Presence of dehydrin-like proteins and levels of abscisic acid in recalcitrant (desiccation sensitive) seeds may be related to habitat

Jill M. Farrant¹*, Norman W. Pammenter², Patricia Berjak², Elizabeth J. Farnsworth¹ and Christina W. Vertucci³

¹Department of Botany, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa
²Plant Cell Biology Research Unit, Department of Biology, University of Natal, Private Bag X10, Dalbridge, 4014, South Africa
³USDA, Agricultural Research Service, National Seed Storage Laboratory, 1111 S. Mason St., Fort Collins, CO 80521, USA

Abstract

The presence of dehydrins could not be demonstrated in axes of mature, undried recalcitrant seeds of the tropical wetland species Avicennia marina, Barringtonia racemosa, Bruguiera exaristata, Bruguiera cylindrica, Bruguiera gymnorrhiza, Ceriops tagal, Rhizophora apiculata, Rhizophora mcrinata and Rhizophora stylosa, but were present in the temperate species Acer saccharinum, Aesculus hippocastanum, Araucaria angustifolia, Camellia sinensis, Castanea sativa, Poncirus trifoliata and Zizania palustris. They were also present in axes of Castanopsis australis (of tropical origin) seeds which underwent development in a temperate climate, and were produced in response to drying in axes of Barringtonia racemosa but not Avicennia marina. The presence of dehydrins was associated with high abscisic acid contents. These proteins may provide protection against low temperatures in temperate seeds and against water loss to which the seeds may be naturally exposed. The presence of dehydrins was unrelated to the evolutionary status of the families studied.

Keywords: abscisic acid, dehydrin, desiccation-tolerance, LEA proteins, recalcitrant seeds.

Introduction

In the seeds of many species (so-called orthodox seeds) the ability to tolerate considerable water loss is acquired during the late stages of development. Recalcitrant seeds, on the other hand, never become as desiccation tolerant, although there is considerable variation among species in the amount of water loss that can be tolerated (Farrant et al., 1988; Vertucci and Farrant, 1995). The recalcitrant seeds of several species do increase in desiccation tolerance during development (e.g. Berjak et al., 1992, 1993; Finch-Savage, 1992; Vertucci et al., 1995) although this is not the case in all recalcitrant seeds (Farrant et al., 1993a). The only characteristic that appears to be common among recalcitrant seeds is their inability to withstand the loss of non-freezable water (Pammenter et al., 1993). However, neither the cause of desiccation sensitivity of recalcitrant seeds, nor the mechanisms whereby orthodox seeds are able to tolerate desiccation, are yet fully understood.

A particular set of proteins termed LEAs (late embryogenesis abundant [Galau et al., 1986]) has been implicated in the acquisition of tolerance to drying in developing seeds (reviewed by Bewley and Oliver, 1992; Vertucci and Farrant, 1995). LEA homologues (dehydrins) are also induced in response to water stress in vegetative tissue (Close et al., 1989, 1993a,b).

The presence of these proteins has been associated with high contents of ABA (Kermode, 1990) and ABA can induce their production (Galau et al., 1986; Finch-Savage et al., 1994). The physical nature of these proteins, together with the situations under which they are expressed, has led to the suggestion that they function in the survival of water stress by acting as protectants (Close et al., 1989; Dure et al., 1989) and/or by stabilizing the subcellular structures in the dry state (Lane, 1991; Close et al., 1993a,b; Dure, 1993).

Indirect evidence for a role of these proteins in the mechanism of desiccation tolerance would be their absence from recalcitrant seeds. However, dehydrin-like proteins have been shown to be present in the recalcitrant seeds of the temperate species Zizania palustris (Bradford and Chandler, 1992; Still et al., 1994) and Quercus robur, Castanea sativa, Aesculus hippocastanum, Acer pseudoplatanus and Acer saccharinum (Finch-Savage et al., 1994). To date there are no reports of any species where dehydrin-like or LEA proteins
have not been detected in mature seeds. The objective of this investigation was to ascertain whether or not LEA-type proteins occur in recalcitrant seeds from a range of climatic zones and habitats, and whether their presence or absence is related to ABA content.

Materials and methods

Species studied

Avicennia marina (Forssk.) Vierh. (Verbenaceae), Bruguiera cylindrica (L.) Bl., Bruguiera exaristata Ding Hou, Bruguiera gymnorrhiza (L.) Lamk., Ceriops tagal (Perr.) C.B. Rob., Rhizophora apiculata Bl., Rhizophora mucronata Lamk. and Rhizophora stylosa Griff. (Rhizophoraceae) are mangroves restricted to intertidal habitats in tropical and subtropical regions (Tomlinson, 1986). Barringtonia racemosa (L.) K. Spreng. (Lethycidaceae) is a tropical wetland and riparian tree (Pooley, 1993). Castanospermum australe A. Cunn. et Fras. (Fabaceae = Leguminosae) is a tree from the coastal forests of tropical and subtropical eastern Australia (Stanley and Ross, 1983). Canellia sinensis (L.) O. Kuntze (Theaceae) is cultivated in the subtropics and throughout the tropics at higher elevation. It is thought to have originated in the lower Tibetan mountains or highlands of south east China (Visser, 1976). Araucaria angustifolia (Bert.) O. Kuntze (Araucariaceae) is a gymnosperm from southern Brazil, northern Argentina and Paraguay (Walters, 1974). Poncirus trifoliata (L.) Raf. (Rutaceae) originated in northern and central China and is planted widely in the southern United States, where it is also used as a citrus rootstock (Bailey and Bailey, 1976); the cultivar Rubidoux was used in these studies. Acer saccharinum L. (Aceraceae), Aesculus hippocastanum L. (Hippocastanaceae) and Castanea sativa Mill. (Fagaceae) are northern hemisphere temperate trees. Acer saccharinum fruits are shed in spring/early summer and the seeds germinate immediately (Olson and Gabriel, 1974), whereas in the other two species seeds are dispersed in autumn and overwinter in a hydrated state. Zizania palustris (Fasset) Dore (Poaceae) is a cold temperate aquatic grass, the seeds of which require stratification to overcome dormancy (Aiken et al., 1988).

Collection of seed material

Embryonic axes of mature seeds only were studied. Seeds of Castanea sativa were purchased from a local supermarket, postharvest treatment unknown. Zizania palustris seeds were stored for one month at 5°C prior to extraction. All other seeds were either collected locally or received within one week of collection. Only newly shed seeds were collected. On arrival at the laboratory seed material was lyophilized and stored at −80°C until extraction. For Avicennia marina and Barringtonia racemosa, proteins and ABA were extracted from fresh seeds and from seeds that had been dried (using silica gel as described by Berjak et al., 1984) for up to 6 days when viability was lost. Aesculus hippocastanum seeds from 1992 and 1994 harvests were investigated. Only a single harvest of all other species was studied.

Extraction and separation of proteins

Freeze-dried samples were weighed, ground to a fine powder and suspended in cold extraction buffer (50 mM Tris-HCl [pH 7.0], 0.7% sucrose, 50 mM EDTA, 0.1 mM KCl, 2% β-mercaptoethanol, 2 mM PMSF [phenylmethylsulphonyl fluoride) in a ratio of 1 ml buffer: 10 mg tissue. After incubation on ice for 10 min, the homogenate was centrifuged at 16 000 × g for 10 min and the protein content of the supernatant was determined (Bradford, 1976). Heat-stable proteins were obtained by incubating the supernatant at 80°C for 10 min. Heat-coagulated proteins were removed by centrifugation. Aliquots containing 85 μg protein were removed from the supernatant and an equal volume of SDS buffer (50 mM Tris-HCl [pH 6.8], 20% glycerol, 10% β-mercaptoethanol and 4% SDS) was added. Samples were heated (100°C, 3 min) and the proteins were separated by SDS-PAGE (Laemmli, 1970). A heat stable extract from mature pea (Pisum sativum) axes (Russouw et al., 1995) was run on each gel as a control and for assessing colour development during Western blotting procedures. Extractions and electrophoreses were performed in triplicate.

Western blotting

Following electrophoresis, proteins were transferred onto a 45-μm pore-size nitrocellulose membrane by standard methods (Harlow and Lane, 1988). Air-dried membranes were incubated for 30 min at room temperature in PBS, pH 7.4, containing 50 mM phosphate, 150 mM NaCl and 5% (w/v) fat-free milk powder. Dehydrin antibody raised against the carboxy terminus of a consensus sequence common to dehydrins (Close et al., 1993a) was added (final dilution 1:1000) and the mixture was incubated for 2 h at room temperature. Membranes were washed three times (10 min each) in PBS containing 0.05% Tween 20. They were then incubated (1 h at room temperature) in PBS buffer and goat anti-rabbit antibody coupled to alkaline phosphatase (dilution 1:500). Membranes were washed as described above with the addition of a fourth wash in 10 mM Tris-HCl (pH 7.4) with 150 mM NaCl. The blot was incubated in 0.1 mM Tris-HCl (pH 9.5) containing 100 mM NaCl and 100 mM MgCl2. For signal development, 0.05% nitroblue tetrazolium (in 70% dimethylformamide [DMF]) and 0.05% 5-bromo-4-chloro-3-indolyl
Table 1. The molecular weights (kDa) of dehydrin-like proteins and ABA content (ng (g dry mass)^{-1}) present in embryonic axes of seeds from a range of recalcitrant species of widely differing natural habitats. A dash (-) indicates that the tissue was not analysed for ABA.

<table>
<thead>
<tr>
<th>Species</th>
<th>Natural habitat</th>
<th>Dehydrin mol wt</th>
<th>ABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avicennia marina — fresh</td>
<td>tropical wetland</td>
<td>absent</td>
<td>50</td>
</tr>
<tr>
<td>Avicennia marina — dried</td>
<td>tropical wetland</td>
<td>absent</td>
<td>50</td>
</tr>
<tr>
<td>Bruguiera cylindrica</td>
<td>tropical wetland</td>
<td>absent</td>
<td>5</td>
</tr>
<tr>
<td>Bruguiera exaristata</td>
<td>tropical wetland</td>
<td>absent</td>
<td>31</td>
</tr>
<tr>
<td>Bruguiera gymnorhiza</td>
<td>tropical wetland</td>
<td>absent</td>
<td>6</td>
</tr>
<tr>
<td>Ceriops tagal</td>
<td>tropical wetland</td>
<td>absent</td>
<td>45</td>
</tr>
<tr>
<td>Rhizophora apiculata</td>
<td>tropical wetland</td>
<td>absent</td>
<td>7</td>
</tr>
<tr>
<td>Rhizophora mangle</td>
<td>tropical wetland</td>
<td>absent</td>
<td>4</td>
</tr>
<tr>
<td>Rhizophora stylosa</td>
<td>tropical wetland</td>
<td>absent</td>
<td>0</td>
</tr>
<tr>
<td>Barringtonia racemosa — fresh</td>
<td>tropical wetland</td>
<td>absent</td>
<td>23</td>
</tr>
<tr>
<td>Barringtonia racemosa — dried</td>
<td></td>
<td>16, 43</td>
<td>290</td>
</tr>
<tr>
<td>Castanospermum australe</td>
<td>tropical forest</td>
<td>50-60</td>
<td>392</td>
</tr>
<tr>
<td>Camellia sinensis</td>
<td>tropical montane/warm temperate</td>
<td>40</td>
<td>252</td>
</tr>
<tr>
<td>Araucaria angustifolia</td>
<td>warm temperate</td>
<td>23, 26, 28</td>
<td></td>
</tr>
<tr>
<td>Poncirus trifoliata</td>
<td>warm temperate</td>
<td>30, 38, 42</td>
<td></td>
</tr>
<tr>
<td>Ziziphus jujuba</td>
<td>cold temperate aquatic</td>
<td>21, 24</td>
<td></td>
</tr>
<tr>
<td>Acer pseudosieboldii</td>
<td>cold temperate</td>
<td>20, 25</td>
<td></td>
</tr>
<tr>
<td>Castanea sativa</td>
<td>cold temperate</td>
<td>46, 180</td>
<td></td>
</tr>
<tr>
<td>Aesculus hippocastanum</td>
<td>cold temperate</td>
<td>12, 14, 18</td>
<td>371</td>
</tr>
<tr>
<td>Aesculus hippocastanum</td>
<td>cold temperate</td>
<td>23, 30, 35-55</td>
<td></td>
</tr>
</tbody>
</table>

ABA analysis

ABA content of the embryonic axes of some species was measured by radioimmunoassay following the protocol described by Farrant et al., (1993b). Two replicate subsamples of 0.2 g were extracted in 5 ml of 70% methanol containing 20 mg l^{-1} butylhydroxytoluene and 50 mg l^{-1} sodium ascorbate. The samples were centrifuged at 20 000 g for 10 min and (^{14}C)-ABA (10 000 dpm) was added to the supernatant to determine recoveries. The supernatant was filtered using a 0.45-μm polytetrafluoroethylene filter and then passed through a C18-Sep-Pak column. The filtrate was reduced to dryness and redissolved in 1 ml 10% methanol in 0.1 M acetic acid (pH 3.5) for purification of the ABA by HPLC. The retention time of ABA was determined by injecting an authentic standard and this was verified by the production of an immunohistogram (Farrant et al., 1993b). Fractions corresponding to the elution time for ABA were used for quantification by radioimmunoassay. These were done in triplicate with serial dilution tests. The data were corrected for cross-reactivity.

Results

Because of the large number of species studied, representative immunoblots of only some species are illustrated. All results are summarized in Table 1. No bands were detected when allele proteins of fresh Avicennia marina and Barringtonia racemosa were exposed to the antibody raised against the carboxy terminus of a consensus sequence common to dehydrins (Fig. 1). The preimmune serum did not detect proteins and those immunoblots are not shown. Embryonic axes of Castanospermum australe had two dehydrin-like proteins with molecular masses of approximately 50 and 60 kDa and axes of Camellia sinensis had one dehydrin with a molecular mass of 40 kDa. These were not detected by the preimmune serum. In axes of Aesculus hippocastanum three bands of molecular mass 12, 14 and 18 kDa were detected by the antibody but not by the preimmune serum (Fig. 1).

In order to ascertain whether dehydrin-like proteins could be induced by dehydration of embryos of Avicennia marina and Barringtonia racemosa, proteins were extracted from axes that had been dried to a range of moisture contents. Dehydration of axes of Barringtonia racemosa from 1.6 to 1.2 g H_2O (g dry mass)^{-1} resulted in the appearance of two dehydrin-like proteins with molecular masses of 16 and 43 kDa, respectively (Fig. 2). Drying below this moisture content did not cause further change to the protein status (Fig. 2). Drying did not induce dehydrin-like proteins in axes of A. marina.
These data, and those from the other species tested, are summarized in Table 1. None of the mangrove species showed proteins that reacted with the antibody in the axes of mature seeds. Undried seeds of *Barringtonia racemosa* did not have dehydrin-like proteins, but two proteins with molecular masses of 16 and 43 kDa were detected when axes were dried. All other species had proteins that were detected by this antibody. *Camellia sinensis*, *Castanospermum australe* and *Castanea sativa* showed only high molecular mass (>30 kDa) dehydrin-like proteins. Axes of *Poncirus trifoliata* and *Aesculus hippocastanum* harvested in 1994 showed bands corresponding to both high and low (<30 kDa) molecular mass dehydrins. However, some of the high molecular mass dehydrins (in the 35- to 55-kDa range) detected in axes of *Aesculus hippocastanum* were also detected by the preimmune serum and may not have been true dehydrins. Only low molecular mass dehydrin-like proteins occurred in *Araucaria angustifolia*, *Acer saccharinum*, *Zizania palustris* and the 1992 *Aesculus hippocastanum* harvest.

The species that did not show dehydrin-like proteins in the axes in fresh material had low contents of ABA (≤50 ng (g dry mass))<sup>−1</sup>. In the other species for which ABA data were obtained, the presence of dehydrin-like proteins was associated with high contents of ABA (>250 ng (g dry mass))<sup>−1</sup>. Dehydration of mature axes of *Barringtonia racemosa* to 1.2 g H<sub>2</sub>O (g dry mass)<sup>−1</sup> resulted in an increase in ABA concentration from 23 to 290 ng (g dry mass)<sup>−1</sup>. Drying did not change ABA concentrations in axes of *Avicennia marina* (Table 1).

**Discussion**

This study has shown that although the presence of dehydrin-like proteins could be demonstrated in the
axes of mature recalcitrant seeds of a variety of species from a range of habitats, these proteins appeared to be absent from the mangroves and fresh seeds of *Barringtonia racemosa*, which are all tropical wetland species. Drying induced two dehydrin-like proteins in *Barringtonia racemosa* but none in *Avicennia marina*. Although the appearance and disappearance of dehydrin-like proteins during development and germination was not followed, the presence of proteins homologous with LEAs in some mature recalcitrant seeds supports the now widely-held view that the presence of LEAs alone is not adequate to confer tolerance to considerable water removal (Blackman et al., 1992; Finch-Savage et al., 1994; Vertucci and Farrant, 1995). However, the converse might be true; the absence of LEAs may imply an inability to tolerate desiccation. The absence of dehydrin-like proteins from *Avicennia marina* at any stage of development is in keeping with the observation of Farrant et al. (1992) that patterns of protein synthesis in this species did not change significantly during development and early germination.

LEAs or dehydrin-like proteins have been identified in a number of non-orthodox seeds (of mostly temperate species) by other workers, frequently using the same antibody used in this study. These data are summarized in Table 2. Where species are common to Tables 1 and 2, there is general agreement on the presence and molecular mass range of the proteins visualized.

The absence of dehydrin-like proteins from seeds of species such as the mangroves, but their presence in others, could have an evolutionary or functional basis. Von Teichman and van Wyk (1991, 1994) have suggested that seed recalcitrance is an ancestral characteristic. The families of the species studied in this report can be arranged in the following order of relative evolutionary status (Cronquist, 1988; von Teichman and van Wyk, 1994): Araucariaceae, Fagaceae, Theaceae, Lecythidaceae, Fabaceae (=Leguminosae), Rhizophoraceae, Hippocastanaceae, Aceraceae, Rutaceae, Verbenaceae, Poaceae. There appears to be no relationship between the presence or absence of dehydrin-like proteins, or the molecular mass distribution, and the evolutionary status of the families to which the species belong. Dehydrin-like proteins are absent from *Avicennia marina* (Verbenaceae, advanced) and present in *Araucaria angustifolia* (Araucariaceae, primitive). Only low molecular mass proteins are present in species from the most advanced (Zizania palustris, Poaceae) and most primitive (Araucaria angustifolia, Araucariaceae) families. However, von Teichman and van Wyk (1994) point out that in some taxa recalcitrance may have arisen secondarily as a reversal (in this study this possibly applies to *Avicennia marina* and *Zizania palustris*), and that the extent to which genetic information for the production of LEAs is present or expressed could have evolved at different rates in various genera within a family and in various species within a genus.

The presence or absence of dehydrin-like proteins in mature recalcitrant seeds could be of functional significance and be associated with the extent of drying or low temperature to which the seeds are exposed. There does appear to be some relationship between low temperature and water stresses (Guy, 1990; Bohnert et al., 1995). Both low temperatures and dehydration induce the expression of dehydrin genes in vegetative material (Neven et al., 1993; Robertson et al., 1994). If the damage caused by low temperatures and by water loss is similar, the occurrence of dehydrin-like proteins in recalcitrant seeds can be viewed in terms of temperature exposure, as well as water loss. The nine species without dehydrin-like proteins in fresh seeds are all tropical wetland species in which seed development and germination occur under conditions where low temperatures are unlikely. Although *Castanopsis australis* is also of tropical origin, the trees from which the seed material for this study were collected were growing in Pietermaritzburg, South Africa. At this location, night-time temperatures in winter (the season during which the seeds develop) frequently drop below 5°C, and occasionally below 0°C.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dehydrin mol wt (kDa)</th>
<th>Comments</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer saccharinum</em></td>
<td>18–20, 30–40</td>
<td>axes and cotyledons</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>20, 60</td>
<td>whole seed</td>
<td>2</td>
</tr>
<tr>
<td><em>Acer pseudoplatanus</em></td>
<td>18–20, 30–40</td>
<td>axes and cotyledons</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>20, 60</td>
<td>whole seed</td>
<td>2</td>
</tr>
<tr>
<td><em>Zizania palustris</em></td>
<td>20, 26</td>
<td>20 kDa band increases with drying</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>increases with maturity</td>
<td>3</td>
</tr>
<tr>
<td><em>Castanea sativa</em></td>
<td>36</td>
<td>cotyledons</td>
<td>1</td>
</tr>
<tr>
<td><em>Aesculus hippocastanum</em></td>
<td>14</td>
<td>axes and cotyledons</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>many preimmune serum bands</td>
<td>1</td>
</tr>
<tr>
<td><em>Porteresia coarctata</em></td>
<td>20</td>
<td>mature dried seeds</td>
<td>2</td>
</tr>
<tr>
<td><em>Quercus rubra</em></td>
<td>12, 20, 32, 56</td>
<td>axes and cotyledons</td>
<td>1</td>
</tr>
</tbody>
</table>

Sources: 1, Finch-Savage et al. (1994); 2, Gee et al. (1994); 3, Still et al. (1994)
Of the warm temperate species, seeds of *Araucaria angustifolia* develop over two years (Walters, 1974) and so will be exposed to chilling temperatures during development. *Camellia sinensis* is of montane origin and would have evolved under conditions of periodic low temperatures. *Poncirus trifoliata* has amongst the highest acquired hardiness of *Citrus* and related genera, and the fruits mature during winter (Yelonsky, 1985). Among the cold temperate species, seed development (the most desiccation sensitive phase) of *Acer saccharinum* occurs during spring (Olson and Gabriel, 1974), when nighttime chilling temperatures are likely to occur. The other cold temperate species over-winter as hydrated seeds and will also be exposed to low temperatures.

Several recalcitrant seed species are known to undergo some water loss and to increase in desiccation tolerance during development (*Camellia sinensis* [Berjak et al., 1993], *Zizania palustris* [Vertucci et al., 1995], *Quercus robur* [Finch-Savage, 1992], *Aesculus hippocastanum* [Tompsett and Pritchard, 1993], *Acer pseudoplatanus* [Hong and Ellis, 1990]), and these all show the presence of dehydrin-like proteins. These proteins may play a role in this increased level of tolerance. *Avenicennia marina* does not have dehydrin-like proteins and the water content and desiccation sensitivity of this species does not change with development (Farrant et al., 1993a). All of the other species in which dehydrin-like proteins could not be detected in the fresh seeds were tropical wetland species. While the changes in water content and in degree of desiccation sensitivity of those seeds during development is not known, it is unlikely that they would be exposed to any marked degree of water loss. Our unpublished observations indicate that there is no change in water content with development of seeds of *Bruguiera gymnorrhiza* and in the inner tissues of the embryo of *Barringtonia racemosa*. The water content of the outer embryonic tissues (immediately adjacent to the fibrous seed coat) does decline from ca 4.0 g H₂O (g dry mass)⁻¹ at histodifferentiation to 1.6 g H₂O (g dry mass)⁻¹ at maturation. However, this is still a high water content and the decline in water content associated with development may not be so severe as to necessitate or induce LEA production.

Post-shedding drying of *Barringtonia racemosa* embryos results in an increase in ABA and the detection of two dehydrin-like proteins. Thus the genetic information appears to be present in this species, but the proteins themselves are accumulated to detectable levels only on exposure to this additional water loss. Dehydration has also been reported to stimulate the accumulation of dehydrins in the desiccation-sensitive seeds of the wetland species *Porteresia coarctata* and *Zizania palustris* (Gee et al., 1994). Thus it does appear that some wetland species do have the potential to express dehydrin-like proteins in response to water stress. This is not the case in seeds of *Avenicennia marina* and it is possible that the genes are not present in that species, or are not activated in response to drying.

ABA has been associated with survival of water stress and also induces the production of dehydrin-like proteins (Galau et al., 1986; Kermode, 1990; Finch-Savage et al., 1994). It is noteworthy that ABA is present in only very low amounts in those species that do not have dehydrins in axes of fresh seeds, whereas high concentrations were measured only in species where dehydrins were present. Furthermore, post-shedding drying resulted in increased axis ABA concentrations and the production of dehydrin-like proteins in *Barringtonia racemosa*. Similar drying of *Avenicennia marina* did not cause increased ABA contents and no dehydrins were detected.

Acknowledgements

The authors would like to thank D. Biggs and L. Hill for technical assistance and Dr I. von Teichman, University of Pretoria, for helpful discussions on evolutionary aspects of seed recalcitrance. Dr T. J. Close, University of California, Riverside, is especially thanked for the donation of the antibody.

References


Dehydrins in recalcitrant seeds — importance of habitat


Received 18 August 1996, accepted 16 September 1996
© CAB INTERNATIONAL, 1996

---

For readers and subscribers in North America...

CAB INTERNATIONAL has just relocated its North American office to New York. If you have any queries regarding your subscription to this journal please feel free to contact Pam Sherman, our North American Market Manager at:

CAB INTERNATIONAL
198 Madison Avenue
New York
NY 10016
USA

Tel: 212 726 6490 or 212 726 6491
Toll-free: 800 528 4841
Fax: 212 686 7993
E-mail: cabi-nao@cabi.org

All matters relating to the scientific content and development of the journal should be directed to either the Editor or Publishing Editor – addresses can be found on the preliminary pages of this issue.