Chloroplast Ultrastructure of Primary Needles in the Adventitious Shoots of Pinus taeda

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ABSTRACT

Chloroplast ultrastructure of loblolly pine was investigated for the primary needles in 3-month-old and 7-month-old adventitious shoots, for those in the seedlings grown for the same length of the time as those shoots, and for 7-month-old brachyblasts. Two media were used to raise the shoots. For ultrastructural studies samples were fixed in 2% glutaraldehyde in 0.2 M sodium cocadylate, post-fixed in 1% buffered osmium tetroxide, dehydrated through an ethanol series, and embedded in Spurr's resin. Sections were stained with 2% saturated uranyl acetate and Reynold's lead citrate. There were no differences in chloroplast ultrastructure due to media. No fundamental differences were recognized in ultrastructure among mature brachyblasts and the primary needles of 7-month-old seedlings or in vitro adventitious shoots, while the last one showed the least development of grana. Chloroplasts showing irregular arrangement of lamellar system with or without development of grana were dominant in 3-month-old in vitro shoots, while such chloroplasts were rarely seen in the seedlings of the same age.

Keywords: Pinus taeda, chloroplast ultrastructure, adventitious shoot

INTRODUCTION

In our laboratory we have found and reported several aberrations of chloroplast structure in tissue culture. In sweet gum (Liquidambar styraciflua) the normal chloroplast structure is not found in adventitious shoots in vitro (Wetzstein and Sommer, 1982). Lee et al. (1985) reported that adventive shoots develop chloroplasts differently from the seedlings under the different light intensity regimes. In slash and longleaf pine callus cultures Birchem et al. (1981) found that chloroplast development was not always complete and that amyloplast-like structures were present instead. Stine (1984) also showed that

chloroplast from adventitious shoots in vitro often contained swollen thylakoids, while those from seedlings included such swollen thylakoids only when they had large starch grains, in Pinus palustris, P. elliottii, and P. palustris x elliottii. Therefore we consider it important to investigate the ultrastructure of chloroplast in in vitro loblolly pine culture.

Chloroplasts, containing chlorophylls and carotenoid pigments, are considered to be one of the most important plant cell organelles, for they are the ultimate suppliers of energy. They are not only sites of photosynthesis; they are also involved in amino acid synthesis and fatty acid synthesis, as well as providing space for the temporary storage of starch.

Studies on the development of plastids in tissue cultures cells have often shown the plastids to resemble those found in quiescent cells (Bornman, 1974). Abberations in the development of plastids in tissues in vitro have been reported by Sjoland and Weir (1971) and by Wetzstein and Sommer (1982). Wellburn (1982) reported etiolated pine plants are not photosynthetically competent, although they could form most of the lamellar system in the dark (Oku et al., 1977), which differs from the angiosperms that cannot synthesize chloroplasts in the dark.

Exogenous kinetin in bean leaf culture increased the number of grana per chloroplast and the size of chloroplasts (Naito et al., 1981; Tsuji et al., 1979). Some tobacco suspension cultures were capable of differentiation of chloroplasts only with the application of a cytokinin (Axelos and Peaund-Lenoel, 1980; Seyer et al., 1975). In Sycamore maple (Acer pseudoplatanus L.) callus it was reported by Sunderland (1966) that NAA and 2,4-D inhibited chlorophyll formation. It was reported

that it seemed chloroplast differentiation was correlated to the differentiation of callus into organs (Barnett, 1978). He found amyloplasts in undifferentiated callus but chloroplasts in stem-like organs originated from callus in Monterey pine. Simola (1982) reported the well-organized chloroplasts were found in the needle-like structures in the callus cultures of Norway spruce.

Pinus taeda embryos formed adventitious shoots in vitro but showed much variation in shoot establishment ability and vigor, depending on medium and exogenous hormone dosages (unpublished data). Electron microscopic studies were done to determine whether there is a difference in chloroplast structure between primary needles from adventitious shoots, primary needles from seedlings, and brachyblasts.

The overall purpose of this study is to compare the chloroplast ultrastructure for different levels of growth regulators and for medium formula.

However, at this time we will report only on the ultrastructure of chloroplasts in adventitious shoots induced on MGE, a medium modified from Gamborg and Eveleigh (1968) and on MLM, a medium modified from Lloyd and McCown (1981), each of which contained 5 mg/1 6-benzylamino-purine (BAP) and 0.1 mg/1 naphthaleneacetic acid (NAA).

Throughout this paper "seedling" stands for a plant with or without a root system formed by the growth of the apical meristem and "shoot" for the analogous structure formed by adventive organogenesis on cotyledons of the embryo, if no additional detail is mentioned.

MATERIALS AND METHODS

Embryos were cultured 3 to 4 weeks for initiation of buds and transferred for the growth of buds to the non-hormonal charcoal medium reduced by half in major and minor salts except iron. Explants were subcultured every 3 to 5 weeks.

The second subculture media included no charcoal. All media contained sucrose and agar at 2% and 6%, respectively. All cultures were placed in the growth room at 23 to 27 centigrade. Illumination was given by cool white fluorescent lamps with an intensity of approximately 1506+540 lux at the level of the explant with a 16-hour photoperiod.

Primary needles from 3-month-old and 7-month-old adventitious shoots were harvested. In addition to 7-month-old brachyblasts, primary needles from the seedlings germinated and grown under the same in vitro cultural environment were used for comparison.

All 7-month-old needles were fixed and embedded in November, 1986 for this investigation. Others were fixed and embedded in May, 1987.

Methods for fixation, embedding, and staining are as follows.

FIXATION

1. 1-mm-long sections were cut from the middle part of the needles and immediately fixed in 2% glutaraldehyde in 0.2 M sodium cocadylate buffer, in vacuo under the room temperature for 12 hrs.

2. Sections were washed 5 mins two times, 30 mins once, and 10 mins once with 0.2 M sodium cocadylate buffer, and then post-fixed for 4 hrs in vacuo at 1-4 C in 1% 0s04 in 0.2 M sodium cocadylate buffer.

3. Sections were washed in the same buffer for 10 mins, followed by a 30 min wash, and finally an additional 10 min wash.

4. The samples were dehydrated using a graded ethanol series by placing them for 15 mins sequentially in 30, 50, 70, 80, and 95% ethanol, and 100% ethanol twice.

EMBEDDING

1. Infiltration was done successively for 4 hrs, 3.5 hrs and 7 hrs, in each of 1:3, 1:1, and 3:1 Spurr's resin (1969): 100% ethanol in vacuo.

2. The embedment media were changed with pure Spurr's resin for 4, 6, and 4 hrs in vacuo.

3. The samples were placed into oven-dry embedding capsules with fresh resin and polymerized for 12 to 24 hrs at 70 C.

TRIMMING AND SECTIONING

The sample blocks were trimmed to form a trapezoid through the middle of the sample. Thin sections were made on a Sorval MT-2 ultramicrotome using a diamond knife.

One micron-thick sections were made for the examination with a light microscope after staining with Paragon 1301 stain (Spurlock et al., 1966). The sections were spread with chloroform vapor prior to being picked up on formvar coated copper grids.

STAINING

The sections were stained for 15 mans with 2% aqueous uranyl acetate, and then rinsed 4 times for 15 secs each with distilled water. Rinsed specimens were stained again with Reynolds lead citrate (Reynolds 1963) for 10 mans in the covered petri dishes free from carbonate by NaOH pellets, followed by 4 rinses of distilled water 15 sec each.

Prepared specimens were examined using Philips 400 transmission electron microscope. The accelerating voltage was set at 80 KV.

RESULTS AND DISCUSSION

Plastids, in addition to cell walls and vacuoles, are specific components of plant cells. They are bounded by an envelope, a pair of unit membranes, and consists of a lamellar system and a matrix substance, stroma, which includes plastid DNA, ribosomes, and occasionally starch grains. Mature plastids are classified on the basis of the kinds of pigments they contain (Whatley et al., 1978). They are etioplast, amyloplast, chromoplast, and chloroplast.

Chloroplasts are usually found disk-shaped with their long axes parallel to the cell wall. The stroma is traversed by an elaborate system of membranes in the form of flattened sacs, thylakoids. A granum is formed when stroma thylakoids organize a structure like a stack of coins. These characterize chloroplasts. The grana are connected with each other by stroma thylakoid. The thylakoid structure is similar to the plastid envelope in that it consists of two unit membranes. The chloroplasts of 7-month-old brachyblast of loblolly pine are shown in Fig. 2. Well-developed grana are conspicuous (Fig. 3). Other cell

organelles in addition to chloroplast are shown in Fig. 1. Starch grains and osmiophilic globules are often found in brachyblast chloroplasts. In Fig. 3 chloroplast ultrastructure is well preserved, and shown are grana, large starch grain, and osmophilic globules.

The chloroplast envelope is also clearly visible. Chlorophylls and carotenoid pigments, which are responsible for photosynthesis, are found embedded in thylakoid membranes (Mohr, 1981).

Chloroplasts in the primary needles of 3-month-old plants are shown in Figs. 4 and 5. Many embryos cultured on MLM produced seedling by the growth of the apical meristem in addition to the adventitious shoots on cotyledons. The primary needles of these seedlings and shoots were recommended materials to investigate chloroplast development.

Fig. 4 shows the fine structure of the primary needle chloroplast in the seedling originated from an embryo which was cultured on MLM including 5 mg/l BAP and 0.1 mg/l NAA. Chloroplasts similar to this were found in the seedlings germinated and grown on either MLM or MGE without growth regulators.

In Fig. 5 is shown a chloroplast in the primary needles of 3-month-old adventitious shoots on the cotyledons from the same embryo that produced the seedling mentioned in Fig. 4. Stroma thylakoids are arranged loosely and irregularly. It is not expected that the seedling and the adventitious shoot originating from the same embryo and grown under the same in vitro conditions would show the ultrastructural differences in chloroplasts.

These differences are supported by Figs. 6 and 7. Fig. 6 is the chloroplast in 7-month-old seedling grown on MGE without growth regulators. Fig. 7 is a highly magnified chloroplast in the shoots of

the same age as that in Fig. 6. These were not differentiated from the same embryo as those in Figs. 4 and 5. In the shoots grana were not as well developed as those in the seedlings. Swollen thylakoids were often found that were rare in the seedlings (Fig. 7). Stine (1984) also showed the similar ultrastructural differences in several pine species.

After the growth of 4 months, chloroplasts developed significantly (Figs. 5 and 7), even though the grana with many stacks of thylakoids were not frequent and some swollen loculi were still present. Even the 3-month-old seedling had formed the grana of up to 12 stacks of thylakoids (Fig. 4).

In sweetgum adventitious shoots, chloroplasts with irregular lamellar system were also reported when grown under various light intensity regimes (Lee et al., 1985). They also found large starch grains in the seedlings but not in the plantlets. However, they did not determine if starch accumulation was cyclic, or whether seedlings and cultured plantlets differed in this respect. Starch grains were shown in 7-month-old adventitious shoots (Fig. 8). The seedlings of this age showed the development of starch grains, but none of 3-month-old seedlings or shoots showed these.

Many in vitro factors, such as kinetin and NAA, affected the chloroplast development (Naito et al., 1981; Tsuji et al., 1979). In some tobacco cultures chloroplasts differentiated only when exogenous cytokinin was applied (Seyer et al., 1975; Axelos and Peaund-Lenoel, 1980). Further studies are required to investigate the effects of growth regulators on the pine chloroplast development in the in vitro shoots. But it seems that there is no significant difference in the chloroplast ultrastructure between two media, MGE and MLM.

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Figs. 1 to 8. Mesophyll cells and chloroplasts in loblolly pine needles. c, cytoplasm; ce, chloroplast envelope; cp, chloroplast; cw, cell wall; g, granum; is, intercellular space; m, mitochondrion; o, osmophilic globule; s, stroma; sg, starch grain; t, thylakoid; v, vacuole.

Fig. 1. General structure of mesophyll cell in brachyblast.

Fig. 2. Detail of chloroplast in brachyblast. Chloroplast with a large starch grain and well-developed grana.

Fig. 3. Higher magnification of brachyblast chloroplast.

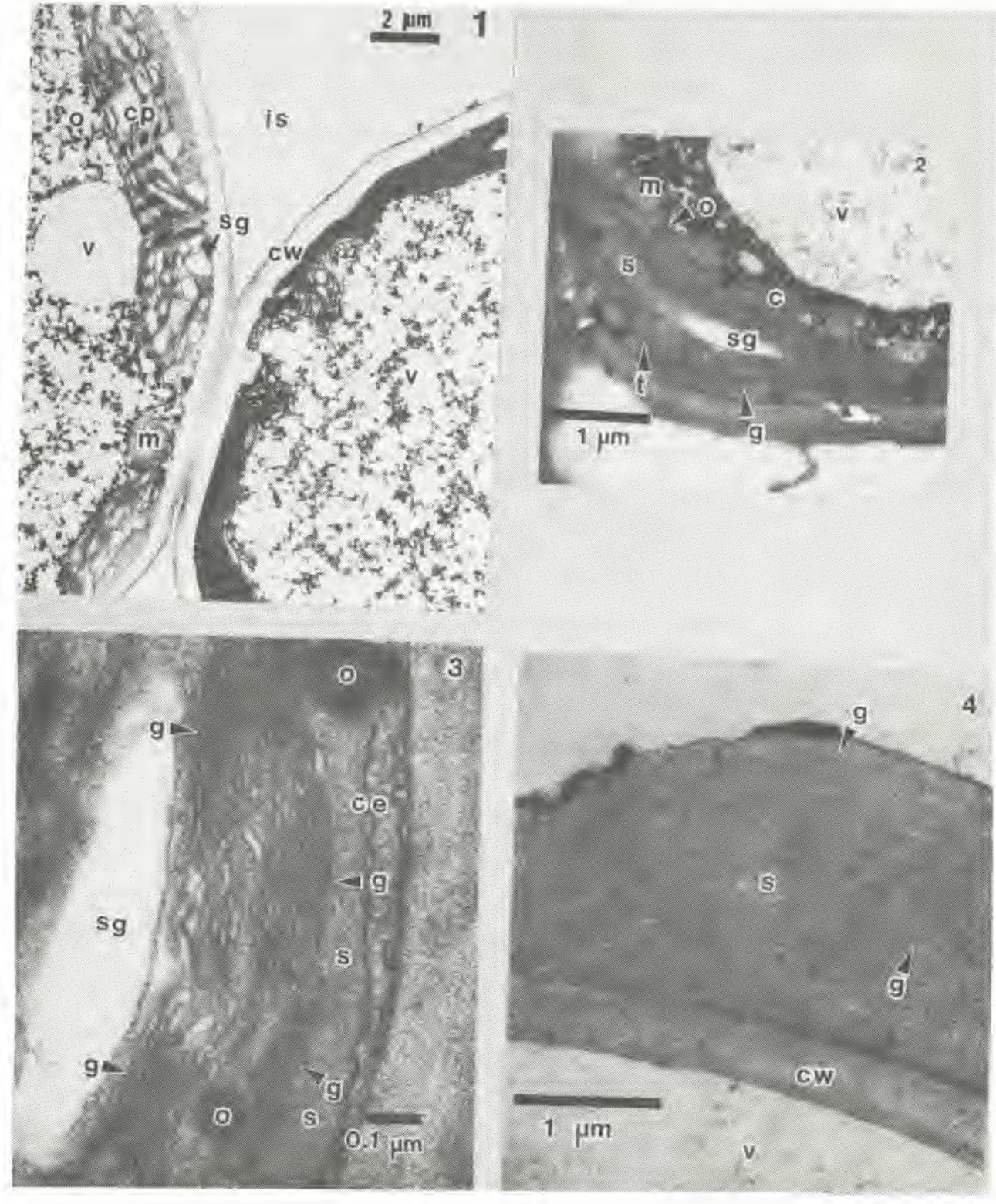
Fig. 4. Chloroplast of primary needle in the 3-month-old seedling originated from an embryo grown on MLM containing 5 mg/1 BAP and 0.1 mg/1 NAA. Well-arranged lamellar system.

Fig. 5. Chloroplast of primary needle in the 3-month-old adventitious shoot induced on the cotyledon of the same embryo mentioned in Fig. 4. Irregular arrangement of lamellar system.

Fig. 6. Chloroplast of primary needle from the 7-month-old in vitro seedling germinated and grown on MGE without growth regulators.

Fig. 7. Chloroplast of primary needle from the 7-month-old adventitious shoot induced on MGE containing 5 mg/1 BAP and 0.1 mg/1 NAA. Well-organized grana but some swollen loculi.

Fig. 8. Chloroplast containing large starch grains in the 7-month-old in vitro shoot.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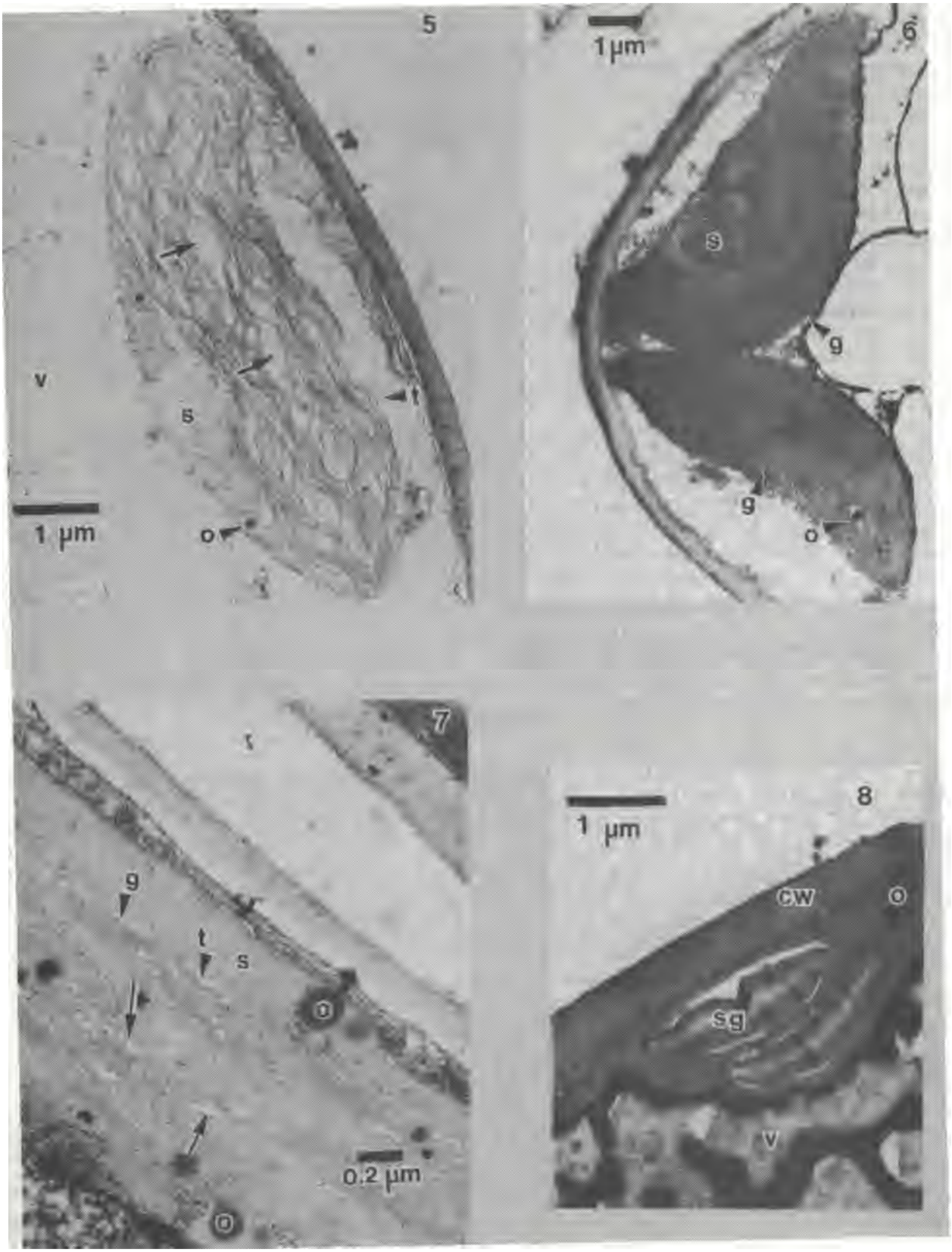
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