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The ultrastructure of plasmodesmata in Tillandsia: New observations

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Botanica. — *The ultrastructure of plasmodesmata in Tillandsia: New observations* (*). Nota di LUIGI BRIGHIGNA (**) presentata (***) dal Socio E. FRANCINI CORTI.

RIASSUNTO. — L'esame delle immagini al microscopio elettronico suggerisce una particolare ultrastruttura dei plasmodesmi che risultano essere, in *Tillandsia usneoides*, di natura membranosa. Viene presentato un modello di plasmodesma che mette in risalto la struttura micellare della membrana costituente l'asse centrale, anche tenendo conto dell'attività fisiologica che queste strutture sono chiamate a svolgere.

INTRODUCTION

In their ultrastructural studies regarding different plant tissues, many workers (Waley *et al.*, 1960; Porter and Machado, 1960; Evert *et al.*, 1961; Frey-Wissling *et al.*, 1964; Dolzmann, 1964; Hepler and Newcomb, 1967; O'Brien *et al.*, 1967; Pickett-Heaps, 1967; 1968; Perrin, 1971; Kwiatkowsk and Maszewski, 1976) pointed out the presence of plasmodesmata. For other researchers (Buvat, 1960; Dolzmann, 1965; Lopez-Saez *et al.*, 1966; Robards, 1968; 1971; Fraser and Gunning, 1969; Burgess, 1971; Brighigna, 1974), the fine structure of plasmodesmata was the object of specific studies.

Most of these researchers construe the origin of plasmodesmata as the result of the inclusion of endoplasmic reticulum fragments by the forming cell plate. Frey-Wissling reports strands of the endoplasmic reticulum, each of them, preventing the coalescence of some Golgi vesicles in the formation of the new cell wall, surrounded by a thin plasmatic layer and constituting the plasmodesmata.

Dolzmann believes that the plasmodesmata are constituted by a central endoplasmic reticulum connected with the surrounding plasma membrane by thin electrondense spokes. Pickett-Heaps points out the presence of plasmodesmata both with (1968) and without (1967) the central strand of endoplasmic reticulum. Robards (1968) suggests one model of plasmodesmata coming from the trapped residues of the spindle fibres in the formation of the cell wall. Fraser and Gunning found plasmodesmata without an internal component such as a desmotubule or a derivate of the endoplasmic reticulum: the plasma membrane is lined with helically arranged particles. Therefore

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these plasmodesmata could have possible functions in intercellular transport which are not well pointed out by Robards (1968). Burgess does not link the processes of cell plate and plasmodesmata formation.

The purpose of the present work is to add details on the ultrastructure of the plasmodesmata to that already suggested (Brighigna, 1974).
The implications of these recent observations made during studies undertaken on some specimens of Tillandsiae are discussed.

Materials and methods

Owing to the particular almost acicular form of the leaf of *T. usneoides*, about 1 mm long segments were prefixed for 40′ in 5 % glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2 (Osinchak, 1964). Subsequently the material was fixed in 2 % OsO_4 in 0.2 M cacodylate buffer, pH 7.2, for 1 hour, dehydrated and then embedded in Epon 812 (Luft, 1961), a mixture being obtained in the ratio A/B = 4/7 + 2 % DMP–30.

Other segments of the same size were prefixed for 20′ in 2.5 % glutaraldehyde and 4 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (Karnovsky, 1965). Then the material was fixed in 2 % OsO_4 in 0.1 M phosphate buffer pH 7.4 for 1 h. 30 sec. The samples were then washed in the phosphate buffer for 30′, dehydrated and embedded in Epon 812.

The embedded material was sectioned by a Reichert OM U2 ultramicrotome using glass blades. The sections, having a thickness varying from 500 Å to 900 Å, were observed with a Philips EM 300 electron microscope at 80 KV, after having been stained for 1 hour in uranyl acetate (Gibbons and Grimstone, 1960) and then with lead citrate (Reynolds, 1963) to obtain a more marked contrast.

Observations and results

The images we present regard the cells of the whole absorbing appartus. Numerous plasmodesmata cross the not cutinized walls which divide these cells, drawing an extensive connecting system. The innermost cell of the apparatus (or the two innermost cells, according to the different cases) are also in connection with the surrounding cells with plasmodesmata gathered into groups (Plate I, fig. 2).

Nor does Robards raise any doubt as to the presence of the endoplasmic reticulum at both terminal poles of the plasmodesmata. Plate I, fig. 3; Plate II, figs. 6–7; Plate III, fig. 9; Plate IV, fig. 11 show the endoplasmic reticulum which penetrates the wall to form the central axis of the plasmodesmata. The central axis is more or less evident; it can be not visible at all as the inside of the plasmodesmata results so electrondense with sufficient homogeneity. The plasma membrane forms the outline of each plasmodesm therefore representing the continuity factor between cells. In Plate I, fig. 3 the endoplasmic

reticulum connected with the plasmodesmata bears ribosomes in some points of its surface. Approaching plasmodesmata the endoplasmic reticulum loses ribosomes. Often at both poles of the plasmodesmata the figure formed by the plasma membrane narrows and comes into contact with the central axil structure (Plate I, fig. 5).

The plasma membrane shows a trilayer structure, the inner osmiophilic layer of which is thicker than the outer.

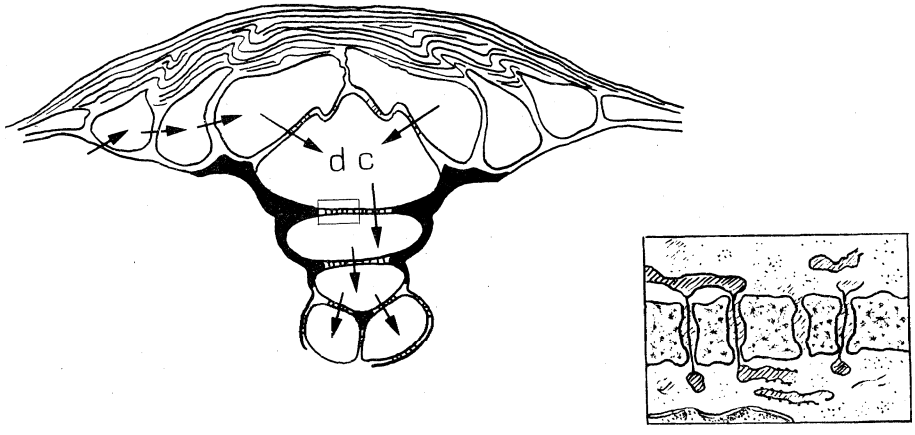

Fig. 1. – Diagram representing the absorbing apparatus of the gen. Tillandsia. The arrows mark the pathway covered by the fluids. d. c. = dome-shaped cell.

The diameter of plasmodesmata varies from 400 Å to 600 Å; only in few cases does it reach 800 Å in the central region. The central axis has a not easily identifiable electrondense structure; it always follows the eventual deviation of the plasmodesmata from the strictly geometric form. Around the plasmodesmata, at both poles, the wall forms a more or less evident edge towards the cytoplasm giving rise to a sketching funnel. Rarely the edge is so asymmetrically marked as to form a beak (Plate I, fig. 4).

In Plate I, fig. 5; Plate II, figs. 6–7, a succession of clear and dark oblique regions can be observed in the central axis of plasmodesmata; this succession can be tested by the oblique sections of a group of plasmodesmata (Plate III, fig. 10). No appreciable difference in the fine structure of plasmodesmata is seen with either of the methods of fixation used.

DISCUSSION

The origin of plasmodesmata is so clearly shown by Frey-Wissling *et al.*, by Porter and Machado, and by Buvat, that their conclusion cannot be discussed. In the works of these researchers we can observe very clearly strands of endoplasmic reticulum which are trapped during the formation of the new cell wall. Therefore, when the endoplasmic reticulum appears as in our case either

in an evident connection with the plasmodesm or directly penetrating it in order to form the central axis, it is logical for us to confirm the conclusions of the above-mentioned authors about the way plasmodesm originates. On the other hand we cannot ignore " a priori " the conclusion reached by Burgess that: " the role of the endoplasmic reticulum is particularly obscure,...". Burgess, in our opinion, comes to a very restrictive conclusion about the fact that the endoplasmic reticulum contributes materials to the cell plate (Cronshaw and Esau). Accepting this role of the endoplasmic reticulum, one cannot at all exclude that peculiar strands of endoplasmic reticulum form the central axis of the plasmodesmata.

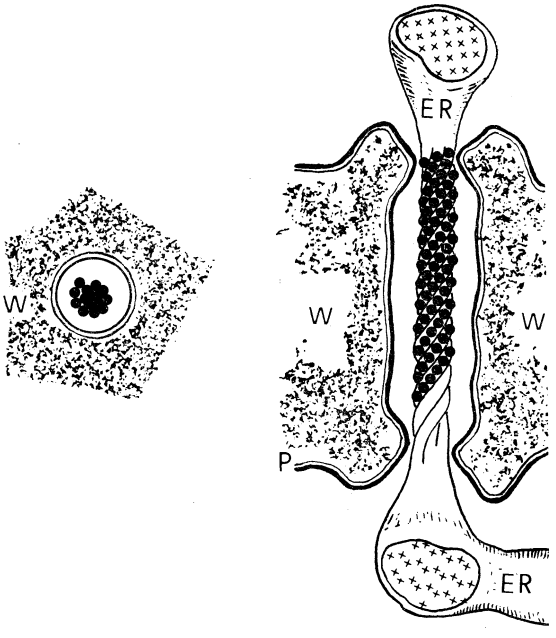

Fig. 12. – Diagram of plasmodesm. The endoplasmic reticulum crosses the plasmodesm constituting the central axis, the micellar components of which are arranged in a helicoidal way. W = wall; P = plasma membrane; ER = endoplasmic reticulum.

We do not understand why, in the delineation of the plasmodesm, the endoplasmic reticulum should be obliged to fuse itself with the plasma membrane.

We have no doubts about this. We accept the claim that the Golgi apparatus principally provides both to the formation of the cell wall and to the increase of the extent of the plasma membrane, respectively with the contents of the vesicles produced and with the membrane delimiting them.

We have already discussed the plasmodesm diagram presented by Robards (1968) lacking an evident functional capacity; the presence of microtubules, which is also pointed out by researchers, cannot be underestimated. During the study, we seldom had the opportunity to see a microtubule through the wall only in the proximity of a plasmodesm and, what is more, not so near as to suggest a correlation between both structures.

The results we have obtained suggest a membranous constitution of the plasmodesmata. Dolzmann, Lopez-Saez, Fraser and Newcomb, and Burgess came to the same conclusion. The central axis shows several properties which are not possessed by a microtubule. " It may stretch, depart markedly from a tubular form (O'Brien and Thimann), and appears to resist fixation in potassium permanganate (Lopez-Saez *et al.*) ": these are several of the reasons enumerated by Burgess to support the claim that the central axis has a membranous nature.

In regard to the morphology of the fully grown plasmodesmata (which could be a justifiable matter of censure) we make every effort to interpret their formation. Nevertheless the connection of the plasmodesmata with the endoplasmic reticulum is evident and it is hard to agree with Robards that the " central core " represents a spindle fibre trapped during the cell plate formation. This fibre should then be able to join its ends with the endoplasmic reticulum: there is no evidence as to how this could be possible. On this subject Burgess (1968), studying the eventual correlation between endoplasmic reticulum and microtubules during mitosis, had the opportunity to see a vicinity of site but not any connection between them. There is no proof that this connection does exist: so Robards (1968) is not able to ascribe any physiological function to a plasmodesm such as the one he had drawn.

According to the above details, we present in this paper a new diagram of plasmodesm strictly related to the one we published in 1974, substantially confirming the coarctation of the endoplasmic reticulum inside the plasmodesm. This action evidences the micellar components of reticulum.

First Robertson and later we ourselves (1974) wrote that the unit membrane (i.e. a strand of endoplasmic reticulum in our case) forced to assume a much smaller diameter consequently evidences the micellar components. The succession of the clear and dark zones in the central axis allows us to assert that the micelles can be arranged in a helicoidal way.

As stated in our first paper micellar structures have a very important task in regulating the exchange of substances between the cells.

In the diagram we have drawn micelles of uniform features with the sole aim of evidencing their helicoidal arrangement in the central axis, excluding a qualitative interpretation which it is beyond our possibility to demonstrate.

A helicoidal structure of the central axis aids the possibility of elongation and gives the plasmodesmata the possibility of exerting a stabilizing influence either on the cytoplasm, around its ends, or on the adherence between the plasma membrane and the cell wall. This capacity of elongation of the whole plamodesm confirms the definite importance of this structure in cell physiology. We consider the sections of plasmodesmata in which we cannot see the central axis as peripheral. Nevertheless Bisalputra describes in a brown alga and Fraser and Gunning in a filamentous green one, plasmodesmata lacking either the endoplasmic reticulum or a " desmotubule " (Robards, 1968).

The presence of many plasmodesmata along the preformed pathway covered by the fluids in the absorbing apparatus of Tillandsiae (Mez, 1904)

strengthen our conviction that they have a definite physiological function. Lopez-Saez denies this role of plasmodesmata which on the contrary for Arisz (1969), in conformity with the theory of symplasm, have a task in intercellular transport. Esau, Cronshaw and Hoefert, as well as Katajima and Lauritis, observed the transit of viruses across modified plasmodesmata, although in this question the activity concerns a pathologic state and, therefore, must be considered with adequate caution. The beak evident in Plate I, fig. 4 could merely be the reply of the plasmodesm structure to the pressure due to the arrival of new materials forming cell plate, but we prefer to consider it as forming a defence whereby the usual structure of the endoplasmic reticulum changes and becomes the micellar central axis.

No pattern of plasmodesm can be rejected since the different ultrastructural features could result from the variant among the studied tissues, cells, their physiological moment and growing stages as well. Nevertheless, all researchers of these structures must attach importance to their common feature in order to arrive, if possible, at a definition of a fundamental model of plasmodesm, the ultrastructural changes in which are referred to different conditions, thus permitting a classification of the plasmodesmata. Only further research will provide the answer as to whether or not such a goal can be attained.

I express my thanks to Professor A. Frey-Wissling for his valuable criticism.

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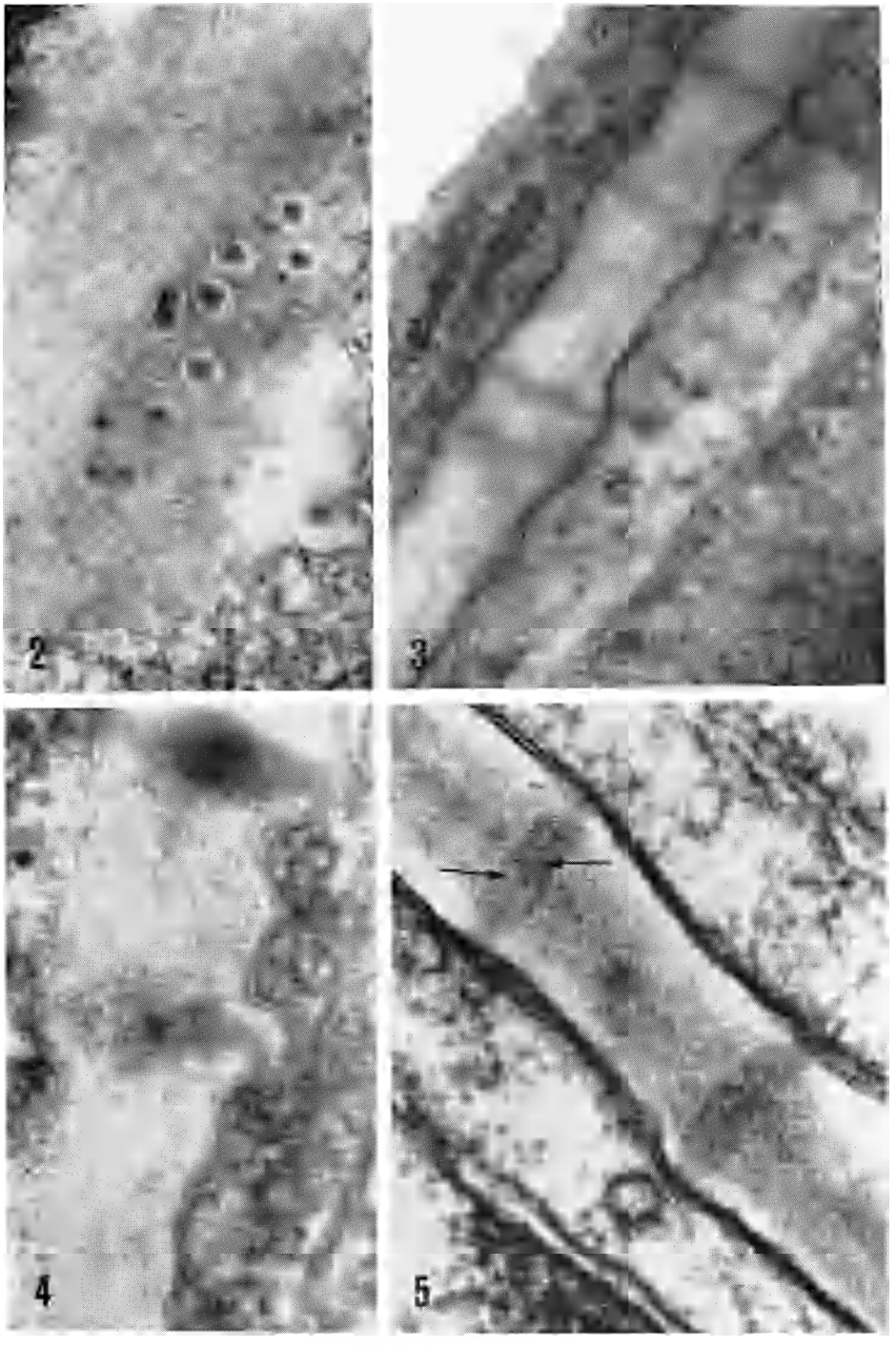
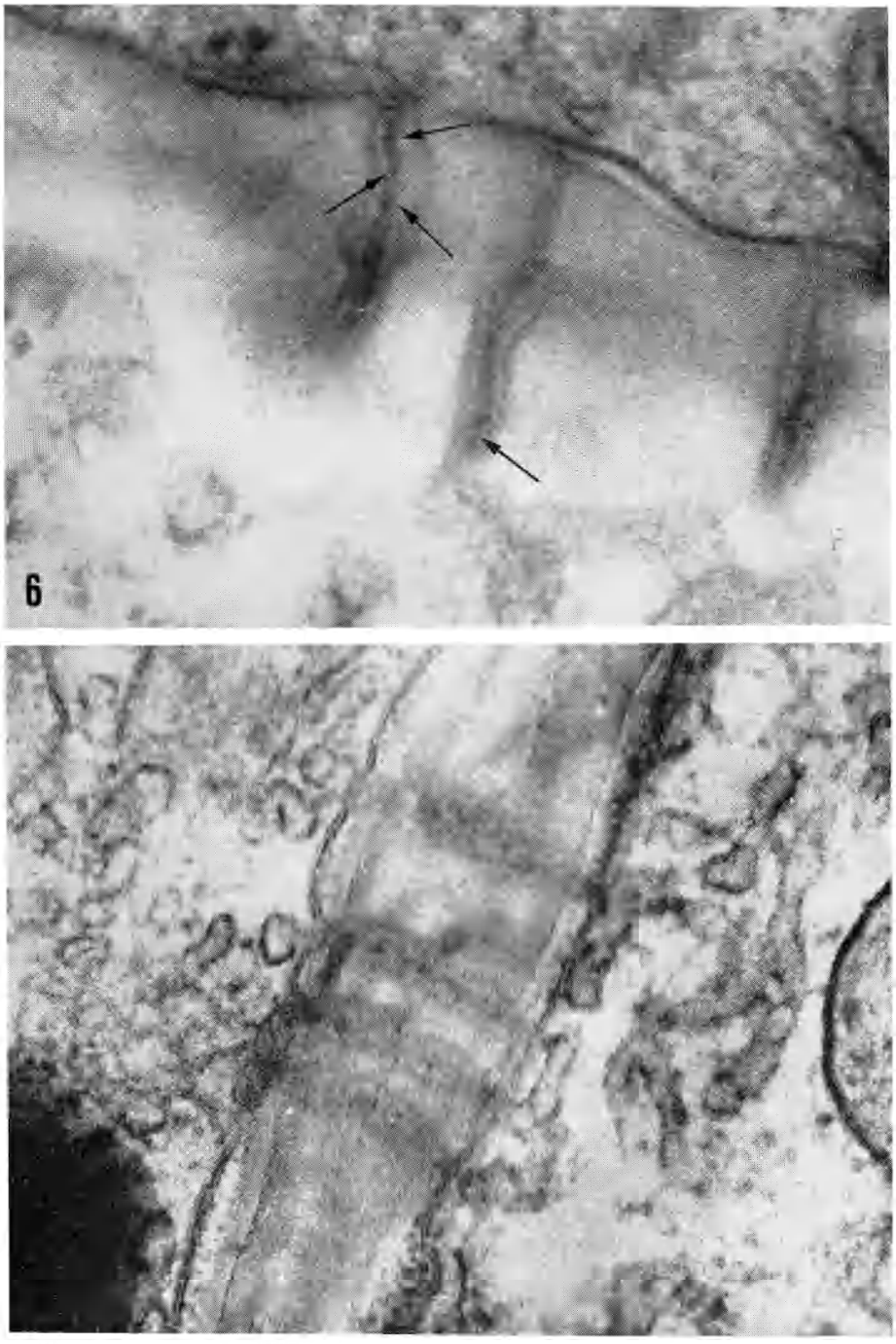
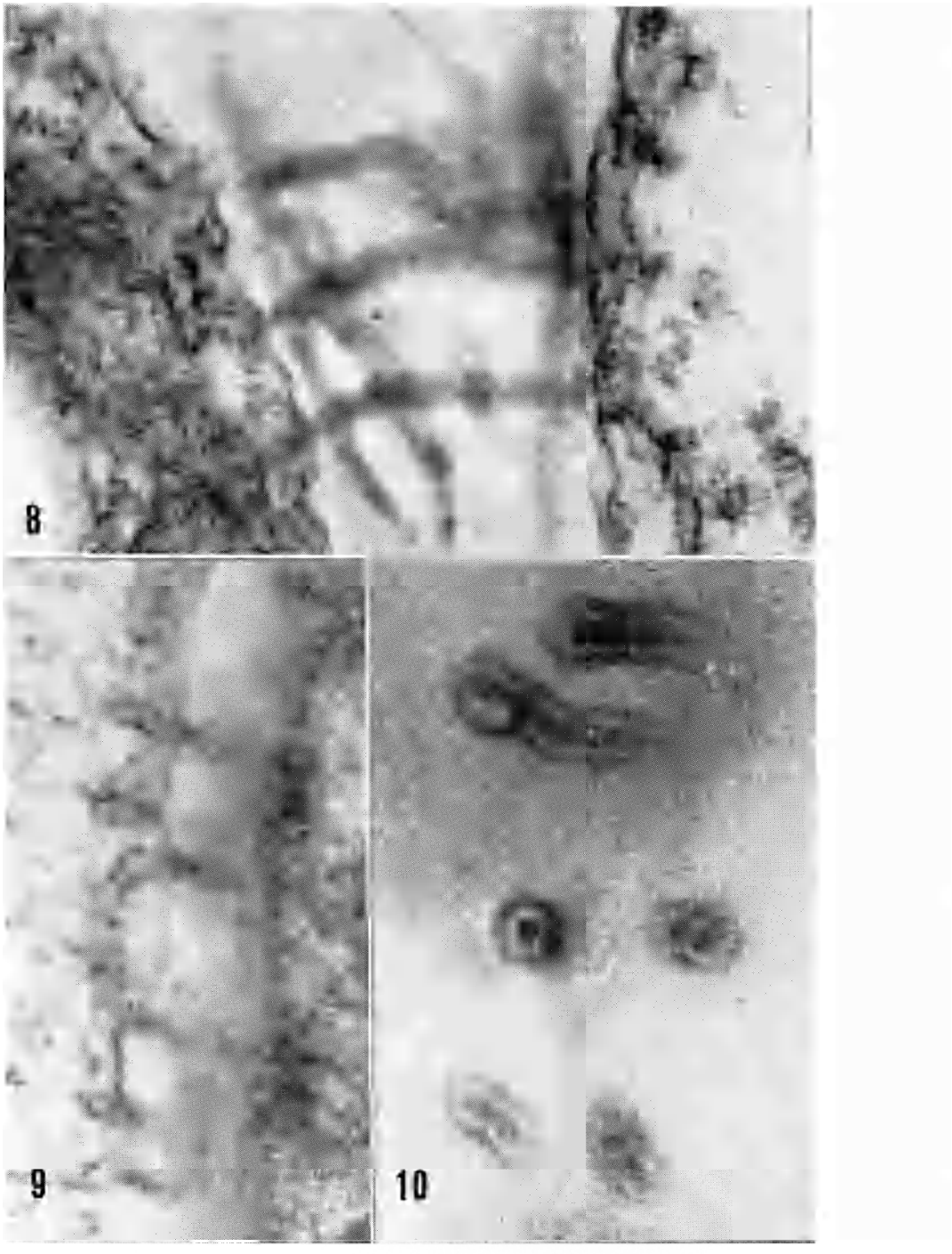


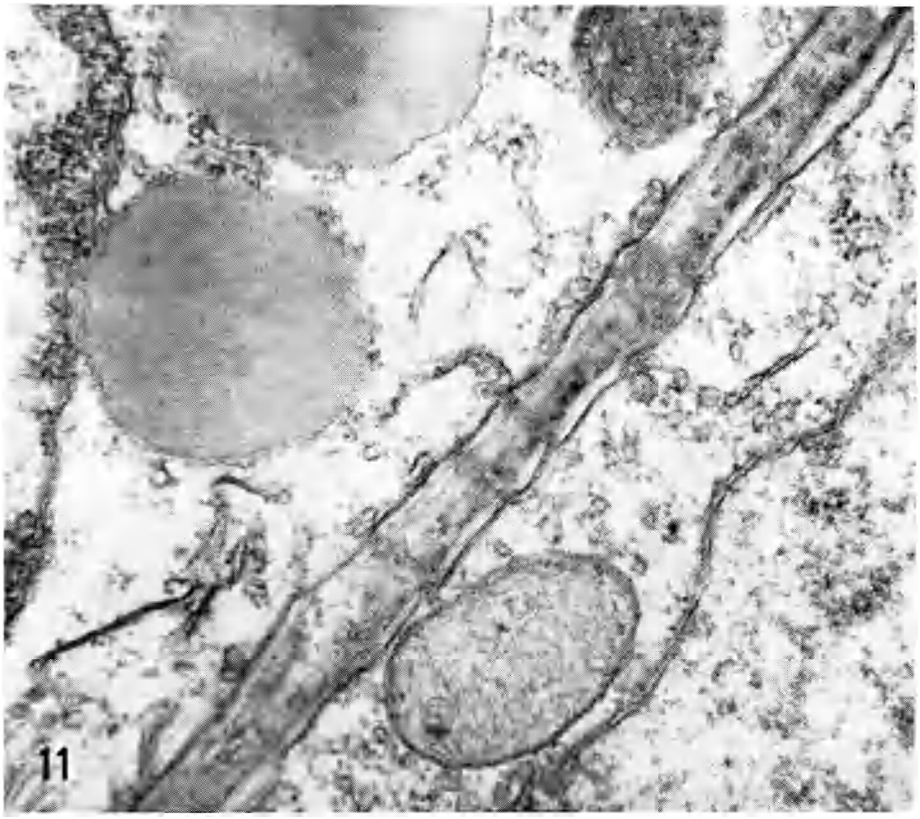
2 (×52.000); 3 (×60.400); 4 (×60.000); 5 (×104.000).



6 (×115.500), 7 (×87.500).



8 (×74.000); 9 (×80.000); 10 (×133.500).



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EXPLANATION PLATES I–IV

Fig. 2. – Transverse section of plasmodesmata. Plasmodesmata gathered into groups connecting the innermost cell with a nearby cell of mesophil.

Figg. 3, 4, 5, 6, 7, 8 e 9 – Plasmodesmata in longitudinal sections. The endoplasmic reticulum is shown, more or less clearly, to form the central axis. This always has a helicoidal structure (arrows). In Plate I, fig. 5, the narrowed plasma membrane at the sides of the plasmodesmata is evident. In the Plate I, fig. 4, the "beak" can be seen.

Fig. 10. – High magnification of plasmodesmata in transverse and oblique sections.

Fig. 11. – Particular view of the wall dividing the dome-shaped from the lower cell: plasmodesmata in longitudinal section.

Figg. 2, 7, 10 e 11. – 5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2; 2% OsO_4 in the same buffer.

Figg. 3, 4, 5, 6, 8 e 9. – 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4; 2% OsO_4 in the same buffer.