藥作誌 (Korean J. Medicinal Crop Sci.) 7(2) : 129 - 137 (1999)

Ultrastructural Difference and Intercellular Transport of Metabolites in Old and New Bulb of *Fritillaria pallidiflora*

Wen Yuan Gao*, Lei Fan and Kee Yoeup Paek**

*Fritillaria pallidiflora*의 신구인경에 있어서 대사물질의 세포간 이동과 미세구조의 차이

高文遠 · 范磊 · 白基燁

ABSTRACT : The structure of amyloplasts and intercellular transport in the old and new bulbs of *Fritillaria pallidiflora* were observed by means of electron microscope. The structure of internal membrane system was different between new and old amyloplasts. The active intercellular transport was observed in both new and old bulbs. The phenomena of encytosis and exocytosis always could be found in the cell membrane, and plasmodesmata established a symplasmic pathway for intercellular transport. Groups of vesicles often located at the ends of plasmodesmata, showing that they participated in the intercellular transport. These results laid a foundation for the further study on the mechanism of growth and development in *Fritillaria pallidiflora*.

Key words : *Fritillaria pallidiflora*, amyloplast, intercellular transport, plasmodesmata

INTRODUCTION

Bulbs *Fritillariae*, derived from the bulbs of various species of the genus *Fritillariae*, has been widely used as an antitussive and expectorant in traditional Chinese medicine for centuries in China, Korea and Japan (Ding et al., 1996). The main constituents in *Fritillaria* are steroidal alkaloids. Verticine and verticinone are the major alkaloids in commonly used *Fritillaria* Herbs (Zhang et al., 1998). Many *Fritillaria* species are currently used as the plant sources, with the amounts and types of *Fritillaria* alkaloids varying in different species (Funda et al., 1997). *Fritillaria pallidiflora* is one of the important species in genus *Fritillaria* that locate in temperate zone. Cultivation of *Fritillaria pallidiflora* is very necessary for producing the required qualified bulbs in demand quantities. Now the yield of *Fritillaria pallidiflora* is still very low, and it needs 4 years to harvest the bulbs for commercial use (Li,

* 충북대학교 첨단원예기술개발연구센터 (Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheong-ju, 361 - 763, Korea)
〈 '99. 4. 21 접수 〉

1985). The study on development mechanism of *Fritillaria pallidiflora* is considered to be the base for increasing the yield.

Fritillaria pallidiflora is a pre-vernal plant, its aerial part grows only 2-3 months each year in spring. During the short growth period every year, the aerial part synthesizes starch and stores the starch in the scales of bulb under ground. In summer, the bulb is in the dormant state about 3 months. The bud will begin to grow in autumn along with the growth of roots. Meanwhile, the new bulb begins to form (Gao et al., 1997). But the bud can not grow out of ground until next spring. In winter, the bulb is in dormant state again because of the coldness (Gao et al., 1998). During most part of the whole year, starch is almost the only nutrient resource. The starch stored in old bulb will be hydrolyzed into sucrose and transported to new bulb. Each year, the new bulb in bigger size grows up along with the degradation of the old bulb (Gao et al., 1995). Bulb is the main storage organ in *Fritillaria pallidiflora*, starch grain is the main organic compounds storing in the bulb. The process of new and old bulb's exchange is mainly the process of starch disintegration in old bulb and starch synthesis in new bulb (Gao et al., 1996). Therefore, study on the mechanism controlling amyloplasts formation and degradation is the key step toward understanding how *Fritillaria pallidiflora* grows and develops.

Several parenchyma cells near the adaxial epidermis degraded at beginning of disintegration in old bulb. Under light microscope, we could find a line of degraded cells (Gao et al., 1995). After that, the other parenchyma cells in the center of scale began to degrade, and the amyloplast (starch grain) was degraded first of all. The present article will deal with the disintegration of starch grain in old bulb, the formation of starch grain in new bulb, and the intercellular transport in new and old bulbs in *Fritillaria pallidiflora*.

MATERIALS AND METHODS

Fritillaria pallidiflora was obtained from Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. The bulbs were cultivated in the green house of Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University. Storage scale samples in old and new bulbs were taken after summer dormancy from the three-years-old plant whose new bulb was forming and old bulb was degrading. The samples were gotten from the center of scales.

Samples were cut into 1-2 ㎟ and fixed immediately in 4% glutaraldehyde in 0.1 mM sodium cacodylate buffer (SCB), pH 7.2, at room temperature for 2 to 3 hrs. Samples were rinsed for 1 h in 3×20 min washes of 0.1 mM SCB at 4℃.

Postfixation was in 1% osmium tetroxide, 0.075 mM SCB overnight at 4℃. After being washed 3 times in 0.1 mM cacodylate buffer for 20 min each, at 4℃, samples were dehydrated in ethanol (25%, 50%, 75%, 95%, and 2×100% steps; 20 min each , at 4℃) and stained in 1% uranyl acetate for 2 hs to overnight in 75% ethanol. Then samples were transferred to ethanol and acetone 1 : 1 and 100% acetone (1 h each step), and embedded in polybed Araldite (P/A, Polysciences, Warren, PA) in steps of 33% (2 to 4 hs), 66% (overnight), 100% (overnight, P/A without hardener), and 100% (2 to 3 hs, P/A with hardener). Samples were cured at 70℃ for 16 to 24 hs.

Ultrathin sections were cut on a LKB ultramicrotome and collected on 0.35% formvar-coated copper slot grids. Sections were poststained 1 min in 1% lead citrate and viewed

on transmission electron microscope (JEOL JEM 100 CX, Germany) at 80 kv.

RESULTS

Disintegration and intercellular transport in old bulb

Most amyloplasts in the old bulb contain one starch grain, which is constructed by the layered deposition of starch around the hilum. The membrane system and stroma are pushed aside to be a shell bounding the starch grain. The shell has a clear structure as the prolamellar body in etioplast, and the thickness of the shell is uneven (Fig. 1 A, B). At some place of the shell, we can find the small vesicles (Fig. 1 A, arrow). During the degrading process of amyloplast, the structure of shell becomes indistinct as the strach grain is hydrolyzed. Meanwhile, the association between the shell and starch grain became loose (Fig. 1 C). At the end of degrading process of amyloplast, the shell is degraded and transported to new organs after hydrolyzation of starch grain. The products from disintegration will be gathered in vascular bundle by intercellular transport, and will be transported to new organs by vascular bundle (Gao et al., 1995). As shown in Fig. 1 D, many vesicles containing particles and filaments are present nearby the cell wall. Lots of particles and filaments were associated with the ends of plasmodesmata that traversed the cell wall (Fig. 2 A). These show that the intercellular transport was active in degrading old bulbs. At the end of disintegration, most cytoplasmic components were degraded and exported, even the cell wall will be broken (Fig. 2 B).

Synthesis and intercellular transport in new bulb

The amyloplast in new bulb is different from the amyloplast in old bub. Its shell contains a dense stroma and a few internal membranes that do not organized into grana and stroma thylakoids, and its structure is not so clear as the structure of amyloplast in old bulb. Rough endoplasmic reticulum (RE), free ribosomes (mostly polysomes), and some small vesicles are closely associated with the plastid shell of amyloplast (Fig. 2 C). Sometimes, we can find a shell bridge between two amyloplasts (Fig. 2 D, arrow), it indicates that substances are exchanging during the process of new amyloplast formation. The intercellular transport in the new bulb is very active. We can find a lot of vesicles containing particles and filaments nearby the cell wall. The phenomena of encytosis and exocytosis occur frequently in the cell membrane and producing many vesicles. At some places between cell membrane and cell wall, numerous small vesicles form a paramural body. There are many particles and filaments in these vesicles. However, no vesicles, particles and filaments are observed in the intercellular space in new bulb (Fig. 3 A). Branched and unbranched plasmodesmata traverse the cell wall, groups of dense particles immediately linked with the ends of plasmodesmata and appeared to pass through (Fig. 3 B). Small vesicles can often be found at the ends of plasmodesmata (Fig. 3 C), and sometime, we can observe groups of small vesicles (paramural body) occur nearby the plasmodesmata (Fig. 3 D). In addition, numerous small vesicles appear immediately above and below the plasmodesmata. The rough ER nearby is dilated and seems to produce small vesicles. Some of the small vesicles are closely associated spatially with the plasmodesmata (Fig. 4 A, B). These phenomena indicate that vesicles participate in the transport by plasmodesmata.

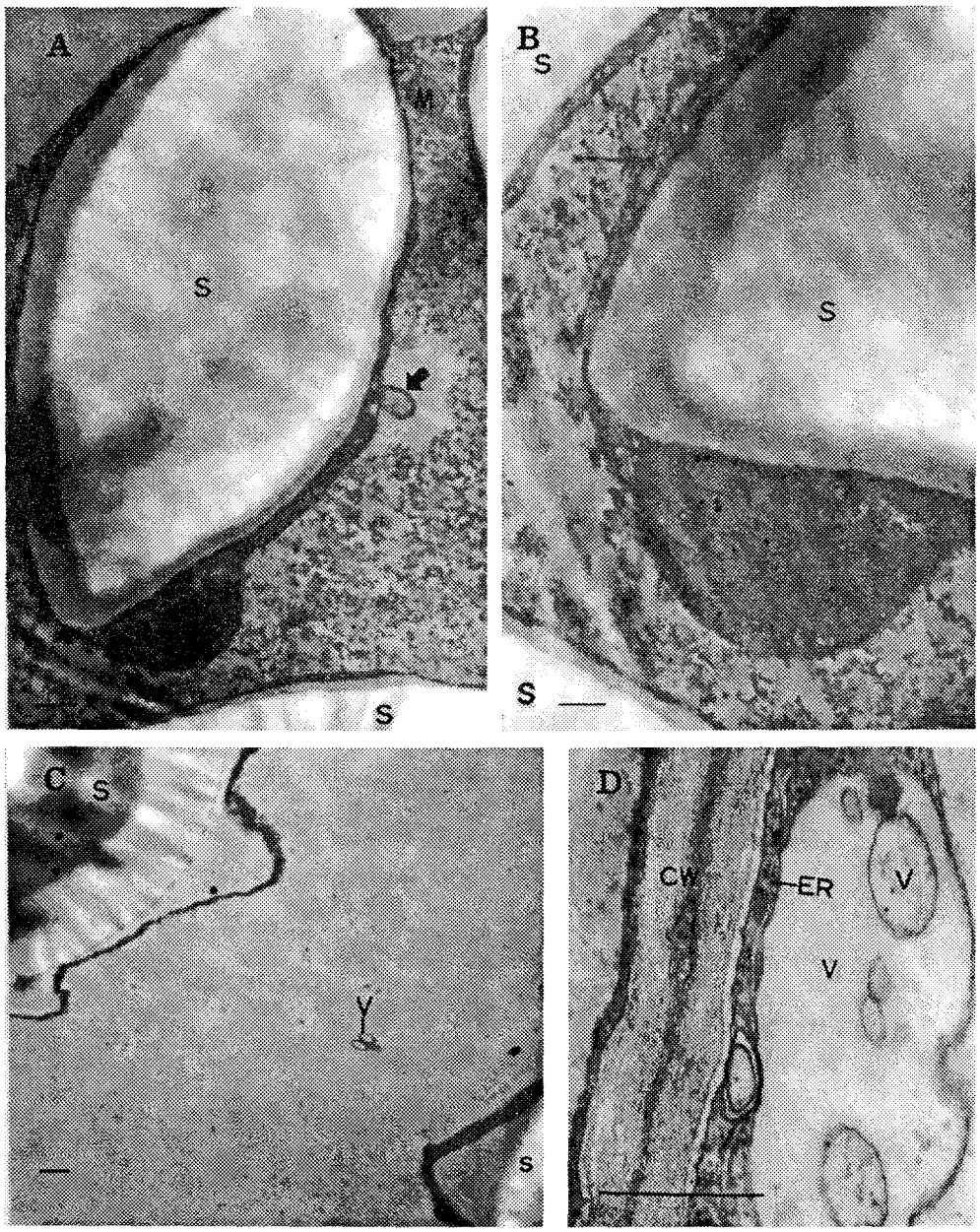

Fig. 1. Transmission electron microscopy of old scale of *Fritillaria pallidiflora.* All scale bars=1.0μm. CW-Cell Wall; D-Dictyosome; ER-Endoplasmic reticulum; M-Mitochondrion; PD-Plasmodesmata; S-Starch grain (in amyloplast) ; V-Vesicle.

(A, B) The amyloplast in old bulb. Its shell had a relatively clear structure. The arrow in Fig. A shows the small vesicle in the shell.

(C) The association between the shell and starch grain becomes loose when the amyloplast begins to be degraded.

(D) The vesicles containing particles and filaments nearby the cell wall.

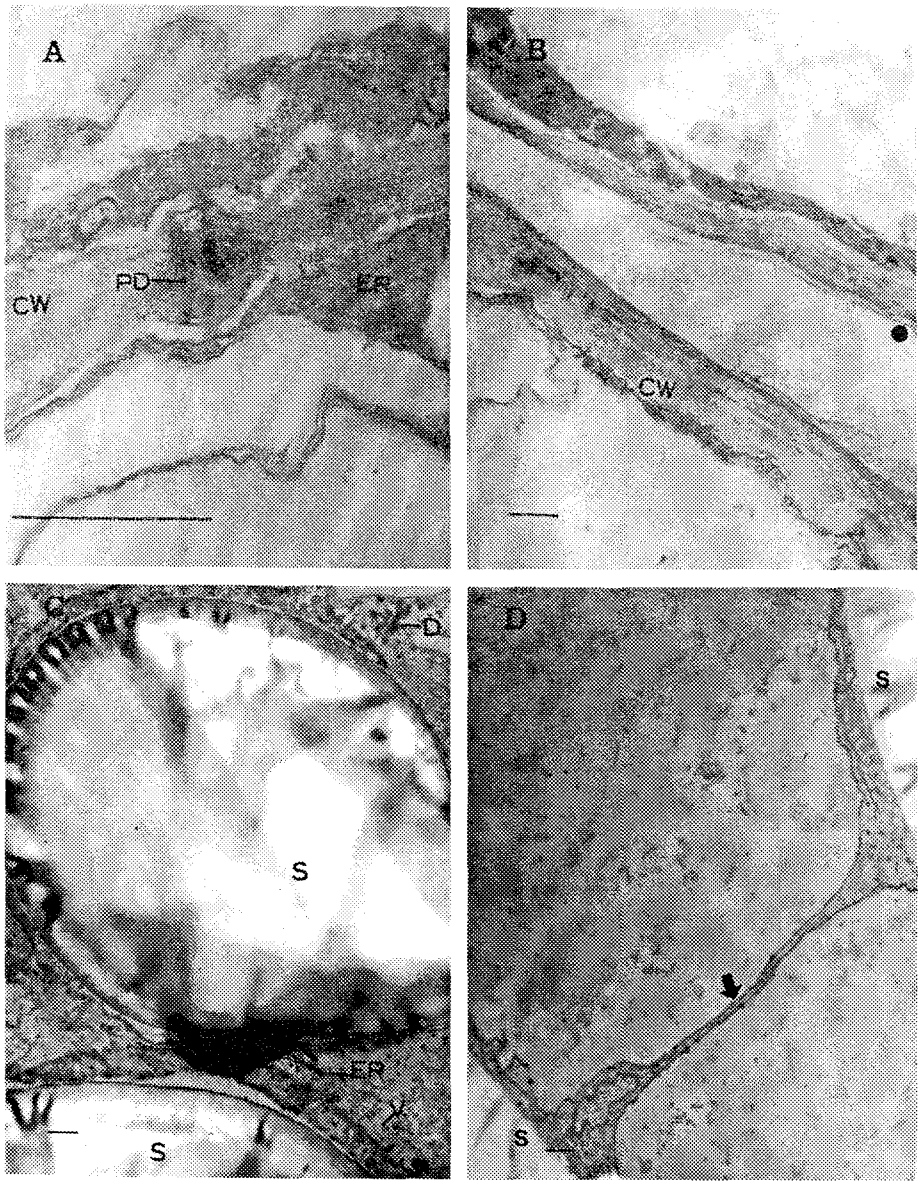

Fig. 2. Transmission electron microscopy of old scale (A, B) and new scale (C, D) of *Fritillaria pallidiflora*. All scale bars=1.0μm.

(A) Plasmodesmata and the associated particles and filaments.

(B) The broken cell wall.

(C) The amyloplasts in new bulb. Its plastid shell contained a dense stroma and a few internal membranes that were not organized into grana and stroma thylakoids. ER, small vesicles and free ribosomes are closely associated with the shell of amyloplsts.

(D) A shell bridge between two amyloplasts.

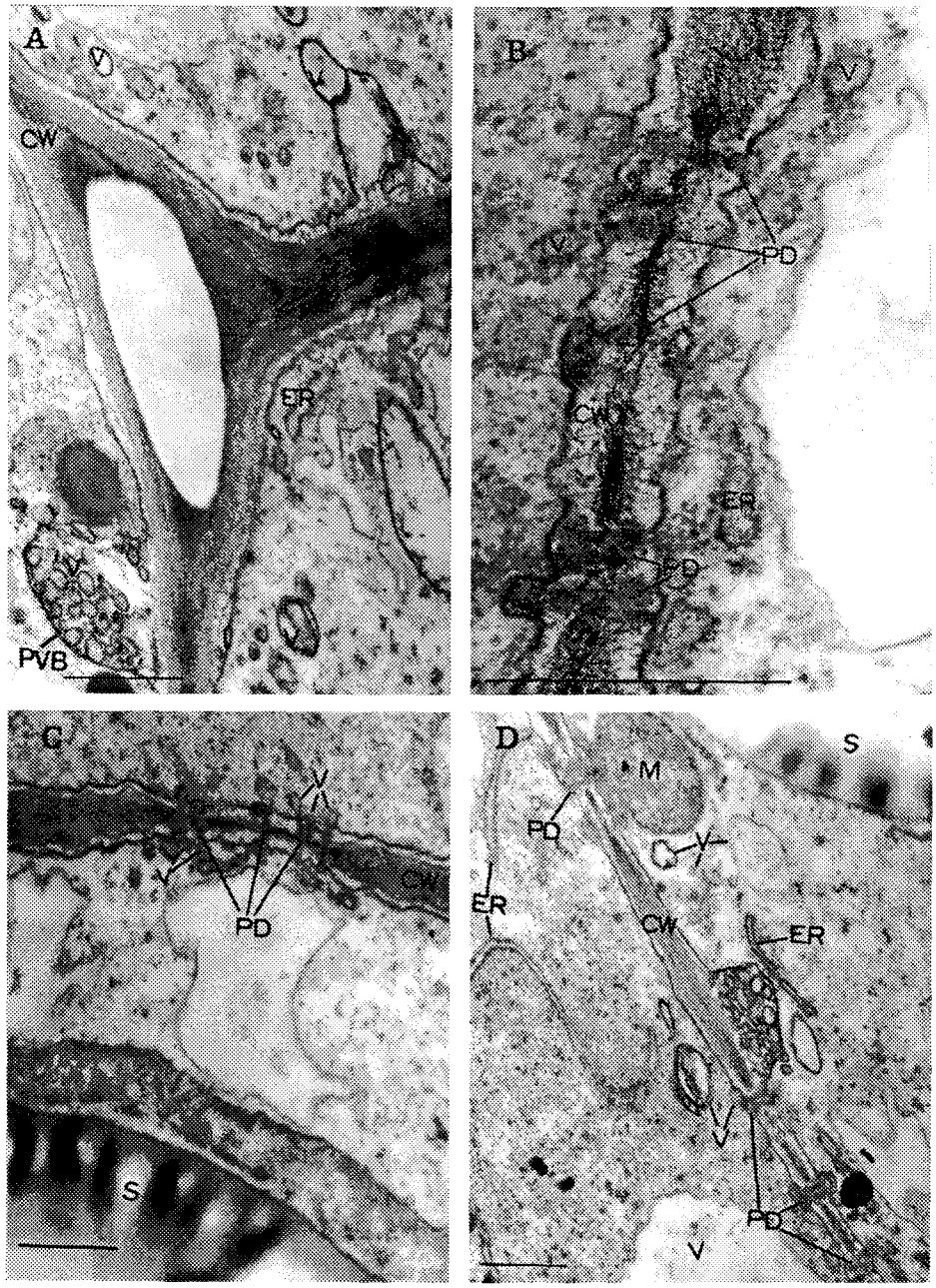

Fig. 3. Transmission electron microscopy of new scale of *Fritillaria pallidiflora*. All scale bars=1.0μm.

(A) No vesicles, particles or filaments were found in the intercellular space in scale of new bulb

(B) Plasmodesmata and the intercellular transport.

(C) Small vesicles at the ends of plasmodesmata.

(D) Groups of small vesicles (paramural body) nearby the plasmodesmata.

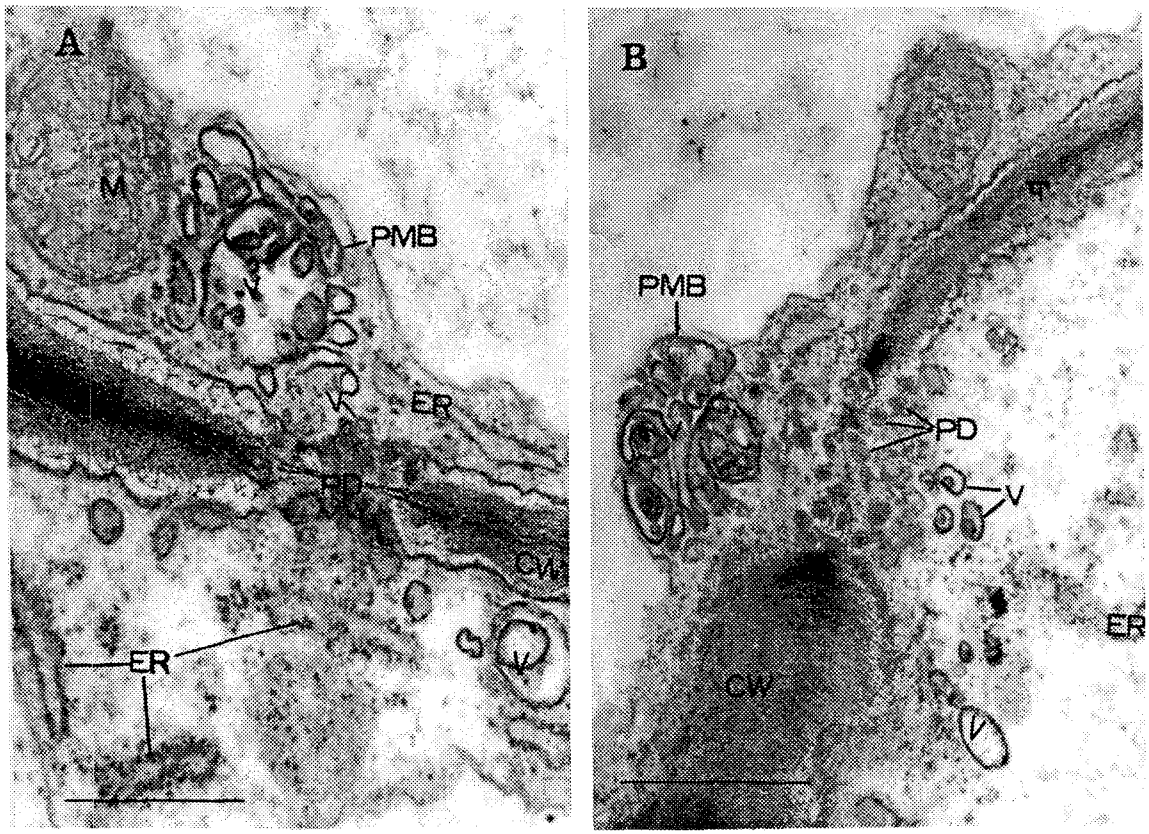

Fig. 4. Transmission electron microscopy of new scale of *Fritillaria pallidiflora*. All scale bars=1.0μm.
(A, B) The relationship between plasmodesmata and small vesicles. Some small vesicles are closely associated spatially with the plasmodesmata. The ER nearby is dilated and seems to produce small vesicles.

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). During the short growth period in spring every year, the aeropart of *Fritillaria pallidiflora* synthesizes starch and store it in the scales of bulb under ground. In the other time of the whole year, starch is almost the only nutrient resource (Gao et al., 1995; 1996). Therefore, analysis of the mechanism(s) controlling amyloplasts formation and degradation are the key steps toward understanding how *Fritillaria pallidiflora* grows and develops. The shell structure of amyloplast always changed at different stages of formation and degradation. The plastid shell may play an important role on the hydrolysis and synthesis of starch. It was demonstrated that cell internal membrane system

such as ER, dictyosomes, vesicles joined in the disintegration of cell inclusions (Lou and Zhang, 1983). The present study shows that rough ER and vesicles are associated with the forming amyloplast, it indicates that the cell internal membrane system plays an important role on the synthesis of cell inclusions as well. In addition, the process of disintegration was ordinal in the old bulb of *Fritillaria pallidiflora*.

Intercellular transport is important for both new and old bulbs. Encytosis and exocytosis, and transport through plasmodesmata are two different pathways for the intercellular transport. Transport by plasmodesmata is a symplasmatic pathway, encytosis and exocytosis include symplasmatic and apoplasmodial pathways. Wang et al. (1983) demonstrated that vesicles participated in intercellular transport in Garlic scape. Robars and Lucas et al. (1990) reported that plasmodesamta establish a symplasmic pathway that interconnects the cytosol of the mesophyll to the long-distance pathway of the phloem, and plasmodesmata were the most likely pathway for the intercellular transformation of metabolites. Lou and Zhang (1983) demonstrated that plasmodesmata would be in an open state while one tissue was degraded 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 across it at the open-state . Our research is concordant with these results. We did not observe the open-state plasmodesmata in the cell of *Fritillaria pallidiflora* bulb, but all the phenomena indicated that plasmodesmata joined the transportation of metabolites between two cells.

In study on the development of laticiferous system in Garlic scape, Zhang et al. (1986) gave evidence that vesicles transport through plasmodesmata in the field is capable of performing such a process : from the parenchyma to the laticifer in loading and from the latter to the former in unloading. From the results we found in *Fritillaria pallidiflora*, we propose that when a vesicle moves to the plasmodesmata, its membrane will dissolve and substances contained in it will transport through plasmodesmata. The ER and dictyosome would produce vesicles to load substances and transport to other place. We think future work will be directed forward understanding this possible involvement.

ACKNOWLEDGEMENT

One of us (Gao, W.Y.) wishes to thank the Korea Science and Engineering Foundation (KOSEF) for financial support, which permitted a stay of two years at Chungbuk National University (CNU). This research was supported in part by the KOSEF through the Research Center for the Development of Advanced Horticultural Technology at CNU.

적 요

*Fritillaria pallidiflora*의 전분체 미세구조와 신구 인경의 세포간 대사물질의 이동을 전자현미경으로 관찰하였다. 기존의 인경과 새로 형성된 인경세포 내 전분체는 내부 막구조에 있어서 뚜렷한 차이를 나타내었을 뿐만 아니라 대사물질의 세포간 이동도 활발히 이루어지고 있음을 관찰할 수 있었다. 엔도사이토시스와 엑소사이토시스 현상이 세포막에서 관찰되었으며 대사물질의 세포간 이동을 위해 원형질 연락에 있어서 염류수송 경로가 형성됨을 알 수 있었다. 또한 일단의 소포체들이 원형질 연락의 말단에 위치하고 있었는데 이는 세포간 물질 이동에 이들이 관여하고 있음을 알 수 있었다. 이러한 결과는 *F. pallidiflora*의 신구 인경형성과 발달에 미치는 기작을 연구하는데 유용하게 이용될 수 있는 것으로 생각된다.

LITERATURES CITED

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

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

Gao, W. Y., Z. L. Li, and P. G. Xiao. 1995. Ultrastructural study on the disintegration in *Fritillaria thunbergii*. Acta Botanica Sinica 37 : 885 - 889.

Gao, W. Y., Z. L. Li, and P. G. Xiao. 1996. Changes of peroxidase and esterases during the process of dormancy relieving in *Fritillaria thunbergii*. Guihaia 16 : 171 - 174.

Gao, W. Y., Z. L. Li, and P. G. Xiao. 1997. Peroxidase assay over the course of dormancy relieving with low temperature in *Fritillaria thunbergii* by electrophoresis. Zhongguo Zhongyao Zazhi 22 : 530 - 533.

Gao, W. Y., Z. L. Li, P. G. Xiao and R. Z. Fu. 1998. Preliminary analysis of isolation of genomic total DNA in *Fritillaria thunbergii* Miq.. Zhongguo Zhongyao Zazhi 23 : 79 - 81.

Li, Z. L. 1985. Study on the cultivation of *Fritillaria*. Chinese Traditional and Herbal Drugs 16 : 17 - 19.

Lou C. H. and W. C. Zhang. 1983. Degradation and transport in plants. Advances in Plant Physiology and Biochemistry in China 2 : 1 - 10.

Mohlmann T., J. Tjaden, G. Henrichs, W. P. Quick, R. Hansler and H. E. Neuhans. 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 A. W. and W. J. Lucas. 1990. Plasmodesmata. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41 : 369 - 388.

Sakai A., K. Yashiro, S. Kawano and T. Kuroiwa. 1996. Amyloplast formation in cultured tobacco cells : effects of plant hormones on multiplication, size, and starch content. Plant Cell Reports 15 : 601 - 605.

Wang Y., C. H. Lou and S. J. Yang. 1983. Cell biological study on the degradation and intercellular transport in Garlic scape. Acta Botanica Sinica 25 : 8 - 13.

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

Zhang W. C., W. M. Yang and C. H. Lou. 1986. The development of laticiferous system in garlic scape. Acta Botanica Sinica 28 : 20 - 25.