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Ultrastructure of Amyloplasts and Intercellular Transport of Old and New Scales in *Fritillaria ussuriensis*

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Amyloplast structure and intercellular transport in old and new scales of *Fritillaria ussuriensis* were observed by means of electron microscope. Most amyloplasts in old scales contained one starch grain, which was constructed by the layered deposition of starch around the hilum. Membrane systems and stroma of amyloplasts were pushed aside to form a shell surrounding the starch grain. In contrast, amyloplast shells in new scales contained a relatively light stroma and few internal membranes that were not organized into grana and stroma thylakoids. Active intercellular transport was observed in both new and old scales. Encytosis and exocytosis were common in the cell membrane and produced many vesicles containing numerous particles and filaments. These results lay the foundation for the further study on the mechanisms of growth and development in

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Fritillariae Bulbs from various species of the genus *Fritillaria* have for centuries been widely used in traditional Chinese medicine as antitussives and expectorants in China, Korea and Japan (Ding et al., 1996). The main active ingredients of commonly-used *Fritillaria* herbs are constituents are steroidal alkaloids, particularly verticine and verticinone (Zhang et al., 1998). Many different *Fritillaria* species are currently used as the plant sources, with the amounts and types of *Fritillaria* alkaloids varying by species (Funda et al., 1997). *Fritillaria ussuriensis*, located in temperate zone, is one of the most important species of genus *Fritillaria*. At present, the yield of *F. ussuriensis* is still very low (Li, 1985), so cultivation of *F. ussuriensis* is necessary to produce the required quantity of bulbs. Studying the development mechanism of this plant should enable an increased yield of *F. ussuriensis*.

Fritillaria ussuriensis is a pre-vernal plant, and its aerial part grows only 2-3 months each year in spring. During the short growth period every spring, the aerial part synthesizes starch and stores it in its bulb scales underground. In summer, the bulb is in a dormant state for about 3 months. The bud begins to grow in autumn along with the growth of roots, but does not emerge until the next spring. Meanwhile, the new scale begins to form (Gao et al., 1997). In winter, the bulb remains in a dormant state because of coldness (Gao et al., 1998). For most of the year, starch is almost the only nutrient resource. The process of new and old scale exchange involves the process of starch disintegration in the old scale and synthesis in the new scale (Gao et al., 1996). As starch stored in the old scale is hydrolyzed into sucrose and transported to the new scale, the new scale grows in size and the old scale shrinks (Gao et al., 1995). Studying the mechanism controlling amyloplast formation and degradation is a key step toward understanding how *F. ussuriensis* grows and develops.

Several parenchyma cells near the adaxial epidermis degraded at the beginning of disintegration of old scale. Under light microscope, we found a line of degraded cells (Gao et al., 1995). Later, other parenchyma cells in the center of the scale began to degrade, with the amyloplast (starch grain) degrading first of all. The present article deals with the disintegration of starch grains in old scales, the formation of starch grains in new scales, and the intercellular transport between new and old scales in *Fritillaria ussuriensis*.

MATERIALS AND METHODS

F. ussuriensis was obtained from the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. Bulbs were cultivated in a greenhouse of the Research Center for the Development of

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Advanced Horticultural Technology, Chungbuk National University. Storage scale samples in old and new scales were taken after summer dormancy from scale centers of three-years-old plants whose new scales were forming and old scales degrading.

Samples were cut into 1-2 mm^3 sections and fixed immediately at room temperature for 2 to 3 h in 4% glutaraldehyde in 0.1 mM sodium cacodylate buffer (SCB), pH 7.2. Fixed samples were rinsed for 1 h with 3×20 min washes of 0.1 mM SCB at 4°C. Post-fixation was in 1% osmium tetroxide and 0.075 mM SCB overnight at 4°C. After being washed 3 times in 0.1 mM cacodylate buffer for 20 min each, at 4°C, each, samples were step-wise dehydrated in ethanol

Figure 1. Transmission electron microscopy of an old scale of *F. ussurriensis*. All scale bars = 1.0 μm. **CW:** Cell Wall; **D:** Dictyosome; **ER:** Endoplasmic reticulum; **M:** Mitochondrion; **PD:** Plasmodesmata; **S:** Starch grain (in amyloplast); **V:** Vesicle. **A, B.** An old scale amyloplast whose shell has a relatively clear structure. There are numerous mitochondria, ER structures, and free ribosomes around the amyloplasts. **C.** A degrading amyloplast which was associated with the ER. Arrows indicate the processes of endocytosis and exocytosis. **D.** Endocytosis and exocytosis (arrow).

(25%, 50%, 75%, 95%, and 2×100% steps; 20 min each, at 4°C) and stained in 1% uranyl acetate in 75% ethanol for 2 h to overnight. Samples were soaked in 1:1 ethanol/acetone 1:1 and 100% acetone (1 h each step), and step-wise embedded in polybed Araldite (P/A, Polysciences, Warren, PA) in steps of 33% (2 to 4 h), 66% (overnight), 100% (overnight, P/A without hardener), and 100% (2 to 3 h, P/A with hardener). Samples were then cured at 70°C for 16 to 24 h.

Ultrathin sections were cut on a LKB ultramicrotome and collected on 0.35% formvar-coated copper slot grids. Sections were posts-tained for 1 min in 1% lead citrate and viewed with a transmission electron microscope (JEOL JEM 100 CX, Germany) at 80 kV.

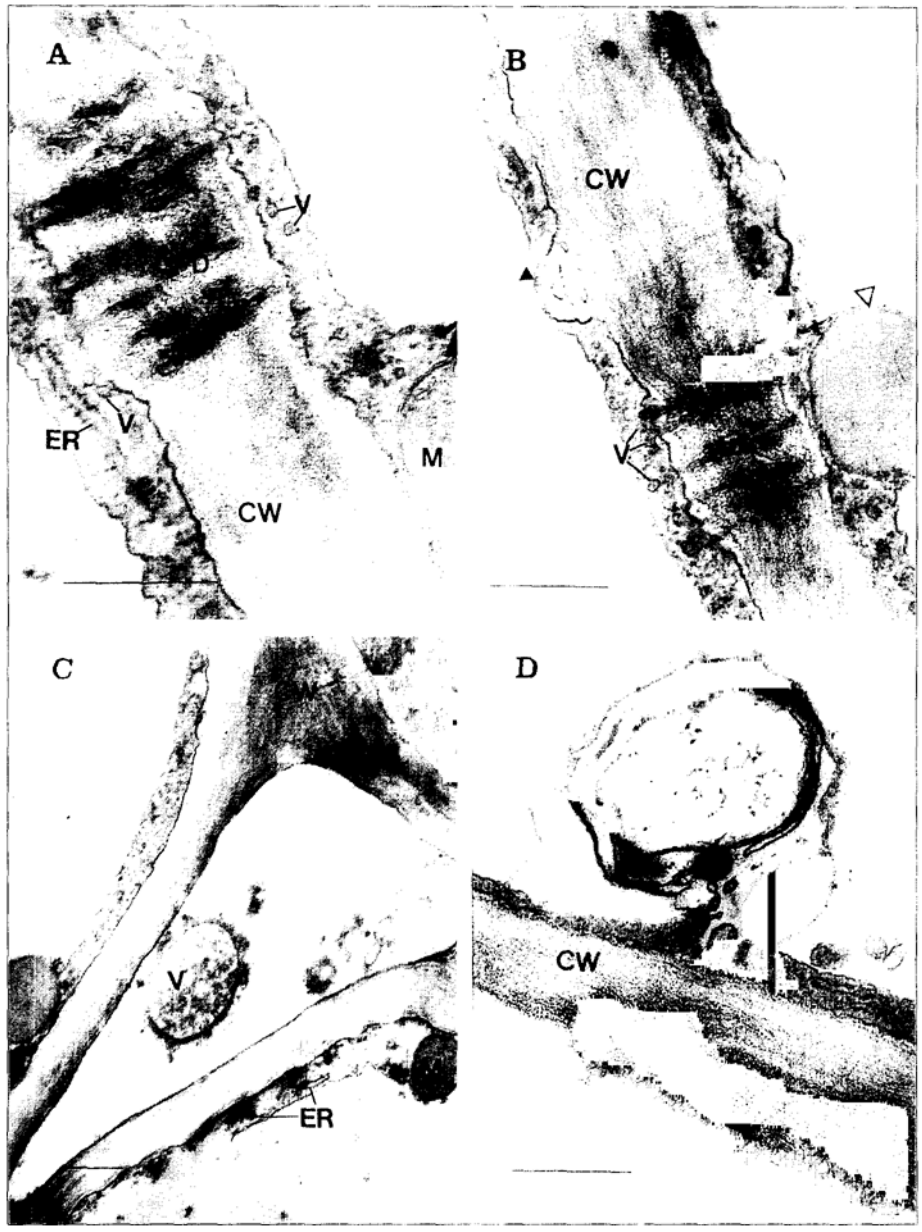

Figure 2. Transmission electron microscopy of an old scale of *F. ussurriensis*. All scale bars = 1.0 μm. **A, B.** Plasmodesmata and intercellular transport. In Figure B, the large arrow shows the dense-appearing spherule near the cell wall, while the small arrow shows endocytosis and exocytosis. **C.** Substances in intercellular space. **D.** A broken cell wall and the phenomena of endocytosis and exocytosis.

RESULTS

Disintegration and Intercellular Transport in Old Scales

Most old scale amyloplasts contained one starch grain, constructed by the layered deposition of starch around the hilum. The membrane system and stroma were pushed aside to form a shell surrounding the starch grain. We found that the thickness of the shell was uneven. Under electron microscope, the cytoplasm around the amyloplasts was dense, and we could observe many organelles including endoplasm reticulum (ER), mitochondria, and free ribosomes in it (Fig. 1, A and B). When the starch grain began to be hydrolyzed, many aggregates and stacks of vesicles,

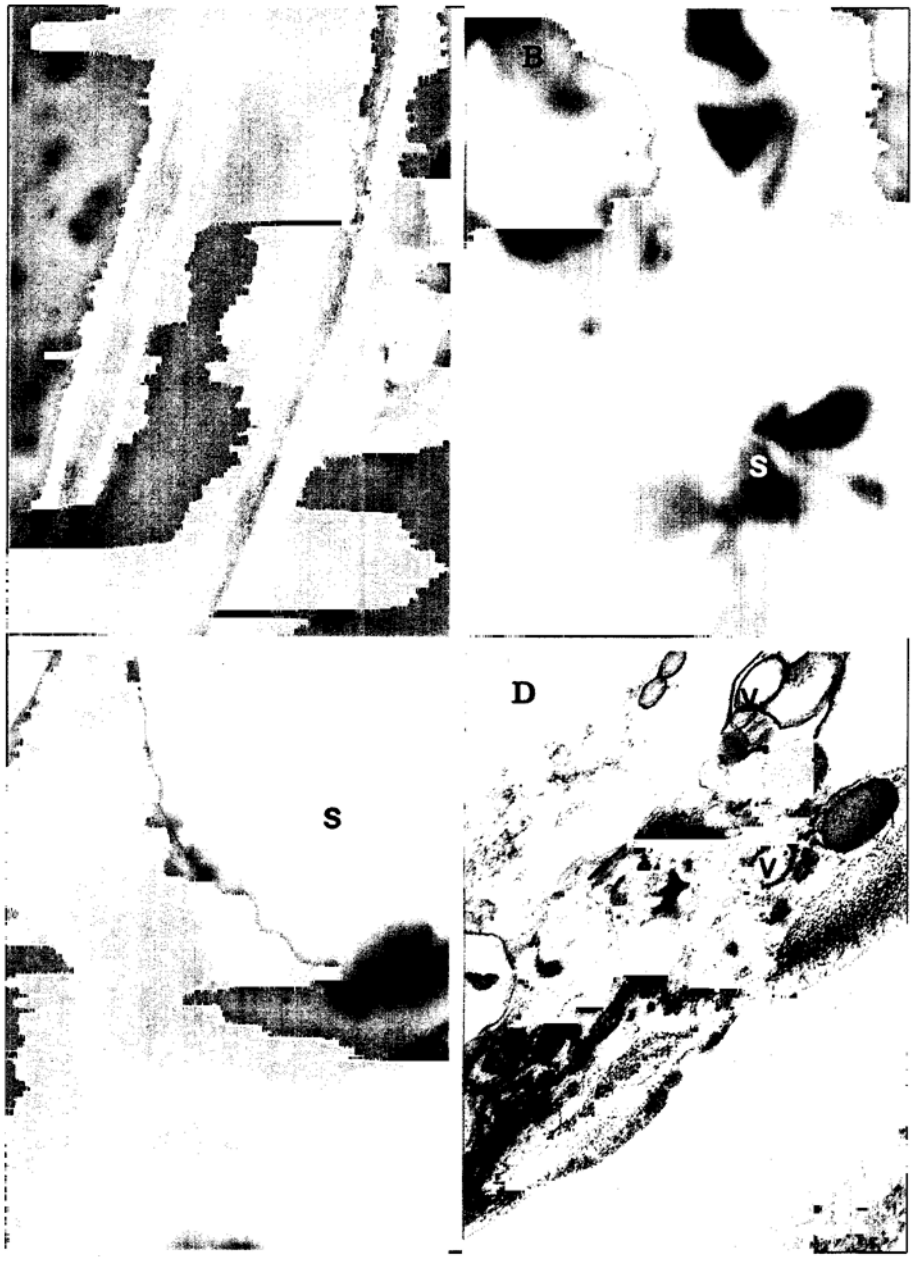

Figure 3. Transmission electron microscopy of old scale (A) and new scales (B-D) of *F. ussurriensis*. All scale bars = 1.0 μm. **A.** A broken cell wall. **B and C.** A new scale amyloplast whose plastid shell contains a relatively light stroma and a few internal membranes that were not organized into grana and stroma thylakoids. There are some mitochondria and ribosomes around the amyloplasts. **D.** Endocytosis and exocytosis. Many vesicles present in the space between the cell membrane and cell wall.

particles, and filaments became distributed throughout the cell. We sometimes found ER around the amyloplasts (Fig. 1C). As shown in Figures 1, C and D (arrow), endocytosis and exocytosis occurred frequently in the cell membrane, producing many vesicles containing particles and filaments. Numerous branched and unbranched plasmodesmata could be found traversing the cell wall. Particles and filaments as well as the small vesicles were closely associated with the ends of plasmodesmata (Fig. 2, A and B), indicating that intercellular transport was very active in the degrading scales. Some dense-appearing spherules were observed near the cell wall (Fig. 2B, large arrow). In addition, the intercellular space also contained some substances, such as vesicles, particles and filaments (Fig. 2C). In the last stage of degradation process, the amyloplast shell was degraded and transported after hydrolyzation of the starch grain. Products from the disintegration were gathered in vascular bundles by intercellular transport and transported to new organs (Gao et al., 1995). After disintegration, most cytoplasmic components and the cell wall had been degraded and exported (Figs. 2D and 3A). In degrading cell walls, we could also identify endocytosis and exocytosis processes (Fig. 2D).

Synthesis and Intercellular Transport in New Scales

Compared with old scale amyloplasts, new scale amyloplast shells contained a relatively light stroma and few internal membranes not organized into grana and stroma thylakoids (Fig. 3, B and C). This was perhaps because the new scales we used was very young, and the amyloplasts were at the developing stage. As observed in the old scales, cytoplasm around the amyloplasts was dense, and we could find mitochondria, ER, and free ribosomes in the cytoplasm (Fig. 3, B and C). Intercellular transport in new scales was also very active. Endocytosis and exocytosis process occurred frequently in the cell membrane, producing many vesicles with plentiful particles and filaments (Figs. 3D and 4A, arrow). It was also observed that many vesicles occupied the space between the cell membrane and cell wall (Fig. 3D). Plasmodesmata traversed the cell wall and established a symplasmic pathway for the intercellular transport. We could find groups of particles and filaments as well as small vesicles closely associated with the ends of plasmodesmata appearing to pass through the plasmodesmata (Fig. 4B). Meanwhile, dictyosomes and ER were very active in secreting numerous

Figure 4. Transmission electron microscopy of a new scale of *F. ussuriensis*. All scale bars = 1.0 μm. **A.** Endocytosis and exocytosis (arrow). **B.** Plasmodesmata and the intercellular transport. Dictyosomes are very active in producing numerous of vesicles. **C.** No vesicles, particles or filaments are found in the intercellular space in scale of new scales..

vesicles to be distributed around the cell (Fig. 4B). Unlike the results observed in degrading old scales, no vesicles, particles or filaments were found in the intercellular space (Fig. 4C).

DISCUSSION

Starch is one of the major storage compounds in plants. In leaves, it accumulates during the day and is nocturnally degraded to supply the stroma and cytosol with the carbohydrates required for various anabolic reactions (Mohlmann et al., 1997). Amyloplasts are non-green plastids that are specialized for the synthesis and accumulation of starch. They are important components of storage organs, and are deeply involved in carbon metabolism in plants (Sakai et al., 1996). In *F. ussuriensis*, starch stored in the amylo-

plasts of old scales is the only nutrient resource for new organs before new bud emerges from the ground, since the plant has no aerial parts to engage in photosynthesis. In addition, starch stored in the amyloplasts is the only organic storage compound in new scales (Gao et al., 1995). In this context, it is important to study the disintegration, transport, and synthesis of starch (amyloplasts) in *F. ussuriensis* from different aspects.

The shell structure of amyloplasts changes at different stages of formation and degradation of starch. The plastid shell may play an important role in the hydrolysis and synthesis of starch. It was demonstrated that cell internal membrane systems such as the ER, dictyosomes, and vesicles participate in the disintegration of cell inclusions (Lou and Zhang, 1983; Zhang et al., 1986). The present study shows that rough ER and vesicles are associated with the degrading amyloplast, probably producing hydrolases for starch hydrolysis. We found significant amounts of ER and dictysomes around the amyloplasts in both old and new scale cells, indicating that the cell internal membrane system plays an important role in the disintegration and synthesis of cell inclusions in *F. ussuriensis*.

Intercellular transport is important for both new and old scales. Endocytosis and exocytosis, and transport through plasmodesmata are two different intercellular transport pathways. Transport by plasmodes- mata is a symplasmatic pathway, while endocytosis and exocytosis are both symplasmatic and apoplasmodial pathways. Wang et al. (1983) demonstrated that vesicles participate in the intercellular transport of Garlic scape. Robards and Lucas (1990) reported that plasmodesamata establish a symplasmic pathway that interconnects the cytosol of the mesophyll to the long-distance pathway of the phloem, and that plasmodesmata formed the most likely pathway for the intercellular transformation of metabolites. Lou et al. (1983) demonstrated that some plasmodesmata were in an open state in degrading garlic stem and the products from the degradation would be transported through the open-state plasmodesmata. They found that plasmodesmata could allow macromolecules or even bigger substances to cross during the open state. We did not observe open state plasmodesmata in *F. ussuriensis* scale cells (Fig. 4B), indicating that the state of plasmodesmata is different in different plants.

In this paper, we first reported the structure of amyloplasts and the intercellular transport in old and new scales in *F. ussuriensis*. The active compounds in the bulbs of *F. ussuriensis* is alkaloids, so, we think the further study is needed to investigate the relationship between starch metabolism and alkaloids accumulation in bulb scales of *F. ussuriensis*.

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LITERATURE CITED

Ding K, Lin G, Ho YP, Cheng TY, Li P (1996) Prederivation and high-performance liquid chromatographic analysis of alkaloids of bulbs of *Fritillaria*. Journal of Pharmaceutical Sciences 85: 1174-1179

Funda B, Bilge S, Mehmet K (1997) Steroidal alkaloids of *Fritillaria imperialis* L.. Journal of Faculty of Pharmacy of Gazi University 14: 17-23

Gao WY, Li ZL, Xiao PG (1995) Ultrastructural study on the disintegration in *Fritillaria thunbergii*. Acta Botanica Sinica 37: 885-889

Gao WY, Li ZL, Xiao PG (1996) Changes of peroxidases and esterases during the process of dormancy relieving in *Fritillaria thunbergii*. Guihaia 16: 171-174

Gao WY, Li ZL, Xiao PG (1997) Peroxidase assay over the course of dormancy relieving with low temperature in *Fritillaria thunbergii* by electrophoresis. Zhongguo Zhongyao Zazhi 22: 530-533

Gao WY, Li ZL, Xiao PG, Fu RZ (1998) Preliminary analysis of isolation of genomic total DNA in *Fritillaria thunbergii* Miq.. Zhongguo Zhongyao Zazhi 23: 79-81

Li ZL (1985) Study on the cultivation of *Fritillaria*. Chinese Traditional and Herbal Drugs 16: 17-19

Lou CH, Zhang WC (1983) Degradation and transport in plants. Advances in Plant Physiology and Biochemistry in China 2: 1-10

Mohlmann T, Tjaden J, Henrichs G, Quick WP, Hansler R, Neuhans HE (1997) ADP-glucose drives starch synthesis in isolated maize endosperm amyloplasts: characterization of starch synthesis and transport properties across the amyloplast envelope. Biochem J 324: 503-509

Robards AW, Lucas WJ (1990) Plasmodesmata. Annu Rev Plant Physiol Plant Mol Biol 41: 369-388

Sakai A, Yashiro K, Kawano S, Kuroiwa T (1996) Amylo-

plast formation in cultured tobacco cells: effects of plant hormones on multiplication, size, and starch content. Plant Cell Reports 15: 601-605

Wang Y, Lou CH, Yang SJ (1983) Cell biological study on the degradation and intercellular transport in Garlic scape. Acta Botanica Sinica 25: 8-13

Zhang A, Wang H, Tang X, Zhang Y, Yi X, Yu K (1998) Isolation and structure elucidation of alkaloids from the bulbs of *Fritillaria wabuensis*. Planta Medica 64: 448-450

Zhang WC, Yang WM, Lou CH (1986) The development of laticiferous system in Garlic scape. Acta Botanica Sinica 28: 20-25