

Some Ultrastructural Observations on Calcium Oxalate Raphide Crystal Idioblasts and Meristematic Cells of the Adventive Root Tips of *Sternbergia lutea* (L.) Ker-Gawl. ex Sprengel (*Amaryllidaceae*)

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Abstract: The adventive root tip cells of a bulbous plant, *Sternbergia lutea* (L.) Ker-Gawl. ex Sprengel (*Amaryllidaceae*), were examined by LM (light microscope) and EM (electron microscope). At the meristematic area of the root tips, which was 1 mm in length, two types of cell were observed: meristematic cells and Ca-oxalate raphide crystal idioblasts. Ca-oxalate raphide crystal idioblasts were observed to lie in two lines between the inner and outer ground meristems. Some similarities and some differences were found between those cells in their ultrastructure. It was seen that the membrane thickness of crystal idioblasts and meristematic cells were the same. They were also found to be similar in cell content, both containing nuclei, cytoplasm, vacuoles, mitochondria and proplastids. However the organization of the cell contents, the numbers, the forms and sizes of vacuoles were different between these cell types. In some meristematic cells and idioblasts of certain roots, some degenerated changes were seen due to necrosis. Those changes were in the form of plasmolysis, karyolysis, karyorrhexis, chromatin roughness, karyopyknosis and organelle loss.

Key Words: *Sternbergia lutea*, adventive root tip, meristematic cell, Ca-oxalate raphide crystal idioblast, degeneration, ultrastructure.

Sternbergia lutea (L.) Ker-Gawl. ex Sprengel (*Amaryllidaceae*)'nin Advetif Kök Uçlarının Meristematik Hücreleri ve Kalsiyum Oksalat Radif Kristal İdioblastları Üzerinde Bazı Ultrasütrüktürel Gözlemler

Özet: Soğanlı bir bitki olan *Sternbergia lutea* (L.) Ker-gawl. ex Sprengel (*Amaryllidaceae*)'nin adventif kök ucu hücreleri ışık ve elektron mikroskobu ile incelendi. Köklerin 1 mm uzunluğundaki meristematik bölgesinde meristematik hücre ve Ca-oksalat rafid kristal idioblastı olmak üzere iki tip hücre gözlemlendi. Ca-oksalat rafid kristal idioblastlarının iç ve dış esas meristemin arasında iki sıra halinde dizilmiş oldukları görüldü. Bu hücrelerin arasında bazı benzerlik ve farklılıkların olduğu saptandı. İdioblast ve meristematik hücrelerin çeper kalınlığı ve hücre içeriği bakımından benzer olduğu, her ikisinin de nükleus, sitoplazma, vakuol, mitokondri ve proplastid içerdiği görüldü. Fakat hücre içeriğinin organizasyonu ile vakuollerin şekli, büyüklüğü ve sayısının her iki hücre tipinde farklı olduğu gözlemlendi. Bazı köklerde her iki hücre tipinde nekroza bağlı dejenerasyon değişiklikleri görüldü. Bu değişikliklerin plazmolizis, karyolizis, karyoreksis, kromatin kabalaşması, karyopiknoz ve organel kaybı şeklinde olduğu saptandı.

Anahtar Sözcükler: *Sternbergia lutea*, adventif kök ucu, meristem, Ca-oksalat rafid kristal idioblast, dejenerasyon, ultrasütrüktür.

Introduction

Numerous studies have been performed on the ultrastructure of the root tip cell of higher plants. In preceding years, root tip meristematic cells (1,2), calyptra cells (3), differentiated cells (4), and the ultrastructural changes during differentiation at the xylem and phloem (5, 6) were examined in detail. In this study, therefore, it was not intended to examine the

ultrastructure of *S. lutea* (L.) Ker.-Gawl. ex Sprengel root tip meristematic cells. During the observations on mitotic division at *S.lutea*'s pollen and root tip cells (7), some meristematic cells were seen to have raphide crystals and therefore their ultrastructure was examined in this study. Druse and raphide crystals which are found in the structure of Ca-oxalate are inclusions that are often seen in higher plants (8, 9, 10) and in fungi (11). Their occurrence and abundance in specific tissues of various

plants are so constant that they are used as a taxonomic tool (9, 10, 12). The Ca-oxalate crystals in the epiderma, mesophyll, cortex, phloem, xylem parenchyma and pericarp of various plants (8, 9,10) were observed by plant anatomists. Presence of the crystals in various tissues of laticifers (13), corm (14) and seeds (15) is known. Although the presence of crystal in various tissues of poisonous plants has been taken into consideration (16), there is no detailed description of idioblast formation in any meristematic cells of root tips. There is very little literature on their presence in meristematic cells (9, 17). In previous studies, Ca-oxalate crystal idioblasts have been usually found in differentiated tissues (12, 13, 14). The ultrastructure of those cells is not well known, there are few studies on it (18, 19, 20). In recent years, the ultrastructure of those cells and Ca-oxalate formation have drawn attention and the number of such studies has increased (19, 20). Such information would greatly increase our understanding of their morphology and functional relationships.

In this study, we described the ultrastructures of calcium oxalate raphide crystal idioblasts in the cell of root tips of *S. lutea*. The ultrastructures of other meristematic cells were also examined for comparison. In addition, their degeneration and necrotic changes were investigated.

Material and Methods

In this study, the root tips of *S. lutea* plants brought from Bozdağ-Izmir and grown in the botanical garden of the Biology Department of Trakya University were used. The material, which was obtained in January and September, in 1997 was preserved in 70% ethyl alcohol after being fixed in Carnoy fluid (3 ethyl alcohol:1 acetic acid). For microscopical preparations, the root tips were dehydrated in ethanol and embedded in paraffin and sections were stained with hematoxylin-eosin and were mounted in Entellan. In addition, the root tips were hydrolysed with 1 N HCl for 10 minutes, and then examined by the Feulgen and aceto-orcein squash method. For a better observation of crystal content in the root cells, some of the roots were preserved in chloralhydrate for 30 minutes, and some roots were treated in acetic acid of 45% for 10 minutes. The roots preserved in acetic acid were examined by staining with aceto-orcein (21). An Olympus Photomicroscope was used for the observation and for photographing the root tip preparations.

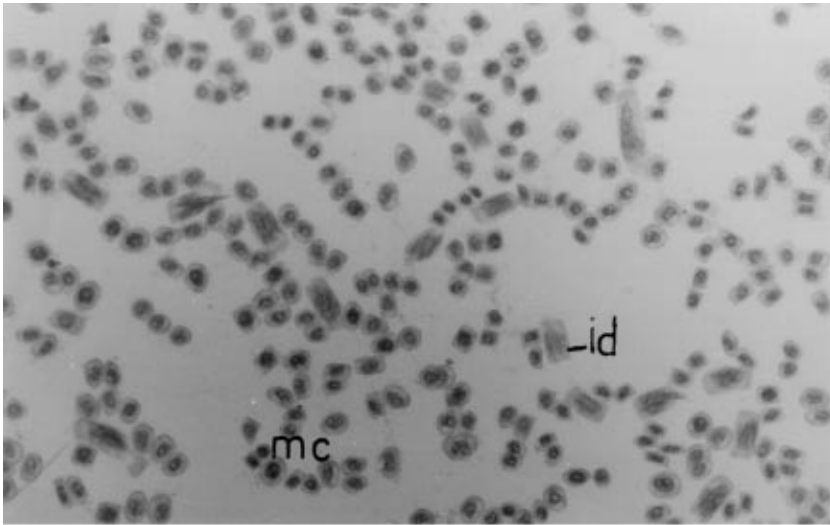
For electron microscopic studies, materials were fixed in 4% phosphate-buffered (PH 7.2) glutaraldehyde at

+4°C for 2 hr. After washing in buffer, the roots were postfixed in 1% OsO₄ (same buffer) at +4°C for 12 hr. This material was dehydrated in a graded acetone series up to 100% acetone and were transferred to a propylene oxide+Epon (2/1, 1/1, 1/2) series for 1 h for each treatment. The materials were saturated overnight on a rotary shaker with Epon mixture and then were embedded in Epon 812 (22). Polymerization was done at 60°C for 24 h. Ultrathin sections were made up with glass knife on a Reichert ultramicrotome, placed on grids and stained with uranyl acetate for 1 h followed by lead citrate for 30 min (22). The sections were examined and photographed with a ZEISS EM-9-S2 electron microscope.

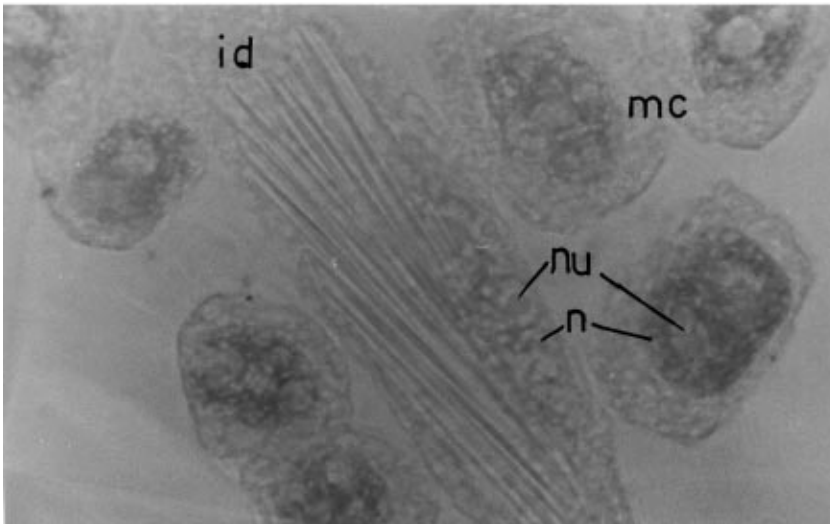
Results

Some Ca-oxalate raphide crystal idioblasts observed at the meristematic area of *S. lutea* adventive root, which is 1 mm in length, were examined by aceto-orcein staining. These idioblasts were different in size (Fig 1). Raphide crystal idioblasts lying along the inner and outer ground of the meristem in two layers were observed in the paraffin sections which were prepared by staining with hematoxylin-eosin (Fig 2).

Electron microscopic studies showed that there were no significant differences between the ultrastructures of the two cell types (Fig 3). It was difficult to determine the place of Ca-oxalate raphide crystals idioblasts by electron microscope because their vacuoles were not very different in appearance from the vacuoles of meristematic cells. The cell contents were almost the same in the two types. These included a nucleus, dividing mitochondria, proplastids and ER (Figs 3, 4). The nucleus of the idioblast included a nucleolus, as in meristematic cells (Fig. 5a). The distinction between them was the organization of their cell contents. In meristematic cells, the nucleus lies in the center, while it is found at the periphery of the cell in idioblasts. The cytoplasm occupies a large space in meristematic cells, but it is found around the cell in idioblasts (Figs 3, 4). The number of mitochondria and proplastids in the mature raphide idioblast was lower than in others (Fig 4). Though no differences were observed in the thickness of cell membranes, some differences were seen in the tonoplasts (Figs 3, 4). Tonoplasts of idioblasts were clearly seen when they were compared to other vacuole tonoplasts (Figs 3, 4). In meristematic cells autophagic vacuoles were also seen (Fig 3). In the young idioblasts, large vacuoles including lamellate particles, and other vacuoles involving crystals were observed (Fig 5). In the young



a)



b)

Fig.1. In the root tip cells of *S.lutea*; meristematic cells and the raphide crystal idioblasts (mc, meristematic cell; id, raphide crystal idioblast; n, nucleus; nu, nucleolus) ; a, x200; b, x1000.

idioblast proplastids, differences were identified, and they were evaluated as crystalloplastids (Fig 4a, b). In addition, a large vacuole containing fibrils of polysaccharide, several paracrystalline bodies (Fig. 5b) and a crystal facet chamber were seen in these cells (Fig. 5c, d). The mature idioblast vacuoles, which included crystals, had characteristics distinct from other vacuoles, such as being sharper and having sharper corners (Fig 4). The shape of vacuoles of meristematic cells was oval (Fig 3). Degeneration was encountered in some cells (Fig 4c). In the cells of some root tips, degeneration due to necrosis was intensive (Fig 6). These degenerative changes

included disappearance of the nucleolus (Fig 6b-e), chromatin roughening in the nucleus (Fig 6e) and dissolution of organelles in some cells (Fig 6a-c). In idioblasts, the disappearance of the nucleolus and diminishing of nucleus were seen (Fig 6d, e). In meristematic cells, karyopycnosis, karyoplasmic vacuolization, organelle dissolution, and karyorrhexis were seen (Fig. 6b, d). In the nuclei of meristematic cells, the withdrawal of the nucleolus toward the nucleus periphery was observed (Fig 6a). In some cells, karyolysis, plasmolysis and breakings were found (Fig 6c, e).

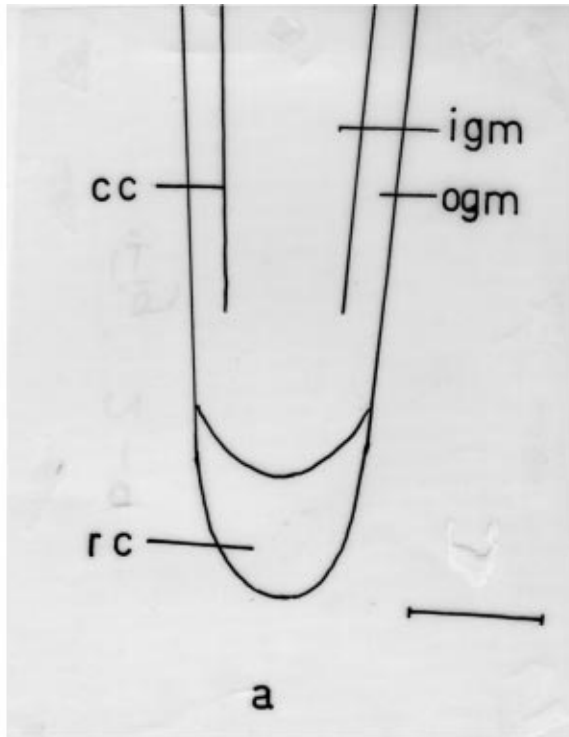
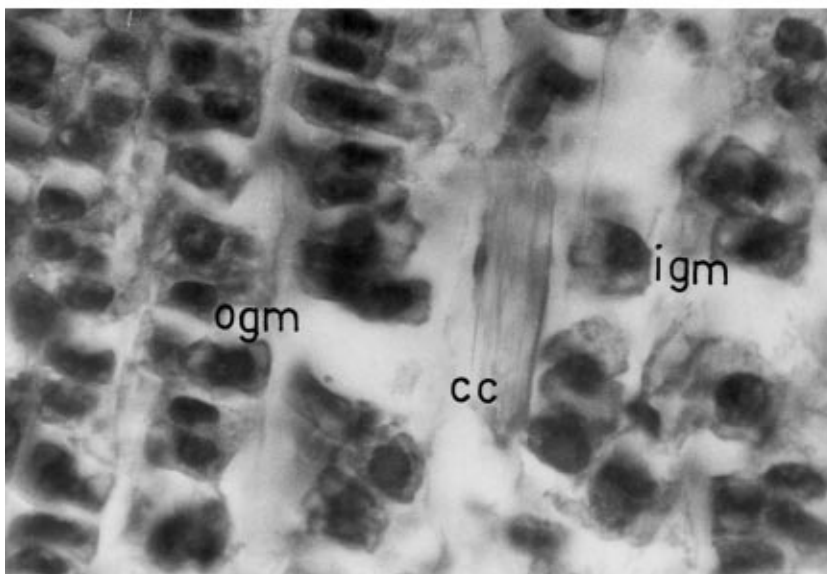


Fig. 2. Median longitudinal section of an adventive root of *S. lutea* showing the region of difference of the apical meristem examined under electron microscope. a. Diagram (scale bar=200 μ m); b. Stained with hematoxylen-eosin (x400); (ogm, outer ground meristem; igm, inner ground meristem; cc, crystalliferous cell; rc, root cap).

a)

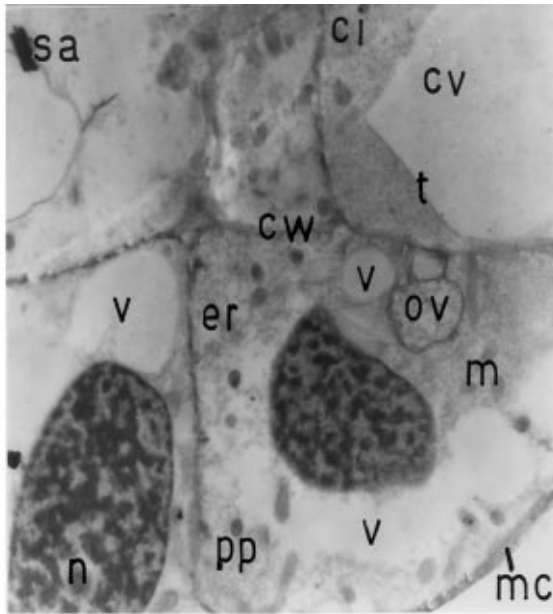


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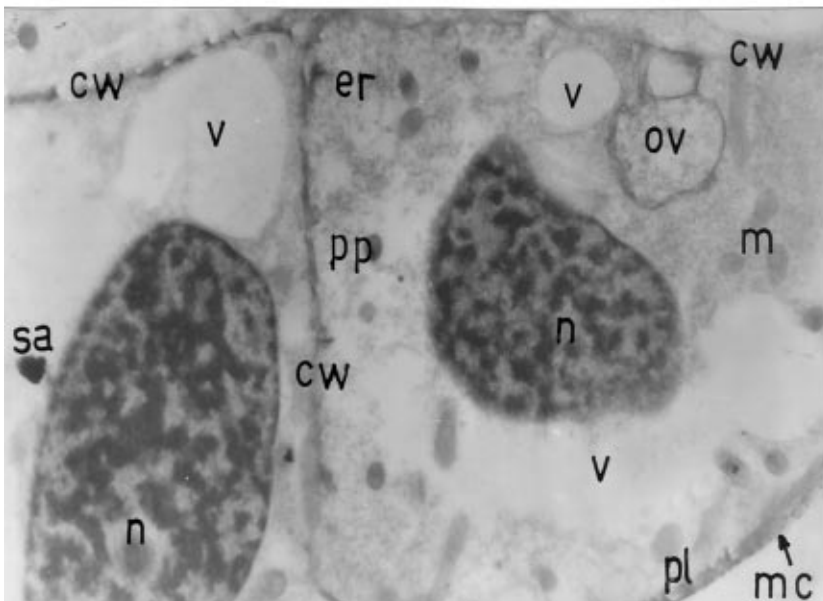
Discussion

Raphide crystal idioblasts observed between the meristematic cells in *S. lutea* adventive root have been reported in the differentiated tissue of various plants (8, 9, 10). Its existence in meristematic cells has been

demonstrated in several studies (17, 19, 20). In 1970, ultrastructural studies were performed by Price (18) on idioblasts in the mesophyll tissue of *Cercidium floridum* Benth. The occurrence of differences in idioblasts during their development was examined in detail in *Capsicum*



a)

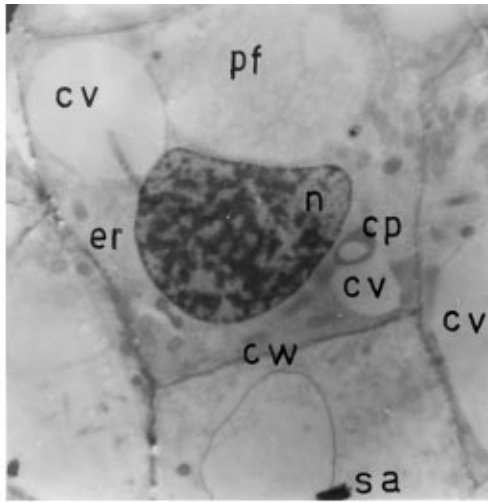


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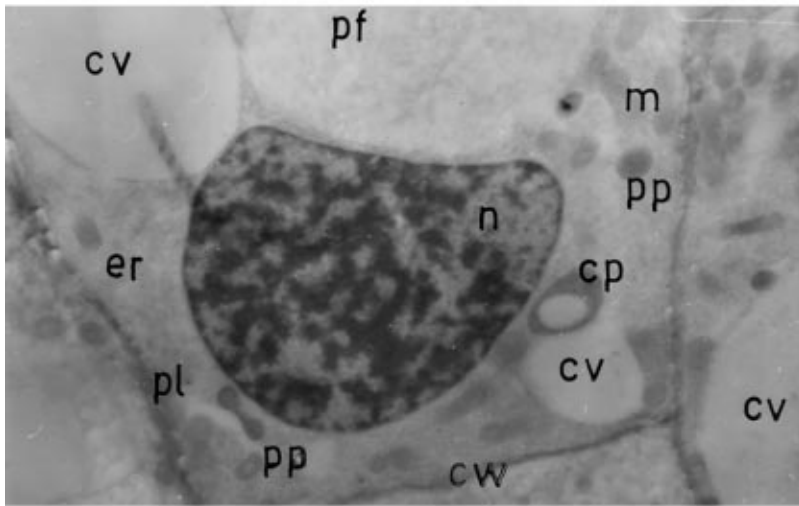
Fig. 3. Electron micrograph of the meristematic cell and raphide crystal idioblast. a, Meristematic cell and idioblast next to it x4800; b, Section of a meristem cell, showing the typical cell structures; x8000. (mc, meristematic cell, ci, crystal idioblast; v, vacuole; ov, otophagic vacuole; cv, crystal vacuole; t, tonoplast; n, nucleus; m, mitochondria; pp, proplastid; pl, plasmalemma; er, endoplasmic reticulum; cw, cell wall; sa, stain artifact;).

annuum L. and *Thypha angustifolia* L. by Horner et al. (19, 20). In the present study, mitochondria and proplastids that are found in idioblasts were observed in *Thypha angustifolia* L. (20). The changing of proplastids into crystalloplastids in the cells of *Thypha angustifolia* L. and *Capsicum annum* L. was observed by Horner et al. (19, 20). In the idioblasts of *S. lutea*, structures similar

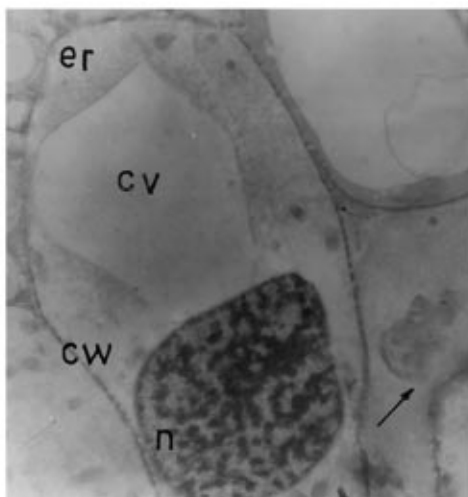
to crystalloplastids were observed. However, it was not possible to examine the changes in those structures. Price (18) compared the druse crystal idioblasts with palisade cells in the mesophyll tissue of *Cercidium floridum* Benth and indicated that the plastids of idioblasts included a few small lipid globules opposing the plastids of the palisade cells, which included starch. Horner et al. (19, 20)



a)



b)



c)

Fig. 4. Electron micrograph of the raphide crystal idioblasts; a,b. Young raphide crystal idioblasts during central vacuole formation, early crystal formation (a, x4800; b, x7200). c. Thin peripheral cytoplasm of mature idioblast contains mitochondria and few proplastids, crystalloplastids, ribosomes and large-cornered vacuole and nucleus with heterochromatine. Cell showing degeneration (arrow) (x4800). (cv, crystal vacuole; pf, polysaccharide fibrils; n, nucleus; m, mitochondria; pp, proplastid; cp, crystalloplastid; pl, plasmalemma; er, endoplasmic reticulum; cw, cell wall; sa, stain artifact;).

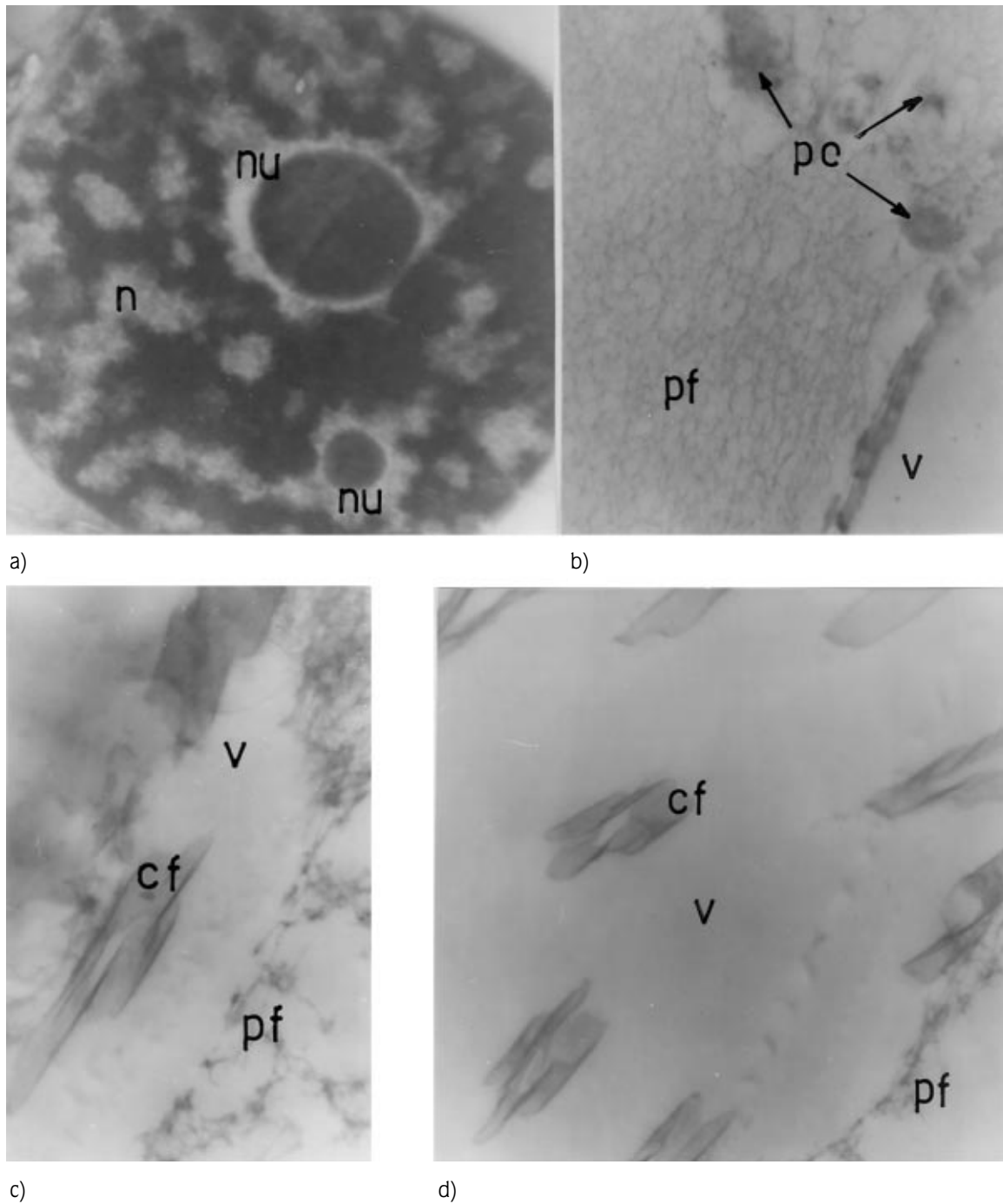


Fig. 5. In young raphide crystal idioblasts; a, Nucleus with two nucleoli x15000; b, Vacuole containing several paracrystalline bodies and fibrils of polysaccharide (arrow) x25000; c,d, A large vacuole containing many crystal facet chamber and fibrils of polysaccharide x65000. (n, nucleus; nu, nucleolus; v, vacuole; pf, polysaccharide fibrils; pc, paracrystalline bodies; cf, crystal faced chamber).

showed that idioblast crystalloplastids contributed to crystal formation. They also added that more studies should be done on the roles of those organelles in crystal

formation. Microbodies and dictyosomes, which have been reported to be found in idioblasts by Horner et al. (19, 20), were not observed in the idioblasts of *S. lutea*.

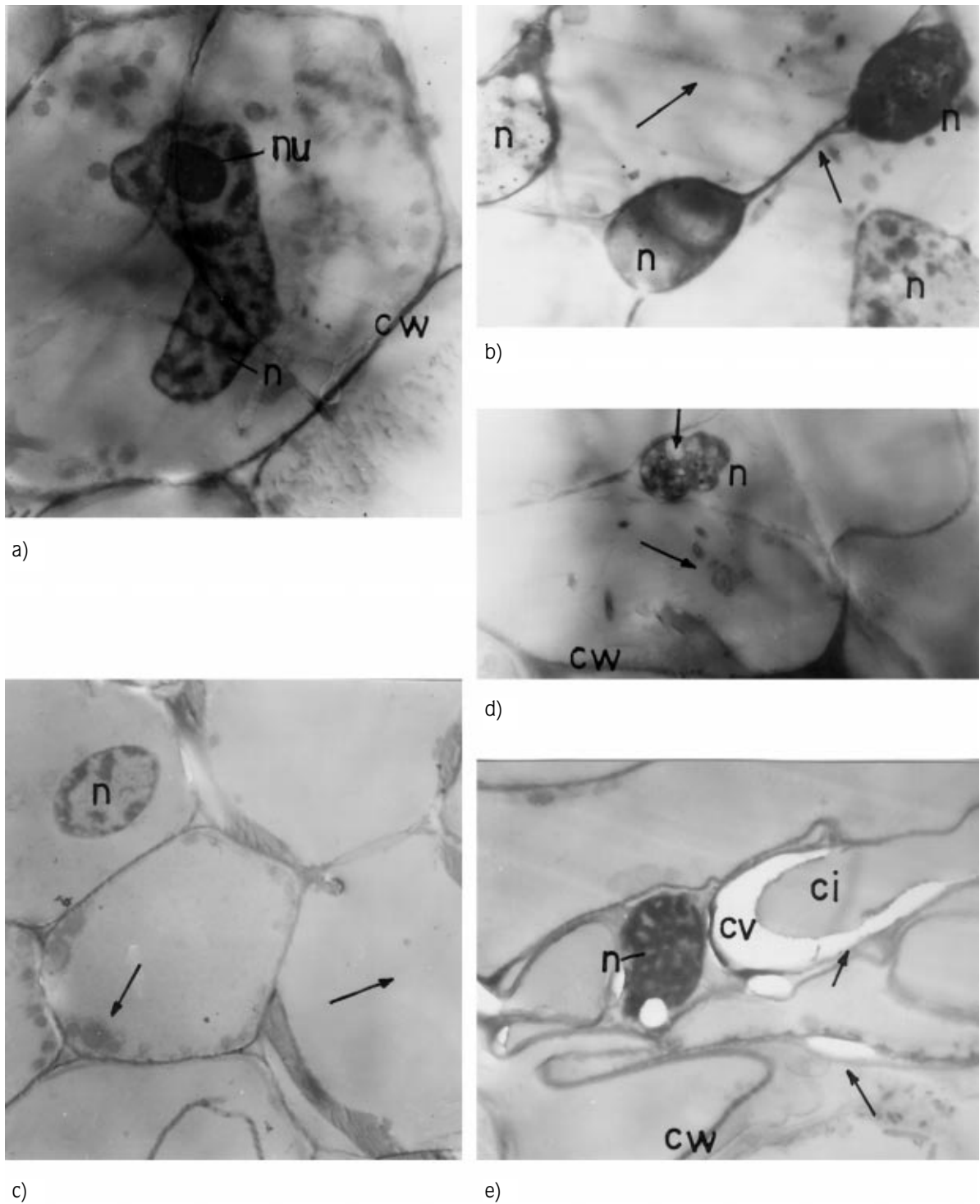


Fig. 6. Meristematic cell and raphide crystal idioblast showing degeneration. (x4800) a, Degenerated meristematic cell with elongated nucleus and disorganised cytoplasm with mitochondria and plastid; b, Showing karyorhexis and plasmolysis (arrow); c, With karyolysis, plasmolysis and organelle loss; d, With karyopycnosis, which showing nucleus including vacuole (arrow) and a high degree of plasmolysis; e, Showing diminished nucleus and kromatin roughness, plasmolysis and broken of the cell (arrow). (n, nucleus; nu, ncleolus; cw, cell wall; mc, meristematic cell; ci, crystal idioblast; cv, crystal vacuole).

Horner et al. (20) claimed that transportation of mucilage vesicles might occur via ER channels, but these authors did not verify their suggestions through cyto-chemical methods. In *S. lutea* idioblasts, some parts of ER channels were seen to be expanded, and vacuole formation was also observed. Moreover, the tonoplast of those cells was darker stained than the other cell vacuoles. Horner et al. (19, 20) and Price (18) also indicated this electron density difference, which is seen in tonoplast. It is not difficult to detect druse crystal idioblasts by electron microscope studies, because their crystal shapes are distinct, and the vacuoles of raphide crystal idioblasts are not so distinct from the other vacuoles. As Horner et al. (20) indicated before, they were difficult to view. As in this study, light-microscope and cytochemical methods can show their location, and they can be examined. In particular, in the first formation phases of raphide crystal idioblasts, it was observed that they were not very different from the meristematic cells in membrane thickness and in cell contents. Other studies also agree on these findings (19, 20). In *S. lutea* raphide crystal idioblasts, nucleus withdrawal towards the periphery of the cell was observed, the cytoplasm was peripheral and the vacuole tonoplast was darker than the others. It was difficult to differentiate idioblasts from meristematic cells, as they are similar. Autophagic vacuoles were seen in meristematic cells. In idioblasts vacuoles including both crystals and lamelled particles were observed. Our opinion, the contents of those two cell types are the same. The only difference we observed was in the organization. Recognizing them involves the consideration of tonoplast, cell and vacuole forms. Parallel to our observations, Bilderback (23, 24) showed the presence of mucilage in tonoplast and also the depositing of mucilage inside the vacuole in *Marsilea vestita* Hook. & Grev. We observed fibrils of polysaccharide in the vacuole, and detected several paracrystalline bodies and raphide crystal facet chambers in the vacuoles of idioblasts (Fig. 5).

Horner et al. (20) indicated that starch and lipids in meristematic cells were not found in mature idioblasts, and those food sources could have been used for oxalate biosynthesis. According to Horner et al. (19, 20) starch may change into acid oxidase and lipid may change into oxalic acid. Thus, the expected microbody is not seen, but crystalloplastids are observed. Horner et al. (19, 20) emphasized the importance of studies on the functions and structures of crystalloplastids. In 1974, Eilert (20) explained that crystalloplastids are degenerated. It has been reported that the proplastids combine with the

vacuoles, and the plastids are considered to be crystalloplastids (20). In *S. lutea*, we also observed that some crystalloplastids were combined with the vacuoles (Fig. 4).

In several studies, the length of plastids in both cell types have been discussed; though in some plants they were same in size, in others they were different (20). In the young raphide idioblast cells of *S. lutea*, crystalloplastids were bigger than the others, but in the mature idioblast cells they were same size as in the others.

Degeneration due to the necrosis normally seen in adventive root of bulbous plants, was also observed in *S. lutea*. Degenerations in cytoplasm and nucleus of both meristematic cells were seen as karyopycnosis and vacuolizations in the nucleus, during the withdrawal of nucleolus towards the nucleus periphery, karyorrhexis, plasmolysis and karyolysis. The degenerative changes in idioblasts observed included the disappearance of the nucleolus, diminution of the nucleus, roughening of the chromatine and degeneration of the cell shapes. These degenerative changes were observed in the leaves of *Cajanus cajan* (L.) Mill. by Prasad et al. (25), in the leaves of *Capsicum annuum* L. by Ilarslan et al. (26) and in the stomiums of the anthers of *Capsicum annuum* by Horner et al. (19). Prasad et al. (25) suggested that environmental factors were responsible for these changes. According to Ilarslan et al. (26) it is because of pathological factors. Horner et al. (19) showed these changes to be due to the physiological conditions. In *S. lutea*, these degenerative changes of the adventive root tip cells are caused by physiological conditions.

In this study, the ultrastructure of raphide crystal idioblasts occurring at the meristematic area of the root tips of *S. lutea* was compared with the meristematic cells and it was intended to show both their similarities and differences. However, it was shown that the vacuoles are not only where raphide crystal idioblasts are deposited, but also where they occur. Moreover, with the degenerative changes of both cells at the macromolecular level, it was shown that cell death exists in both cell types.

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