Semi-viviparous embryo development and dehydrin expression in the mangrove *Rhizophora mucronata* Lam.

Flora AbdulRahman Ismail · Lisette M. C. Nitsch · Mieke M. C. Wolters-Arts · Celestina Mariani · Jan W. M. Derksen

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**Abstract** *Rhizophora mucronata* Lam. is a tropical mangrove with semi-viviparous (cotyledon body protrusion before shedding), non-quescent and non-desiccating (recalcitrant) seeds. As recalcitrance has been thought to relate to the absence of desiccation-related proteins such as dehydrins, we for the first time systematically described and classified embryogenesis in *R. mucronata* and assessed the presence of dehydrin-like proteins. Embryogenesis largely follows the classic pattern till stage eight, the torpedo stage, with the formation of a cotyledonary body. Ovule and embryo express radical adaptations to semi-vivipary in the saline environment: (1) A large, highly vacuolated and persistent endosperm without noticeable food reserves that envelops the developing embryo. (2) Absence of vascular tissue connections between embryo and maternal tissue, but, instead, transfer layers in between endosperm and integument and endosperm and embryo. Dehydrin-like proteins (55–65 kDa) were detected by the Western analysis, in the ovules till stage 10 when the integuments are dehisced. An additional 50 kDa band was detected at stages 6–8. Together these results suggest a continuous flow of water with nutrients from the integument via the endosperm to the embryo, circumventing the vascular route and probably suppressing the initially induced dehydrin expression.

**Keywords** Dehydrins · Embryo development · Mangroves · *Rhizophora mucronata* · Semi-vivipary

**Introduction**

Most angiosperms disperse their seeds in a dormant, dry and resting, condition, so called orthodox seeds. In contrast, viviparous plants develop embryos that do not enter dormancy nor desiccate and germinate whilst attached to the maternal plant. Members of the Rhizophoraceae show continuing embryo growth prior to dispersal, albeit not vivipary *in sensu stricto*. Instead, they develop a cotyledon body that protrudes from the seed, with the radicle germinating and protruding only after shedding. We characterize this process as “semi-vivipary” in this work. The semi-viviparous seed, which remains attached to the mother plant, is termed a “propagule”. Embryos of such species either emit or modify a number of post-fertilization events. In angiosperms, this behaviour is restricted to some mangroves and members of the sea grass genera *Amphibolis* and *Thalassodendron* (Elmqvist and Cox 1996). Mangroves are woody species found in brackish or saline tropical and subtropical tidal areas. Representative genera of semi- or true-viviparous mangroves include *Rhizophora*, *Bruguiera*, *Ceriops* and *Kandelia* of the tribe Rhizophoraceae, family Rhizophoraceae (Tomlinson 2000).
In orthodox seeds, quiescence is acquired during the later stages of maturation, at the same time that a considerable quantity of water (~90%) is gradually lost by desiccation (Finkelstein et al. 2002). Desiccation tolerance acquisition is marked by a variety of factors such as an increase in low molecular weight solutes and the appearance of dehydrins, the D11 family (Dure 1993a, 1993b) of late embryogenesis abundant (LEA) proteins (Battaglia et al. 2008; Close 1997; Finkelstein et al. 2002; Ingram and Bartels 1996; Wise and Tunnacliffe 2004). Dehydrins are detected in several tissues during stress and development. They are suggested to be involved in resisting environmental stresses by stabilizing proteins and membrane structures at low water conditions (Allugulova et al. 2003; Arora et al. 1997; Campbell and Close 1997; Ingram and Bartels 1996; Rorat 2006; Wise and Tunnacliffe 2004). Dehydrins are in general thermostable, hydrophilic and contain at their carboxy-terminus a “K-motif”, which in angiosperms includes the amino acid residue consensus sequence, EKKGMDKIKEKLPG (Close 1996).

Absence of dehydrins in mature mangroves seeds has been related to vivipary (Farrant et al. 1996); however, there are no reports on their expression during embryogenesis. Presently, we systematically described and staged embryo development in Rhizophora mucronata and assessed the presence of dehydrins in ovules and embryos through development from fertilization to the point of emergence from the micropyle.

Materials and methods

Plant material

Flowers and ovaries from R. mucronata Lam. were collected during February–March from Mbweni fishing village, 40 km from Dar es Salaam, Tanzania. In R. mucronata, several stages of development (from flower bud to propagule) can often be found on a single plant. The trees grow almost exclusively in single stands, and samples were collected from ~25 trees at a single site to ensure that developmental stages were representative of the local population. Synchrony of each developmental stage is at best an estimate, since the sample population is wild and the environment unsettled.

Chemicals

Unless stated otherwise, all chemicals (P.A. grade) were from Merck (Darmstadt, Germany). The polyclonal anti-dehydrin anti-serum and peptide–salt containing the dehydrin K-segment consensus peptide were kind gifts from Dr T. Close (Riverside, CA, USA).

Light microscopy

All materials were prepared according to standard techniques. Ovules were immediately dissected with much of the testa removed before fixation using 2% glutaraldehyde and 1% paraformaldehyde in buffer (100 mM PIPES pH 7.0, 2% sucrose, 0.05% CaCl₂·2H₂O, and 0.1% Tween 20). The samples were infiltrated with Paraplast via a water–ethanol–xylene series. Sections (5–7 μm thick) were cut using an AO 820 microtome (American Optical, Ramsey, MN, USA), were de-paraffinized, rehydrated and stained with 0.1% toluidine blue O in borate buffer (pH 8). In addition, 1-μm thick sections were made using Spurr’s resin-embedded material (see below) and stained with 1% aqueous toluidine blue. Micrographs were made using a Leitz Orthoplan microscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with a CoolSnap digital camera (Roper Scientific, Tucson, AZ) and Meta Vue (Universal Imaging Corporation, West Chester, PA, USA) imaging software.

Electron microscopy

Aldehyde-fixed ovules were post-fixed in 2% OsO₄ in double distilled water, dehydrated in a water–ethanol series and embedded in Spurr’s resin (Polysciences Inc., Warrington PA, USA; Spurr 1969). Ultrathin sections ~90 nm thick were made using a Sorvall MT-5000 ultramicrotome (Analytical Instruments LLC, Golden Valley, MN, USA) and glass knives, with sections mounted on Formvar-coated copper grids. Sections were stained with 2% uranyl acetate for 20 min and lead citrate for 5 min according to Reynolds (1963). Examination and photographing occurred with a JEOL 100CX II transmission electron microscope operated at 60 kV.

Protein extraction

Proteins were extracted from freeze-dried ovules/seeds at different stages of development (Table 1) using the procedure of Farrant et al. (1996) for recalcitrant seeds. The extraction buffer was made up of 50 mM Tris–HCl (pH 7.0), 0.7 M sucrose, 50 mM EDTA, 0.1 M KCl, 2 mM PMSF (phenylmethylsulphonyl fluoride) and 2% β-mercaptoethanol. The samples were ground with extraction buffer (1 ml:10 mg), agitated on ice for 30 min and then centrifuged at 16,000×g at 4°C for 10 min. One additional extraction of the pellet was done. Supernatants were precipitated with 0.1 M ammonium acetate overnight at −20°C. The next day, precipitates were washed with ammonium acetate, dried with acetone and ethanol and resuspended in PBS at pH 7.5. Heating of the extracts did not change the detection of dehydrin-like protein bands and was therefore omitted. For protein quantification we used
Table 1  Morphogenesis staging in *R. macronota* ovaries from anthesis through propagule maturation

<table>
<thead>
<tr>
<th>Stage</th>
<th>Ovary Ø*</th>
<th>Field markers b</th>
<th>Histology c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.4 or less</td>
<td>Anthesis: flower open</td>
<td>Degeneration of nuclerus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calyx: yellow, 2 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corolla: white, 3 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stamens: present, beige</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovary: yellow</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovules: four, translucent pale green</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.4–0.6</td>
<td>Anthers and corolla detached</td>
<td>Fertilization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calyx: yellow</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovules: four, translucent pale green</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.6–0.7</td>
<td>Calyx extension by ovary growth</td>
<td>Early embryogenesis, few-celled globular embryo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovules pale yellow, opaque</td>
<td>Cellular endosperm. Compressed cells with thickened walls surround the embryo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unfertilized ovules smaller than fertilized ones</td>
<td>Start of suspensor formation at the micropylar side</td>
</tr>
<tr>
<td>3</td>
<td>0.8–1.0</td>
<td>Calyx drawn flat (approx. 180°)</td>
<td>Embryo at globular stage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovule colour light peach opaque</td>
<td>Compressed cells with thickened walls differentiate into transfer cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unfertilized ovules smaller than fertilized ones and brown</td>
<td>Development of the transfer cell layer in the integument</td>
</tr>
<tr>
<td>4</td>
<td>1.0–1.2</td>
<td>Calyx pushed out further, bending towards petiole</td>
<td>Embryo polarization with cell divisions in the distal region (away from micropyle)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calyx colour: green</td>
<td>Endosperm with transfer layer surrounding suspensor and embryo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovary colour: yellow-green</td>
<td>Fully developed transfer cell layer in the integument</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovule’s micropylar end open with endosperm visible</td>
<td>Multiseriate suspensor and surrounding transfer cells in endosperm fully developed</td>
</tr>
<tr>
<td>5</td>
<td>1.2–1.4</td>
<td>Ovule expansion in proximal region</td>
<td>Embryo club shaped. Distal region with relatively small and rapidly differentiating cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endosperm visible outside micropyle area</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.4–1.6</td>
<td>Ovary longer than wide</td>
<td>Embryo becoming heart-shaped and developing into a torpedo shaped embryo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovary colour: green</td>
<td>Meristem initiation, site marking future root meristem evident at proximal side</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endosperm clearly protruding from micropyle</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.6–1.8</td>
<td>Ovary wall darkening in proximal region close to petiole</td>
<td>Embryo attains a torpedo shape and emerges from the micropyle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Root and shoot meristem present</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.8–1.9</td>
<td>Hypocotyl obvious outside micropyle</td>
<td>Development of the cotyledonary body</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calyx completely bent towards petiole</td>
<td>Hypocotyl growth through endosperm</td>
</tr>
<tr>
<td>9</td>
<td>1.9–2.0</td>
<td>Ovary colour: dark and with brown spots</td>
<td>Suspensor degenerates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hypocotyl emerged from ovary wall but remain attached to ovary and maternal plant</td>
<td>Fully developed embryo with fused cotyledons pushed out by the endosperm</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
<td>Large green propagules gradually extending</td>
<td>Drying and degeneration of integuments</td>
</tr>
</tbody>
</table>

* a Ovary size classes from field measurements expressed in mm  
  b Field indicators for stage identification  
  c Taken from light microscopic images of endosperm and embryo sections (see also: Juncosa 1982)

the SDS-compatible staining protocol from Dieckmann-Schuppert and Schnittler (1997).

Western blot analysis

Approximately 1.5 μg total protein was separated using SDS-PAGE. Pairs of loaded gels of 12.5% were run at 100 V for 2 h. One gel was electro-blotted onto a 0.45-μm pore size nitro-cellulose membrane (Optitran BA-S 85, Whatman-Schleicher & Schuell GmbH, Dassel, Germany) and dried between two Whatman filter papers for Western blot analysis. As a negative control, 5 μmol tobacco lipid transfer protein (LTP; Nieuwland et al. 2004) having none of the dehydrin K-segment sequence was spotted on the
The counter gel was stained with Coomassie brilliant blue (CBB) for confirmation of protein loading. Blots for Western analysis were washed twice with TBS (pH 7.5). Non-specific binding was blocked with 10% skimmed milk powder in TBS overnight. The primary antibody (Close et al. 1993) directed to the K-segment consensus peptide was 1:1000 diluted in TBS with 5% skimmed milk powder. Incubation was conducted overnight at 4°C. Then, the blots were washed three times for 5 min in TBST (TBS with 0.1% Tween-20) and incubated with the secondary antibody, an anti-rabbit immunoglobulin G conjugate with horseradish peroxidase (Sigma–Aldrich, Zwijndrecht, The Netherlands) diluted 1:20,000 with TBS with 5% skimmed milk powder for 60 min. Finally, blots were washed thrice for 5 min with TBST. As a control, anti-dehydrin serum blocked with the consensus peptide was used. Proteins were detected using a West Pico Chemiluminescence kit (Pierce, Rockford, IL, USA), according to the manufacturer’s recommendations. The film was developed using a CEApro processing machine (CEA GmbH, Hamburg, Germany). The Western analysis was repeated three times and a representative gel is shown.

Results

Morphological characterization

The ~2-mm long flowers of *R. mucronata* Lam. (Fig. 1) are hypogynous. The ovary consists of two carpels. Starting at anthesis, the following field parameters were used to arbitrarily stage further development: (1) Presence and colour of flower parts, i.e., calyx, corolla and stamen. (2) Ovary size and colour. (3) Presence, colour and size of ovules. (4) Visibility of endosperm development. (5) Hypocotyl emergence. The null stage was identified as a recently opened flower with corolla and stamens still attached. A schematic representation of the successive stages together with the major histological events is presented in Table 1 and Fig. 2. The duration of the different stages was variable, but stage 9 was always completed within 13 weeks after anthesis.

Megagametogenesis

Microscopic examination of *R. mucronata* ovaries at anthesis (stage 0) showed two locules with two ovules each. Only one of the four ovules will ultimately develop into a propagule. At stage 0 (Fig. 3a, b), a bitegmic ovule with the embryo sac surrounded by the remnants of a largely degenerated nucellus was clearly recognized. These in turn are enclosed by the inner integument, except at the micropylar region (Fig. 3b, c), where the pollen tubes penetrate the inner integument to reach the embryo sac (Fig. 3c). Some of the embryo sacs appear to have collapsed by this stage, and their ovules degenerate soon afterwards. The distinction between inner and outer integuments is no longer evident at middle stages of embryo development. Integumentary cells appear to remain viable in developing ovules.

Embryogenesis

Only a single fertilized embryo sac develops; all others rapidly degenerate. The loose attachment of the embryo sac to the integuments greatly facilitates microscopic preparation of the early stages (Fig. 3d), but may have led to embryo loss during sectioning and/or fixation. In addition to asynchronous ovule development (Juncosa 1982b), ovule loss may also contribute to the poor understanding of early embryo stages and the sparse documentation of early fertilization stages in this species. Stage 1, anthesis till fertilization, was completed within 7 days, its duration depending on the time of fertilization after anthesis.

During embryogenesis, the endosperm rapidly proliferated, fully surrounding the embryo at stage 2 and separating the embryo from the integument and the micropyle (Fig. 3d). No vascular connections with the maternal tissue were present. The expansion of the endosperm forces the micropyle to open at stage 4, leading to the emergence of the embryo from the micropyle at stage 6 (Fig. 2j). Large flattened cells surround the embryo in stage 2 (Fig. 3d). They develop into the typical layer of labyrinthine transfer cells seen at stage 5 (Fig. 3c, f, inset). With further outgrowth of the embryo, the transfer cells appear to lag during the later development until they degenerate together.
with the suspensor at stage 8. Another layer of transfer cells with thick labyrinthine walls surrounding the endosperm was visible at stage 4 (not shown). This stage was completed within 6 weeks. From the first observations on stage 2, the endosperm appeared cellular and highly vacuolated with scant cytoplasm. Only in the outer endosperm cell layer and in the flattened transfer cells around the embryo (Fig. 3d, e) was denser cytoplasm seen. No indications for the presence of endosperm storage in the form of amyloplasts, lipid globules or protein bodies could be inferred from the light and EM observations.

*Rhizophora mucronata* embryos have a multiseriate suspensor which is first evident at stage 2 (Fig. 3d) and reaches its full development at stage 5, persisting until the emergence of the cotyledon body from the micropyle at stages 7–8 (Fig. 2i). The suspensor points towards the micropyle. At stage 8, the suspensor starts to degenerate. Suspensor cells are relatively large when compared to those of the embryo proper, with distinct globular organelles. As these organelles are not osmiophilic and stain densely with Fast Green FCF (data not shown), they probably represent protein bodies.

The zygote of *R. mucronata* undergoes a series of cellular divisions that result in a globular embryo proper and a suspensor. The division pattern in development does not seem very strict and therefore cannot be attributed to a specific pattern of embryo development (see also Juncosa 1982a). At stage 2, the embryo is clearly globular, and events in embryogenesis such as differentiation of the suspensor became evident (Fig. 3d). By stage 4 (Fig. 3e), the embryo is highly polarized with larger, more highly vacuolated cells in the embryo proper and more amyloplast-containing cells at the suspensor pole. As a result of cell divisions in the chalazal region (opposite the micropyle), the embryo progressively changes its shape into a club during stages 4 and 5. A fully developed transfer cell layer of endosperm surrounds the club-shaped embryo at stage 5 (Fig. 3f). At stage 6, divisions in localized regions of the club-shaped embryo (Fig. 3g) indicate the sites where the meristems will form. Two sites at the outermost layer of the distal region mark the future cotyledons. As cotyledons develop, the region between them marks the site of future shoot meristem formation. Simultaneously, at the centre of the pro-embryo, another region of rapidly dividing cells becomes evident that will form the future apical root meristem. With further divisions, the shape of the embryo is altered from that of a club to that of a heart. This stage is generally reached within 9 weeks. Further cell divisions at stages 6 and 7 give rise to the typical form of the Rhizophoraceae embryo, which forms a typical toroidal cotyledonal body (Fig. 3h) with elongating cotyledons at the beginning of stage 8 (Fig. 3i). During stage 8, the cotyledonal body and hypocotyl develop, and the embryo is pushed out by the endosperm. Stage 8 is completed within 11 weeks. At the end of stage 9, the cotyledons are fused and the embryo emerges. The seed remains attached to the ovary via the hypocotyl. As the integuments degenerate and dry, the large green propagule becomes visible at stage 10.
Fig. 3 Light microscopic images from toluidine blue stained sections of developmental stages 0–8 of *R. micronata* embryogenesis. Unless otherwise indicated, all sections were made at the near median longitudinal plane. **a** Ovule at stage 0 showing inner (*i*) and outer (*o*) integument, nucellus (*nu*) and micropyle (*m*) with micropylar canal (*arrow*). The embryo sac apparently has collapsed. **b** Similar image as in **a** showing a large embryo sac (*es*) with egg apparatus (*ea*) at the micropylar pole. The nucellus (*nu*) is laterally displaced and inconspicuous. **c** At stage 1, two synergids (*s*) with filiform apparatus (*f*) and egg nucleus (*arrow*) are clearly visible. Remnants of the nucellus (*nu*) remain around the egg apparatus, with an apparent pollen tube (*unlabelled arrowheads*) located in the nucellus (*nu*). **d** Endosperm (*en*) with globular embryo (*e*) prepared from a stage 2 ovule. At the micropylar end (*m*), the beginning of the suspensor (*unlabelled arrowhead*) can be seen. Endosperm cells around the embryo have a thick-walled and compressed appearance, whereas the outmost endosperm cells appear to remain small with dense cytoplasm. **e** Embryo (*e*) at stage 4 shows the relatively large cell cells of the suspensor (*unlabelled arrowhead*) towards the micropyle. Suspensor cells contain less cytoplasm but more amyloplasts than cells in the embryo proper. Large transfer-like cells (*i*) with denser cytoplasm surround the embryo. **f** Near cross-section of the ovule at stage 5. Transfer cells (*t*) with thick walls (') around the embryo (*e*). TEM image (inset) clearly shows the labyrinthine character of the thick transfer cell walls. **g** At stage 6, the embryo is club-shaped with relatively small cells forming at the proximal side (*p*). **h** Oblique section through a heart-shaped embryo (*e*) at stage 7 showing toroidal primordium (*white dots*) that will form the cotyledonary body; *unlabelled arrowhead* depicts suspensor pole. **i** At stage 8, cotyledons (*c*) are well developed; shoot (arrow) and root (pointer) apical meristems can be recognized. Suspensor is still visible (upper left). **a**, **b**, **i**: Bars 200 μm; **d**, **g**, **h**: Bars 100 μm; **c**, **e**, **f**: Bars 50 μm

Protein analysis

Western blot analysis indicates protein bands with affinity to the K dehydrin motif between 55 and 65 kDa in ovules and seeds at all stages of development (Fig. 4). An additional band at approximately 50 kDa was consistently observed in stages 6–8. In stage 9, when the embryo emerges from the micropyle, the 65 kDa band remains evident and the 50 and 55 kDa bands become variable and less obvious. The 65 and 55 kDa bands were also found in leaf tissue, together with an additional prominent band at 34 kDa. Protein integrity was confirmed by Coomassie Brilliant Blue staining (not shown).

Discussion

The abscission of corolla and stamens at fertilization formed a clear and reliable mark for the onset of embryogenesis. Ovary size was used to further characterize
The typical layer of the labyrinthine transfer cells surrounding the embryo seen at stage 5 (Fig. 3e, f, inset) appear to degenerate together with the suspensor at stage 8. Due to its transient character, this transfer layer may easily escape the attention. This particular layer of transfer cells may represent a unique development for R. mucronata, as no such layer was recorded in R. mangle (Juncosa 1982b; Wise and Juncosa 1989). As discussed by Wise and Juncosa (1989), transfer cells at the border of suspensor and embryo do occur in many species, though they may have different origins, as for example in Medicago (Sangduen et al. 1983) where they originate from the suspensor. The presence of transfer cell layers between the embryo and the endosperm, whilst vascular connections are absent, shows the emergence of an alternative transport route for water and solutes via the endosperm to the embryo that does not conform to the regular vascular route. Absence of vascular connections, along with the development of two transfer layers associated with the vacuolated endosperm, suggests that a continuous flow of water with nutrients may flow from the integument through the endosperm to the embryo, which would facilitate semi-vivipary. A storage function for the endosperm is then obsolete. The presence of protein bodies in the suspensor cells at stage 7 may indicate their dysfunctional character as protein and lipid bodies are also known to accumulate in the suspensors of defect Arabidopsis embryos (Schwartz et al. 1994).

**Dehydrins**

Using an anti-serum for the K-motif of dehydrins, proteins with a molecular weight of between 50 and 65 kDa were detected in both leaves (not shown) and ovules (Fig. 4). Because we did not confirm their true dehydrin character by cDNA or functional analysis, the proteins are referred to as dehydrin-like (Close et al. 1993). The proteins detected here were considered dehydrin-like, based on the immunological affinity with the typical K-motif, which serves as a marker for dehydrins (Close 1997). As the embryos do not undergo dormancy typical of other angiosperms, a possible role for their presence in ovules might include osmotic protection from infiltrating salt, adding to other observed salt stress adaptations in the form of well developed transfer tissues and absence of direct vascular tissue connection with the mother plant (Wise and Juncosa 1989).

Absence of dehydrin-like proteins is not a general feature of recalcitrant seeds, as their absence has been reported to occur in recalcitrant seeds of a number of species from temperate climates and in tropical species, i.e. Castanospermum australe, when growing in temperate climates (Farrant et al. 1996). They have, however, been reported absent in species from tropical wetlands, including mangroves from the genera Avicennia, Bruguiera and
Rhizophora and some other species (Farrant et al. 1996; Han et al. 1997; Kermode 1997). Recently, Panza et al. (2007) showed the presence of dehydrins in the extremely recalcitrant seeds of the tropical palm tree Euterpe edulis. Our observation of dehydrin-like proteins during seed development in Rhizophora mucronata suggests that they may also be present in some other tropical species that lack seed dormancy.

With maturation at stage 9, some dehydrin-like proteins seem to disappear. However, the 65 kDa band remained and was also present in leaves, suggesting that this dehydrin-like protein was not developmentally regulated but more generally expressed, e.g., as an adaptation to salt stress. In conventional (orthodox) embryos, dehydrin expression may already be initiated at the heart stage (e.g., Zimmerman 1993; Lin et al. 1996; Sankara Rao 1996), but does not cease as the embryo grows and finally becomes desiccated. The early presence of dehydrin-like proteins well before desiccation suggests that they may also play additional roles. The appearance of the 50-kDa protein in R. mucronata ovules coincides with the heart-shaped embryo (stage 6) and may mark the onset of a conventional developmental programme, which may become suppressed with the onset of semi-vivipary.

In conclusion, R. mucronata embryogenesis shows a series of developments that express a radical adaptation to an extreme form of recalcitrance, to which also the disappearance of especially the 50-kDa dehydrin-like protein might be attributed.

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