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Cellular organisation in meiotic and early post-meiotic rice anthers

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Abstract

We have used fluorescent, confocal laser and transmission electron microscopy (TEM) to examine cellular organisations, including callose (1,3-β-glucan) behaviour, in meiotic and early post-meiotic rice anthers. These features are critical for pollen formation and provide information to better understand pollen sterility caused by abiotic stress in rice and other monocotyledonous species. Among organelles during meiosis, abundant plastids, mitochondria and nuclei of the anther cells show distinctive features. Chloroplasts in the endothecium store starch and indicate a potential for photosynthetic activity. During meiosis, the middle layer cells are markedly compressed and at the tetrad stage are either vacuolated or filled with degenerating electron-opaque organelles. Viable mitochondria, stained with Rhodamine 123, are seen in the endothecium and tapetum, but the mitochondria in the middle layer are not stained during meiosis. The radial walls of the tapetum are disorganised and degenerating, indicating the formation of a syncytium; pro-orbicules are located at the locular walls at the tetrad stage. Immunohistochemical studies show that the sporogenous cells are entirely enveloped by a thick callosic layer at early meiosis. Cell plate callose was assembled in a plane between the dyad cells. In the tetrads, however, callose formed only at the centre, showing that the tetrad microspores are not enveloped but separated by callose walls. Thick, undulating electron-opaque walls around the tetrads indicate the beginning of exinous microspore wall differentiation.

Keywords: Anther; Callose; Dyad; Immunolabeling; Meiosis; Rice; Tetrad microspore

1. Introduction

Meiosis is the central event in the transition from meiocyte to microspore, producing a haploid, gamete-producing generation from the diploid sporogenous cells. When their differentiation is complete, the sporogenous cells become spherical meiocytes, which are transformed into dyads and then tetrads by two distinct episodes of meiotic division. The cellular organisation of meiotic anthers expedites the physiological route to normal pollen grain formation (Pacini, 1994; Raghavan, 1997). The important events in this process are the formation of unique organelles and locular structures and the initiation of pollen wall construction. Among the specialised events that occur during or just after meiosis are: tapetum cytoplasm differentiation, formation of pro-orbicules in the tapetum cytoplasm and wall patterning in the microspores. Along with other organelles, mitochondria play a significant role in anther development. In higher plants, mitochondrial function meets the changing respiratory demands of different tissues. Mitochondrial function is perturbed in many sterile male lines. Thus, an understanding of cellular physiology is important to elucidate the cause of pollen failure by abiotic stress.

Abbreviations: DDG, 2-deoxy-D-glucose; TUNEL, TdT-mediated dUTP nick-end labelling.

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The formation and dissolution of the special callose walls are conspicuous events in the centre of the anther locule during microsporogenesis. Generally, callose is synthesised at the plasma membrane as part of normal development and also in response to environmental stimuli (Mascarenhas, 1975; Stone and Clarke, 1992). During microsporogenesis, callose isolates the sporogenous cells to ensure their genetic independence and establish their autonomy as the first cells of a new generation; it also acts as a mould for the future pattern of the primexine (Izhar and Frankel, 1971; Raghavan, 1997). The timing of callose deposition and its dissolution by the tapetally secreted callase (1,3-β-glucanase) seems to be critical for fertile pollen formation (Bhandari and Khosla, 1995; Worrall et al., 1992). Pollen abortion and defective pollen walls have been observed when callose is prematurely degraded by 1,3-β-glucanase (Worrall et al., 1992). Despite the importance of callose in microspore formation, the spatial and temporal organisation of callose in rice anthers has not been reported.

This study documents the ultrastructural organisation and spatial and temporal distribution of callose during the transition from meiocyte to tetrad in rice anthers.

2. Materials and methods

2.1. Plant materials

Oryza sativa L. *cv. Doongara*, a low-temperature sensitive Australian rice cultivar, was selected and grown in hydroponic culture medium as previously described (Mamun et al., 2005). Panicles were collected from the plants at an auricle distance of <-10 mm to 0 mm, which corresponds approximately to the transition from meiocytes to tetrad microspores in rice anther development, and were placed in B & K medium (Brewbaker and Kwack, 1963) in separate vials until the anthers were transferred to fixative for microscopy.

2.2. Mitochondrial activity

Anthers were stained with Rhodamine 123 (Molecular Probes, Eugene, OR, USA) at 10 mg l^{-1} as previously described (Mamun et al., 2005). A Zeiss LSM 410 confocal attached to a Zeiss Axiovert 200 microscope, or a Zeiss LSM 5 Pascal confocal microscope attached to a Zeiss Axiovert 200M (Carl Zeiss, Germany), was used to collect longitudinal optical sections of the anthers. Rhodamine 123 was illuminated by the 488 nm line of an argon laser. All images were captured and processed digitally.

2.3. Transmission electron microscopy

Glutaraldehyde and paraformaldehyde fixed anthers were embedded in spur's resin, and sections were cut in series with a microtome and stained with uranyl acetate and lead citrate (Mamun et al., 2005). The sections were examined with a Zeiss 902 electron microscope at 80 kV in the Electron Microscope Unit, The University of Sydney, Australia.

2.4. Aniline blue staining of callose

The tips of anthers at different stages of meiosis were excised and placed on a glass slide in a drop of Brewbaker and Kwack medium (Brewbaker and Kwack, 1963) containing 0.025% (w/v) aniline blue. A cover slip was placed over the anthers and the contents of the locules were extruded by gentle pressure on the cover slip surface. The anthers were then examined under UV illumination using a Zeiss Axiophot microscope. Images were taken on Ektachrome 400 ASA colour reversal film (Eastman Kodak) and the photographic slides were digitised on a Nikon LS-1000 Super Cool Scan (Nikon Inc., Tokyo Japan).

2.5. Immunogold labelling of callose

To prevent further callose synthesis in the anthers, fresh florets were dissected out and kept in B & K media supplemented with 0.1 mM DDG in 0.1 M phosphate buffer, pH 6.9. The anthers were fixed in freshly prepared 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 6.9) overnight and post-fixed in 1% osmium tetroxide for 2 h at 4 °C. After dehydration in an ethanol series and infiltration with ethanol: medium grade LR white resin (ProSciTech, Thuringowa Central, Queensland, Australia), the specimens were embedded in medium grade LR white resin in gelatin capsules and polymerised in a vacuum oven at 65 °C. Ultrathin sections were collected on hexagonal gold grids coated with 1% formvar. The grids were transferred to 30 μl of blocking solution consisting of 4% BSA in PBS for 1 h, followed by incubation for 3 h at room temperature in 30 μl mouse monoclonal anti-1,3-β-glucan antibody (Biosupplies Australia Pty. Ltd.) at 1:100 dilution in PBS containing 1% BSA. After six washes with 30 μl drops of PBS for 5 min, each grid was placed in a 30 μl drop of goat anti-mouse IgG conjugated with 10 nm colloidal gold (Amersham International Plc., Buckinghamshire, England) at 1:30 dilution in PBS and incubated overnight at 4 °C. The sections were then washed twice in buffer followed by a final wash in distilled water, post-stained in 2% aqueous uranyl acetate and Reynold's lead citrate, and examined by transmission electron microscope as described earlier.

3. Results

3.1. Anther wall

Transverse EM sections of anthers show dramatic changes in organisation from onset of meiosis (Fig. 1A) through to the release of individual microspores after meiosis into the fluid-filled space of the locule (Fig. 1B). However, the outer two cell layers of the anther wall changed little between the meiocyte and tetrad stages. The meiocyte cells show nuclei with scattered chromosomes indicating obvious DNA replication (Fig. 1A). At this stage, early deposition of special callose wall is also seen in different places around the meiocyte cell wall (Fig. 1A) that gradually envelopes the meiocyte during meiosis. Callose, along with scattered wall materials (seemingly breakdown products of callose), is seen around the newly formed tetrad microspores (Figs. 1B and 5E). The epidermal cells retain their characteristic central vacuole and narrow band of cortical cytoplasm (Fig. 1C). The plastids within the epidermis become larger and differentiate more into amyloplasts (Fig. 1C) and the mitochondria become scattered throughout the narrow cortical cytoplasm (Fig. 1C, D). Nuclei with prominent nucleoli are also visible and considerable stacks of rough ER are often observed near the cell cortex and adjacent to the nucleus (Fig. 1D). The cuticle on the outside wall of the epidermis appears smooth at this stage (Fig. 1D).

The endothecium cells contain mitochondria and many chloroplasts (Fig. 1E). Numerous chloroplasts in the endothecium are visible by red autofluorescence (Fig. 4C). These chloroplasts contain a considerable number of starch granules (Figs. 1E and 2A, B) and most of them develop extensive grana and thylakoids (Fig. 2A) during the meiotic period. Each endothecium cell has two or three medium-sized vacuoles