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Development of cycad ovules and seeds. 1. Implication of the ER in primary cellularisation of the megagametophyte in *Encephalartos natalensis* Dyer and Verdoorn

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Abstract Very little is known about the pre- and post-shedding megagametophyte development and utilisation of accumulated reserves, respectively, in cycads (Zamiaceae). In the present study on developing ovules of the recalcitrant-seeded species, *Encephalartos natalensis*, cells of the megagametophyte were found to become progressively packed with starch and protein as the two main storage reserves, a limited number of discrete lipid bodies, and occasional mitochondria all of which appeared to be embedded in a homogeneous matrix. ER-derived vesicles (and not Golgi-derived vesicles) appeared to be the principal contributors of the primary cell wall components, pectin and xylan, during megagametophyte cellularisation. This was confirmed by the use of enzyme-gold localisation. High-pressure freezing (HPF) and freeze substitution (FS) of samples the following season showed that while the apparently featureless cytomatrix of the megaspore was an artefact of conventional fixation, there was still an insignificant occurrence of Golgi bodies during primary wall formation. When enzyme-gold localisation was employed on the HPF-FS material, label for pectin and xylan was found only in the regions of ER and vesicles and not in any of the few Golgi bodies or their associated vesicles. Immunocytochemistry revealed that pectin and xylan were restricted to the ER and not to any vesicles or to the occasional Golgi body that was found. This suggests that the ER exclusively, is involved in the deposition of these primary cell wall components during the cellularisation of the *E. natalensis* megagametophyte. While cellularisation took place over approximately 1–2 weeks, subsequent development of the megagametophyte cells involved the accumulation of storage reserves, this phase lasting approximately 8 months—after which the seeds were shed whether pollination/fertilisation had recently occurred, or not.

Keywords Cycad · Enzyme-gold localisation · Histochemistry · Immunocytochemistry · Ovule ontogeny · Recalcitrant · Ultrastructure

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Introduction

Cycads have been described as the "coelacanths of the plant world", the "dinosaur plants", and "living fossils" (Giddy 1984; Jones 1993). Allied to their primitive nature, cycads (Gymnospermeae) display many unusual and some unique features in their reproductive systems, cyanobacterial symbiosis and toxins (Osborne 1995) making them one of the most interesting plant groups for research.

Although there has not been much research on cycad seeds thus far, there was a perception that they may be recalcitrant (Forsyth and van Staden 1983; Dehgan and Schutzman 1989) mainly based on the fact that the seeds are 'wet'. Woodenberg et al. (2007) have since shown that the seeds of *Encephalartos natalensis* and *E. gratus* are desiccation-sensitive (recalcitrant), with both the embryo and megagametophyte losing viability when subjected to dehydration. It is not surprising that these seeds emerged as desiccation-sensitive as the embryos continue to develop after seed-shed, reaching a germinable size only 4–6 months after abscission from the cone (Giddy 1984). While embryo development is largely a post-shedding event, the megagametophyte of cycads, which functions as the nutrient reserve tissue of the seed, is formed before