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Embryology of Flowering Plants Applied to Cytogenetic Studies on Meiosis

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1. Introduction

The present chapter has three specific goals: (1) to discuss the incongruencies in terminology applied to meiosis and to pollen grains, (2) to describe cellular and subcellular aspects of plant meiosis and pollen, and (3) to review cytogenetic studies in plant meiosis involving embryological approaches.

The life cycle of plants is constituted by two generations: sporophytic and gametophytic. Gametophytic generation is the sexual generation. Differently from meiosis in animals, which gives rise to gametes, meiosis in plants originates spores. Heterospory occurs in some pterydophytes and in seed plants. This consists of the formation of two types of spores in separate sporangia (androsporangium and gynosporangium). In angiosperms, when meiosis occurs in anther sporangia, the spores are called androspores. When it occurs in seminal rudiment sporangia, they are called gynospores. The sporogenesis develops in a complete endosporic manner. In the case of gynospores formed, generally only one is viable in each sporangium. The viable spore germinates and, after three mitotic divisions, forms the female gametophyte, which develops in the sporangium tissue - the nucellus. During the development of the male gametophyte, the first mitosis occurs inside the sporangium, the other ones may occur after male gametophyte release. The androgametophyte is called pollen grain and the gynogametophyte is called embryo sac. The two sperm cells formed in the second mitosis of the male gametophyte, are the male gametes. They are present in the tricellular pollen grain or after mitosis of the generative cells during the pollen tube germination. The female gametes are called egg cell and central cell. In this way, sexual

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generation in flowering plants is reduced to few cells and nutritionally dependent on the sporophyte. All the structures of the plant body, except the pollen grain and the embryo sac belong to the sporophytic generation.

We must not confuse or mix the different plant generations based on Hofmeister's alternation of generations (1851). When we analyze meiosis we are analyzing the reproductive results of the sporophyte, with the genetic recombinations present in the spores. No relation with sexuality was needed to obtain the expected result from a sporophytic generation – only SPORITY – androspores or gynospores, maintaining the reference to its localization in the androceum or gynoeceum in the word etymology, but without a sexuated connotation.

On the other hand SEXUALITY is present in the gametophytes, a generation that produces the male and female gametes, in individuals that are separate and thus unisexuated. They form, through fertilization, the new sporophytic generation (embryo) and the xenophytic generation (endosperm). The xenophyte is an accessory generation responsible for nourishing the embryo that is being formed, not directly connected to the reproductive cycle, since it never manages to produce its own reproductive structures.

In this chapter we will discuss the terminology applied to meiosis and to pollen grains, in accordance with the spority and sexuality of generations, the cellular and subcellular aspects of these structures, and cases of cytogenetic studies involving embryological approaches.

2. Terminology applied to meiosis in plants and pollen grains

The scientific investigation of plant sexuality began in the 17th century with Rudolph Camerarius (1694) in his work De Sexu Plantarum Epistola. Camerarius was the first to prove the existence of sex in plants. His discovery was a Copernicus-like event for Botany, and for several fields of biology (Zàrsky & Tupy 1995). According to Cocucci (1969), Camerarius work was preceded by Nehemiah Grew (1682) that described the pollen, and Marcello Malpighi (1687) that described ovary and ovules, nowadays, known as seminal rudiment. Camerarius mistakenly identified sexuality in sporophytic structures, since he established that the stamina and pistils were male and female "organs". Carolus Linnaeus established a classification system that includes two basic principles: the use of Latinized or Latin words to name groups of organisms, as well as the use of categories that distribute the organisms from large to limited groupings. Linnaeus (1810) created the "Sexual System" positioning the plants with flowers in twenty-four classes based on the number of stamina and pistils. According to Quammen (2007) descriptions of flowers were compared to sexual relations among human beings, causing polemic and scandal in the society of his time. The *Fuchsia* flower, for instance, was classified in this system containing eight male stamina around a female pistil, belonging to class 8, described as 'eight men in the nuptial chamber of only one woman'.

In 18th century, Hedwig (1784) thought the antheridia of mosses to be equivalent to anthers (Wagenitz 1999). In the 19th century, Hofmeister (1851) published the theory of alternation of generations, accepting that there could be two generations for briophytes and pterydophytes, one of them sporophytic and the other gametophytic.

Darwin (1877) was the first to discuss the presence of different floral types in plants of the same species, and called the flower hermaphrodites, female and male. The terminologies adopted by Darwin, attributing sexuality to flowers, are used in biology even today, in a large part of scientific production. (Richards 1997; Ainsworth 1999; Barrett 2002; Mitchell & Diggle 2005; Karasawa 2009).

The Hofmeister's work, considered as genial as that of Mendel or Darwin, was not widely disseminated in scientific circles (Kaplan & Cooke 1996). Scholars in the field of biology, taking Darwin as their primordial, guiding assumption (Cohen 2010), commonly observe a flower from the perspective of Camerarius and Linnaeus, as a sex organ, attributing sexuality to the sporophyte. The terms female flower and male flower are wrong, since they include both sporophytically originated structures, to which no sex is ascribed (calyx, corolla, androecium and gynoecium), and gametophytic structures that express sexuality such as the female gametophyte (embryo sac) and the male one (pollen grain). Further criticism of the term hermaphrodict is that every gametophyte in angiosperms and gymnosperms is unisexual, and in turn, hermophroditism is a condition present until isospore pterydophytes along the evolutionary scale of plants, in which a single spore gives rise to a prothallus with male and female gametangia. (Cocucci 1969; 1973; 1980; Cocucci & Hunziker 1994; Cruden & Lloyd 1995; Cocucci & Mariath 1995).

The terms 'female', 'male' and 'hermaphrodite' (Camerarius 1694; Linnaeus 1754, 1810; Darwin 1877) should be replaced by "imperfect pistillate", "imperfect staminate" and "perfect", respectively (Cocucci & Mariath 1995; Cocucci 1980; Greyson 1994; Cruden & Lloyd 1995). These terms are utilized by the APG (2007). This is due to the fact that the flower constitutes a sporophytic structure holding one or two gametophytes that depend nutritionally from the sporophyte (Coccuci 1973).

Based on these assumptions, Cocucci & Mariath (1995) propose to name flowers as: "monosporic" when only one kind of sporangium developed, producing androspores or gynospores; and "bisporic", when the flower presents the two types of sporangia, producing androspores and gynospores. The present study adopts the terms perfect flower, imperfect pistillate and imperfect staminate, due to their frequent use in current scientific literature, avoiding an inappropriate sexual connotation.

Despite the conceptual issue of considering flowers as a structure that includes tissues of the sporophytic and gametophytic generation, studies on their evolution have developed greatly, and are currently one of the topics discussed in the fields of evolution, ecology and genetics. Charles Darwin (1877) recognized that plants with seeds have an incredible diversity of reproductive systems, a 'sexual diversification' that is determined by ecological and genetic factors that are one of the core problems of evolutionary biology. The integration of phylogeny, ecology and studies on population genetics has supplied new ideas regarding the selection mechanisms that are responsible for the greatest evolutionary transitions between the modes of reproduction (Barrett 2002).

However, in our view, this so-called 'sexual diversity' is actually the expression of the 'spority' of the antophytes, a diversification that includes flowers with different morphologies, and may carry both functional sporangia, androgynosporangiates (perfect flower), or only one of these two types, androsporangiate (androic) or gynosporangiate (ginoic), or other combinations of these sporangia (monoic, dioic, trimonoic, andromonoic and gynomonoic).

As regards the gametophytic generation, based on the heterospored pterydophytes, the gametophytes are always unisexuate, and they are never hermaphrodite or bisexuate. Cocucci & Mariath (1995) and Cocucci (2006), within the sphere of studies on plant reproduction and improvement, propose to limit the sexual terminology to the gametophytes, erradicating the sexual terminology applied to sporophytes.

3. Meiosis and pollen grain: Cellular and subcellular aspects

The life cycle of plants consists of two generations: a sporophytic, diploid, and a gametophytic, haploid (Hofmeister 1851; Cocucci 1969; Cocucci & Mariath 1995). In seeds plants, gametophytic and thus sexuated generation begins from a spore that develops a multicellular structure called gametophyte through mitotic divisions. If this process occurs in the androecium, the male gametophyte is called androgametophyte, microgametophyte, androphyte or pollen grain. If it occurs in the gynoecium, the female gametophyte is called gynogametophyte, megagametophyte, gynophyte or embryo sac. Therefore, in the seed plants, the gametophytic sexuated generation is small and depends nutritionally on the sporophyte, and the morphological structures, except the embryo sac and the pollen grains, belongs to the sporophytic generation (Cocucci 1969; Cocucci & Hunziker 1994; Cocucci & Mariath 1995).

As a case study we shall analyze *Passiflora elegans* Master (Passifloraceae), a species of passion flower native to Brazil, regarding aspects of the development of androsporogenesis and androgametogenesis, divided into four stages described below.

Stage I – Sporangium and archesporial cells. The completely formed young anther has four sporangia, two of them in the ventral-lateral regions and the other ones in the dorsal-lateral regions (Figure 1 A). The epidermal cells, as well as the endothecium and middle layers, present vacuolated cells with conspicuous nuclei (Figure 1B). The epidermis and endothecium are indifferentiated at this stage. The middle layers present nuclei with portions of condensed chromatin and small vacuoles in the cytoplasm. The tapetal cells are radially elongated, multinucleated, their nuclei have conspicuous nucleoli, the cytoplasm is dense, with the presence of small vacuoles (Figure 1B). The archesporial cells are the largest cells of the sporangium, the nuclei are hyaline, with conspicuous nucleoli, and few portions of condensed chromatin, the cytoplasm presents storage lipids granules (Figure 1B) which, through their oxidation, ensure the energy needed to trigger the meiotic process.

Stage II – Meiosis and the end of sporophytic generation. Concomitantly, when meiosis begins, deposition of a callose wall begins on the inner side of the primary wall of the androspore mother cell. Individual isolation of the young androspores occurs through the formation of a callose wall, only after the tetranucleated phase, thus characterizing cytokinesis as being of the simultaneous type (Figure 2A-H). The tetrads present a tetrahedric spatial arrangement (Figure 2E,G-H). The sporoderm begins to be deposited around the future androspores, already in the tetrad phase, through the formation of the primexine that is initially internal to the callose wall. This wall is constituted by polysaccharide and protein.

The deposition of sporopollenin inside the tetrad occurs after the primexine wall is formed, and it was confirmed with Auramine O (Figure 2G). The reticulated aspect of the future exine is noted when it is submitted to analysis with differential interference contrast. (Figure 2H).

In monocotyledons, the most common cytokinesis is of the successive type, as demonstrated in *Pitcairnia encholirioides* (Bromeliaceae) (Figure 3A-C). After telophase I, the androspore mother cell gives rise to a dyad of androspores (Figure 3B). A callose wall is syntethized between the dyad of androspores. Meiosis II takes course forming the androspore tetrads, mostly with an isobilateral arrangement (Figure 3C).

Stage III – Beginning of the gametophytic phase (unicellular gametophyte). The young androspores are released from the tetrads inside the anther loculus and take on a spherical shape (Figure 2I). The androspores present a large hyaline nucleus, with a conspicuous

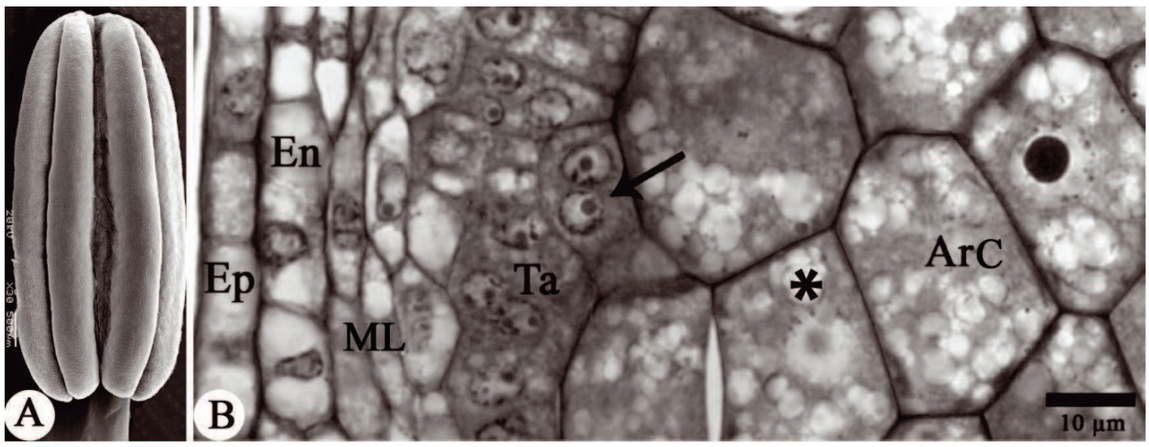

Fig. 1. Young anther of *Passiflora elegans* at the pre-meiotic stage. (A) Tetrasporangiated anther under scanning electron microscopy. (B) Cross-section of anther in pre-meiosis with archesporial cells. (Ep) epidermis; (En) Endothecium; (ML) middle layer; (ArC) Archesporial cell; (*) storage granules with lipidic nature.

nucleolus, and a large vacuole inside them (Figure 2I), signaling the beginning of gametogenesis. Sporoderm formation begins during the tetrad phase and in the free androspore phase the exine presents two stracta (ectexine and endexine) (Figure 4A). The ectexine, in *Passiflora elegans,* does not present a basal layer, so that the collumelae are plunged directly into the endexine. The intine, as well as the exine, are thick in *P. elegans* and are constituted by three chemically distinct pectic-proteic-cellulosic strata (Figure 4a). The cytoplasm presents a dense aspect and concentrates its largest volume at the polarized nucleus. After polarization, the androspore nucleus undergoes mitotic division forming the vegetative cell (VC), and the generative cell (GC) (Figure 4B). The tapetal cells, which previously were elongated and organized around the loculus, present irregular contours and degrade at the end of meiosis.

Stage IV – Bicellular pollen grain (bicellular gametophyte). Once the mitotic division has occurred, the central vacuole becomes smaller and the vegetative nucleus returns to the median portion of the cytoplasm, while the generative cell is kept in a parietal position. The wall separating the two cells is continuous with the intine. The vegetative cell includes the generative cell, acquiring a shape that ranges from lenticular to sickle-like (Figure 4B). From this phase on, the vegetative cells accumulates a large quantity of starch in their cytoplasm. This starch is distinctly hydrolyzed during the differentiation of pollen, so that different species can present pollen with or without starch during anthesis. In cases in which the starch is hydrolyzed, the cytoplasm is reactive to the PAS reaction while in the others, without hydrolysis, there is a weaker PAS reaction of the cytoplasm. The bicellular mature pollen grain, in cross-section, presents a hyaline vegetative nucleus, with a rounded shape and a conspicuous nucleolus. The generative cell is found immediately next to it and has an elongated shape (Figure 4B), in longitudinal section and a roundish cross-section, and a nucleus with portions of condensed chromatin (Figure 4B). Depending on the plant analyzed, the pollen grain is released in this bicellular form, completing the formation of gamete cells along the germinated pollen tube, as a result fo the mitosis of the generative cell (Figure 4D).

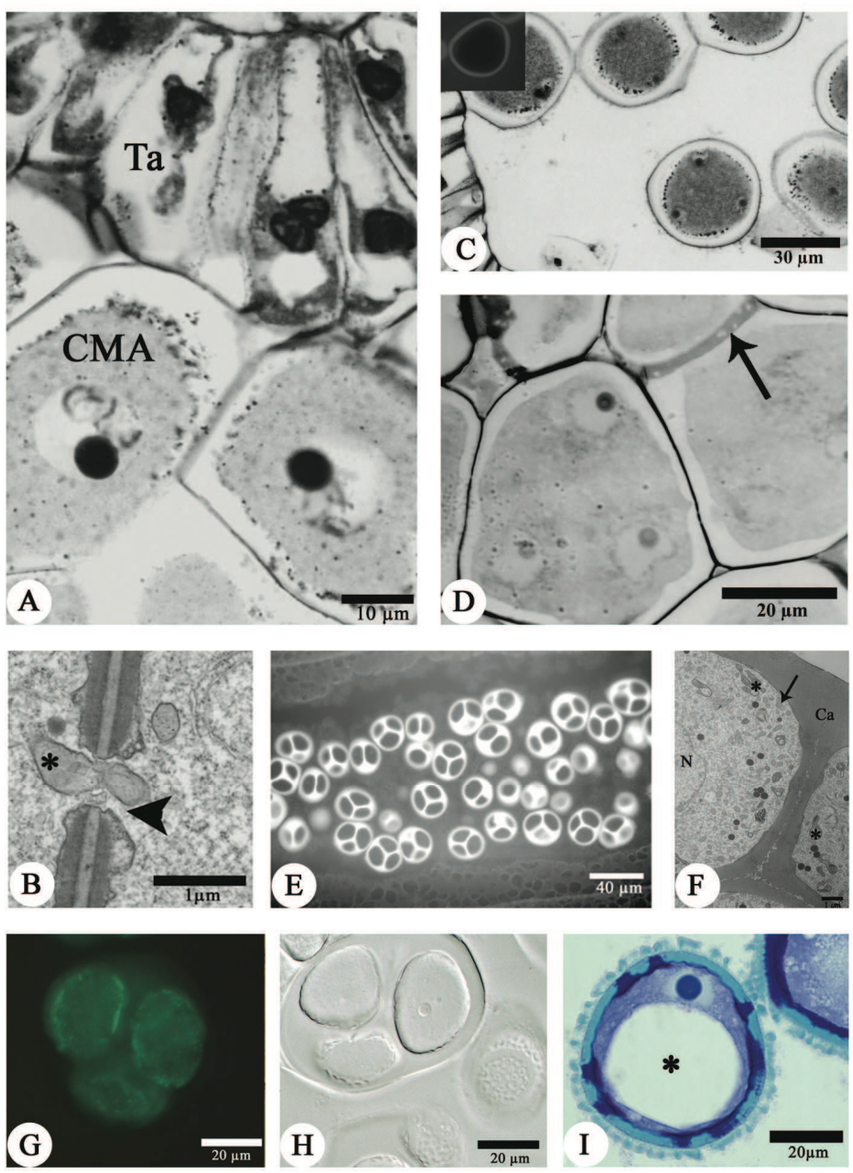

Fig. 2. Young anther of *Passiflora elegans* in meiotic and post-meiotic stage. (A) Androspores mother cells in prophase I. (B) Electron micrography of a cytomitic channel between androspores mother cells with the transit of plastids during meiosis. * means plastid, and arrowhead means cytomitic channel. (C) Meiocytes in telophase II. Insert reveals positive reaction to callosis with aniline Blue under fluorescence microscopy. (D) Cytomitic channels between meiocytes (arrow). (E) Tetrad after simultaneous cytokinesis, with callose between the androspores, marked with Aniline Blue under fluorescence microscopy. (F) Electron micrography of the callose wall involving the androspores in the tetrad. * means plastid, and arrow means mitochondria. (G) Androspores tetrad with exine deposited in the primexine, below the callose walls. Tetrad stained with Auramine O and observed under fluorescence microscopy. (H) Androspores tetrad in differential interference contrast, showing the reticulated aspect of the exine. (I) Androspore released from the tetrad in a vacuolated state (asterisk) stained with Toluidine Blue O.

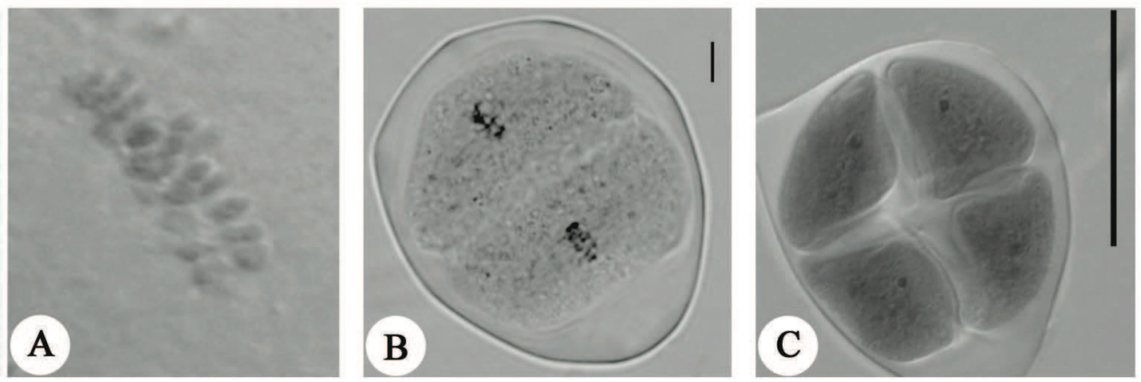

Fig. 3. Successive cytokinesis in P*itcairnia encholirioides*. (A) Androspore mother cell, metaphase I (B) Androspores dyad in metaphase II . (C) Androspores tetrad at the end of meiosis. Scale: 3A-B =20µm; 3C=50µm.

4. Cytogenetic studies involving embryological approaches

4.1 Cytogenetic and embryological analyses of sporophytic sterility and gametophytic sterility, in flowering plants

Staminal sterility, also known in agronomic and biotechnological spheres as "male-sterility", consists in the lack of some stage of androsporogenesis and androgemetogenesis, which leads to the non-formation of a viable androphyte. A viable androphyte is considered to be a pollen grain morphologically typical and metabolically active that can emit a pollen tube (Dafni & Firmage 2000; Duarte-Silva et al. 2011). "Male sterility" would be a more appropriate term to designate only sterility events related to failures in androgametogenesis, without attributing sex to sporophytic phases (Cocucci & Mariath 1995). Since the term is used in the literature equally for any failure in the development of the anther and of the pollen grain, in the present study we will adopt the term 'staminal sterility' for failures in the initial stages of androsporogenesis (sporophytic sterility) and 'pollen sterility' for failures in stages after sporoderm formation (gametophytic sterility). The detection and investigation of staminal sterility in edible and medicinal plants are highly important, since they help improve the plant, because they are naturally free of self-pollination and therefore more easily manipulated in reproductive system experiments to produce strains with a given character of interest (Bhat et al. 2005). The study of androsporogenesis and androgametogenesis in cases of staminal sterility can determine the stage when spore development (in the sporophytic phase) or the pollen grain (in the gametophytic phase) cease. Besides, together with the tools of cytogenetics, immunocytochemistry, and transmission electron microscopy, it allows inferring the cause of this failure. Once the critical stage for the occurrence of staminal sterility has been identified, molecular biology studies can be performed to detect the genes involved in the expression of sterility. Embryological studies to investigate staminal sterility are common in widely sold edible plants, such as soy, wheat, rice, beans and maize, and little studied in medicinal plants used in the phytotherapeutic industry (Duarte-Silva et al. 2010).

Staminode formation is caused by different embryological events, motivated by equally diverse environmental and genetic factors. Different genes act on each stage of embryo development, and their failures may lead to the same result: stamen and pollen sterility.

Mutants *bam1* and *bam2,* of *Arabidopsis* do not develop the endothecium, middle layer and tapetum cells, and the mother cells of androspores, in turn, degenerate before meiosis (Hord et al., 2006).

Many cases of staminal sterility are related to tapetum problems. The function of the tapetum is related to the the synthesis of enzymes needed to separate the androspores from the tetrad and also to nourish the pollen grains (Goldberg et al., 1993). Tapetum degeneration occurs normally at the late stages of pollen grain development and it has been considered a process of programmed cell death or apoptosis (Papini et al., 1999; Li *et al.* 2006). However, apoptosis, early or late, leads to staminal sterility events, as in rice (*Oriza sativa* L.) where tapetum degradation occurs late, obliterating the anther locule and causing the androspores to collapse (Li et al., 2006). Already in mutant BR 97-17971, of *Glycine max*,

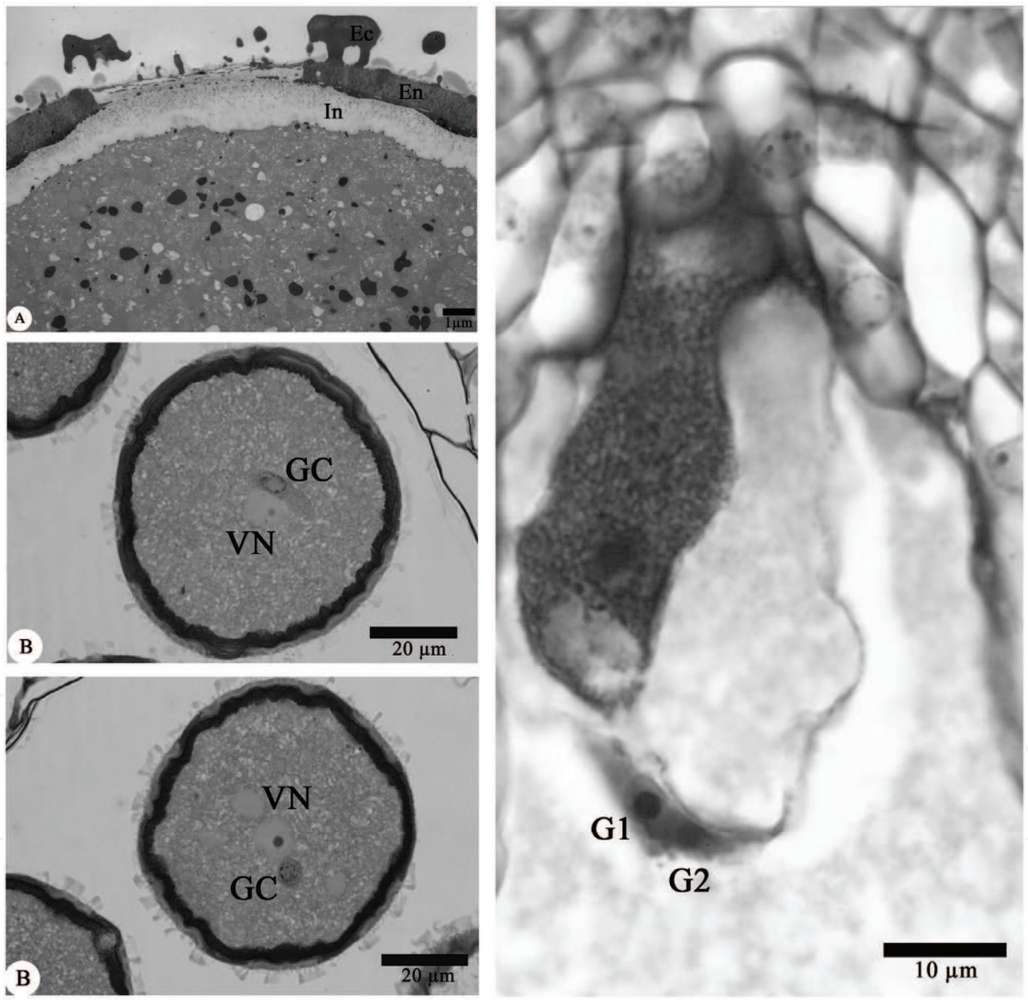

Fig. 4. Pollen grain of *Passiflora elegans.* (A) Eletron micrography of sporoderm (B) Slice of mature pollen grain with degenerative cell in a longitudinal section encompassed by the vegetative cell. (C) Slice of mature pollen cell at cross section. Generative cell enveloped by the vegetative cell. (D) Gametic cells (G1 and G2) after discharge of the pollen tube. (Ec) ectexine; (En) endexine; (In) Intine; (NV) nucleus of vegetative cell; (GC) generative cell, (G1 and G2) male gametes., (Si) Synergid , (EC) Egg cell.

early degeneration of the tapetum occurs in telophase II of meiosis, with the absence or failure of cytokinesis of the androspore mother cells, forming a cenocyte, or, sometimes, a tetrad with some degenerated androspores (Bione et al., 2002). Another tapetum-related event is the abnormal vacuolation of tapetal cells in *Bidens cervicata* Sherff. (Asteraceae) which leads to the disintegration of the sporogenic tissue (Sun & Ganders 1987). In the case of maize (*Zea maiz* L., Poaceae), mutants for the *ms 23* and *ms 32* genes presented periclinal divisions in the tapetum layer followed by their non-differentiation, causing the cell death of the sporogenic tissue in prophase I of meiosis (Chaubal et al., 2000). In *Helianthus annus* L. (Asteraceae) disordered periclinal divisions occur, showing signs of disorganization in the organelles and in the cell wall. Thus, the androspore tetrads disintegrate (Horner, 1977).

Problems in the formation of the androspore tetrad also lead to sterility. In bean plants (*Phaseolus vulgaris* L., Leguminosae) abortions occur in the tetrad stage due to cytoplasmatic connections, which are maintained between the androspores, indicating incomplete or aberrant cytokinesis (Johns et al., 1998). A similar event occurs in pistillated flowers of *Valeriana scandens* (Caprifoliaceae). The androsporogenesis of pistillated flowers is the same as in perfect flowers until the androspore tetrad phase. In this stage the exine is deposited in the androspores, both in the primexine wall and in the aperture regions, forming connections between the androspores; as the callose degrades through the callase enzyme, the androspores are released in the locule in a tetrad shape; an exine continuum is formed among them; they undergo vacuolation and begin androgametogenesis, but become senescent, and only the exine of the sporoderm remains collapsed in the locule of the anther in anthesis (Figure 5A-B) (Duarte-Silva et al. 2010).

A mutant of strain 6492 of Arabidopsis (Brassicaceae), in the tetrad stage presents eight microspores enveloped by callose (Peirson et al., 1996). Two other mutants (strains 7219 and 7593) present tetrads with other sizes of microspores, with failures in callose production and in vacuole development, and also multinucleate cenocytes (Peirson et al., 1996). Disorders in the synthesis and degradation of callose, and in the timing at which these processes occur, lead to sterility. Mutant *ms32* of *Arabidopsis* presents early degradation of callose right after meiosis, because of the formation, in the same stage, as a large amount of rough

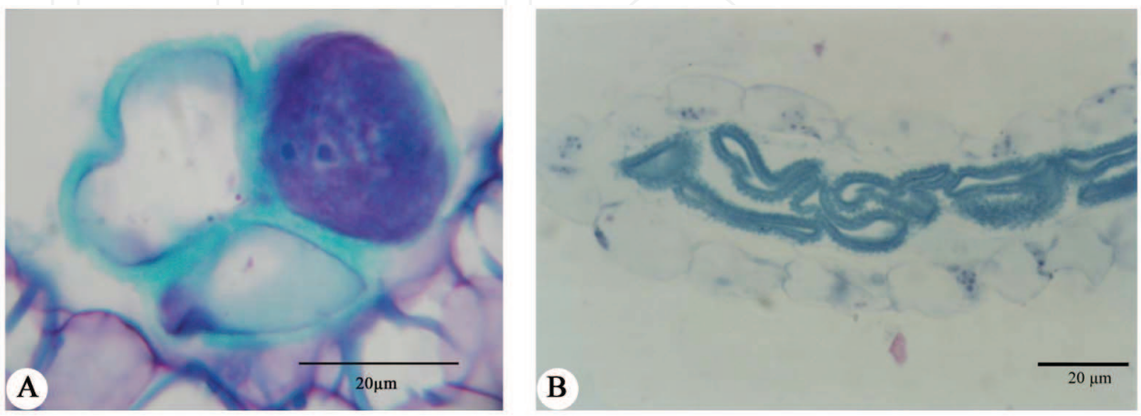

Fig. 5. (A) Malformed tetrad of *Valeriana scandens* (Caprifoliaceae). Observe the continuum of the exine between the tetrad androspores. (B) Sterile pollen in the anther in anthesis.

endoplasmic reticulum in the tapetum cells, probably responsible for callase synthesis and/or secretion in the anther locule (Fei & Sawhney 1999). On the other hand, in the mutant for gene *cals*5 of the same species, callose deposition practically does not occur in the mother cell of androspores and in the tetrad, causing androspore degeneration (Dong et al., 2005). Once the androspore tetrads have been formed, there are cases in which the androspores interrupt their development at this stage. In *Allium schoenoprasum*, two mutants for staminal sterility (*wi* e *st1*) do not present dissolution of the callose after microspore tetrad formation, causing the non-deposition of sporopollenin (Engelke et al. 2002).

Pollen grain abortion can also occur in the sporoderm development phase. The mutant *nef1* of *Arabidopsis thaliana* presents pollen grains with primexin and sporoderm without sporopollenin, synthetized and accumulated in the locule of the anther (Ariizumi et al. 2004). In mutants *cesa1* and *-6* of *Arabidopsis*, cellulose synthesis deficiency in the sporoderm leads to abortion of the pollen grain (Persson et al. 2007). Mutants *rip1* of *Oryza sativa* developed sterile pollen grains in consequence of no intine formation (Han et al. 2006). Also, in *Oryza sativa,* the presence of empty pollen grains is the result of allele interactions of loci *S-a*, *S-b* and *S-c* which lead to abnormalities in the migration of the androspore nucleus to the cell periphery, where asymmetric mitose occurs and the consequent formation of vegetative and generative cells (Zhang et al. 2006). In the same study, stained, but non viable pollen grains are listed among the failures of generative cell migration and of the vegetative nucleus to the center of pollen, as well as failures in sperm cell formation (Zhang et al. 2006). In *Arabidopsis*, the bicellular androphyte goes into programmed cell death after a deficiency in the division of the generative cells to form the gametic cells, due to the lack of the key enzyme 'SerinePalmitoyltransferase' (Teng et al. 2008).

The synthesis of reserve substances is a critical event in androphyte development. The synthesis and accumulation of starch were investigated in androphytes of *O. sativa* and six genes that act on the sucrose-starch metabolic pathway were identified (*Rsus, OSINV2,OsPGM, OsUGP,OsAGPL3* and *OsSSI*). Mutants for these genes are deficient in the production of amyloplasts and constitute a sterile class called HL type (Kong et al. 2008). *Arabidopsis* sporophytes recessive for the gene (*atatg6/atatg6*) develop morphologically typical androphytes that are incapable of emitting a pollen tube (Fujiki et al. 2007). On the other hand the transgenics of *Brassica campestris*, that do not have gene *BcMF2*, presented, in the sporoderm, the pectic proportion of the overdeveloped exintine and the underdeveloped endintine, causing in vitro development of balloon-shaped pollen tubes (Huang et al. 2009).

4.2 Cyperaceae and their uncommon microsporogenesis

4.2.1 Ultrastructural and structural studies of pseudomonads in cyperaceae

Androsporogenesis in the Cyperaceae family follows a distinct pattern from that found in other Angiosperm groups. After meiosis, three of the four nuclei formed take up a defined region of the cell. They are isolated, degenerate and only one becomes the functional androspore. The four nuclei present a polarized distribution in an asymmetric cell known as pseudomonad (Figure 6A-B) (Selling 1947; Erdtman 1971; Strandhede 1973). In the

development of the pseudomonad, all the cells come into contact with the tapetum inside the sporangium, in an arrangement known as peripheral (Kirpes et al. 1996). The pear-shaped pseudomonad has the broader region oriented toward the tapetum (abaxial region) and the narrower one oriented toward the center of the anther locule (adaxial region) (Figure 6b) (Strandhede, 1973). This spatial distribution, referring to the adaxial and abaxial aspects of a plant organ, may lead to mistaken interpretations, considering a entire sporangium, half of it oriented toward the adaxial aspect of the growth axis (rachila), while the other half is oriented toward its abaxial aspect. We suggest the orientation of these structures to the contiguous region, referring to the tapetum or locule.

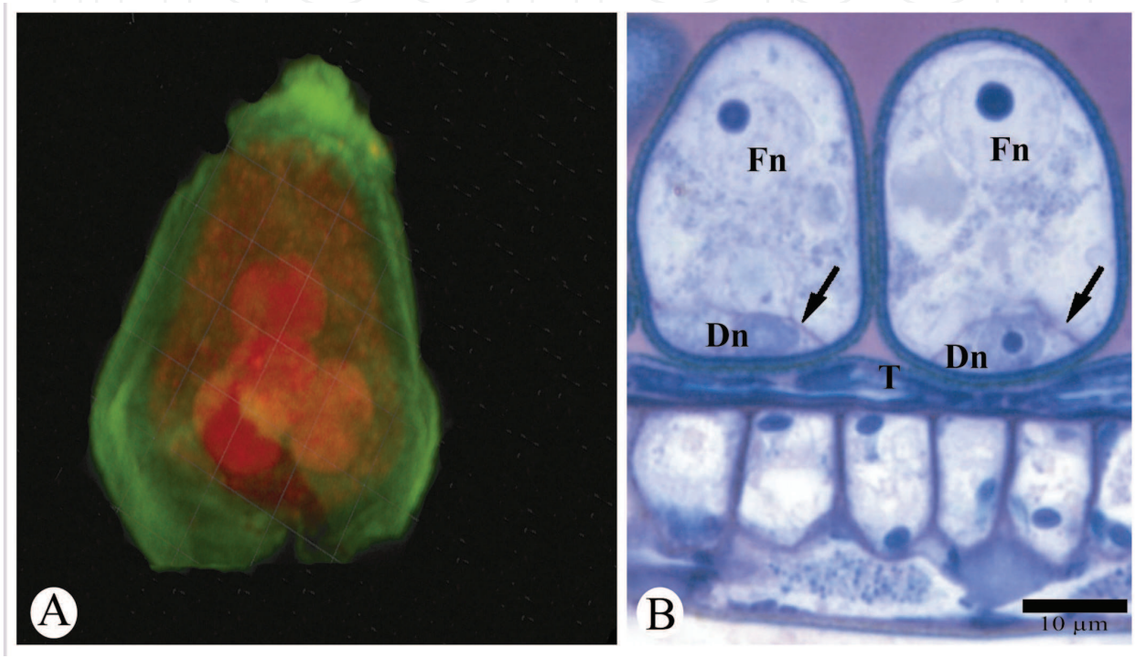

Fig. 6. (A) *Rhynchospora pubera* pseudomonad in confocal scanning microscopy showing the four nuclei in tetrahedral arrangement. The degenerative nuclei are located in a broad region of the cell, while the functional ones is placed in the central region. (B) Pseudomonad in contact with tapetum. The functional nucleus oriented toward the anther locule and the degenerative nucleus isolated by a thin inner cell wall (arrow). (Dn) degenerative nuclei; (Fn) functional nucleus; (T) tapetum.

In most genera of the Cyperaceae family, polarization follows a single pattern. The degenerative nuclei of the pseudomonad are oriented toward the locule, while the functional nucleus is oriented toward the tapetum region (Furness & Rudall 1999). This pattern of nuclear placement led to propose the "tapetum-pore" hypothesis. According to this hypothesis, the determining factor of the functional domain of the pseudomonad is due to the presence of the pollen pore close to the tapetum region (Strandhede 1973; Kirpes et al. 1996). This hypothesis can be applied to species of genus *Eleocharis* as shown in this chapter (see below). However, it cannot be applied to all Cyperaceae, since in *Rhynchospora* species the degenerative nuclei are found to be tapetum oriented and the functional nucleus is locule oriented (Figure 6B).

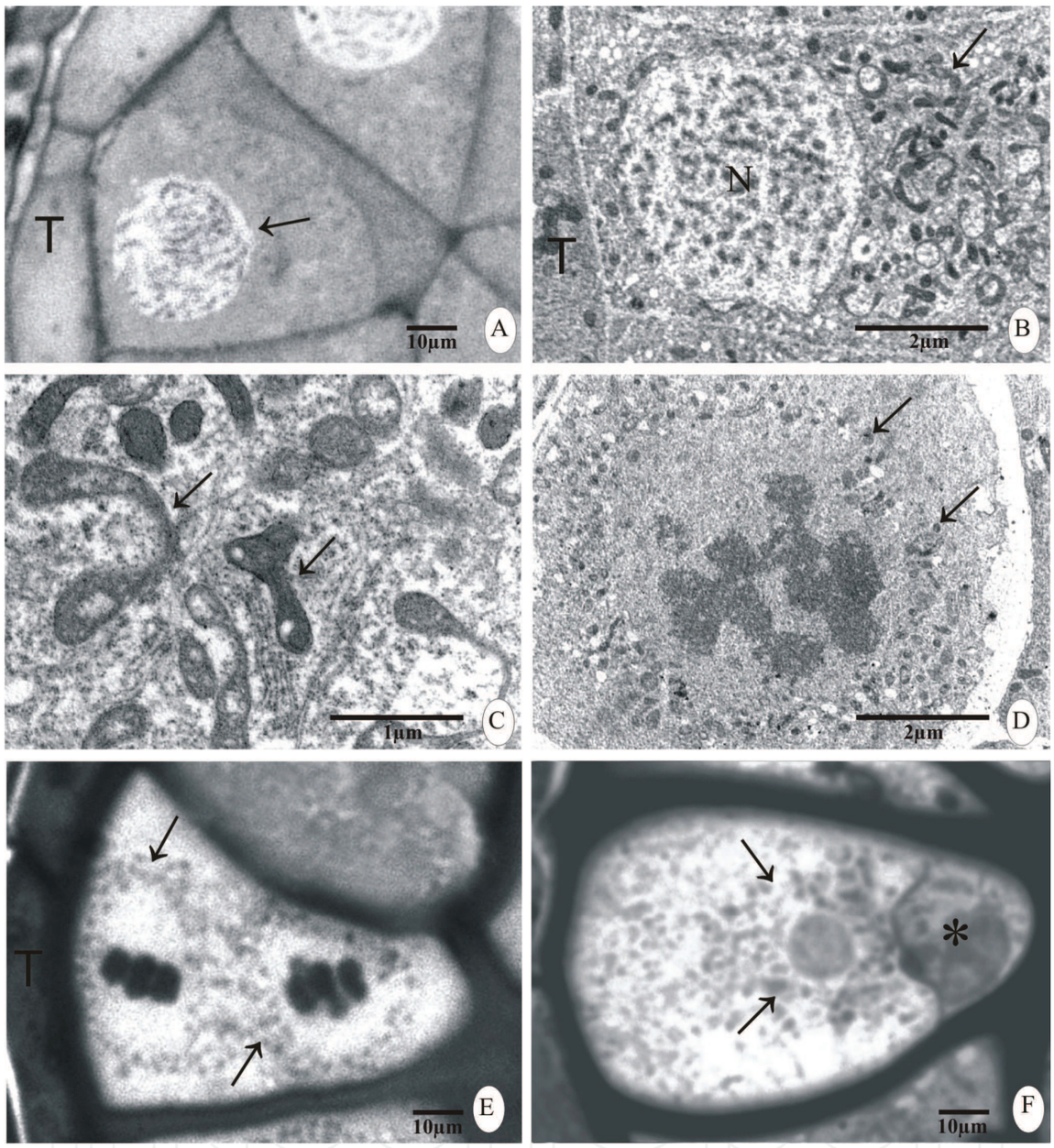

Fig. 7. Pseudomonads of *Eleocharis sellowiana*. (A) The nucleus is present in basal region of the androspore mother cell (arrow), during initial prophase. Toluidine blue 100X. (B) Electron micrograph of prophase I showing the organelles (arrow) located in the apical cytoplasm. (C) Detail of the figure 8B. Note organelles (arrow) in process of division, most of them are proplastids, mitochondria and rough endoplasmic reticulum. (D) Electron micrograph showing metaphase I. Arrows indicate organelles organized surrounding chromosomes. (E) Androspore mother cell during metaphase II exhibiting a cord of organelles (arrows) surrounding chromosomes. Toluidine blue. (F) Photomicrograph showing pseudomonad during final meiosis. The degenerative nuclei are at the cell apex (*). The arrows indicate remained organelles involving the functional nucleus. Toluidine blue. Tapetal cells (T). Nucleus (N).

In *Rhynchospora pubera* the androspores mother cell presents organelles with a polarized distribution, oriented towards the locule region, and the nucleus oriented towards the tapetal region (San Martin et al. 2009). These authors suggest that the pseudomonad undergoes a polarization process even before meiosis occurs. However, in *Carex blanda*, meiosis occurs with the nucleus of the androspore mother cell in the center of the cell and the three degenerative nuclei migrate to the pseudomonad region oriented toward the locule (Brown & Lemmon 2000). The reason why there is more than one type of polarization in the pseudomonads of Cyperaceae is still unknow. However, some cytoplasmic evidences indicate that this organization may be influenced by the behavior of organelles and cytoskeleton.

In *Eleocharis sellowiana*, as well as in other Cyperaceae, the androspores appears asymmetrically organized, culminating in pseudomonads polarized as found in *Carex*. However, in the early stages of meiosis the organelles develop an atypical arrangement around the chromosome complement. At prophase I, the nucleus occupy the basal region of the cell (Figure 7A-B) and the organelles increase in size and in number (Figure 7C). At metaphase I the organelles surround the chromosomes establishing a dense "Organelles Cord" (Figure 7D). This arrangement has not been documented for other Cyperaceae species, although it was reported in *Malva silvestris* (Kudlicka and Rodkiewicz 1990) and *Lavatera thuringiaca* (Tchórzewska *et al*. 2008). This "organelles cord" is held up sourrounding the chromosomes set until telophase I, when the phragmoplast is established. As meiosis progresses, the regions occupied by the phragmoplast are occupied by organelles cord, being one for each chromosome complement. These are held up until metaphase II / anaphase II (Figure 7E). There is evidence of organelles cord being maintained in one of the four nuclei after telophase II, and it is possibly important to select the functional nucleus. The functional one appears surrounded by an organelles cord in the pseudomonads in *E. sellowiana* (Figure 7F). The biological role of the organelles cord found in the *E. sellowiana* androspore mother cell remains uncertain, but the data suggest that it could influence in the delimitation of functional and degenerative nuclei at the transition from anaphase II to telophase II.

In *Rhynchospora pubera*, polarization occurs independent of the presence of organelles cord, and in the pseudomonads the functional nucleus appears directed to the anther locule. Toward the asymmetric and simultaneous cytokinesis, the degenerative nuclei are isolated after phragmoplast fusion and formation of an electron dense pectic wall (Figure 8A-B) (San Martin et al. 2011 in press). The degenerative nuclei are thus isolated with portions of cytoplasm containing organelles (Figure 8A-B). This shows the presence of two cell domains: one of them functional and the other degenerative. In this way, four androspores are formed, but three are eliminated (Figure 6A-B, 8A). This entire ensemble is contained in a single sporoderm with a maturating exine, constituted by tectum and columelle. (Figure 8A). In the cytoplasm of the functional domain, there are lipid droplets, which is not seen in the degenerative domain (Figure 8A). Vacuoles, mitochondria and other organelles are found both in the functional and in the degenerative domain (Figure 8A-B). Folded of rough endoplasmicc reticulum membranes are present in the functional cytoplasm, in some cases associated with lipid droplets (Figure 8C). The cytoplasm portions that envelop the degenerative androspores show signs of shrinkage, which does not occur in the cytoplasm of the functional androspore (Figure 8A-B). The degenerative androspore nuclei are small with more packed chromatin, seen both at light microscopy and at transmission electron microscopy. The chromatin in the functional nucleus is unpacked, indicating high metabolic activity (Figure 6B, 8A-B).

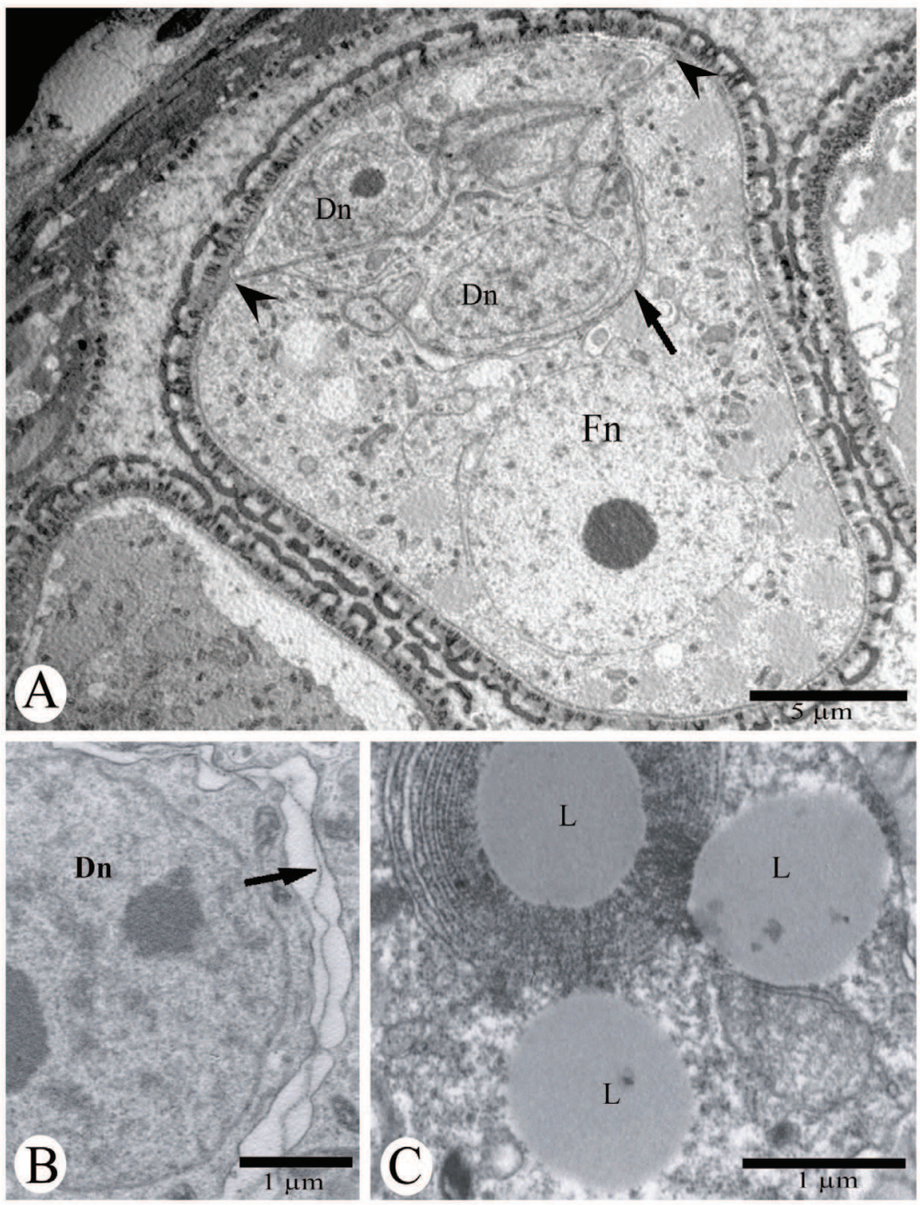

Fig. 8. Transmission electron micrographs of the pseudomonad of *Rhynchospora pubera*. (A) General view. Degenerative nuclei with chromatin packed and isolated by a wall (arrow), which establishes contact with intine (arrowhead). In the functional nucleus chromatin is unpacked. (B) Detail of inner cell wall isolating the degenerative androspores, arrows show the electrondense string in the inner of phragmoplast. (C) Free lipid droplets associated with folded membranes in the cytoplasm of functional androspore. (Dn) degenerative nucleus; (Fn) functional nucleus; (L) lipid droplet.

The degenerative androspores present ultrastructural characteristics of programmed cell death, highlighted by the packed chromatin, cytoplasmic shrinkage and vacuolation (Coimbra et al., 2004). On the other hand, the functional androspore shows characteristics of a cell during a phase of high molecule synthesis, evidenced by the unpacked chromatin,

lipid droplets accumulation and presence of a well-developed rough endoplasmic reticulum. A crucial aspect in pseudomonad development in Cyperaceae is the occurrence of an asymmetrical cytokinesis after meiosis. This enables cell polarization and the isolation of the three degenerative androspores. In the same way, it allows the signalization to the programmed cell death, in degenerative domain, does not influences in the development of the functional domain.

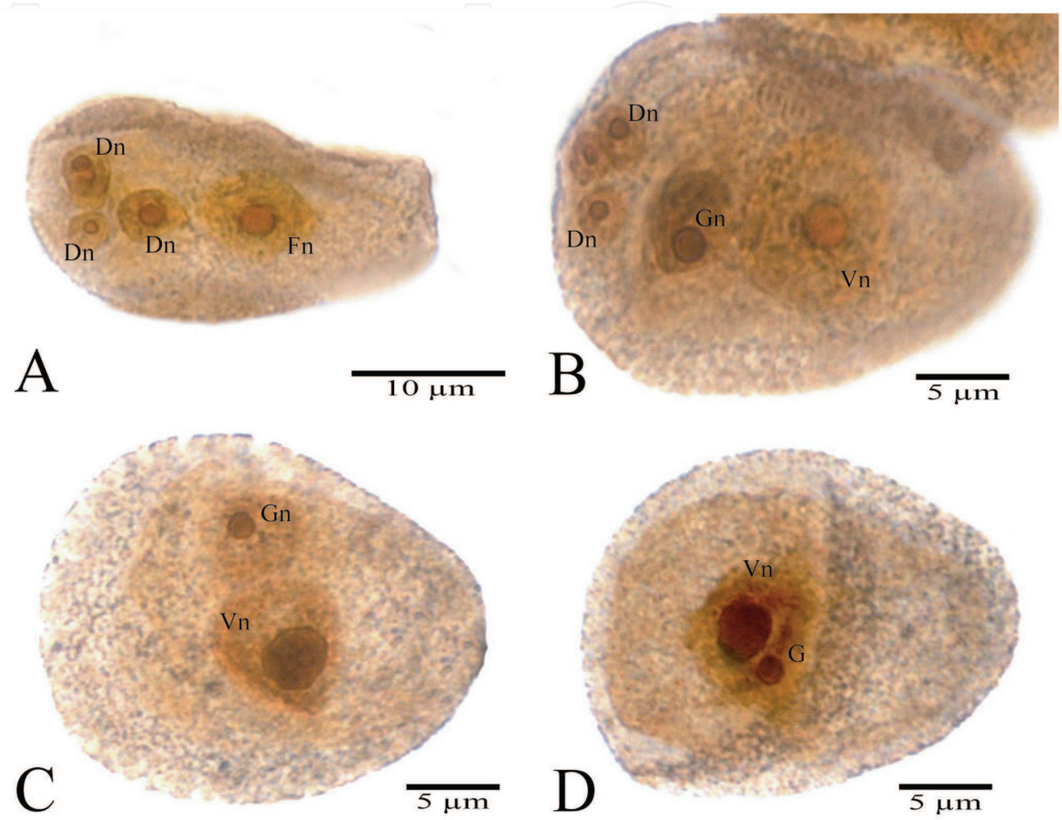

Fig. 9. Squash technique of the pseudomonad of *Rhynchospora pubera* stained with silver impregnation (AgNO3) to evidence ribonucleoproteins. (A) Four pseudomonad nuclei, three degenerative and one functional. (B) Gametophyte after first mitosis, showing the vegetative nucleus and the nucleus of the generative cell, in the presence of degenerative sporophytic nuclei. (C) After the complete PCD of the non-functional microspores, the generative cell and the vegetative nucleus are placed together in the central region of the pseudomonad. (D) Mature androphyte with vegetative cell and one gametic cell after generative cell mitosis. (Dn) Degenerative nucleus; (Fn) functional nucleus; (Gn) generative nucleus; (G) gametic cell; (Vn) vegetative nucleus.

Even in the presence of the degenerative androspores, the functional androspore undergoes mitosis giving rise to the vegetative and degenerative cell (Figure 9A-B), forming the young male gametophyte. After the complety elimination of the degenerative androspores , the generative cells gives rise to the gametic cells, through mitosis. Thus, the pollen grain (mature male gametophyte) is released from the anther in its tricellular form (Figure 9C-D).

The evolutionary history and adaptive advantage of developing a single functional androspore, instead of the four usual ones, remain unknown. A hypothesis would be that

the increased volume of the cytoplasm would also increase the adaptive character of the cell (Ranganath & Nagashree 2000). This idea can be supported by the similarity of gynospore formation, where three of the four products of meiosis degenerate, resulting in an increased volume of the functional gynospore that will give rise to the female gametophyte.

Even so, some questions remain unanswered about androsporogenesis in the Cyperaceae family: 1) What is the biological reason for the degeneration of three of the four nuclei formed after meiosis? 2) Why does polarization occur differently in different genera? 3) Is the selection of functional and degenerative nuclei a chance phenomenon or not? 4) Does selection occur in favor of the functional nucleus or of the degenerative ones? 5) At what time during meiosis does the cellular signalling system act on the choice of nuclei?

4.2.2 Post-reductional meiosis in cyperaceae

Another poorly understood cytological feature about representatives of Cyperaceae, as well as, of holocentric chromosomes, is the post-reductional or inverted meiosis. This meiotic behavior was reported in *Carex* (Heilborn 1928) and it has been considered typical from members of Cyperaceae.

The inverted meiosis was also reported to *Luzula*, Juncaceae (Nordenskiöld 1951), and *Cuscuta*, Convolvulaceae (Guerra & Garcia 2004), as well as for some insect groups, but in the last case it was always associated with sex chromosomes and not with autosomes (Solari 1979; Pérez et al. 1997; Bongiorni et al. 2004). Besides holocentric chromosomes presented kinetic activity diffused along its major axis at mitosis (Nagaki et al. 2005), there is evidence of kinetic activity only in the terminal region of each chromatid, with spindle fibers attached directly to each one of the four chromatids (Vieira et al. 2009; Guerra et al. 2010). This arrangement could allow the formation of "box structures" of the bivalents (see Pazy & Plitmann 1987, Vanzela et al. 2000 and Guerra et al. 2010). However, there is no convincing image in the literature that shows a "box structure" directed to inverted meiosis, with the four chromatids completely individualized. Thus, we do not consider this enough to define the existence of inverted meiosis. In fact, it is very difficult to show if segregation of sister chromatids or homologous chromosomes has occurred using only conventional staining, unless there is a chromosome marker that allow to visualize each one of the chromatids. It is possible to univalents and sex chromosomes of insects (Hughes-Schrader & Schrader 1961; Vieira et al. 2009). The last author questioned the wide use of inverted meiosis for holocentric autosomes because reduction of part of the chromatid always occurs when there is chiasmate in bivalents, independent of orientation. This has been well documented in insects (Bongiorni et al. 2004; Nokkala et al. 2006), but not mentioned in plants (Nordenskiöld 1951, Strandhede 1965; Vanzela et al. 2000).

There are at least two excellent examples of inverted meiosis in plants. In the first case, Pazy (1997) showed the separation of sister chromatids in B-chromosomes in anaphase I of *Cuscuta babylonica*. In the second case, Da Silva et al. (2005) reported the separation of sister chromatids for all homologous of *Eleocharis subarticulata* (Cyperaceae). However, the two cases can be considered special situations due to the presence of a univalent B-chromosome and of a karyotype with multivalents rearranged by disploidy, respectively. But independent of these arguments, this atypical meiotic behavior has been referred only to organisms with holocentric chromosomes (Viera et al. 2009). Here we show evidence of reductional, or normal meiosis, in at least one chromosome pair of *Rhynchospora pubera*.

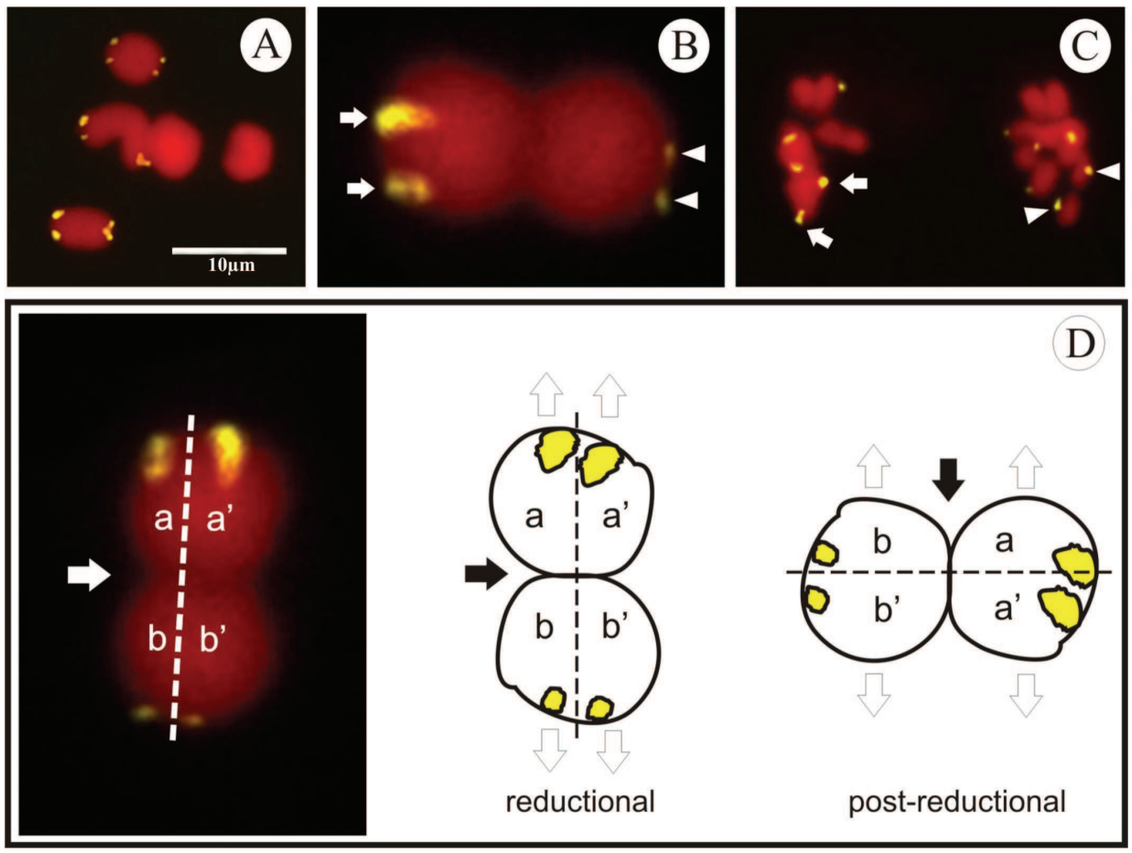

Fig. 10. Meiotic cells of *Rhynchospora pubera* hybridized with biotiniled 45S rDNA probe and detected with avidin-FITC. (A) Metaphase I showing pairs 1, 2 and 4 with terminal hybridizations signals. (B) Pair 2 isolated. Observe sister chromatids laterally joined while homologous are joined end-to-end. This bivalent shows a heteromorphism in the hybridization signals size (arrows and arrowshead). (C) Anaphase I and (D) Scheme with the second pair. Both images show segregation of homologous chromosomes and not sister chromatids. Arrows in (C) point out homologous of pair 2 with major signals and the arrowheads indicate the other homologous with minor hybridization signals. Thus, at least to pair 2 the meiosis was reductional and not inverted. The scheme in (D) was elaborated from pair 2 (on the left). The normal sense is the segregation of the homologues (center image), and not the segregation of sister chromatids in the first anaphase (on the right).

This event is enough to question the occurrence of inverted meiosis in all chromosomes and species of Cyperaceae. *R. pubera* exhibits six chromosomes with terminal 45S rDNA sites (Vanzela et al. 1998). Fortunately, we found one individual with a polymorphism in pair 2, which presents one of the homologous with a major hybridization signal after FISH with 45S rDNA probe. This event allowed accompanying the bivalent segregation in anaphase I and found the separation of homologous chromosomes, and not of sister chromatids (Figure 10).

This evidence raises doubts about some published images on bivalents in "box", which were interpreted as a conclusive diagnosis of post-reductional meiosis of all bivalents (Vanzela et al. 2000). At this time, there are no certainties as to whether all bivalents or part of them are oriented together or re-oriented independently, i. e., the post-reductional meiosis may occur but, is it necessary that all autosomes always behave the same way?

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6. Conclusion

Firstly, based on Hofmeister's theory (1851), the life cycle of plants consists of two generations: sporophytic and gametophytic. In flowering plants, gametophytic generation is reduced to pollen (male gametophyte) and embryo sac (female gametophyte). In the sphere of studies on plant reproduction, is necessary limit the sexual terminology to the gametophytes, erradicating the sexual terminology applied to sporophytes, specially, the terms: male, female and hermophroditic flowers. In the second, the study of cellular and sub-cellular aspects of meiosis can improve the comprehension of plant meiosis process, particularly in cases of staminal or pollen sterility, as well as, in studies of uncommon types of meiosis, illustrated by pseudomonads in Cyperaceae.

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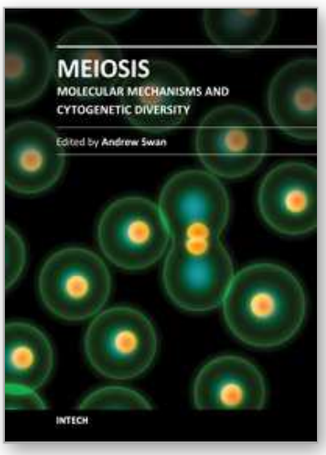

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Meiosis, the process of forming gametes in preparation for sexual reproduction, has long been a focus of intense study. Meiosis has been studied at the cytological, genetic, molecular and cellular levels. Studies in model systems have revealed common underlying mechanisms while in parallel, studies in diverse organisms have revealed the incredible variation in meiotic mechanisms. This book brings together many of the diverse strands of investigation into this fascinating and challenging field of biology.

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