cells in photos 28 and 29. There is a possibility that these structures are a transitional element of membranes.

A number of fine fibrillar structures were seen on the plasma membrane and they were oriented in Z-helix about at 45°, or 90° in respect to the longitudinal axis of the cell. The orientation of these was coincident with that of microfibrils in S<sub>2</sub> or S<sub>3</sub> layers of tracheids. Similar structures have been reported in *Fagus* (OHTANI and ISHIDA, 1976). They might be possibly microtubules. They were observed often in many fusiform cells using SEM.

Many small vacuoles in dormant fusiform cells observed by SEM corresponded to that of TEM. Tonoplasts of the vacuoles were well preserved and they were

not breakable.

The authors observed some cellular organelles using SEM and obtained valuable informations about the three dimensional features and seasonal changes of them.

It may be concluded that in growing season, tracheids are highly vacuolated, and ray parenchyma cells are rich in various organelles; i. e., rod-like, branched and nodular mitochondria, disc-like plastids, scattered lipid droplets and sheet-like rough ER which are all involved in net-like cytoplasmic ground substances. In dormancy, tracheids have many small vacuoles. In ray parenchyma cells, amyloplasts and lipid droplets are increased in number, being the latter arranged in a particular manner.

### References

- 1) BARNETT, J. R.: Seasonal variation in the ultrastructure of the cambium in New Zealand grown *Pinus radiata* D. DON. *Ann. Bot.*, **37**, 1005-1011 (1973).
- 2) CATESSON, A. M.: Cambial cells. in "Dynamic aspects of plant ultrastructure", ROBARDS, A. W., ed., McGraw-Hill Book Co., 358-390 (1974).
- 3) CRONSHAW, J.: Cytoplasmic fine structure and cell wall development in differentiating xylem elements. in "Cellular ultrastructure of woody plants", CÔTÉ, W. A. Jr., ed., Syracuse Univ. Press, N. Y., 99-124 (1965-a)
- 4) CRONSHAW, J.: The organization of cytoplasmic components during the phase of cell wall thickening in differentiating cambial derivatives of *Acer rubrum*. *Can. J. Bot.*, **43**, 1401-1406 (1965-b).
- 5) ECHLIN, P. and BURGESS, A.: Cryofracturing and low temperature scanning electron microscopy of plant material. *IITRI/SEM*, **1**, 491-500 (1977).
- 6) ITOH, T.: On the ultrastructural of dormant and active cambium of conifers. *Wood Research*, **51**, 33-45 (1971).
- 7) KEITH, C. T. and GODKIN, S. E.: Fixation of juvenile cambium from two conifers species for ultrastructural study. *Wood and Fiber*, **8**, 177-200 (1976).
- 8) MAITRA, S. C. and DEEPESEH, N. DE.: Role of microtubules in secondary thickening of differentiating xylem element. *J. Ultrastruct. Res.*, **34**, 15-22 (1971).
- 9) MARCHANT, R. and ROBARDS, A. W.: Membrane systems associated with the plasmalemma of plant cells. *Ann. Bot.*, **32**, 457-471 (1969).
- 10) MURMANIS, L. and SACHS, I. B.: Seasonal development of secondary xylem in *Pinus strobus* L. *Wood Sci. and Technol.*, **3**, 177-193 (1969).
- 11) MURMANIS, L.: Structural changes in the vascular cambium of *Pinus strobus* L. during an annual cycle. *Ann. Bot.*, **35**, 133-141 (1971).
- 12) MURMANIS, L.: Particles and microtubules in vascular cells of *Pinus strobus* L. during cell wall formation. *New Phytol.*, **70**, 1089-1093 (1971).
- 13) MURMANIS, L. and SACHS, I. B.: Cell wall formation in secondary xylem of *Pinus strobus* L. *Wood Sci. and Technol.*, **7**, 173-188 (1973).
- 14) NORTHCOTE, D. H. and LEWIS, D. R.: Freeze-etched surfaces of membranes and organelles in the cells of pea root tips, *J. Cell Sci.*, **3**, 199-206 (1968).
- 15) OHTANI, J. and ISHIDA, S.: An observation on perforation plate differentiation in *Fagus crenata* BL., using scanning electron microscopy. *Res. Bull. College Exp. For., Hokkaido Univ.*, **33-1**, 115-126 (1976).
- 16) ROBARDS, A. W. and KIDWAI, P.: A comparative study of the ultrastructure of resting and active cambium of *Salix fragilis* L. *Planta*, **84**, 239-249 (1969).

- 17) ROBARDS, A. W. and KIDWAI, P.: Vesicular involvement in differentiating plant vascular cells. *New Phytol.*, **68**, 343-349 (1969).
- 18) SRIVASTAVA, L. M.: On the fine structure of the cambium of *Fraxinus americana* L. J. *Cell Biol.*, **31**, 79-93 (1966).
- 19) TIMELL, T. E.: Ultrastructure of the dormant and active cambial zones and the dormant phloem associated with formation of normal and compression woods in *Picea abies* (L.) KARST. Technical publication No. 96, State Univ. of New York, College of Environmental Science and Forestry, Syracuse, N. Y. (1973).
- 20) WODZICKI, T. J. and HUMPHREYS, W. J.: Cytodifferentiation of maturing pine tracheids: the final stage. *Tissue and Cell*, **4** (3), 525-528 (1972).
- 21) WODZICKI, T. J. and BROWN, C.: Organization and breakdown of the protoplast during maturation of pine tracheids. *Amer. J. Bot.*, **60** (7), 631-640 (1973).
- 22) WODZICKI, T. J. and HUMPHREYS, W. J.: Maturing pine tracheids. *J. Cell Biol.*, **56**, 263-265 (1973).

## 要 約

樹木の形成層のような軟組織を變形することなく走査電子顕微鏡 (SEM) で観察することは極めて困難と考えられており、従って、これについての SEM 観察結果の報告はほとんどみられない。一方透過電子顕微鏡 (TEM) では、試料を包埋することにより軟組織の變形を少なくすることが可能なために、その観察報告は数多くなされてきている。

本研究は、トドマツ (*Abies sachalinensis* MAST.) の形成層並びに木部細胞の細胞器官の形態を TEM 観察の結果と比較対照しながら、アルデヒド-オスミウム酸二重固定、臨界点乾燥法等を用いて SEM により観察を行なったものである。観察対象は、活動期と休止期における放射柔細胞と分化中の木部紡錘型細胞 (仮道管) である。

得られた結果を要約すると次の通りである。

1) 活動期の形成層付近の放射柔細胞 (放射組織始原細胞, 分化中および壁形成を終えた細胞を含む) では、ミトコンドリア、プラスチド、脂質粒、rough ER、液胞、原形質膜、核などの形態が SEM で観察された (Photo 1~11)。ミトコンドリアは大部分が直径 0.5~1  $\mu$  で長さが約 2  $\mu$  の小棒状であるが、時折 3~7  $\mu$  の長いもの、枝分れしたものや結節状のものが存在する。プラスチドは直径 3~5  $\mu$  の円盤状で、脂質粒は直径 0.5~2  $\mu$  の球状である。rough ER は表面がざらざらした不定形の大きな膜で、液胞は小さい平滑な袋状のトノプラストからなっている。原形質膜は細胞壁と接して存在し、他の細胞内の膜と連結している。核の表面には円形に孔が存在している。ゴルジ体は SEM では確認することができなかった。

休止期には核、ミトコンドリア、プラスチド、脂質粒が SEM で観察された (Photo 12~16)。アミロプラストはデンプンの発達に伴い卵型となり、脂質粒は細胞内部全体を外側から被うように配列する。

2) 活動期の分化中仮道管の細胞器官は、大部分が活動期の放射柔細胞のものと形状が同じであるが、rough ER は確認できなかった。S<sub>2</sub>, S<sub>3</sub> 形成段階の仮道管では、原形質膜上 (内

側) にマイクロチューブールや Paramural bodies と一致するような構造の存在が SEM で示された (Photo 17~25)。

休止期の未分化の仮道管は、多くの小液胞を有するのが特徴であり、それらとその内部物質や管状の膜構造が SEM で観察された (Photo 26~29)。

### Explanation of photographs

**Note:** Longitudinal direction of wood is vertical in all photographs. Photos 5 and 9 were taken from the specimens prepared through the freeze-fracture.

**Key to labeling:** L; lipid droplet, M; mitochondrion, N; nucleus, P; plastid, Pl; plasma membrane, S; starch grain, Vm; vacuolar membrane, W; cell wall.

#### Photos 1-11; Xylem ray parenchyma cells near the cambium in growing season.

- Photo 1.** Cell organelles as well as nucleus in a differentiating ray parenchyma cell. An elongated nucleus is placed at the left in this photograph. Global lipid droplets, rod-like mitochondria and disc-like plastids are shown.
- Photo 2.** Mitochondria of  $0.5\ \mu\text{m}$  in diameter and  $2\ \mu\text{m}$  in length (arrow) and cytoplasmic ground substances. Mitochondria are found on the net-like matrix of cytoplasmic ground substances.
- Photo 3.** A branched mitochondrion (arrow) and cytoplasmic ground substances.
- Photo 4.** A nodular-shape mitochondrion (upper arrow) and a branched one (lower arrow).
- Photo 5.** A freeze-fractured tangential surface of a ray parenchyma in the cambial zone showing the inner part of nucleus and transverse section of a mitochondrion (arrow).
- Photo 6.** TEM micrograph showing plastids, mitochondria and lipid droplets. They are corresponding well to those of SEM micrographs (cf. photos 2-5).
- Photo 7.** A disc-like plastid (arrow) of about  $3\ \mu\text{m}$  in diameter. Mitochondria and lipid droplets within cytoplasmic ground substances are also shown.
- Photo 8.** A large granular membrane of rough ER.
- Photo 9.** A freeze-fractured tangential surface of a ray parenchyma cell near the cambial zone. A pouch-like vacuolar membrane (arrow) is connected with the plasma membrane at the left in this photo.
- Photo 10.** The plasma membrane closely attached to the cell wall (arrow) in a mature ray parenchyma cell.
- Photo 11.** A surface of nucleus in a ray parenchyma cell in the cambial zone. Nuclear pores are arranged circularly (arrow).

#### Photos 12-16; Xylem ray parenchyma cells near the cambium in dormancy.

- Photo 12.** Cell organelles as well as nucleus in a mature ray parenchyma cell. The arrangement of lipid droplets, large starch grains and dense cytoplasmic ground substances is shown. Compare with photo 2.

- Photo 13.** A TEM micrograph showing cell organelles and nucleus. They are corresponding well to those of SEM micrograph (cf. photo 12).
- Photo 14.** Oval-sphere-like amyloplasts of 3-5  $\mu\text{m}$  in diameter.
- Photo 15.** Starch grains in amyloplasts.
- Photo 16.** Numerous lipid droplets exposed by removal of the cell wall in this side. They are densely arranged along the cell wall.

**Photos 17-25 ; Differentiating tracheids in growing season.**

- Photo 17.** The vacuolar membrane and the cytoplasm placed between the vacuolar membrane and the plasma membrane in a tracheid undergoing  $S_2$  layer formation.
- Photo 18.** Many small vesicles on the plasma membrane in a tracheid in the late stage of secondary wall formation.
- Photo 19.** A TEM micrograph showing large vacuoles and the peripheral cytoplasm in tracheids in the early stage of secondary wall formation.
- Photo 20.** The vacuolar membrane and small vesicles on the plasma membrane in a tracheid undergoing  $S_2$  layer formation.
- Photo 21.** A higher magnification view of the area arrowed at the lower right in photo 20. The vacuolar membrane is broken in places. Mitochondria (arrow) are shown between the vacuolar membrane and the plasma membrane.
- Photo 22.** A higher magnification view of the area arrowed at the left in photo 20. Steep Z-helix fibril-like structures and five mitochondria are placed on the plasma membrane. The plasma membrane is broken at the left in this photo, where the cell wall is seen.
- Photo 23.** A higher magnification view of the area arrowed at the upper right in photo 20. The vacuolar membrane is connected with the plasma membrane through the pouch-like membrane. Fibril-like structures of steep Z-helix are also seen.
- Photo 24.** Flat S-helix fibril-like structures and the small vesicles on the plasma membrane in a tracheid undergoing  $S_3$  layer formation.
- Photo 25.** A vesicle (arrow) attached to the plasma membrane in a tracheid undergoing  $S_3$  layer formation.

**Photos 26-29 ; Undifferentiated tracheids in dormancy.**

- Photo 26.** Many small vacuoles.
- Photo 27.** A TEM micrograph showing many small vacuoles. They are corresponding well to those in photo 26.
- Photo 28.** A field emission SEM micrograph showing tubular membranes connected each other and the plasma membrane.
- Photo 29.** A field emission SEM micrograph showing tubular membranes (arrow) of about 500-1000  $\text{\AA}$  in diameter.











