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THE ULTRASTRUCTURE OF THE TAPETUM OF TILLANDSIA ALBIDA MEZ ET PURPUS

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Abstract

Tillandsia albida Mez et Purpus subsists exclusively in a limited area near Mezquitlan, Mexico, from where we collected several plants of this epiphyte. Since literature on micromorphological studies in reproductive structures in Tillandsia is lacking, we have undertaken an ultrastructural study of the anther tapetum, a tissue that is important for the maturation of the microspore. Three consecutive functional phases have been identified in the development of the tapetum: the first comprises differentiation and maturation; in the second, ecocrine secretion of a hydrophilic substance that affects the walls precedes a merocrine secretion whose function is to digest the callose that surrounds the tetrads; and the third consists of the progressive degradation of the tapetum and its consequent holocrine secretion into the loculi. The tapetum of T. albida is secretory and develops rapidly; furthermore, the premature disruption of the symplastic connections leads to a loss of synchrony in the development of the cells of the tapetum.

Key Words: Tillandsia, anther, microsporogenesis, tapetum, ultrastructure.

Tillandsia is the most significant genus among the Bromeliaceae on account of its epiphytic strategy. At least 480 species have been identified to date. Large number of species and the broad distribution of many of them, as well as the vastness of their range, testify to a remarkably efficacious mechanism of sexual reproduction in the taxon. Nevertheless, there are some Tillandsia species that have an extremely narrow distribution or are even present in only a single or limited habitat. Tillandsia albida Mez et Purpur is a rare Mexican species that inhabits only a single, circumscribed station; the Barranca of Mezquitlan in the environs of the homonymous Mexican city (Smith & Downs 1974). In this region the species survives owing to its very active vegetative propagation.

We directed our attention to the reproductive organs, taking into consideration that in the literature there are no studies of the tapetum of the Bromeliaceae. In this paper, we report our ultrastructural observations on the development of the anther tapetum before anthesis, a tissue that is of importance in the maturation of the male sporogenic tissue.

1. Received for publication: October 26, 1992.
Fig. 1A, B

Wil et al., 1990

Cells have numerous elongated internal vacuoles.
Material and Methods

The anther used in our investigation were obtained from plants of Tillandsia albida Mee et Purpus that had been collected in Mexico in 1986 in the course of a scientific expedition financed by the Department of Plant Biology and Tropical Herbarium of the University of Florence. The specimens are growing in the greenhouses of the Botanical Garden of this university.

On account of the acroetal development of flowers, it is possible to identify stamen at different stages of development by simultaneously sampling buds growing at different heights. Pieces of anther approximately 2 mm in length were pre-fixed in a solution of 2.5% glutaraldehyde and 4% paraformaldehyde adjusted to pH 7.4 with phosphate buffer, fixed in 2% OsO₄ in the same buffer, and then dehydrated and embedded in epoxy resin (Luft 1961). Ultrathin sections obtained with a Reichert OM U3 ultramicrotome were stained with uranyl acetate (Gibbons & Grimstone 1960) and subsequently with lead citrate (Reynolds 1963). The observations and recording of images were performed with a Philips EM300 transmission electron microscope at 80 kV.

We must add that fertile parts of Tillandsia give only modest quality samples for TEM investigation, exactly as it happens to the vegetative shoot (Dolzmann 1964, 1965, Brighigna et al. 1981).

Observations

Within the theca of the very young anther, at which time the barely differentiated fertile cells have already laid down a thin parietal layer of callose on the outer side of the plasma membrane, the cells of the tapetum assume a prismatic form with very thin walls (Fig. 1A). Along the radial walls frequent plasmodesmata maintain symplastic connections between the cells of the tissue. The cytoplasm is highly electron-dense because of the presence of numerous ribosomes. The nucleus is large and lobate. The mitochondria are small and elongated. The plastids are also small; they contain only a few granules of starch, and their internal membranous system consists mainly of small vesicles. The dictyosomes, located for the most part in the cytoplasmic periphery, are constituted of a few budding cisternae. The vacuole is already prominent, but fragmented into numerous components. All these characteristics remain thus until the meiotic prophase of the pollen mother cells.

Fig. 1A, B — (A) tapetum; (P) plastid; (V) vacuole; (M) mitochondria; (N) nucleus; (MT) mechanical tissue; (ST) sporogenous tissue. A. The tapetum at the time when the sporogenic tissue is still undifferentiated. The tapetal cells, which have a bidentate shape, may be distinguished from those external, which are smaller and flat. The sporogenous cells reveal organelles at an early stage of development. Noteworthy in the tapetal cells are the fragmental vacuoles (note the presence of fusion figures), the prominent lobate nucleus, and the plasmodesmata that connect the cytoplasm. The sporogenous cells are already wrapped in a thin layer of callose of low electron density. x 8,100. B. The tapetum during the meiotic prophase of the mother cells of the microspores. The sporogenous cells have assumed an elongated shape here, except in areas in which plasmodesmata are present, they show prominent parietal swellings. Their vacuoles are larger and have diverse contents, while the cytoplasm is very dense. In the lower cell, two nuclei are seen. The sporogenous cell is wrapped in a thick layer of callose. x 4,600.
By the time the pollen mother cells have accomplished the deposition of the thick callistic sheath, the cells of the tapetum have elongated and assumed a radial alignment so as to maintain contact with the sporogenetic tissue (Fig. 1B). An impressive phenomenon observed in these cells concerns the irregular thickening of the walls which remain thin only in the area where a larger number of plasmodesmata are grouped together (Fig. 1B). The thickenings are more accentuated on the side of the sporogenetic tissue (Fig. 1B) and reveal a disordered disposition of electron-dense fibers within the clear matrix (Fig. 2A). The cytoplasm matrix is more electron-dense, especially at the periphery and the cells are now binucleate. The nuclei have large, spongy nucleoli, and close to them are swarms of electron-dense granules. Because of the electron density of their matrix, the mitochondria are not easily identified in the cytoplasm, but have dilated cristae. The plastids are elongated and, although they gather the vesicles near the internal membrane, now possess some osmiophilic globules; one or two starch granules constitute their carbohydrate stores. The dictyosomes cisternae produce, by budding, large, clear vesicles that move to the periphery and come into contact with the plasma membrane (Fig. 2B).

Two other aspects characterize this phase. Vacuoles enter into contiguity with the more prominent parietal thickenings of the inner periclinal wall that connects the cells of the tapetum with the sporogenetic ones (Fig. 2A); the RER profiles too are frequent in the proximity to the plasma membrane, which they border (Fig. 2C). The vacuoles tend to reunite into larger elements that have contents of variable electron density.

The locular cavity is formed as a result of the disappearance of the tangential internal walls of the tapetum cells (Fig. 2D). The maturing fertile tissue at this stage comprises tetrads that are free within the locules but sheathed by a thick layer of callus. The radial walls matrix of the tapetum cells has lost osmiophilis so that the walls give the appearance of clefs between the cytoplasm. Within those clefs, numerous aggregates of diverse shape are present, all highly electron-dense (Figs 2D, 3A); osmiophilic material is also visible on both sides of the residual segments of the central middle lamella (Fig. 3A).

The dissolution of the radial walls leads to the disappearance of cytoplasmic bridges, and thus the loss of synchrony in the development of the cells of the tapetum becomes evident. Some of the cells degenerate rapidly, while others live and continue their normal activity and development (Fig. 3B).

Fig. 3A-D — (C, callose; P, plastids; N, nucleus; V, vacuole; W, wall; D, dictyosomes; RER, rough endoplasmic reticulum). A. The tapetum during the prophase of the mother cells of the microspores. Details of a tapetum cell. Medium-sized vacuoles are in contiguity with the more prominent parietal thickenings on the side of the prothalamus cells, which are wrapped in the callous sheath. Note the dense cytoplasm, the highly-sclerified shape of the plasma membrane, and the fusion figures that characterize the vacuoles. x 11,200. B. The tapetum during the meiotic prophase of the mother cells of the microspores. Details of a tapetum cell. This segment of the radial wall has a very irregular profile and is traversed at its thickest point by plasmodesmata. Clear Golgi vesicles are moving towards the wall. Note the contact of one vesicle with the plasma membrane. x 29,700. C. The tapetum during the meiotic prophase of the mother cells of the microspores. Details of a tapetum cell. The RER profiles are numerous near the wall, and the cytoplasm is very electron-dense. x 29,700. D. The tapetum during the first stage. The digestion of the walls is already advanced, as evidenced by the intercellular electron-dense aggregates. The cells are binucleate; their vacuoles are predominantly composed of spherical elements. x 5,300.
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Before modification, long profiles of dictyosomes are grouped in the loculus. The cytoplasmic plasma membrane is visible along the radial wall (Fig. 4C, 5A). The dissolution of different deglobularized reticulum is visible in the cytoplasm, which is not covered by membranes.

The proenvelope formed by small vesicles and plastids is also visible. Organelles like cristae and osmiophilic membranes are visible in the exine of the tapetal cell.

At the first cell of the tapetal cell, the tapetum is rough endoplasmic reticulum. The electron-dense matrix is visible in the exine of the tapetal cell.

Fig. 3A-C - The first cell of the tapetum, the exine of the tapetal cell is visible.
Before other changes intervene in the sporogenic tissue, the tapetal cells undergo further modifications. In the cytoplasm, the matrix is no more homogeneously dense; bundles of ER long profiles are largely distributed. Furthermore, the clear vesicles budding from the dictyosomes have disappeared; in the central region of the plastids, the membrane are grouped together in a single, compact, central pack (Fig. 3C).

In the second phase, an extensive network of the endoplasmic reticulum, whose elements are dilated, is notable; fusion shapes characterize the vacuome (Fig. 4A). On the side of the loculus, the plasma membrane is markedly bordered with small and large scallops (Fig. 4A). The cytoplasm is enclosed by the endoplasmic network increase osmophiles and the tapetal cytoplasmic masses, which lack a parietal skeleton, impart an extremely sinuous shape to the plasma membranes (Fig. 4B).

The appearance of a large number of small, electron-dense bodies located predominantly along the radial plasma membrane characterizes a subsequent phase of the tapetal cells (Figs. 4C, 5A). This phase begins simultaneously with the end of the tetrad stage, marked by the dissolution of the callosic layer. Several vacuoles contain large membranous tangles at different degree of osmophila (Fig. 5B). Within the remaining plastids, the electron-dense globules reveal a concentric lamination that begins from the periphery (Fig. 5A). The cytoplasm, which is compressed within the extremely extensive network of the reticulum, consists of many small portions of strong electron density.

The process of maturation of the tapetum is completed with the appearance of chaotic disorder within the cytoplasmic masses (Fig. 5C), in which a disordered cluster of tubules and small vesicles represent the residue of the endoplasmic reticulum and Golgi apparatus. The plastids have disappeared, while the remains of the mitochondria (the most persistent organelles) are small, round bodies, almost always delimited by a single membrane and rarely showing cristae. Areas of variable shape and marked rarefaction appear within the cytoplasmic masses.

Subsequently, the cells of the tapetum transform into spongy aggregates of variably osmophile masses. Around them, in the locular fluid, a flocculent material appears (Fig. 5D).

At the stage of the mature pollen grains, the residua of the tapetum consist of black globules of various dimensions that are both adherent to the mechanical tissue and attached to the exine of the grains (Fig. 5E).

Discussion

The first observation that we may draw from our investigations is that in Tillandsia albida the tapetum is glandular and has an accelerated development (in relation to the life of

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Fig. 1A-C — (4, aggregate; MT, mechanical tissue; V, vacuole; L, locule; N, nucleus; D, dictyosome, RER, rough endoplasmic reticulum; M, mitochondrion; P, plastid). A. The tapetum during the tetrad stage. Details of a tapetal cell. Residual segment (arrow) of the midline lamina, whose profiles are marked by accumulations of electron-dense material, persist within the radial intercellular space, together with highly electron-dense aggregates. x 31, 200. B. The tapetum during the tetrad stage. One cell, whose cytoplasm is strongly osmophilic, is between two cells at earlier stage of development. x 4,200. C. The tapetum during the tetrad stage. The presence of long, parallel profiles of RER gathered in broad bundles characterizes the cytoplasm of the cells in this phase. The dictyosomes are abundant and compact. x 14,900.
the sporogenic tissue) with respect to the cases reported in the literature. In other words, *Tillandsia* seems to be one of those genera of monocotyledons which are characterized by a lower evolutionary stage (Pacini et al. 1985, Chiarugi 1927), in so far as the function of the tissue under examination is concerned.

On the other hand, being that the glandular tapetum is the most widespread among plants living in xeric environments (Pacini et al. 1985), this characteristic of *Tillandsia albida* may not be correlated with the evolutionary stage, but rather with its environmental life-strategy. In fact, one might think that the liquid that is collected in the locule covered by the glandular tapetum insulates the fertile cells.

The morphological variations observed in the development of the tapetum has permitted us to identify three functional phases.

The first, which can be defined as that of differentiation and maturation, comprises the assumption by the cells of a radially-oriented, elongated form and the investment of a rich, protein (enzymatic) resource suitable for a subsequent phase of secretory activity.

This first stage terminates approximately when the mother cells of the microspores come to be surrounded by a thick sheath of callose.

The second stage is characterized by aspects that indicate marked synthetic activity (by the ribosomes) and a very likely glandular activity that acts on the walls and causes their swelling and digestion. In this sense, the contribution of the vesicles of the dictyosomes indicates a granulocrine activity, but the absence of other vesicles may indicate that the product of the activity of the ribosomes (lytic enzymes) reaches the walls by means of an exocrine mechanism. The bundles of RER distributed in the cytoplasm indicate a further, more complex, protein-synthesizing activity, and contemporaneously there are signs of direct granulocrine secretion toward the locule. This activity ends simultaneously with the disappearance of the callose sheath around the tetrads, wherein the microspores have completed their endogenous exine layer.

The third stage comprises the production of small, electron-dense masses in the course of progressive cellular degeneration, which leads to generalized lysis. This is concurrent with the first phase of maturation of the free pollen grains.

During the first stage, the cells of the tapetum are attached to one another by cytoplasmic junctions (plasmodesmata), which assure synchronous differentiation. This relationship continues even when the walls undergo the process of swelling. This swelling, whether it derives its supply of new material through the marked activity of the dictyosomes, or is attributed, at least in part, to a loss of compactness of the amorphous matrix (as indicated by the markedly disordered disposition of the electron-dense fibrillar component) does not affect those segments of the walls that are traversed by grouped plasmodesmata.

Fig. 4A-C — (V, vacuole; ER, endoplasmic reticulum; N, nucleus; M, mitochondria; P, plastid; I, locule; Mt, mechanical tissue). A. The tapetum during the young microspore stage. Details of a tapetal cell. It completely lacks the inner tangential wall, and the plasma membrane profile reveals several marked scallops. The cytoplasm is crossed by a widespread network of stunted endoplasmic reticulum. × 7,000. B. The tapetum during the young microspore stage. Details of a tapetal cell that demonstrates the marked sinuosity of the intercellular spaces. The ER is laden with contents of medium electron density. × 9,000. C. The tapetum at the microspore stage. The cytoplasm contained within the compact ER network is markedly eosinophilic. The appearance of small, eosinophilic bodies (arrows) which make contact with the plasma membrane is significant. × 8,000.
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Plasmodesmata are initially present also between the cells of the tapetum and those of the sporogenic tissue, but the initiation of the deposition of callose by the latter interrupts the junctions. An analogous behaviour has been observed by Pacini & Juniper (1979) in the secretory tapetum of *Olea europaea*.

The irregular, lobate form of the nucleus and its duplication testify to the secretory type of the tapetal cells and to the necessity for rapid, efficient interrelations between the nucleus and cytoplasm, which are achieved by an increment in the surface area of exchange. The sponginess of the prominent nuclear masses and the swarms of electron-dense granules extruded by them are related to the large increment of the number of ribosomes required for the protein synthesis. The spatial contiguity of some of the vacuoles with the most marked parietal swellings may be interpreted as a sign of enzymatic activity directed at the walls. Nonetheless, one cannot disregard the possibility that such parietal swellings represent a transfer modification as a function of transportation over a short distance toward the fertile tissue, analogous to what Marquardt et al. (1968) suggests about the parietal swellings of the tapetum of *Paeonia*.

The second functional stage of the cells of the tapetum is characterized by a premature loss of synchrony. In fact, the cells are at different stages of development and have varying cytoplasmic osmophilic, probably as a result of the disappearance of symplastic connections (plasmodesmata). The digestion of the substances that constitute the walls is evidenced by the low osmophilic spaces between the radial plasma membranes and by the fact that the internal tangential plasma membrane directly delimits the locular cavity because of the disappearance of the walls.

The enzymatic attack on the walls, which is indicated by the presence in the peripheral cytoplasm of cisternae of ER parallel to the plasma membrane, leads to the appearance of diverse electron-dense materials distributed in the intercellular space. Although we cannot exclude the implication of a variety of substances, accurate observation of the different electron-dense bodies has led us to consider the majority of them as successive stages in the aggregation of materials of parietal origin. This is likely because electron-dense debris deposited on fragments of median lamella and porous granular aggregates predominate at first; then, the small, compact masses, some of which are sectioned again diametrically by the residua of the median lamella, are more numerous. Fig. 6 is a reconstruction of the dynamics of formation of the electron-dense bodies.
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Undoubtedly, the degradation of the walls discharges into the loculus a sugar supply, which is available for the fertile cells. However, since Eschlin (1971) denies the possibility that molecules larger than the trioses can penetrate the callose filter and Southworth (1971) has demonstrated the incorporation of glucose in the callose wall (which acts as a barrier) only at a later time would the cytoplasm of the fertile cells benefit from most of the products of the parietal hydrolysis of the tapetum. Thus, it is the locular fluid that is initially enriched by the sugar supply.

The appearance of broad bundles of parallel ER profiles indicates prominent protein synthesis, which must be associated with the secretory activity that the cells of the tapetum initiate. This synthesis of protein precedes the manifestation of the wide network of dilated reticulum (Fig. 4B) and the subsequent osmiophilic alteration of the interconnected cytoplasm (Fig. 5B), and is hence connected to the autolytic mechanism that perhaps provides a role also for the dictyosomes in the distribution of the enzymes. The merocrine activity, which is indicated by the vesicles that make contact with the plasma membrane and subsequently open, discharging their contents to the outside, is manifestly simultaneously with the autolytic phenomenon and is connected with the concurrent degradation of the callose wall of the tetrad, at the conclusion of which the microspores are liberated. Of the synthesis of callose, precise ultrastructural proof is lacking in the literature. The production of callose by the tapetum has been described by Mepham & Lane (1969), but the literature does not go beyond the citation (Pacini & Juniper 1979) of the noteworthy presence of elements of RER.

Fig. 6 — Pathway of the formation of electron-dense aggregates of parietal origin.
The disappearance of amylaceous reserves and the high number of mitochondria completes the description of the intense cellular activity of this phase.

The third functional stage of the tapetum is characterized initially by the appearance of small, electron-dense bodies located along the plasma membrane. Their morphology and location identify them as orbiculi (Echlin 1971, Heslop-Harrison & Dickinson 1969). Their appearance occurs simultaneously with the reduction of the osmiophilia of the plastid matrix and of their globules and with the presence in the peripheral cytoplasm of vacuoles that also contain small, electron-dense masses and membranous tangles.

In reference to the formation of the orbiculi, Brooks & Shaw (1968, 1971) have shown that the xanthophylls of plastidial origin are precursors of the sporopollenin, which is one of the essential components of the orbiculi.

In Tilletia, the formation of orbiculi is manifested at the end of the tetrad stage of the sporogenic tissue, which is analogous to what has been observed in Lilium by Heslop-Harrison & Dickinson (1969). Moreover, these authors assign the role of precursor of the orbicicular sporopollenin to the carotenoids of the chloroplasts of the tissues most external to the tapetum, denying it to the cells of the tapetum themselves.

Lipids and carotenoids of the plastids found in the tapetum also contribute to the formation of the pollen grain (Hesse 1989, Echlin & Goldin 1968).

After the formation of the orbiculi and in concomitance with the separation of the pollen grains that are liberated within the anther, the degenerative process of the tapetum appears generalized and irreversible. The mitochondria, which have lost almost all their crests, are nevertheless the most persistent class of organelles, maintaining a limiting membrane for a fairly long time; this appearance has also been observed in Antirrhinum majus by Lombardo & Caramo (1976). The plastids, on the other hand, rapidly tend to commix with the cytoplasmic mass.

This cytoplasmic degeneration itself represents an act of secretion that may be defined as holocrine.

With the generalized osmiophilia of the cellular materials, a fine, floccular, electron-dense content is scattered into the lumen. This represents the last secretory action of the hydrophilic material.

When the pollen grains achieve complete maturation (the stage of the young gametophyte), residual electron-dense and amorphous tapetal globes of variable size and shape appear. They are adherent to the mechanical tissue and are distributed randomly among the isolated grains, more or less in contact with the ectine.

This material represents the lipophilic tapetal residue, which, as is known, is the principle component of the pollen grain.

In conclusion, the tapetum of T. albida, even if it has peculiar ultrastructural aspects such as the premature loss of synchrony in the function of its cells, follows a development that is analogous, in its fundamental lines, to other secretory tapeta. Nonetheless, it is worth noting the rapidity with which its functions are carried out as compared to the sporogenic processes. In plants under extreme environments, such as T. albida, rapid execution of biological processes is probably one of the exponents of survival.

The tapetum has been identified as the locus of a possible cause of infertility by Chiurugi (1927) in Achillea clavuata (Asteraceae), in which the occasionally unsuccessful degeneration
of its periplasmal tissue leads to the digestion of the pollen grains in a pollen sac or in an entire flower.

The premature cellular asynchrony that we have observed in T. albidus and the secretory phase that is premature with respect to the development of the fertile cells may be judged to be causes for a different type of tapetal dysfunction, and thus does not facilitate the normal development of some of the pollen grains, as the high percentage of nonviable grains indicates. This condition is undoubtedly of determinant significance in the difficulty of the species in enlarging its area of distribution.

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