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Short communication

Early meiosis in *Rhynchospora pubera* L. (Cyperaceae) is marked by uncommon ultrastructural features

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Abstract

The family Cyperaceae has an unusual microsporogenesis in which tetrad formation does not occur. In addition, other cytological features are important, such as the occurrence of holokinetic chromosomes and post-reductional meiosis. We have examined the ultrastructural features of the pollen mother cell (PMC) of *Rhynchospora pubera*. Anthers of several sizes were analyzed using light and transmission electron microscopy. The PMC before meiosis presented a central nucleus and a regular profile of the nuclear envelope. During prophase I, the nucleus was in the abaxial region of the cell. This cellular polarization was accompanied by other marked ultrastructural features in the nuclear envelope. Morphological changes involved dilations of perinuclear cisterns and polarization of the nuclear pore complexes. The results show that polarization occurs in the initial phases of microsporogenesis in *R. pubera*, unlike other plant species.

Keywords: Cyperaceae; Microsporogenesis; Nuclear envelope; PMC; Transmission electron microscopy

1. Introduction

The initial stages of microsporogenesis are marked by several cytoplasmic and nuclear changes, the most common being an increase in cell size and number of organelles, intense nuclear activity, and modifications in the chemical composition of the cell wall (Scott et al., 2004). These structural and biochemical changes can differ among angiosperm groups and species, and even tissues of the same anther. Microspores of *Pinus banksiana* Lamb (Li and Dickison, 1987) and *Lycopersicon esculentum* Mill (Polowick and Sawhney, 1992) have grooves and invaginations in the nuclear envelope. The development of tetrads in *Tradescantia virginiana* L. occurs inside the vacuoles in the plasmodial tapetum (Furness and Rudall, 1999), and pollen kit substances accumulate unevenly on the surface of early microspores in Chinese cabbage (Xie et al., 2005).

Microsporogenesis begins with meiotic division of the pollen mother cell or PMC (Bedinger, 1992), and produces a tetrad of haploid cells that are released as free microspores by callase action (McMcormick, 1993). Each microspore undergoes an unequal division, giving rise to the vegetative and generative cells (Tanaka, 1997). Interestingly, the representatives of the family Cyperaceae display an unusual microsporogenesis, where tetrads are absent and a single functional product called a pseudomonad is formed (Brown and Lemmon, 2000; Furness and Rudall, 1999). Furthermore, this family is characterized by other uncommon cytological features, such as the occurrence of holokinetic (holocentric) chromosomes (Guerra et al., 2006; Vanzela and Colaço, 2002) and post-reductional meiosis (Da Silva et al., 2005).

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Rhynchospora pubera is a Brazilian species considered to be a model for the study of holokinetic chromosomes, since it possesses few ($2n = 10$) and large chromosomes and regular meiosis with 5 bivalents (Luceño et al., 1998). However, information about its microsporogenesis, as well as in other Cyperaceae, is fragmented. Besides, the ultrastructural features of the pre-meiotic cells have not been documented. This study set out to determine the cellular changes that occur in early microsporogenesis of *R. pubera*.

2. Material and methods

Ten individuals of *R. pubera* L. (Cyperaceae) were collected in Recife, Pernambuco in Northeast Brazil and were kept in a greenhouse of the Laboratório de Biodiversidade e Restauração de Ecossistemas at Universidade Estadual de Londrina, Brazil. Vouchers are kept at the FUEL herbarium.

Anthers were collected, and fixed by immersion in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 24 h at room temperature, with constant mixing in a cell shaker, before being grouped by size. These samples were washed in sodium cacodylate buffer, post-fixed with 1% osmium tetroxide, washed again, and dehydrated in a graded ethanol series. Samples were immersed in propylene oxide, treated in a graded propylene/Araldite® series and embedded in Araldite® resin.

Anthers were sectioned using an ULTRACUT (Leica). Semi-thin sections (ca. 2 μm) were stained with toluidine blue and examined to determine the anther stages. Images from suitable stages were acquired using a Leica DM 4500 B microscope equipped with a DFC 300FX camera and the Leica IM50 4.0 software, in ∼20 from each phase. Ultrathin sections (ca. 70 nm) were stained with 9% uranyl acetate and lead citrate (Reynolds's solution) and analyzed using a FEI Tecnai 12 transmission electron microscope at 80 kV. The images were acquired with the Soft Imaging System.

3. Results and discussion

The young anthers of *R. pubera* were formed from 5 distinct tissues, each of them containing a single layer of cells: epidermis, endothecium, middle layer, tapetum and sporogenous tissue (Fig. 1A). The last is composed by pollen mother cells. This arrangement indicates that the anther morphology of *R. pubera* follows the common pattern found in higher plants (Bedinger, 1992). Along the pre-meiotic stages, meiocytes appear surrounded by a single callose wall (Fig. 1B), which effectively isolates each PMC (Enns et al., 2005; Mascarenhas, 1989; McMcormick, 1993). The PMCs were pear-shaped, positioned adjacent to other cells and with the tapetum cells (Fig. 1B). In this stage, plasmodesmata were absent, unlike in rice, where plasmodesmal connections between PMCs and tapetum cells are frequently observed (Mamun et al., 2005). Some organelles, e.g., plastids and mitochondria, were difficult to characterize. In this case, the term "chondrioma" can be used to denote the organelles of uncertain ontogeny (Mamun et al., 2005). Large lipid droplets were also evident (Fig. 1B, C). A large nucleus with unpacked chromatin was more centrally located (Fig. 1B, C), which was bordered by a regular nuclear envelope (Fig. 1C). These morphological features are common in other groups of monocots, such as the Poaceae (Kirpes et al., 1996). The central position of the nucleus in pre-meiosis has been reported for other Cyperaceae groups, such as the *Eleocharis* and *Carex* (Brown and Lemmon, 2000; Furness and Rudall, 1999; Kirpes et al., 1996), as well as in other plant groups, e.g., yellow passion fruit (Souza and Pereira, 2000). The centrally located nucleolus is surrounded by an electron-lucid halo (Fig. 1C). The cytoplasm of tapetum cells showed an electron density similar to that of PMCs, with numerous organelles sparsely distributed, indicating a regular metabolism (Fig. 1D).

In prophase I, the PMCs of *R. pubera* were also pear-shaped (Fig. 2A), similar to those microsporocytes found in other Cyperaceae species (Brown and Lemmon, 2000; Furness and Rudall, 1999; Kirpes et al., 1996; Moar and Wilmshurst, 2003; Ranganath and Nagashree, 2000). The PMCs were bordered by tapetum cells at the abaxial position, but PMCs side by side showed plasmodesmata connecting them (Fig. 2B). Cytoplasmic connections between PMCs, which were sufficiently broad to permit the passage of organelles and chromosomal materials, were reported in *L. esculentum* (Mill) − Solanaceae (Polowick and Sawhney, 1992). These connections can be important in maintaining meiotic synchrony among PMCs of *R. pubera*, as suggested by Heslop-Harrison (1966) for other plant species. Plasmodesmata have been described also between PMCs and tapetum cells of *Tillandsia albida* (Mez and Purpus) − Bromeliaceae and *Lobivia rauschii* (Zecher) − Cactaceae (Papini et al., 1999), but these connections are lost along the maturation process. In *R. pubera*, plasmodesmata were not seen between PMCs and tapetum cells, suggesting that a more physical independence could culminate in an asynchrony of cellular maturation. This was seen when we compared the electron density patterns between PMCs and tapetum cells. In the early stages (Fig. 1D), the two cells displayed similar electron density, while in the late stage (Fig. 2B, C), the tapetum cells were more electron-dense. In addition, vacuolation and cytoplasmic shrinkage were seen in tapetum cells (Fig. 2B). These ultrastructural features can be a sign indicative of the initial stage of programmed cell death (Papini et al., 1999; Pennell and Lamb, 1997; Wu and Cheung, 2000). The cytoplasm of PMCs exhibited a larger number of organelles at the adaxial position, but with mitochondria and plastids undifferentiated and several vacuoles randomly distributed (Fig. 2B, C, E). Small and few lipid droplets were observed (Fig. 2E) as compared with earlier stages. The nuclei appeared at the abaxial region with more condensed chromatin and nucleoli peripherally located, independent of cell polarization (Fig. 2A–C). The opposite positioning of the organelles and the nucleus at early meiosis of *R. pubera* seems to be different from that described for *Carex blanda* Dewey (Brown and Lemmon, 2000). This species shows the prophasic nuclei in the central region of the cell. According to Ranganath and Nagashree (2000), cell and organelle polarizations are uncommon before meiosis I. However, our results indicate that in *R. pubera* cellular asymmetry and the polarization of organelles occur in early meiosis.