# STRUCTURAL CHANGES OF THE GUARD CELL MOTHER CELL OF SOYBEAN COTYLEDON DURING THE EARLY GERMINATION PERIOD

TA-CHING CHOU[1] and KUO-CHUN LIU[1]

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**Abstract:** The upper epidermal layers of soybean seeds and germinating were studied at different time intervals with light microscope and transmission and scanning electron microscopes. There was no stoma on the upper epidermis of mature soybean seeds before seed germination. Instead, guard-cell mother cells were present among other epidermal cells. Each guard-cell mother cell forms two connected guard cells within 24 h and the stomatal pores appear at about 72 h after planting. The ultrastructural changes of the guard-cell mother cells were studied during the early germination period at 24 h interval for 3 days. The stored food particles including starch grain, lipid droplets and protein bodies in the cytoplasm of the guard-cell mother cells and guardcells were studied histochemically.

## INTRODUCTION

Plant stomatal structure and function have been one of the major research interest among physiologists for many years. The developmental pattern and functional mechanism of the stomata of the leaves of dicotyledon plants are well documented (Inamdar *et al.*, 1986; Kothari and Shah, 1975; Wilkinson, 1979). Only a few reports related to the morphology and development process of cotyledonary stomata (Reddy and Shah, 1979; Sakurai *et al.*, 1986). Yamamoto *et al.* (1984) reported the stomatal density and differentiation in stomatal distribution of soybean plant. However, there is no report on development of the cotyledonary stomata of the soybean plants. This report presents the events observed on the cotyledonary stoma of soybean seed during the germination process.

Soybean is an important food ingredient of oriental people. The major constituents of nutrients of soybean seeds are protein and lipid. There is a large number of research reports on nutritional value of soybean (Kao, 1986). Yet there is no histological evidence report on the storage condition in the seed of these two important ingredients. Dean and Krober (1950) showed that the translocation of food ingredients of polysaccharides, lipids and protein of soybean seedling. Histochemical localization on the food particles of peanut cotyledon was reported by Hu and Xu (1990). Therefore, part of this report is to present the results of a histological localization of the major food ingredients. The results of a comparative study of the developmental changes of the stomata and during the early germination period on both light and electron microscopic level, and the nature of the food particles stored in the epidermal cells are presented.

(1) 周大慶、劉國鈞, Department of Biology, Science College, Tunghai University, Taichung, Taiwan, Republic of China.

As happened to most of the research projects that at the beginning one or a few questions need to be answered, but the process always leads to more questions. In our case, during germination the division of the guard-cell mother cell and the reapperance of the organelles which are present during seed formation need to be elucidated in further research.

## MATERIALS AND METHODS

Soybean, *Glycine max*, seeds were obtained from the Asia Vegetable Reseach and Development Center, Tainan, Taiwan. They were soaked and sterilized in 70% ethanol for 1 min and then were planted to 4 to 5 cm deep and spaced 3 to 4 cm between on sand bed in a greenhouse. The greenhouse temperature was around 28°C during the study period.

After planting, seeds were sampled every 24 h for 3 days. The upper epidermal layers of the cotyledons were peeled off with a razor blade and a pair of fine tweezers. They were mounted on glass slides for light microscopic observation. Samples were also prepared for both TEM and SEM observations.

For electron microscope observation, samples from dry seeds and germinating seeds were double fixed in 4% glutaraldehyde and 1% osmium tetraoxide in phosphate buffer at pH 7.3 for 1 h. After rinsing with the same kind of buffer they were dehydrated with a series of ascending concentration of ethanol, from 50% to 100%, at a 10% increasing in concentration in each change.

The samples were then treated with propylene oxide and embedded in Spurr's resin After polymerization, the samples were sectioned around 70 nm in thickness with glass knife on a Richert Ultracut microtome. Sections were stained with both uranyl acetate and lead citrate (Reynolds, 1963). Observations were made and photographs were taken with a Hitachi H-600 electron microscope at 75 KV.

For scanning electron microscopic observation, samples were fixed and dehydrated with ethanol as for TEM study and then treated in a critical point dryer, coated in an Eiko IB-2 coating apparatus for 4 min, examined and photographed with a Hitachi S-2300 scanning electron microscope at 15 KV.

For histochemical detection of carbohydrates resin sections of cotyledon were treated in 0.5% periodic acid in 0.3% nitric acid for 10 min, rinsed in running water for 1 min, and then stained in Schiff's reagent for 30 min. After being washed in sodium metabisulfite 3 times, 2 min for each time, they were rinsed in running water for 5 mins. For detection of lipids sections were rinsed in 70% ethanol for 1 min stained in 1% fresh Sudan black B in 70% ethanol for 45 min, then rinsed in 70% ethanol and water. For detection of proteins, sections were treated in 7% acetic acid for 1 min and then stained in 1% Coomassie brilliant blue R in 7% acetic acid for 20 min at 60°C. After being treated in 0.1% acetic acid for 1 min again, the sections were rinsed in running water for 5 min. Sections stained with the various reagents were air dried at room temperture. Observation and photography were made with light microscope.

## RESULTS

### Stomata development in cotyledon epidermis

The epidermal layer of the cotyledons of mature soybean seed showed no stoma. The locations, where the future stomata occupy, show only guard-cell

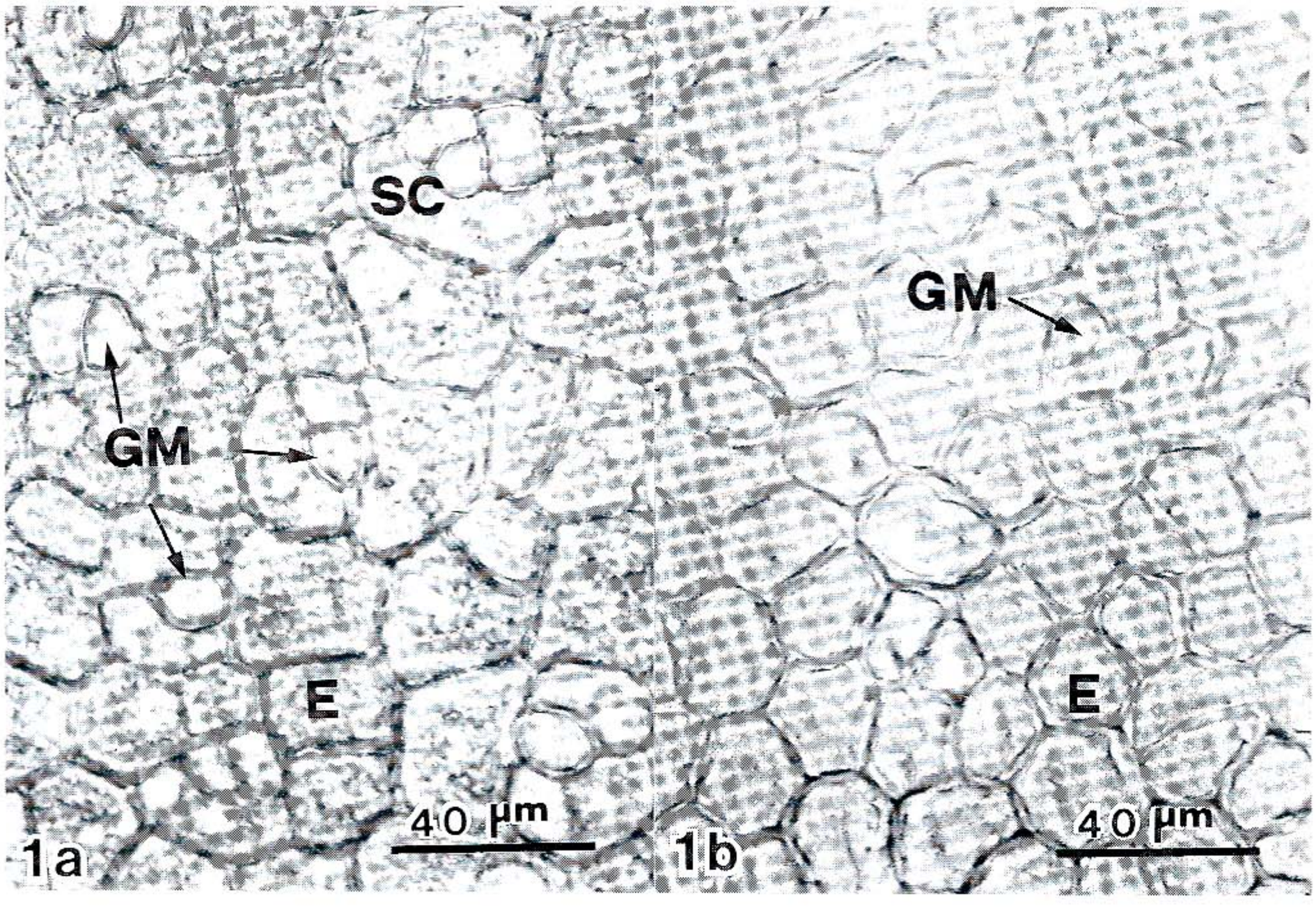


Fig. 1. (a) Light microscopic picture of the upper epidermal layer of soybean cotyledon after soaking in water. It shows that the guard-cell mother cells (GM) were surrounded by subsidiary cells (SC) and the regular epidermal cells (E). No stoma is present at this time. (b) The lower epidermal layer, shows fewer guard-cell mother cells.

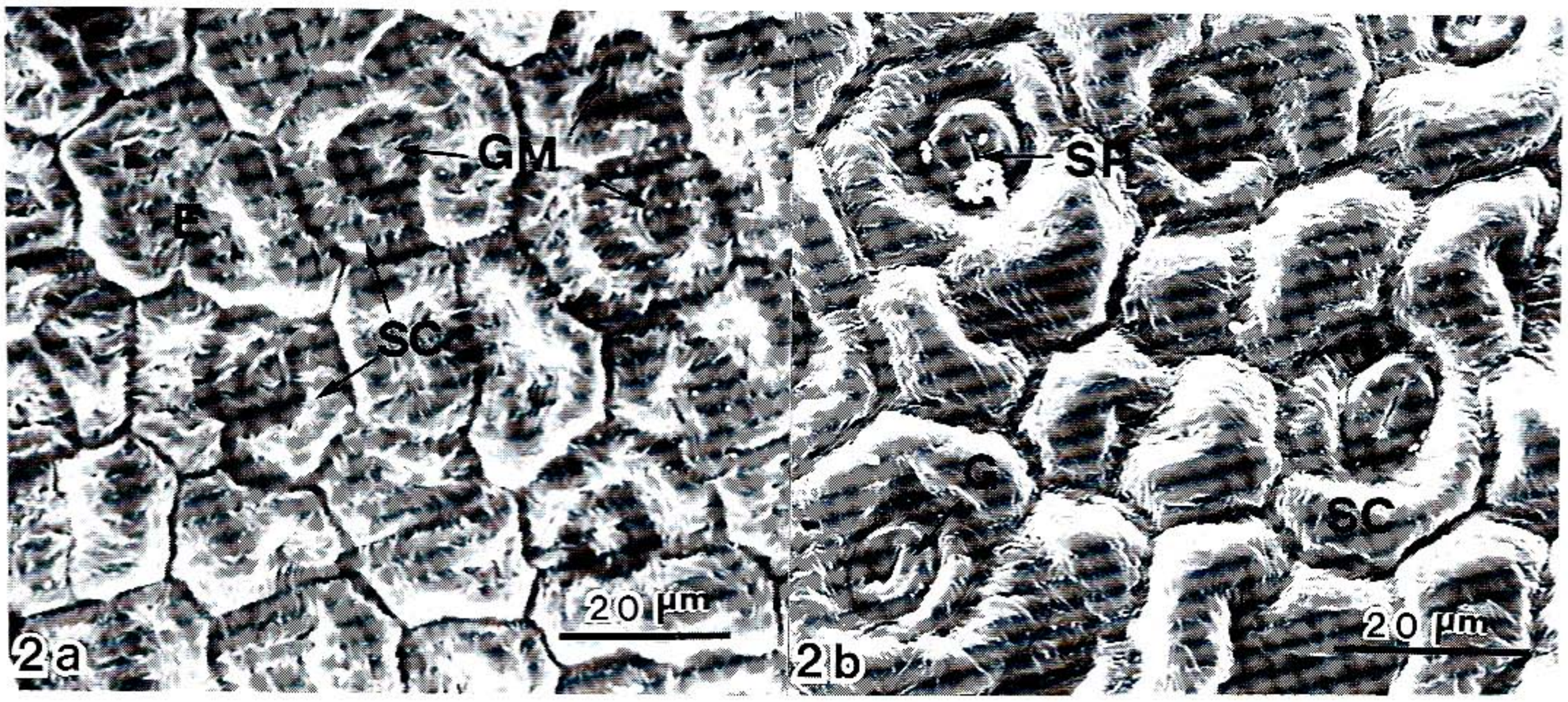


Fig. 2. (a) The upper epidermal layer of soybean seeds before planting shows guard-cell mother cell (GM), subsidiary cells (SC) and epidermal cell (E). (b) The guard cells (G) and stomatal pore (SP) were evident about 72 h after planting.

mother cell (Fig. 1). Guard-cell mother cells are the smallest cells of the epidermal layer. Each guard-cell mother cell is surrounded by subsidiary cells. The number of subsidiary cells around each guard-cell mother cell varies from two to three.

Under SEM, the surface of dry soybean seed is rough and the guard-cell mother cells are lower than the level of other epidermal cells (Fig. 2a). Samples of 24h after planting, guard-cell mother cells raised to the level of ordinary epidermal cells from their original position, and two guard cells were formed, but they were connected with the common cell wall. Samples of 48 h after planting, show the two guard cells and the split in between where the stomatal pore will form. After 72 h the two connected guard cells divided and the stomatal pores formed. A functional stomatal apparatus is completed (Fig. 2b).

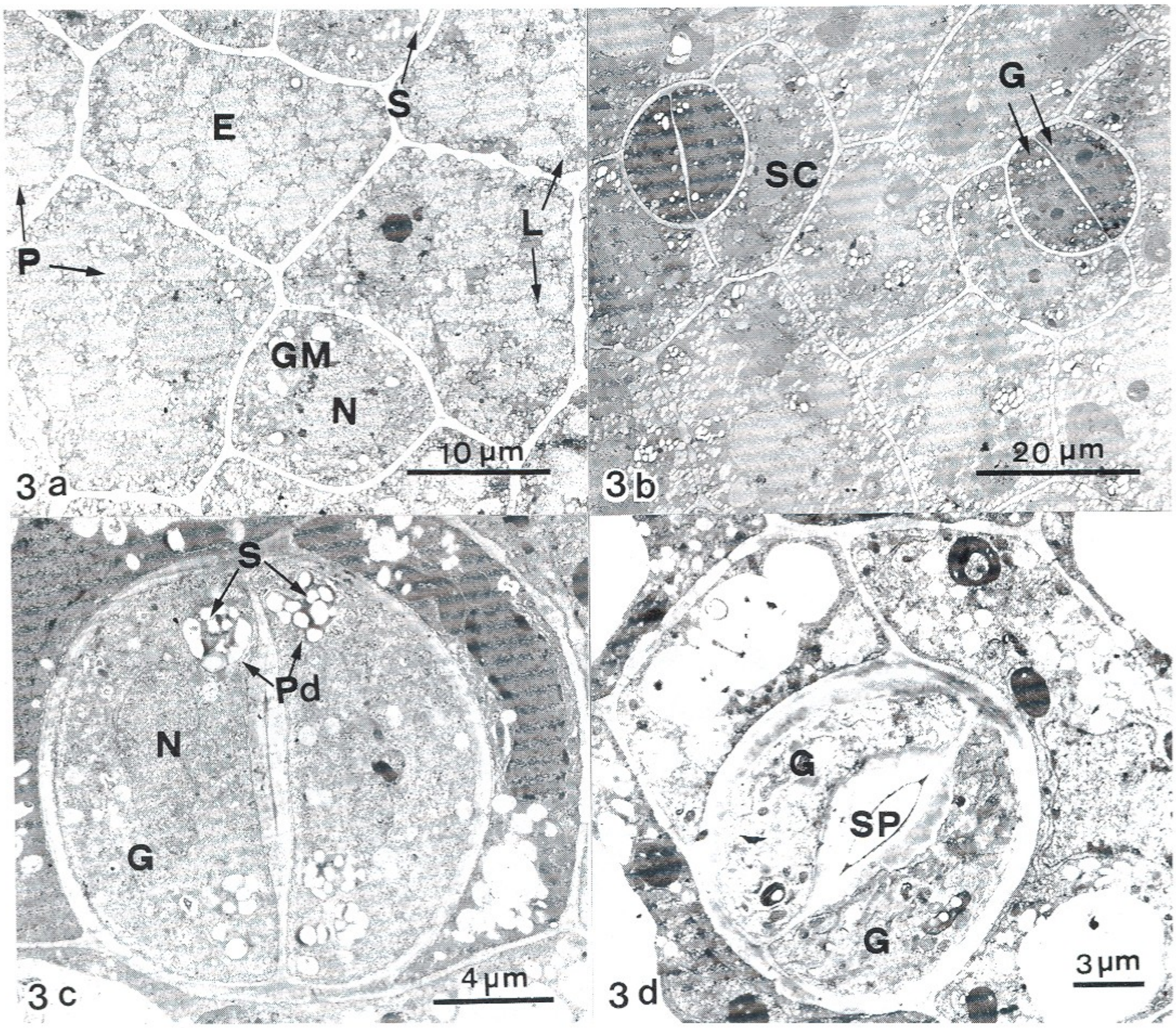


Fig. 3. (a) Transmission electron micrograph of epidermal cells from the soybean cotyledon before planting. It shows the nucleus (N) of guard cell mother cells (GM). The epidermal cells (E) are filled with protein bodies (P), lipid droplets (L) and starch grains (S) in plastids. (b) Twenty-four hours after planting, the guard cell mother cells divided to two guard cells (G), subsidiary cells (SC). (c) Forty-eight hours after planting, the cell wall between the guard cells (G) started dividing. (d) The stomatal pores (SP) are already formed indicated that a functional stoma is completed.

Before planting epidermal cells of the cotyledon are all polygonal in shape. The guard-cell mother cell is the smallest cell among the epidermal cells. There are many storage food granules in the epidermal cells (Fig. 3a). At this stage besides nucleus and plastids no evident of other organelles can be identified. Twenty-four hours after planting the guard-cell mother cell showed as two connected kidney-shaped cells. At this time the stomatal pore has not formed (Fig. 3b).

Forty-eight hours after planting, the stomatal pore has not been opened but the cell wall between the two guard cells started to separate from each other (Fig. 3c). Mitochondria and endoplasmic reticulum can be identified in the cytoplasm; lipid droplets are fewer in number and smaller in size as compared with those of dry seed.

Seventy-two hours after planting, the stomatal pore opened and functional stomatal apparatus were completed (Fig. 3d). At this stage the seedlings emerged from the soil about 1 to 2 cm in hight.

**Identification of food particles in epidermal cells of soybean cotyledon**

Using PAS and Coomsaaie brilliant blue R stianing method, carbohydrate stained in red color, only shows in cell wall material and in plastids as starch grains; lipid droplets were stained as blue areas around the round and gray protein bodies (Fig. 4a). Using Sudan black B and Coomassie brilliant blue R,

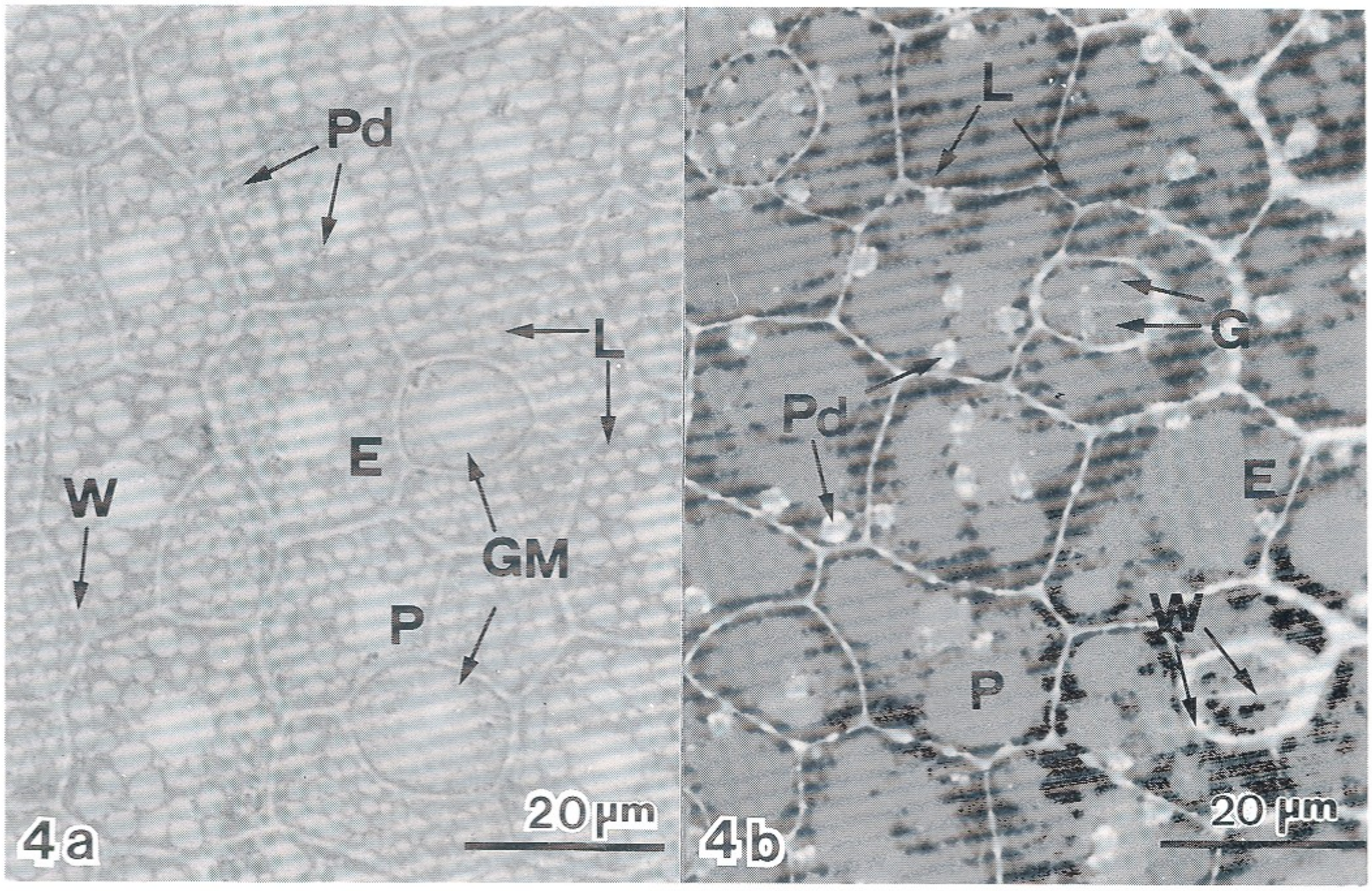


Fig. 4. (a) Section from sample of dry soybean cotyledons, guard-cell mother cell (GM) and other epidermal cells (E) stained with Schiff's reagent and Coomassie brilliant blue R, cell wall (W) and plastids (Pd) red, protein bodies (P) grey. (b) Section from sample of 72 h after planting, pairs of guard cells (G) and epidermal cells (E) stained with Sudan black B and Coomassie brilliant blue R reagent, lipid droplet (L) black and protein bodies (P) blue.

Carbonhydrate shows pale in color appeared in cell wall and plastids; lipid droplets were stained black around the large blue gray colored protein bodies (4b). Comparison between epidemal cells of dry seed and 72 h sample showed that the stomatal mother cell of dry seed is a single cell (Fig. 4a) and in 72 h sample are two guard cells (Fig. 4b). In dry seed sample the lipid droplets and protein bodies are evenly distributed, and in 72 h sample protein bodies are coagulated into large protein bodies and the lipid droplets are distributed in between.

## DISCUSSION

It was observed that most of the stomata of germinated soybean are on the upper epidermal layer. This is contrary to the fact that the stomata of foliage leaves of soybean plant as well as other plants are on the lower epidermal layer of leaves. The meaning of this phenomenon is not clear. The coincidence of stomatal development from the guard-cell mother cell of the cotyledon with the fast development of the seedling could be closely related to the respiration, food source utilization and photosynthesis after emergence.

The ontogeny and development of stomata of different plants species are well documented (Rasmussen, 1981; Sack, 1987; Willmer, 1983). However, there is no literature mentioned about the guard-cell mother cell of soybean seed cotyledon before germination period. The guard-cell mother cell will not divide into two guard cells untill the start of germination. We call this phenomenon "guard-cell mother cell dormancy". As far as we know, soybean is the first plant observed which shows cotyledon stomatal dormancy before germination. In dry seeds the surface of the epidermal layer is wrinkled. This could be caused be dehydration during seed riping. After planting the guard-cell mother cell raises to the level of ordinary epidermal cells.

The histochemical study showed that, after stained with Schiff's reagent, the cell wall material and the starch grains in leucoplasts showed positive PAS reaction. The starch grains in leucoplasts can also be verified by electron microscopy. After stained with Coomassie brilliant blue R, only the protein bodies showed positive reaction. Those are the dominant granules which are stored food particles in the cotyledon. The size of the protein bodies are variable. They may represent different section profiles. Some of the larger ones measured from one half to one third of the diameter of the cell. Recent report showed that the protein bodies are formed by filling the vocuoles during seed development (Zheng *et al.*, 1990). Lipid is another major component of soybean seed and was identified as electron dense granules due to its osmophilic nature. They are present as numerous small droplets around and between protein bodies. The histological identification of the soybean seeds was comparable to the results reported on peanut (Hu and Xu, 1990).

Based on histological and electron microscopic observation, the chemical nature of the granules in the cells of soybean seed were localized. Our results show that proteins and lipids are stored as food materials in the soybean cotyledon, and that carbohydrates are primarily the structural components of cell wall. The starch grain-containing plastid became chloroplast in the guard cells later on.

The observations made among numerous epidermal samples indicate that stomatal dormancy is a nondisputable fact. The guard-cell mother cell became two guard cells only 24 to 48 h after planting and a functional stomatal apparatus

was formed only 48 to 72 h after planting. According to this fact one mitosis should take place in the guard-cell mother cell before 24 h after planting. This was not clearly demonstrated because too many storage food particles were present in the cytoplasm. This is under investigation in our laboratory by trying to digest the food particles.

## ACKNOWLEDGEMENT

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# 大豆萌芽初期子葉表皮保衛細胞母細胞的結構變化

周大慶　劉國鈞

## 摘　要

成熟的大豆種子在未萌發之前，子葉表皮上並無氣孔的構造，僅有保衛細胞母細胞分布在上皮細胞之間。當播種經過 24 小時之後，保衛細胞母細胞分裂成兩個保衛細胞，兩保衛細胞間之細胞壁尚未分開，無氣孔出道。

本研究利用光學和電子顯微鏡，觀察大豆種子在萌發的早期，在第 24、48 和 72 小時階段，子葉表皮保衛細胞母細胞分化形成保衛細胞及氣孔的外部形態和微細構造的變化，同時利用組織化學定性的方法研究保衛細胞母細胞分裂成兩個保衛細胞的過程和一般表皮細胞中所含之糖類，蛋白質和脂質的分布變化。