PLASTIDIAL DYNAMICS DURING POLLEN DEVELOPMENT IN 
*TILLANDSIA ALBIDA* MEZ & PURPURS (BROMELIACEAE) 
BEFORE ANTHESIS1

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Abstract

Since the literature regarding the changes that occur in the plastids organization during the pollen development is not adequate, we present our ultrastructural observations on the behaviour of the plastids during the ontogeny of the pollen grain of the monocotyleden *Tillandsia albida* Mez & Purpur (Bromeliaceae). In *T. albida* the plastidal inheritance was maternal, of the Lycopericium type, and the male gametophyte at the time of dispersion was bicellular. Only one amyloplastos was observed, important in quantity. The amyloysis process took place before anthesis and preceded the protein synthesis and the building of the membranous material that were necessary to prepare the gametophyte for a fast fertilization process.

Key Words: *Tillandsia*, pollen development, plastids, ultrastructure.

The ultrastructural organization which characterizes the different ontogenetic stages of the male gametophyte of higher plants is well investigated (Heslop-Harrison 1968, Pacini & Juniper 1979, Brighigna et al. 1981, Croft et al. 1985), but the changes in the structure of plastids during microsporogenesis are specifically discussed only for a few of plants (Pacini & Franchi 1988, Pacini et al. 1992). During ultrastructural studies in the tissues involved in the microsporogenous process of *Tillandsia*, a genus of epiphytic neotropical monocotyledonous family Bromeliaceae, we observed the behaviour of plastids during the maturation of the pollen grain of *Tillandsia albida* Mez & Purpur, a rare mexican species. We present here an account of amyloplastos and amyloysis.

Material and Methods

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

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On account of the acropetal development of flowers in the inflorescence, it is possible to test stamens at different stages of development by simultaneously sampling buds occurring at different heights. Anther pieces, approximately 2 mm in length, were pre-fixed in a mixture of 2.5% glutaraldehyde and 4% paraformaldehyde adjusted to pH 7.4 with phosphate buffer, post-fixed in 2% OsO₄ in the same buffer. The fixed material was dehydrated and embedded in epoxy resin (Luff 1961). Ultrathin sections cut on a Reichert OM U3 ultramicrotome were stained with uranyl acetate (Gibbons & Grimstone 1960) followed by lead citrate (Reynolds 1963). The observations and photographs were done on a Philips EM 300 transmission electron microscope at 80 kV. 2 μ sections were tested with PAS reaction after aldehyde blockade to localize the total polysaccharides.

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

Observations

Initially spongy parenchyma showed young small plastids, cylindrical in shape, showing highly osmiophilic matrix characterized by the presence of many peripheral vesicles. Frequent were the images of the organelle's division such as constriction after the appearance of a thin central cord (Fig. 1A). These poorly differentiated plastid lacked starch and were similar to those present in the surrounding tissues.

The plastids in the micropore mother cell were located in the cytoplasmic periphery. They had a markedly ameboid shape contrary to the established form and figure and often showed cup-like invaginations that included fractions of the cellular cytoplasm. They do not contain any polysaccharide material (Fig. 1B) and had increased in number due to divisions already mentioned.

Successively, during the last phases of the meiotic division that precede the tetrad stage, the plastids had assumed a very different shape; these were like big irregular sacks that enclosed 3-4 bags (sectors) delimited by membranes which had a pronounced asymmetrical stratification in three layers. The content of the bags was more electron-transparent than the matrix of the plastid and was even electron-transparent in some places. Small flat thylakoidal vesicles and accumulations of spongy dark material were present in the matrix between the bags (Fig. 1C). Observation of thin sections under light microscopy showed that even the cytoplasm of the mother cells had a positive reaction to the PAS test.

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Fig. 1A-D — (C, callose; E, exine; F, intine; M, mitochondria; N, nucleolus; P, plastid; W, wall) A. Undifferentiated spongy parenchyma. The plastids are small, elongated and contain many peripheral vesicles into the very osmiophilic matrix. Images of division by constriction (arrow) can be observed. x 13,400. B. Micropore mother cell. The plastids have an accentuated ameboid shape. Within their matrix starch is not present. x 13,400. C. Micropore mother cell during meiosis. The plastids contain 3-4 bags (sectors) delimited by membranes and containing scarcely electron-dense material. In the matrix small, flat thylakoids and a sponge-like material is visible among the bags. The formation of the cellular plate is well visible (arrow). x 7,300. D. Tetrad stage. The columella-like of the exine are already visible. The bags (sectors) within the plastids have lost their electron-transparent content and appear to contain an amorphous material of heterogeneous density and medium electron density. x 12,700.
possible occurring in a procedure and then being done with a silver nitrate solution after treatment with TEM 300 solution after 1 hour.

showing frequent division of a thin wall material to the periphery, and often do not divide

stage, quick that matrical than the lakeoidal been the given the

W, walls thick into spheroids x 13,400, trace and is visible somehow at conot 700.

Fig. 1A-D
Later, during the late tetrad stage, when the intine becomes multilayered and the columnella of the exine were evident, the bags inside the plastids had lost the electron-transparent areas and contained, instead, an amorphous material of heterogeneous granularity and of medium electron-density (Fig. 1D).

During the first (laccal) haploid mitosis, the generative cell that was attached to the intine lacked plastids. The cytoplasm of the vegetative cell, instead, showed numerous big leucoplasts, completely filled with starch grains of variable electron-density (Fig. 2A), and several lipid droplets.

After the detachment of the generative cell from the intine, the plastids in the vegetative cytoplasm started to break into fragments (Fig. 2B), and the carbohydrates stored in the resulting little organelles started degeneration progressively.

Subsequently, when the generative cell had assumed an elongated shape, these small plastids, that showed just a little content of starch and more osmiophilic matrix (Fig. 2C), were profiled with long RER elements. At the same time, the small vacuoles had come into contact with the lipid droplets thus forming aggregates of electron-dense material in the form of myelin-like sheets (Fig. 2D). The progressive disappearance of the carbohydrate storages (polysaccharides and lipids) was contemporaneous to the increase of the myelin-like forms. This phase preceded anthesis.

Discussion

Only one amylogenesis was observed during the ontogeny of *T. albida* pollen grain. This datum is concordant with what has been reported for *Tillandsia palidiflora* (Ihes 1951), but it does not follow the scheme valid for monospermoids which undergo more than two amylegenetic phases (Pacini & Franchi 1988). Since *Tillandsia* retain the male gametophyte at the bicellular stage, it is very likely that the greater number of amylogenesis observed in the other monospermoids is related to the formation of the three-celled male gametophyte that characterizes these plants in which as many as two haploid divisions occur before the pollen grain maturation. The occurrence of one only amylogenesis has to be considered as the signal of the rapid and early maturation of the pollen.

At the end of the meiotic division in *T. albida*, plastids abandon their amoboid shape, characteristic of the growth in absence of light (Thomson & Whatley 1980), and envelope portions of cytoplasm into a limited number of membrane-bound bags. Since the carbohydrates are necessary to the micropore for the synthesis of the intine, which occurs

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**Fig. 2A-D** — (E, exine; OC, generative cell; I, intine; L, lipid droplet; M, microbody; N, metacolon; P, plastid; RER, rough endoplasmic reticulum; SK, skirn; V, vacuole). A. After the first haploid division the generative cell does not contain plastids. Many big leucoplasts filled with starch grains are present in the vegetative cytoplasm. Lipid droplets are also present. Arrows indicate the very thin wall of the generative cell. x 5,600. B. When the generative cell has detached from the grain wall the plastids of the vegetative cytoplasm show constriction images. x 6,900. C. Pollen after the elongation of the generative cell. The vegetative cytoplasm has a more osmiophilic matrix and is profiled with RER elements. The presence of starch granules is reduced. Arrows indicate the very thin wall of the generative cell. x 20,000. D. Same phase as C. The lipid droplets of the vegetative cytoplasm come frequently into contact with small vacuoles, thus forming an accumulation of strongly electron-dense myelin-like sheets, in various shapes. x 27,900.
The small (2C) nuclei form tetrads.

The pollen grain

flattens

towards the male

genesis

and the outer

envelope

decays

(schizogenous, P)

towards the vegetative

plastids

and dense cytoplasm.

In the filament, the pollen grains indicate to develop a more

dense cytoplasm.

Fig. 2A-D
when the tetrad is still enveloped by the colpus barrier, we believe that an enzymatic
activity of the plastids on the cytoplasmic portions segregated into the bags could recover molecules
useful for that purpose. Generally, during the earlier stages of the sporogenesis, plastids lack
any storage products (Van Went & Cresti 1989). In contrast, plastids in *T. pallidifloraens*
(Hasl 1991), show ultrastructural complexity and stromatic accumulations of polysaccharides,
thus confirming the peculiarities of these epiphytic plants. On the other hand, the cytochemical
test showed the presence of soluble glucosides even in the cytoplasm of the mother cells,
evidently a surplus (of sporophytic origin) is not consumed for the internal metabolism or for
the synthesis of the callosic layer. After the formation of the callosic plaque, that acts as a
molecular filter (Southworth 1971, Brigighina & Papini 1993), the passage of carbohydrates
of exogenous origin to the micropore is interrupted. It is therefore, the locule, delimited by
glandular tapetum, that functions as a temporary storage site for the exogenous carbohydrates included those transported across the tapetum from the most external tissues
(chlorenchyma) to the inner part of the anther lobe (Pacini & Franchi 1988, 1991, Brigighina
& Papini 1993).

The disappearance of the content of the bags and successively of the bags themselves,
occur during the formation of pectocellulosic layer of the intine in pollen grain wall.

In *T. albida*, the starch accumulation in the plastids of the vegetative cell of young
bi-cellular pollen grain is evident. This phenomenon might be connected with the antecedent
demolition of the tapetum as has been suggested by Huijuan & Yanzhi (1994) for *Gentiana
macrophylla*. All the glucosides materials, originated from the sporophytic tissue and
previously stored in the locale fluid, is now transported inside the grain for polymerization
inside the plastids and/or immobilized as lipid droplets in the cytoplasm. These storage
products are successively used (beginning with starch and ending with lipids) as metabolic
'fuel' for the several proteic syntheses that are necessary to prepare the pollen for its
fertilization activity, and as the required material for the formation of the pollen tube.
The appearance of the myelin-like sheaths inside the vacuoles of the vegetative cell occurs
contemporaneously to the demilition of the plastids and follows the consumption of the lipidic
stores. This indicates that the cell prepares in advance the membraneous stores, to provide
for the growth of the pollen tube that is characteristically rapid (a few hours time) in many
Tilliandus (Francini Corti 1981).

If the absence of digestion of the plastidial starch has to be considered as a cause that
leads to the male sterility in *Tobacco* cybrid (Pollak 1992), the exhaustive degradation of
glucosidic stores in *T. albida* makes us think that at least from this point of view the
development of its pollen should regularly continue.

Our investigations allow us to make a final consideration: the total absence of plastids
in the generative cytoplasm of *T. albida* indicates that the plastidial heredity is maternal and
of the Lycopersicon type (Schroeder 1984, 1985).

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