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9. ABSTRACT

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Mature Raphid and Raphid Idioblast Structure in Plants of the Edible Aroid Genera *Colocasia*, *Alocasia*, and *Xanthosoma*

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ABSTRACT

Mature raphid idioblasts examined in this study appear to consist of a bundle of raphid crystals contained within a polysaccharide matrix. The cytoplasm consists of a thin parietal layer with few discernible organelles. These cells appear to be true idioblasts, and no intercellular connections were observed between these cells and adjacent parenchyma cells. Dissolution of the middle lamella occurs in *Colocasia* and to some extent in *Xanthosoma*. The mechanism of crystal release in the five species studied is the same, e.g. a swelling of a polysaccharide material which breaks the idioblast wall and forces the release of the raphides. In *Colocasia* this results in a forceful ejection with release of a relatively few number of crystals at any one time. *Xanthosoma* and *Alocasia* do not exhibit a forceful ejection of their crystals, but a less forceful release of a crystal mass which then disassociates to free the individual crystals. The raphides differ in structure and size, however, they have three structural features in common. First, the crystals have two distinct points, one tapering to an elongate point, the other abruptly pointed. Second, the crystals have surface barbs with tips oriented away from the tapering point. And third, deep grooves are present along the length of the crystals. The possible relationship between raphid structure and the acrid nature of the idioblasts is discussed.

INTRODUCTION

Calcium oxalate salts in a number of crystalline forms are found in the majority of plant families (Esau, 1965), among them the family Araceae. The specialized cells which contain the crystals are called crystal idioblasts. In raphid idioblasts the calcium oxalate is in the form of a bundle of isolated, needle-shaped crystals.

Ingestion of fresh plant material containing raphid idioblasts usually results in severe irritation of the throat and mouth. Three reasons for this irritation have been suggested: (1) forceful ejection by the idioblast of the raphides into mouth and throat tissues (Haberlandt, 1914; Black, 1918; Middendorf, 1968); (2) chemical irritation by a curare like drug associated with the crystal (Barnes and Fox, 1955); or (3) mechanical irritation by the raphid crystal itself (Haberlandt, 1914; Black, 1918).

It is important that we have an understanding of the acrid nature of raphid idioblasts as they occur in several important tropical food crops. Recent ultrastructural studies of raphid idioblasts have not related structural characters to acridity (Mollenhauer and Larson, 1966; Arnott and Pautard, 1970; Ledbetter and Porter, 1970; Schotz, Diers, and Bathelt, 1970). The plants we describe here all produce an irritation of the mouth and throat. They are grown both as commercial and subsistence crops in many tropical areas. Among the edible aroids, *Colocasia esculenta* (taro) is the most important starch source in the Pacific region (de la Pena, 1970). *Xanthosoma sagittifolium* (tannia or yautia) and *X. brasiliense* (belembe or Tahitian spinach) are grown in upland areas of many tropical islands (Plucknett, 1970). *Alocasia* (ape), probably because of its acrid nature, is grown as a subsistence crop where taro or tannia is in short supply. Corms, petioles, or leaves of all three genera require cooking before they are eaten.

MATERIALS AND METHODS

Corms, stems, petioles, and leaves of *Alocasia cucullata* Schott (Lyon Arboretum Ascension Number L-70.255), *A. macrorrhiza* (L.) Schott (L-68.188), *Colocasia esculenta* (L.) Schott, cultivars *Lehua Keokeo* (L-68.141), *Piko Kea* (L-68.120), and *Taro Jen* (L-68.428), *Xanthosoma brasiliense* (Desf.) Engl. (L-69.009), and *X. sagittifolium* (L.) Schott (L-69.011) of the Family Araceae were collected from the plantings at the Harold L. Lyon Arboretum of the University of Hawaii. Plants were grown with supplemental broadcast lime and 5-10-10 pelleted fertilizer by the non-paddy system using 4 mil black polyethylene sheeting as a mulch. The soil was of the Tantalus series, a member of the medial over cindery, isothermic family of the Typic Dystrandepts.

For transmission electron microscopy (TEM) 1 mm slices of the material were fixed at 4 °C for 4 h with the formaldehyde-glutaraldehyde fixative of Karnovsky (1965) in 0·2 M cacodylate buffer, rinsed with buffer and post-fixed for 2 h at 4 °C with 1 per cent OsO_4 in buffer. After washing in buffer, the material was dehydrated in a graded series of ethanol, treated with propylene oxide, and embedded in Spurr low-viscosity embedding medium (Polysciences, Inc.). Thin sections were obtained with a diamond knife on a Sorvall Porter-Blum MT-2B Ultra Microtome, stained with 2 per cent aqueous uranyl acetate and lead citrate (Reynolds, 1963) and viewed and photographed at 50 kv with the Hitachi HS-8 electron microscope of the St. John Plant Science Laboratory Electron Microscopy Facility.

For scanning electron microscopy (SEM) fresh or Ag-Pd alloy vacuum coated material was viewed and photographed with a Cambridge Stereoscan S-4 operated at 5 or 10 kv. Energy dispersive X-ray analysis was performed on fresh material with the Cambridge S-4 operated at 20 kv and a Nuclear Diodes EDAX 707.

For light microscopy, fresh, 3 per cent formalin fixed, or thick sections of TEM embedded material were viewed and photographed with a Zeiss Standard RA microscope equipped with a Photomicrographic Camera I. The PAS, periodic acid-Schiff's base, reaction for polysaccharides (Jensen, 1962), mercuric bromphenol blue stain for protein (Mazia, Brewer, and Alfert, 1953), phloroglucinol-HCl reaction for lignins, and 0·05 per cent aqueous toluidine blue-o as a general stain were used. The tetrazolium reaction was used to test for dehydrogenase activity (Jensen, 1962), guiacol-H_2O_2 was used to test for peroxidase activity (Jensen, 1962), and iodine-potassium iodide or phosphotungstic acid was used to test for alkaloids (Lee, 1937; Johansen, 1940).

OBSERVATIONS

Colocasia esculenta (taro)

The raphid idioblasts of taro are obtuse ended, spindle shaped cells about 150 μm long and 50 μm in diameter. The same cell type is found in the rhizome, petiole, and leaf. In the petiole, the idioblasts often transverse the aerenchyma and protrude into the air canals (Plate 1A). In the rhizome and leaf they appear adjacent to small intercellular spaces (Plate 1B).

In all tissues, the idioblasts are easily dislodged from the supporting tissue. If rhizome material is diced on a microscope slide in a drop of water, the idioblasts float free and eject crystals through the ruptured ends (Plate 1E). Those cells which do not immediately eject, can be induced to do so by application of slight pressure to an applied cover slip. In sections of fresh petiole tissue in water, the idioblasts eject crystals into the air canals (arrow, Plate 1A). Freezing in liquid nitrogen and thawing had no apparent effect on raphid ejection. Boiling, however, stopped further ejection.

As indicated by Haberlandt (1914), under the light microscope the walls of the obtuse ends appear thin (Plate 1D). In thin sections viewed by TEM a gradual thinning of the wall can be observed (Plate 1F). The thickness of the wall in the end nubs is less than 0·1

μm. Histochemical tests of these idioblasts with the PAS (periodic acid-Schiff's base) reaction for polysaccharides revealed the presence of polysaccharide material surrounding and within the crystal bundle of raphides (Plate 1B, C). The wall of the idioblast was also PAS positive. No reaction for phenolics or lignin was observed in the walls or cytoplasm. A light blue colour reaction with mercuric bromphenol blue indicated the presence of protein within the cytoplasm of these cells. Tests for dehydrogenase activity, peroxidase activity, and alkaloids were negative.

The raphides in taro are long and needle-like, about 60 μm in length and less than 2·0 μm in thickness at their widest point (Plate 1J). The two ends of the raphides differ in structure, one is abruptly pointed (Plate 1G) and the other tapers to an elongate point (Plate 1H, I). Evidence of grooves along the length of the raphides can be observed in Pt-Pd shadowed replicas (Plate 1G) and in SEM (Plate 1I) or direct preparations (Plate 1H). Barbs with tips oriented away from the narrow tapering point occur along the length of the raphid (Plate 1I). These barbs are on ridges on either side of the grooves similar to that reported on *Xanthosoma sagittifolium* raphides (Sakai, Hanson, and Jones, 1972).

The raphides are found in the central area of the cell. The raphid material is normally not retained in the sections, and only a hole remains to indicate the position and form of the crystals. Because of this, the crystal area of the sections is often badly torn. The crystals in cross sections have an 'H' shape (Plate 2A). The longitudinal grooves evident in Plate 1G, H, I, appear to connect some cross sections leaving two isolated halves. In other sections, especially near the tip, little evidence of the groove can be seen (Plate 3F).

The wall of the raphid idioblasts is thick and appears layered (Plate 2B). The layering is due to differences in the orientation of successive layers of fibrillar material of the wall (Plate 3A, B). This wall structure is similar to that described for collenchyma (Wardrop, 1969; Chafe, 1970) and epidermal (Chafe and Wardrop, 1972) cell walls. Dark staining electron opaque material also occurs in wall layers near the cell lumen (arrowheads, Plate 3A, B). The layers of cell wall corresponding to the middle lamella and the first formed primary wall appear loosely structured (ml, Plate 3A, B). Attachment of an idioblast cell to adjacent cells does not appear strong. Also, no plasmodesmata were observed between idioblasts and adjacent cells.

A tonoplast and plasma membrane were observed in cells (Plate 3B). However, recognizable organelles were seldom observed. The majority of the idioblast is filled with material which corresponds to the PAS positive material and probably also corresponds to the 'carbohydrate matrix' of Arnott and Pautard (1970) (Plate 2A, B). This material appears amorphous in some areas and fibrillar in other areas (Plate 2B). Some of the fibrillar appearing areas (indicated by an ×, Plate 3C) adjacent to the raphides may be artifacts caused by mechanical disruption after fixation was complete, possibly in dehydration.

Each crystal is surrounded by a structure (Plate 3C) which Arnott and Pautard (1970) termed the 'chamber membrane'. Between this membrane and the crystal is a layer of electron translucent material (arrowheads, Plate 3C). This may correspond to the 'crystal sheath' of Rosanoff (1865, in Price, 1970). However, Price described this layer as electron dense in druse idioblasts of palo verde leaves. Arnott and Pautard (1970) have described numerous microtubules in the carbohydrate matrix adjacent to the chamber membrane. We have not, however, observed microtubules in the mature idioblasts of our study. Layers of membraneous material were observed (Plate 3D, E). These appear similar to some immature forms of raphides described by Arnott and Pautard (1970). In some instances evidence of barbs could be observed in cross sections as protusions of the chamber membrane (arrows, Plate 3C). Ejected crystals in cross section (Plate 3F) show a chamber membrane still surrounding some of the crystals. The 'carbohydrate matrix' still appears to be associated with these ejected crystals.

Alocasia

In *Alocasia macrorrhiza* three types of raphid idioblasts and two sizes of raphid crystals can be observed. Two types of idioblasts and both sizes of raphid crystals occur in the petiole while only one type of idioblast and the larger crystal occur in the rhizome.

In air canals of petiolar tissue, long (about 2000 μm), extended, sacklike raphid idioblasts are infrequently observed (Plate 4D, E). These idioblasts can be observed in longitudinal sections of petiolar tissue due to their orientation. The bundle of large crystals, which are usually centrally located in the idioblast, are not ejected from these cells. Apparently some mechanical disruption of the tissue is required for the release of these raphid crystals.

The most common idioblast type in petioles are small blunt ended cells about 90 μm long and 40 μm in diameter. These cells extended from the aerenchyma into the air canals (Plate 4A). These idioblasts, like taro idioblasts, contain large amounts of PAS positive material (dark areas of Plate 4F). However, the manner of crystal ejection differs. In *Alocasia* swelling of the polysaccharide material breaks the cell wall near the base of the cell (Plate 4B). After the cell wall ruptures, the polysaccharide material apparently continues to swell, forcing itself and the crystals from the cap-like cell wall portion (Plate 4B, C). The polysaccharide material continues to swell and disperses, freeing the crystals. A similar type of raphid idioblast has been observed with scanning electron microscopy in *Alocasia cucullata* (Plate 4G, H). Because of the increased depth of field it is possible to show the frequent occurrence of these idioblasts protruding into the air canals (Plate 4H). Fresh sections containing the idioblasts have dehydrated slightly in the vacuum of the SEM. However, their general morphology can be observed.

Observation of the raphides from the smaller petiolar idioblasts of *A. macrorrhiza* in the SEM revealed grooves and barbs along the raphid similar to that found in taro (Plate 4I). The raphides are also of similar length and width. However, a thickened region near the centre of the raphid can be observed with the light microscope. This thickened region has been observed only in *Alocasia* raphides (see Plate 7B) and not in *Colocasia* or *Xanthosoma* raphides. The walls of the *Alocasia* petiole raphid idioblasts do not show the layering as seen in taro (compare Plate 5A and Plate 3A). The middle lamella between idioblast cell and adjacent cells does not appear loosely structured; here also, no plasmodesmata were observed. The area of apparent cell wall rupture during raphid release appears thinner and deformed (Plate 5A, indicated with an arrow).

In the mature petiolar raphid idioblasts, a tonoplast and plasma membrane were detected, but only a few organelles were observed. The greater portion of cell contents of these idioblasts is filled with the PAS positive material which appears amorphous to fibrillar in structure. The crystals occur predominantly in areas which appear more electron dense than surrounding regions (Plate 5C). These raphid crystals are also H-shaped in cross section and surrounded by a chamber membrane (Plate 5D). The polysaccharide matrix surrounding the chamber membrane appears more fibrillar in areas adjacent to the membranes. This does not appear to be a fixation artifact as in the taro idioblasts; in the *Alocasia* petiole idioblast these fibrillar areas are observed consistently.

In rhizome tissue the raphid idioblasts appear large and similar to adjacent parenchyma cells. The PAS positive material occurs as light and dark staining zones (Plate 6A). These idioblast cells are not released in diced rhizome tissue. Instead only the mucilage material and crystal bundle are released, similar to the smaller petiolar idioblasts. In the transmission electron microscope the lighter bands of PAS material appear vesiculate (Plate 6C). The vesicles contain electron dense material (arrowheads, Plate 6E). In several instances the vesicles appeared two-parted (Plate 6E). The large crystals occur predominantly in these vesiculate areas (Plate 6B), apparently within enlarged vesicles. The chamber mem-

brane appears to surround the raphid directly with little or no material between it and the raphid. However, the polysaccharide matrix does not lie close to the chamber membrane and a clear area occurs between the vesicle edge and the chamber membrane (Plate 6B). The crystals again have the grooves in cross section although the H-shape appears distorted probably by some disruption of the plastic when the crystal material was lost from the section.

The PAS positive material and the crystals apparently occupy the vacuole and do not fill the cell. The polysaccharide material has a distinct boundary on the side towards the former cytoplasmic area (arrows, Plate 6A, C, E). However, there is little cytoplasm. Of all the raphid idioblasts observed the cytoplasm in these rhizome idioblasts appears the most degenerate. The plasma membrane also did not appear well structured. The walls of these raphid idioblasts are not layered (wri, Plate 6D). However, there is some evidence of a loose structured middle lamella (ml, Plate 6D).

The crystals in these idioblasts are of the large size similar to the crystals in the large, extended, sack-like cells of the petiole. They are about 3 μm in width and 130 μm in length. These crystals are apparently of the same structure as the smaller crystals in blunt ended cells in the petioles. Both crystals have a thick structured region near the centre of the crystal (Plate 7B). They both also have two types of points. A narrow tapering end and an abruptly pointed end (Plate 7A). The barbs on the large crystals are visible under the light microscope (Plate 7B). Again they appear to have their tips oriented away from the narrow tapering end. Tests of the rhizome idioblasts for alkaloids were negative.

Xanthosoma

Aerenchyma of the petiole of *Xanthosoma sagittifolium* contains only one type of raphid idioblast which appears similar in shape (though shorter, about 60 μm) to the small raphid idioblasts in *Alocasia* petioles. However, crystal release in *Xanthosoma* occurs through rupture of the blunt end of the idioblasts and extrusion of the central bundle of crystals with the surrounding material (arrow, Plate 7C). In rhizome idioblasts the mechanism of crystal release also appears to occur through wall rupture as no intact idioblasts are observed when diced material is placed in water; only masses of crystals are observed. In some instances a limiting membrane, presumably the tonoplast, appeared to surround these crystal bundles (arrows, Plate 7D).

In cross sections stained with PAS, the rhizome raphid idioblasts appear similar in size to surrounding starch-containing cells. The crystals occur in a bundle near the centre of the cell and are surrounded by polysaccharide material (Plate 7E). Transmission electron microscopy of these rhizome raphid idioblasts show the raphides located near the centre of the cell with a band of polysaccharide material surrounding this bundle (Plate 7F). The cytoplasm appears degenerate; however, a tonoplast may be present (arrow, Plate 7F). The walls are not layered (w, Plate 7F). The crystals are again H-shaped in cross section with each surrounded by a chamber membrane (Plate 8A). Tests for alkaloids of these rhizome raphid idioblasts also were negative.

As was reported by Sakai *et al.* (1972), examination of the raphides from the petioles of *X. sagittifolium* with the scanning electron microscope revealed the presence of barbs extending from the ridges on either side of the grooves. These barbs are oriented away from the tapered point towards the blunt pointed end. A similar structure was observed for raphides of *X. brasiliense* (Plate 8B).

Energy dispersive X-ray analysis of the raphid idioblasts of *X. sagittifolium* confirmed the presence of large amounts of calcium in the idioblasts. A distribution analysis for calcium (calcium K^{α}, 3100–3500 ev) revealed a concentration within the raphid idioblasts (Plate 8C, D).

DISCUSSION

The mature raphid idioblasts of this study appear to consist primarily of a bundle of raphid crystals contained within a polysaccharide matrix. The cytoplasm consists of a thin parietal layer with few organelles. The cells give no reactions for dehydrogenase or peroxidase activity and a weak reaction for proteins. They appear degenerate when compared with mature raphid idioblasts of *Oenothera* (Schotz, Diers, and Bathelt, 1970) or *Eichhornia crassipes* (Arnott and Pautard, 1970). However, the plasmalemma and tonoplast appear intact in most observations. The cells may be living with low metabolic activity. Cheavin (1938) considered raphid idioblasts to be dead cells filled with a mucilage material capable of swelling, rupturing the cell wall, and releasing the raphides. However, other workers (see review in Arnott and Pautard, 1970) have observed resorption of calcium oxalate, which we presume must be by living cells. The idioblast cells are difficult to fix and shrinking or swelling of the carbohydrate matrix after fixation with glutaraldehyde and osmium could not be prevented. Thus some disruption of the cytoplasm may have occurred during the dehydration steps of specimen preparation and some of the degenerate appearance of the cytoplasm may be artifact.

The raphid-containing cells appear to be true idioblasts, and no intercellular connections have been observed between these cells and adjacent parenchyma cells. In *Colocasia* this lack of plasmodesmata along with the dissolution of the middle lamella allows for the release of the raphid idioblast from the surrounding tissue. The solubilization of the calcium pectate is apparently caused by excess oxalate during the formation of the raphides. Currier (1956) reported that 'competing' substances act in solubilizing the middle lamella by the formation of insoluble calcium oxalates. We have also observed some breakdown of the middle lamella in *Xanthosoma*, but not in *Alocasia*. In both of these genera we have not observed separation of the raphid idioblasts from the surrounding cells.

The cell wall in *Colocasia* appears to be well developed for forceful raphid release. The tapered shape of the cell with thickened lateral walls causes pressure from the swelling polysaccharide material to be released by rupture of the end nubs. A possible wall modification for raphid release was also observed in *Alocasia*. In this genus there appears to be a thin region of the wall in the area of wall breakage. In *Xanthosoma* no evidence of wall modification was observed. However, this may be due to not obtaining the critical sections.

The raphides of all three genera appear similar with two opposite longitudinal grooves and barbs with tips oriented away from a tapering end and towards an abruptly pointed end. However, there are differences in size, and in *Alocasia*, raphides of the large idioblasts of the petiole and normal idioblasts of the stem are much larger than all other raphides observed. Both the large and small raphides of *Alocasia* also showed thickenings near their centres which were not present in raphides of the other genera studied.

Developmental studies (Arnott and Pautard, 1970; Mollenhauer and Larson, 1966; Ledbetter and Porter, 1970; Schotz, Diers, and Bathelt, 1970) have shown that the formation of the raphid is closely controlled by the idioblast and the shape of the crystals is probably decided by the shape of the chamber membrane formed by the cell. In the three genera studied here, the chamber membrane appears closely appressed to the space left by the mature crystal. In *Colocasia* the chamber membrane also appears to bulge in regions of probable barbs. An unusual situation occurs in the large crystal containing idioblasts of *Alocasia*. Only a few crystal chambers are filled by the large crystals with other crystal chambers containing a fibrillar electron opaque material. Apparently the formation of the large crystals resulted in a reduction in the number of crystals.

The mechanisms of crystal release in the five species studied here is the same, e.g. a swelling of a polysaccharide material which breaks the idioblast wall and forces the re-

lease of the raphides. In *Colocasia* this results in a very forceful ejection with the release of a relatively few number of crystals at any one time. *Xanthosoma* and *Alocasia* do not exhibit a forceful ejection of their raphid crystals, but a less forceful release of a crystal mass which then disassociates to free the individual crystals. The forceful ejection of raphid crystals from idioblasts into tissues of the mouth and throat was suggested by Haberlandt (1914) as the principle means of irritation in *Pistia statiodes*. A similar means was described by Black (1918) in *C. esculenta* and by Middendorf (1968) in *Diffenbachia* and *Monstera*. This forceful ejection of crystals does not fully account for the irritation produced by ingestion of uncooked tissues of *Xanthosoma* and *Alocasia* and another source of the irritation is hereby suggested.

Barnes and Fox (1955) suggested that the irritation in *Diffenbachia* was caused by a toxic material associated with the crystal. They did not detect a specific toxin but suggested that it may be a curare-like substance since raphides often were ingredients of curare mixtures. However, curare is primarily an alkaloid and to our knowledge alkaloids have not been reported in any quantity from the family Araceae and, furthermore, the presence of alkaloids is not noted in chemical analyses of *Colocasia*, *Xanthosoma*, or *Alocasia* (de la Pena, 1970; Standal, 1970; Ezumah, 1970). Also, our preliminary chemical tests for alkaloids using phosphotungistic acid and iodine-potassium iodide (Lee, 1937; Johansen, 1940) failed to detect their presence. We thus feel that the physical characteristics of the crystals themselves are responsible for the irritation in *Colocasia*, *Alocasia*, and *Xanthosoma*.

All needle-like crystals would presumably cause irritation, however, the raphides of *Colocasia*, *Alocasia*, and *Xanthosoma* exhibit three structural features which may intensify and prolong this irritation. First, the crystals have two distinct points, one tapering to an elongate point, and the other abruptly pointed. Second, the crystals have surface barbs oriented away from the tapering point. And third, deep grooves are present along the length of the crystals. In penetrating a mouth or throat epithelial cell the tapering point would tend to enter first. Once there, the crystal could not be easily dislodged because of the presence and orientation of the barbs. The crystal would tend to continue into the cell. The lateral grooves as suggested earlier (Sakai *et al.*, 1972) would act similar to blood channels in preventing sealing of the wound around the raphid.

All raphid crystals do not show these three morphological features. In *Yucca brevifolia* and *Eichornia crassipes* the raphides appear square to rectangular in cross section (Arnott and Pautard, 1970). No SEM was done with the *Yucca* or *Eichornia* raphides, so the presence of surface barbs is not known. We also do not know if these raphid crystals produce an irritation reaction upon injestion.

In Hawaii, *Alocasia macrorrhiza* was only eaten in times of great scarcity and only after thorough cooking (Neal, 1965). Our studies have shown that the crystals in this plant are over ten times as large as those found in the other two genera which are commonly eaten. Other structural features, however, do not differ greatly among the three genera. Also the stem of *Alocasia* can remain acrid after cooking for up to twelve hours (D. Anderson, personal communication). This would suggest that a chemical was not responsible for the irritation. Since calcium oxalate is a very insoluble salt, much cooking would probably be required to dissolve the raphid crystals. Thus, the raphides in *Alocasia* would remain after cooking for long periods while the smaller raphides in *Colocasia* and *Xanthosoma* would dissolve sooner. Studies are presently being undertaken to determine the effects of cooking on raphid crystal structure.

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EXPLANATION OF PLATES

PLATE I

Mature raphid idioblasts and raphides of *Colocasia esculenta*

A. Idioblast with ejected raphides (arrow) projecting into petiolar air canal; cv. *Taro Jen.*

B. Epon longitudinal section of idioblast projecting into intercellular space in corm; stained with PAS for polysaccharides. Starch is labelled s; cv. *Taro Jen.*

C. Epon cross section of idioblast in petiole; stained with PAS; cv. *Taro Jen.*

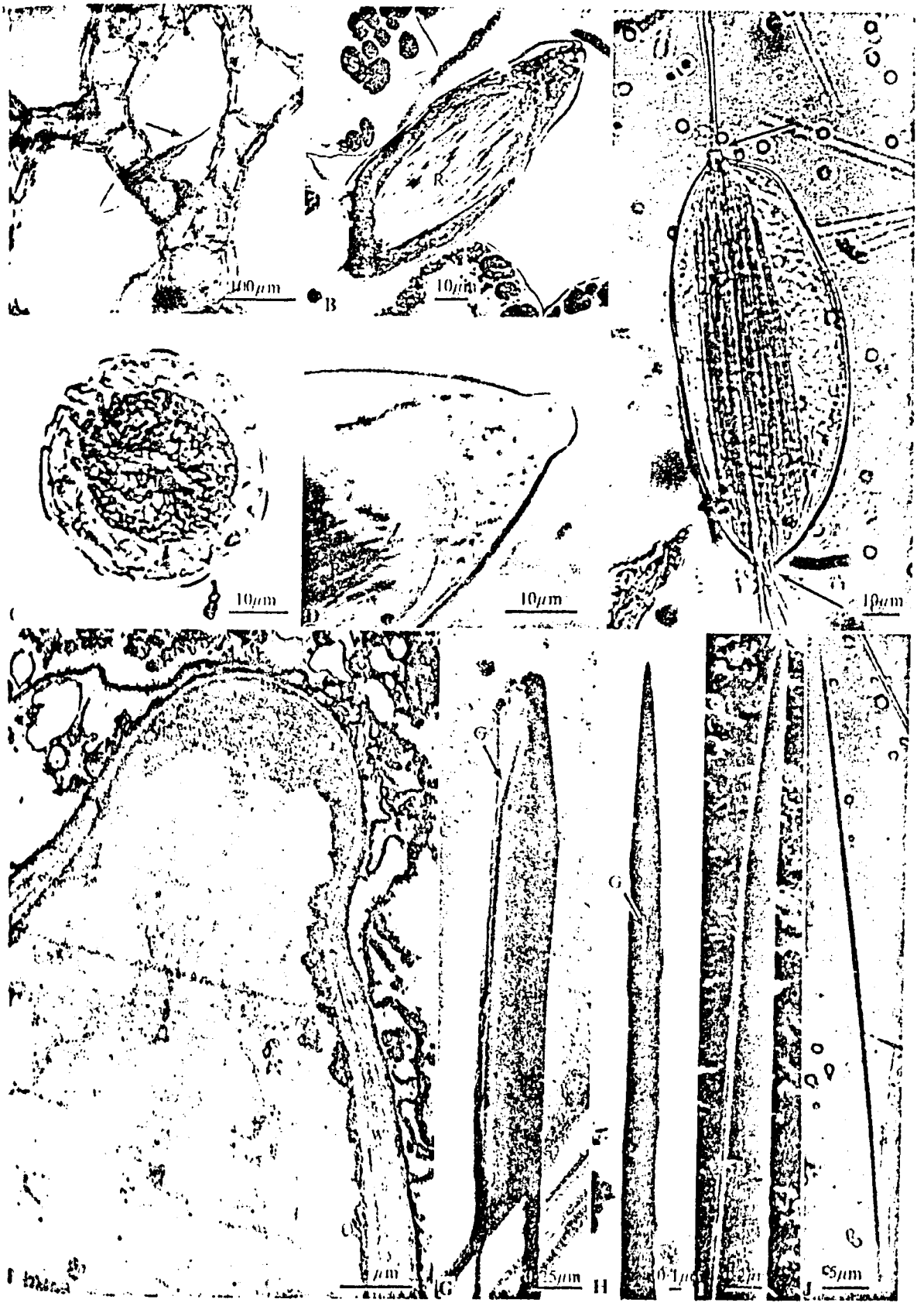
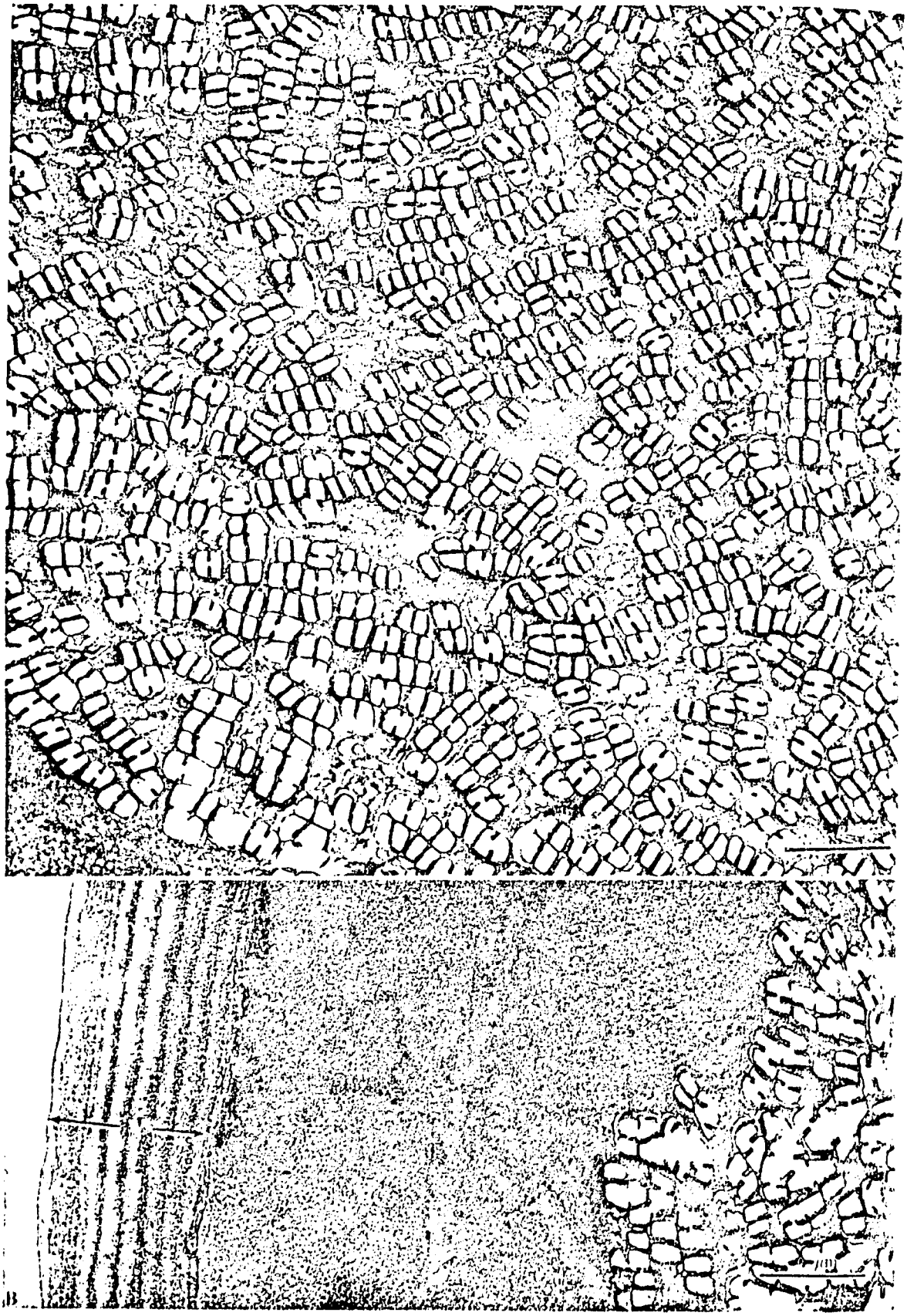


PLATE 1



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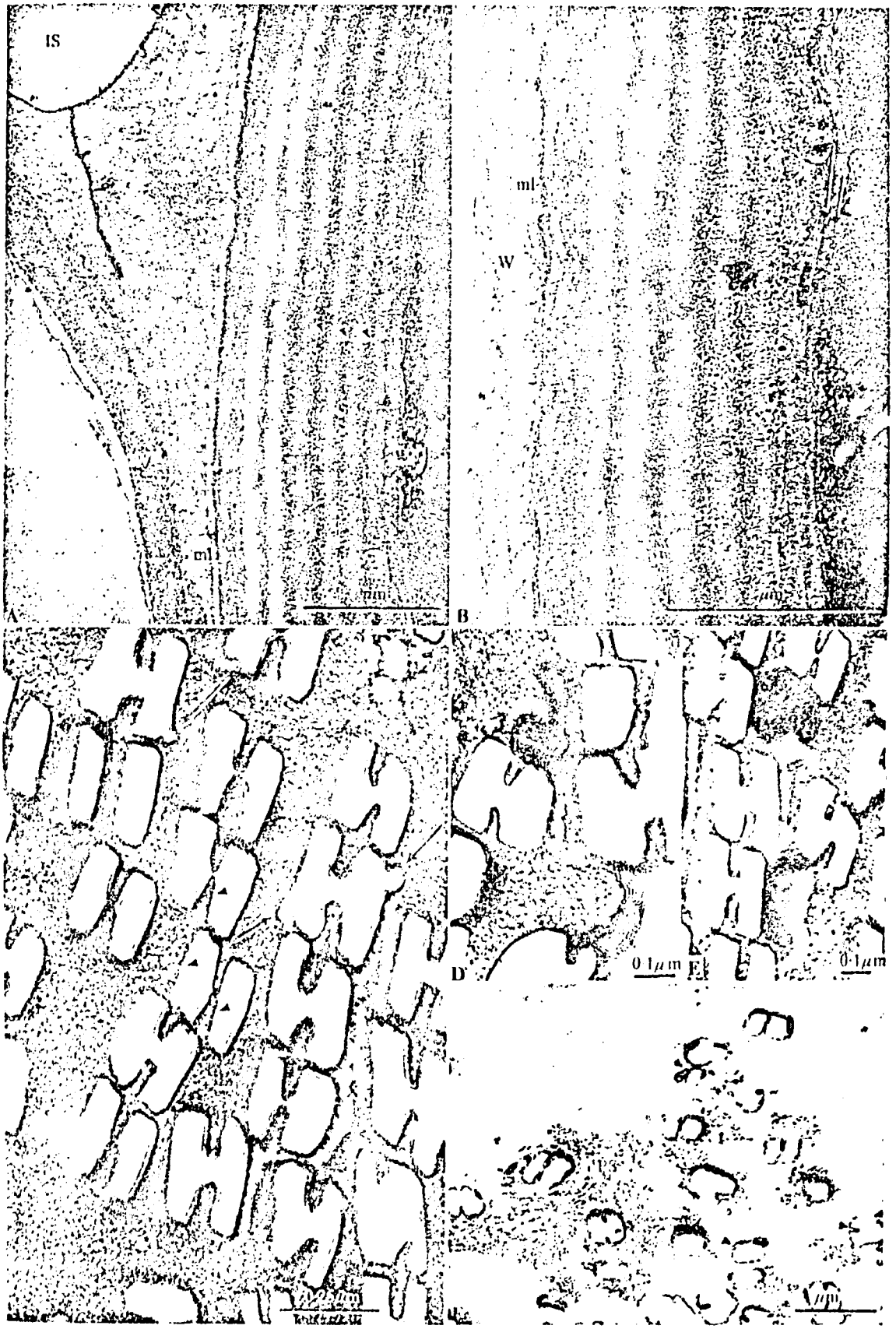

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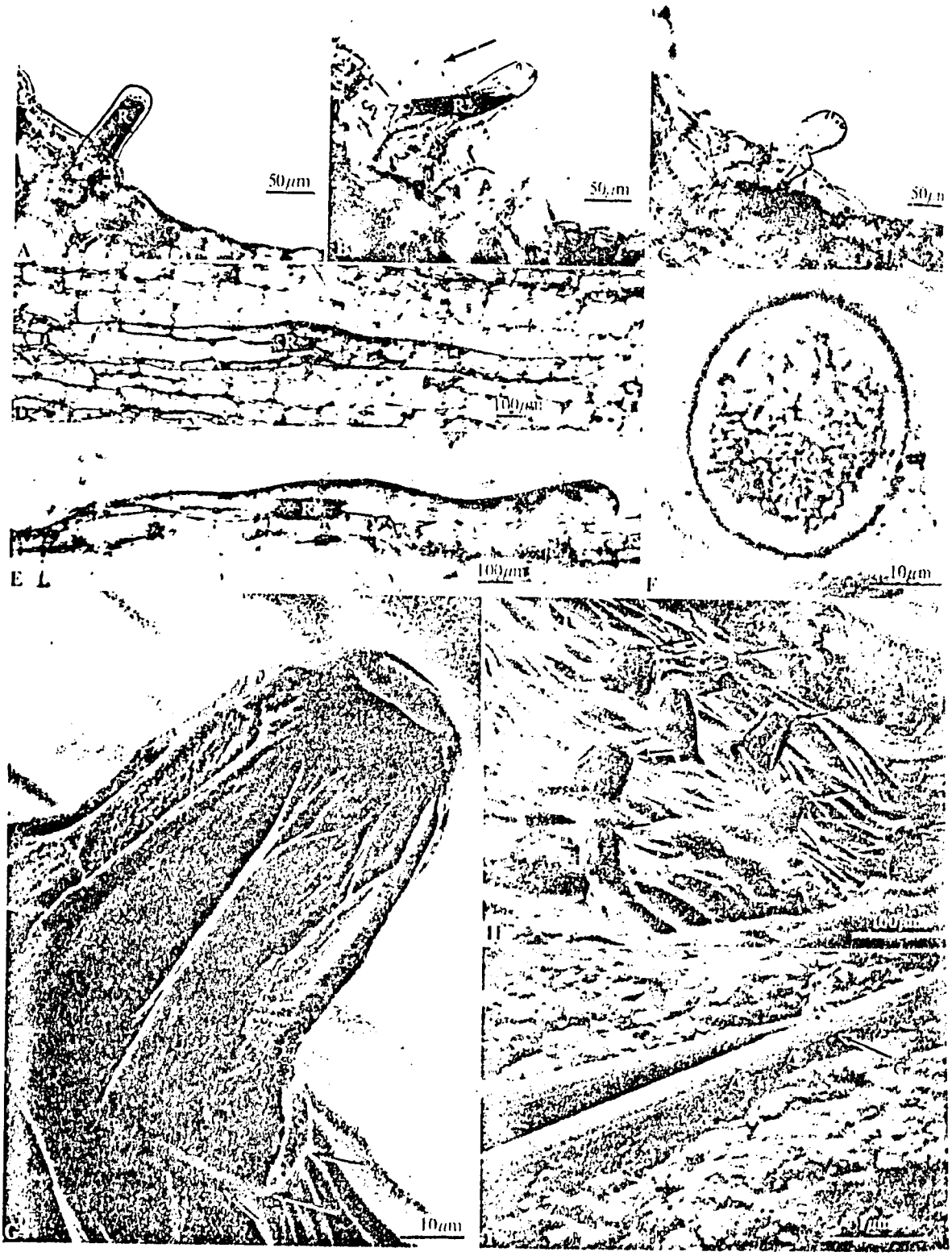
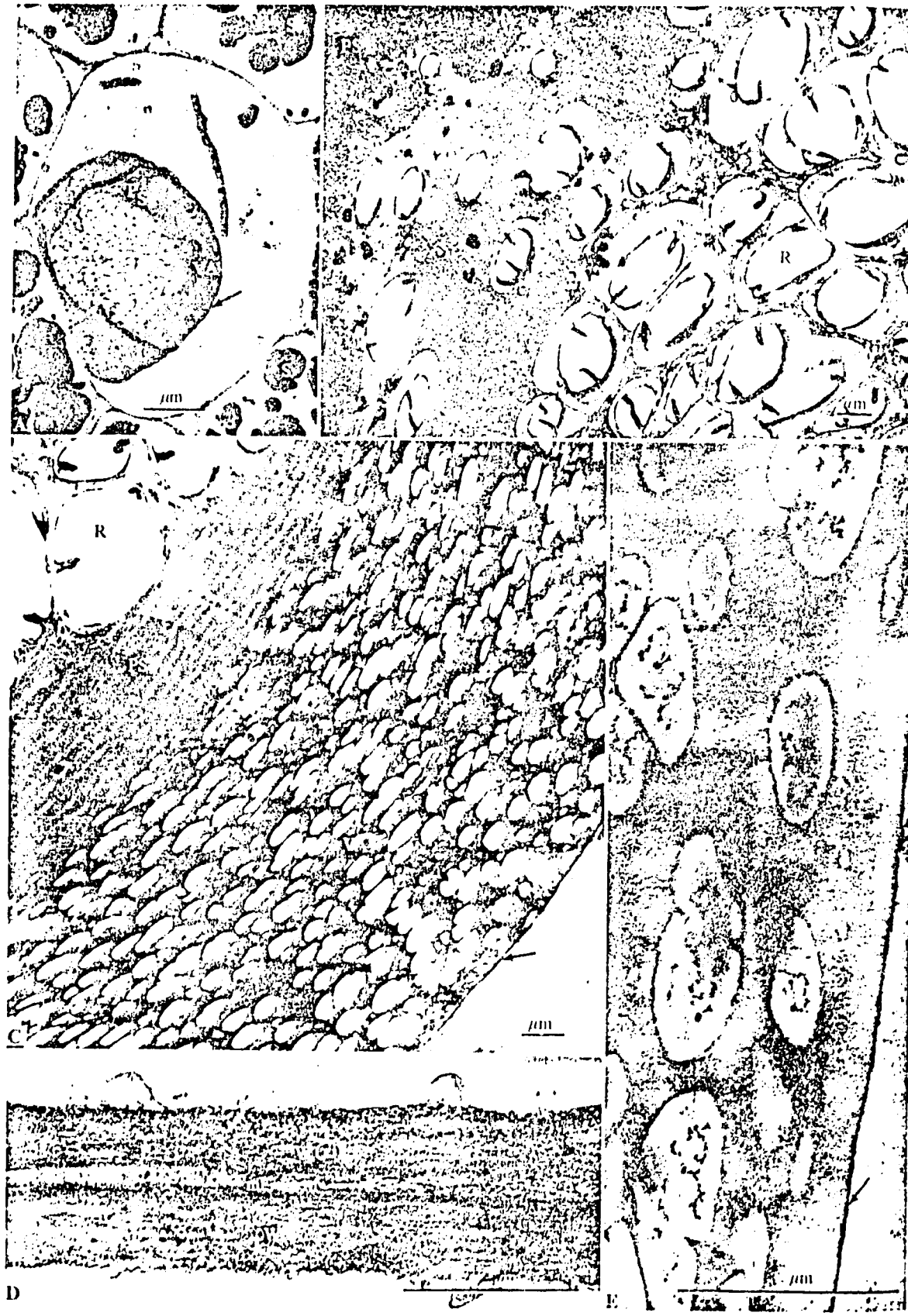


PLATE 4

PLATE 5



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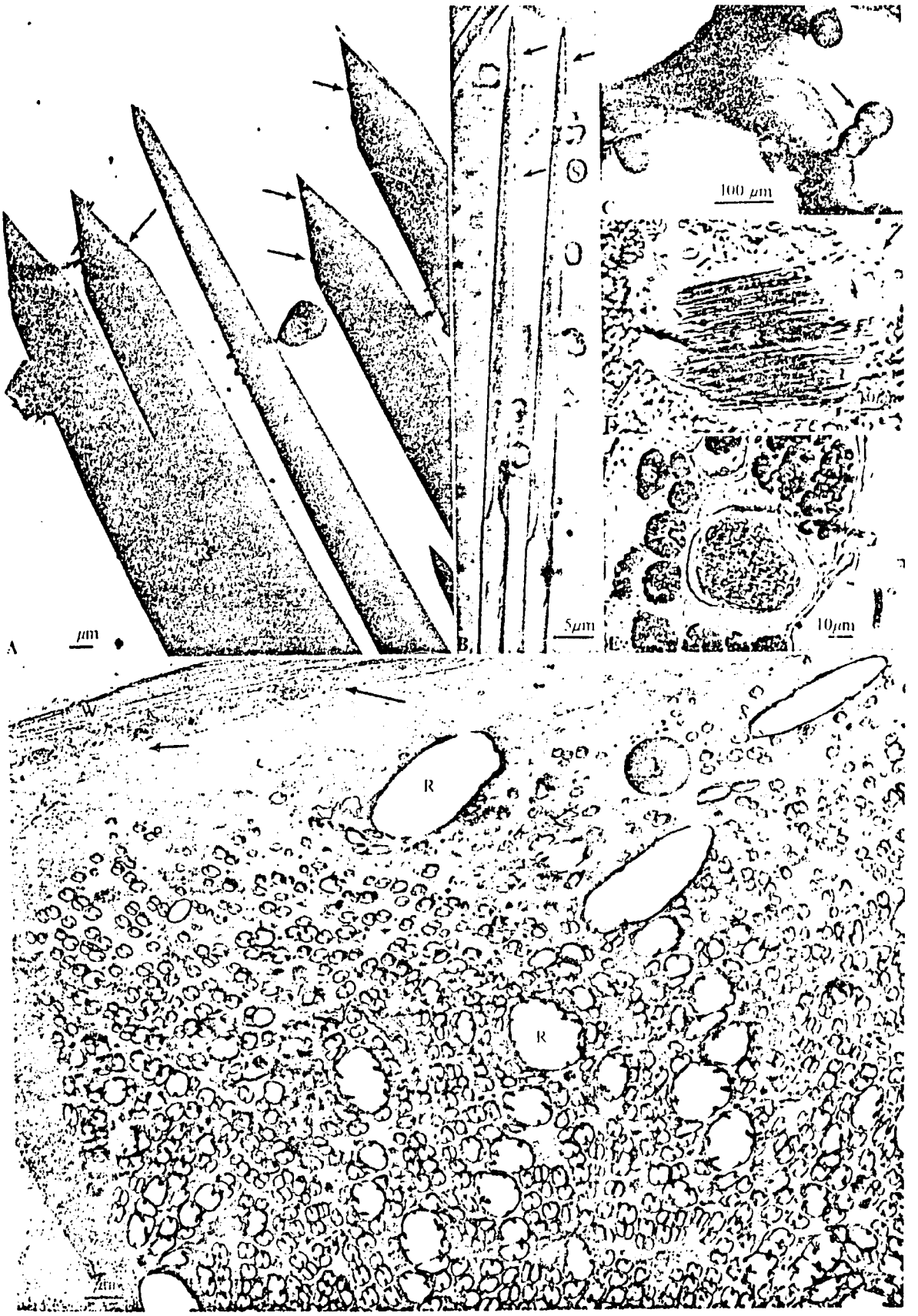

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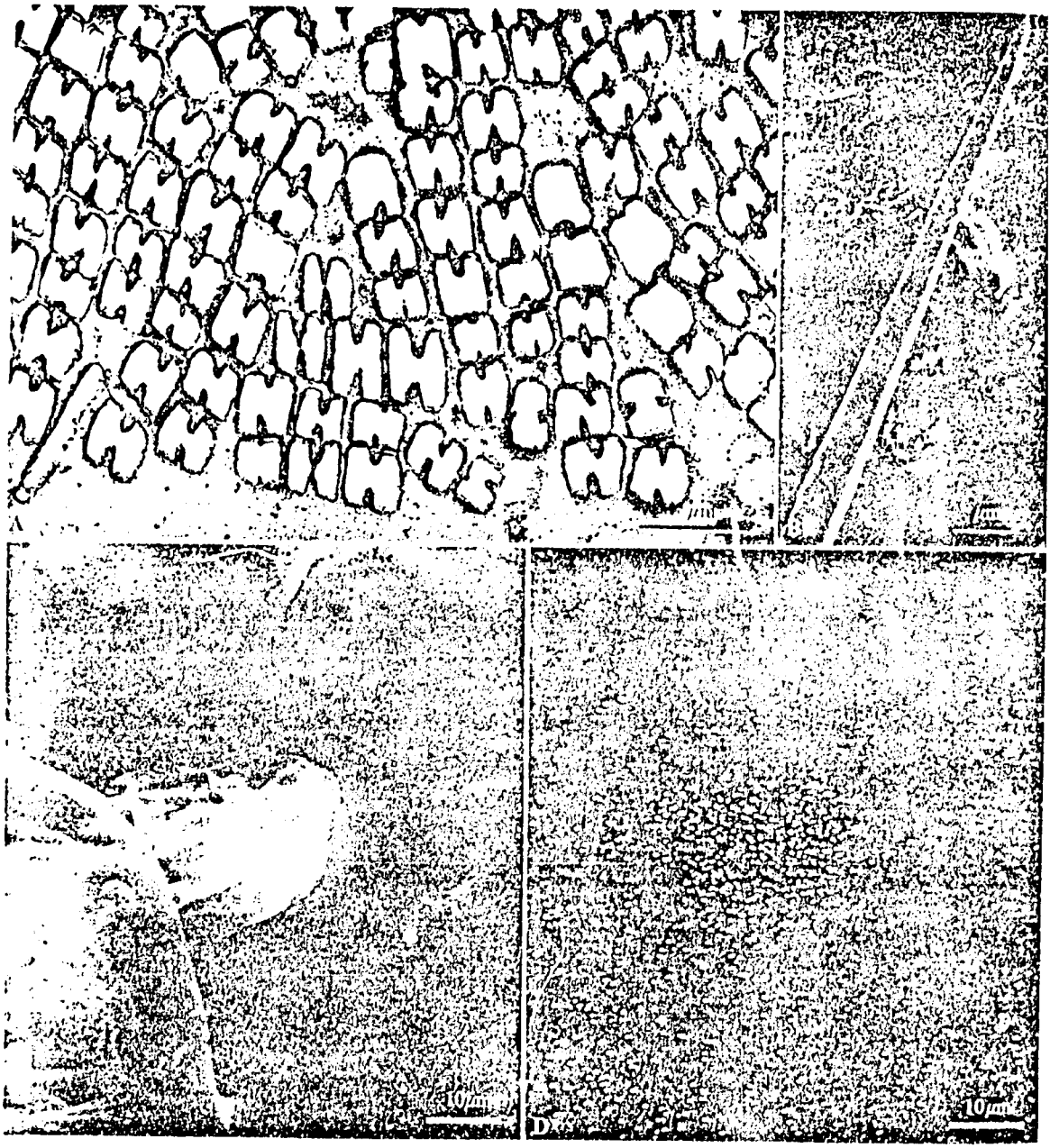

PLATE 8

D. Tip of freshly mounted idioblast from rhizome. Raphides (R) to left appear within vacuole; cv. *Taro jen.*
E. Fresh mounted idioblast from rhizome. Entire cell is released from tissue and raphides are ejected from both ends (arrows); cv. *Piko kea.*
F. TEM of tip of idioblast from petiole. Note the thinning of the walls (W) in the tip; cv. *Taro jen.*
G. TEM of Pt-Pa shadowed carbon replica of raphid near abruptly pointed end. Note evidence of groove (G); cv. *Lehua keokeo.*
H.–I. TEM (H) and SEM (I) of raphides from petiole. Note groove (G) and barbs (B); cv. *Taro jen.*
J. Fresh raphid from rhizome showing needle-like structure; cv. *Taro jen.*

PLATE 2

A.–B. TEMS of cross-sections of raphid idioblast from rhizome of *C. esculenta* cv. *Lehua keokeo.* Note the H-shaped raphides in cross section, the thin layer of cytoplasm (c) adjacent to the wall (W), and the granular to fibrillar polysaccharide matrix surrounding the bundle of raphides.

PLATE 3

TEMS of sections of raphid idioblasts and raphides of *C. esculenta*

A.–E. Sections from rhizome of cv. *Lehua keokeo.*
A. Section showing layered wall of idioblast, middle lamella (ml), and adjacent cell wall (W). Note the thin fibrillar nature of the middle lamella adjacent to the intercellular space (IS) and the dark staining layers (arrowheads) of cell wall.
B. Section showing portion of idioblast and adjacent cell. The plasma membrane (pm) and tonoplast (t) do not appear well structured and the middle lamella (ml) appears loose and fibrillar. Note the layers of cell wall are more electron opaque near the cytoplasm (arrowheads).
C. Enlarged section of raphides showing bulges in the chamber membrane around probable barbs (arrows). Arrowheads indicate electron translucent 'sheath'. Disrupted polysaccharide matrix at × is probably artefact.
D.–E. Sections of membraneous material within the raphid bundle.
F. Section of ejected raphides from petiole, cv. *Taro jen.* Note the chamber membrane (arrowheads) and polysaccharide matrix (PS) still surrounding some crystals.

PLATE 4

Photomicrographs and SEM of *Alocasia* raphid idioblasts and raphides

A.–F. Sections from petiole of *A. macrorrhiza.*
A. Protrusion of a small raphid idioblast into the adjacent air canal.
B. Ejection of raphides (R) from a similar cell. Note that the idioblast cell wall breaks near the base and raphides are ejected in a bundle apparently surrounded by a membrane (arrow).
C. An empty idioblast after the raphides were ejected.
D.–E. Large raphid idioblast containing bundle of large raphides. Attachment of the idioblast to the wall of the air canal is to left.
F. Cross section of small idioblast containing raphides (R), stained with PAS. Note the dark staining polysaccharide around the raphides.
G.–H. SEM of idioblasts protruding into petiolar air canals of *A. cucullata.* The fresh idioblasts have partially dehydrated in the vacuum of the microscope. Arrows in G show the base of the idioblast where wall rupture may occur.
I. Small petiolar raphides of *A. macrorrhiza.* Note the protruding barbs (arrowheads) and groove (G).

PLATE 5

TEM of petiolar raphid idioblasts of *A. macrorrhiza*

A. Section showing idioblast (lower cell), an adjacent cell, and intercellular space (IS). Note the middle lamella (ml) is not loosely structured and the cytoplasm of the idioblast appears degenerate. Arrow points to possible area of wall rupture.
B. Section through an idioblast showing raphides on right and wall (W) on left. Note the large amount of material in the vacuole and the thin parietal layer of cytoplasm (C).
C. Section through the bundle of raphides showing the presence of electron opaque material associated with the raphides.
D. Enlarged portion with crystals showing the presence of a chamber membrane around the crystals.

PLATE 6

Photomicrographs and TEM of idioblasts from rhizome of *A. macrorrhiza*

A. Photomicrograph of idioblast stained with PAS. Note the general lack of cytoplasm and the dark staining polysaccharide matrix around the raphid bundle (R). A vesiculate area (VA) in the polysaccharide matrix (PS) was only observed in these idioblasts. Starch grains (S) are present in adjacent cells. Arrows in A, C, and E point to probable tonoplast.

B. Section through raphides (R) showing the polysaccharide matrix (PS) and the vesiculate area separated from the chamber membranes by a clear space. The crystal chambers were probably deformed when crystals were removed in sectioning.

C. Enlarged view of raphides (R), polysaccharide matrix (PS) and vesiculate area (VA).

D. Section of walls (W) between raphide idioblast (ri) and adjacent cell. Note the porous nature of the middle lamella (ml).

E. Section through edge of vesiculate area. Some evidence of a tonoplast appears at edge (arrow). Note the electron opaque contents (arrowheads) and two-parted nature of the vesicules which are probably undeveloped crystal chambers.

PLATE 7

Photomicrographs and TEM of raphides and idioblasts of *A. macrorrhiza* and *X. sagittifolium*

A.–B. Raphides from rhizome of *A. macrorrhiza*.

A. TEM of raphides showing the tapering end and the abruptly pointed end. Arrows point to probable barbs.

B. Photomicrographs showing the structured central region of the raphid and barbs (arrows) with tips oriented away from the tapering end.

C. Petiolar idioblasts of *X. sagittifolium*. One (arrow) is extruding raphides through the blunt tip.

D. An extruded bundle of raphides from the rhizome of *X. sagittifolium*. Note the presence of the tonoplast (arrows) around the bundle.

E. Raphid idioblast from rhizome of *X. sagittifolium*, stained with PAS showing a cross section of the raphid bundle (R) and starch grains (S) in adjacent cells.

F. TEM of idioblast from rhizome of *X. sagittifolium*. Note the normal appearing wall (W) and the presence of a tonoplast (arrows). The section has torn in some areas of raphides (R). A lipid droplet is also present (L).

PLATE 8

Raphides and raphid idioblasts from *Xanthosoma*

A. Enlarged view of raphides of *X. sagittifolium* showing the H-shaped cross section.

B. Raphid from *X. violaceae*, showing the tapering end. Note barbs with tips oriented away from this end, and groove.

C.–D. SEM and elemental distribution pattern for calcium of idioblast of *X. sagittifolium*—a situation expected since the raphides are of calcium oxalate.