The recalcitrant plant species, *Castanospermum australe* and *Trichilia dregeana*, differ in their ability to produce dehydrin-related polypeptides during seed maturation and in response to ABA or water-deficit-related stresses

Bin Han¹, Patricia Berjak², Norman Pammenter², Jill Farrant³ and Allison R. Kermode¹

¹ Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6 Canada
² Department of Biology, University of Natal, Durban 4001, South Africa
³ Department of Botany, University of Cape Town, Cape Town 7700, South Africa

Received 12 February 1997; Accepted 15 April 1997

Abstract

In contrast to seeds of orthodox species, those of recalcitrant species do not acquire desiccation tolerance during their development and are shed from the parent plant at high water contents. Dehydrin production in seeds of recalcitrant species was examined during development and germination, in response to abscisic acid (ABA), and following the imposition of various water-deficit-related stresses, including desiccation, water stress, high salt, high osmolarity, and low temperature. Two tropical species exhibited a differential capacity to produce dehydrin-related polypeptides during seed maturation. Dehydrins were present in axes and cotyledons of *Castanospermum australe* seeds during mid-maturation and at maturity. In *Trichilia dregeana*, no dehydrin-related polypeptides were detected in the mature seed. During the development of *C. australe* seeds, the nature of the dehydrin-related polypeptides accumulated in the cotyledons and axis changed and new polypeptides were detected in the mature seeds that were not present during mid-maturation. The dehydrins present in cotyledons of mature seeds (31, 37 and 40 kDa) were still detectable after germination (i.e. in untreated seedlings). These dehydrins became less abundant in the cotyledons of *C. australe* seedlings following ABA and all stress treatments except cold, although most of the dehydrins were still detectable. An exception was the desiccation-treated seedlings, in which no dehydrins were detected. In the roots of *C. australe* seedlings, no dehydrins were found after germination nor were they induced in the root by ABA or any of the stress treatments imposed on seedlings. Seedlings of *Trichilia dregeana* did not produce dehydrins in the roots or cotyledons when exposed to ABA or water-deficit-related stresses.

Key words: Dehydrin, ABA, desiccation, recalcitrant, seed.

Introduction

The terms ‘orthodox’ and ‘recalcitrant’ are used to describe the storage behaviour of seeds. Orthodox seeds are shed from the parent plant at low moisture contents, having undergone maturation drying prior to this event, and can generally be further dried to moisture contents in the range of 1–5% without damage. In this dehydrated state, the seed can resist the vicissitudes of the environment, and unless dormant, will resume full metabolic activity, growth and development when conditions conducive to germination are provided. Because of these properties, such seeds can be stored for long periods. Recalcitrant seeds, on the other hand, do not undergo maturation drying, and are shed at relatively high moisture contents. Such seeds are highly susceptible to desiccation injury, and thus are not storabile under conditions suitable for orthodox seeds (reviewed in Vertucci and Farrant, 1995; Smith and Berjak, 1995). Furthermore, the seeds of many recalcitrant species are sensitive to chilling injury at lowered temperatures (Farrant et al., 1988).

The cause of desiccation sensitivity of recalcitrant seeds, and indeed the mechanism of desiccation tolerance in...
seeds of orthodox species, is not well understood. In orthodox seeds, a group of proteins is synthesized during mid- to late-maturation that are presumed to play a protective role during desiccation. This group of proteins, termed dehydrins (a subset of the proteins termed LEAs; Late Embryogenesis Abundant or RABs; Responsive to ABA), have some common features in their structure that may be important for their putative protective function. Precocious appearance of the proteins and their mRNAs can be induced in cultured immature embryos by ABA treatment. Specifically, it has been hypothesized that, during development, high concentrations of ABA induce the accumulation of these polypeptides and hence prepare the embryo for desiccation or possible cellular disruption upon subsequent rehydration (reviewed in Bray, 1991, 1993; Chandler and Robertson, 1994; Kermode, 1990, 1995, 1997; Bewley and Oliver, 1992). The proteins are characterized by a conserved 15-amino acid, lysine-rich sequence near the carboxyl terminus. The consensus polypeptide forms an amphipilc α-helix which may serve among other functions as an ion trap in dehydrating cells, sequestering ions as they become concentrated (Close et al., 1993a; Dure, 1993).

Dehydrin genes exhibit a flexible expression repertoire, being responsive to both developmental and environmental cues (reviewed in Thomas et al., 1991). In addition to being expressed under abnormal (stress) conditions, they exhibit temporal regulation during seed development where expression is most intense during mid- to late-development. Hence, the protective role of dehydrins in the survival of water loss is purported to be dual: during maturation drying of the developing seed and following germination/growth of the mature seed (i.e. in seedlings or plant vegetative tissues undergoing mild water stress).

The question arises as to whether the desiccation sensitivity of recalcitrant seeds is at least partially the result of an insufficient accumulation of dehydrins, or whether other factors (including a lack of protective sugars) are more important. Other features that may be part of the basis of desiccation-intolerance include an inability to repair desiccation-induced damage upon subsequent rehydration and an inappropriate proportion or distribution of freezable and non-freezable (bound) water within the seed (Berjak et al., 1992; reviewed in Bewley and Oliver, 1992; Vertucci and Farrant, 1995). An interesting hypothesis is that dehydrins have detergent and chaperone-like properties and may interact with compatible solutes to serve as structural stabilizers of macromolecules under conditions of water deficit (Close, 1996). Dehydrins have been reported to be absent during the late stages of development of recalcitrant mangrove (Avicennia marina) seeds, a finding suggested to account for the lack of desiccation tolerance of this species (Farrant et al., 1992, 1996). In grains of Zizania palustris, a seed that exhibits characteristics intermediate between recalcitrance and orthodoxy (Bradford and Chandler, 1992; but see also Vertucci et al., 1994; Probert and Brierley, 1989), dehydrins can be detected by the maize dehydrin antiserum in both embryos and seedlings (Bradford and Chandler, 1992). The Z. palustris embryos and seedlings are also capable of ABA accumulation during limited dehydration. The intolerance of Z. palustris seeds to dehydration at low temperature does not seem to be due to an absence of dehydrins or an inability to accumulate ABA (Bradford and Chandler, 1992). This indicates that the presence of dehydrins alone is not sufficient to prevent desiccation injury (Blackman et al., 1991; Bradford and Chandler, 1992). Finch-Savage et al. (1994) detected dehydrins in mature seeds of five desiccation sensitive (recalcitrant) trees (all temperate species), and Lea mRNA can also be induced in stored recalcitrant seeds of Quercus robur L. (English oak) by ABA and limited drying treatments. Since desiccation tolerance is a quantitative feature (Vertucci and Farrant, 1995) the amount of dehydrins/LEAs, or the rate at which the proteins accumulate, may determine the level of tolerance. Accumulation of other protectants is likely to be required in addition to LEA proteins (Blackman et al., 1992) since interactions in combination may be necessary to stabilize macromolecules under conditions of water deficit (Close, 1996; Ingram and Bartels, 1996).

Although all recalcitrant seeds are considered to be desiccation sensitive, the degree of water loss tolerated varies with the species; hence, dehydrin gene expression needs to be investigated in a wider range of recalcitrant seed types. Further, temporal studies to examine changes in dehydrin production during development and following germination are necessary. This is especially important since there is often no clear-cut event delineating the end of seed development and the start of germination in recalcitrant species (Vertucci and Farrant, 1995). Moreover, even though seeds of recalcitrant species may be capable of dehydrin synthesis during their development, a decline in the synthesis of dehydrins (or an inability to produce specific dehydrins) following the transition to germination/seedling growth may be an important factor contributing to losses in viability after seed shedding.

Using a dehydrin antibody (Close et al., 1993a), dehydrin-related proteins were compared in immature and mature seeds of two tropical species that are recalcitrant—Castanospermum australe and Trichilia dregeana. The responses of seeds and seedlings to ABA and water-deficit-related stresses were further examined.

Materials and methods

Plant material

Trichilia dregeana seeds were collected at mid-maturation and at maturity from trees growing in Durban (rainfall 1018 mm
per year, mean temperature 20 °C with a mean winter minimum about 10–12 °C and a mean summer maximum about 30 °C). Seeds of *C. australe* were collected at mid-maturation and at maturity from trees growing in Pietermaritzburg, which is hotter in summer and cooler in winter than Durban and has slightly lower rainfall. The average minimum temperature during fruiting of *C. australe* (May to June) is approximately 8 °C. Axes and cotyledons were obtained from immature seeds at mid-maturation, from mature seeds and from seedlings with radicle lengths of approximately 25 mm (obtained by germinating mature seed as outlined below). Some immature seeds and seedlings of *C. austral* and *T. dregeana* were first subjected to the various treatments outlined below.

**Seed germination**

Mature seeds were surface-sterilized in a solution of 1% hypochlorite, 0.01% SDS for 10 min, rinsed three times in sterilized water, and seed coats removed under aseptic conditions in a flow hood. Seeds were placed in Petri dishes containing sterile filter paper moistened with 10 ml sterile water and germinated at 26 °C in the dark until they had acquired radicle lengths of approximately 25 mm. Seedlings were then used for the various treatments.

**ABA treatments**

Immature seeds and seedlings were incubated under ambient laboratory conditions for 7 d in different concentrations of ABA (10^{-2} M–10^{-6} M) in Petri dishes containing sterile filter paper and 10 ml ABA (sterilized by passing through a 0.22 μm filter).

**Water stress treatment**

Immature seeds and seedlings were subjected to water stress by equilibrating them in a desiccator for 7 d under ambient laboratory conditions over 25% glycerol (osmotic potential −12.6 MPa), which produced a relative humidity in the chamber of approximately 91%.

**Desiccation treatment**

To impose a more severe water stress, immature seeds and seedlings were dried slowly by placing them in a desiccator over stirred saturated salt solutions. The drying regime used for immature seeds and for seedlings was as described in Kermode and Bewley (1985). This involved placing seeds or seedlings for 1 d sequentially in desiccators containing stirred saturated salt solutions maintaining RH values of 92, 86 (2 d), 74, 65, 44, and 22%.

**High salt and high osmolality treatments**

Immature seeds and seedlings were placed for 7 d under ambient laboratory conditions in Petri dishes containing sterile filter paper moistened with either 10 ml 0.2 M NaCl (high salt treatment) or 10 ml 0.3 M mannitol (high osmolality treatment). Both solutions were previously sterilized by passing through a 0.22 μm filter.

**Cold treatment**

Immature seeds and seedlings were placed in Petri dishes containing sterile filter paper moistened with 10 ml of sterile water, and then maintained at 4 °C for 2–7 d.

**Water-control treatment**

As a control for the ABA and water-deficit-related stresses, immature seeds and seedlings were placed under ambient laboratory conditions in Petri dishes containing sterile filter paper moistened with 10 ml of sterile water for the same length of time as the ABA or stress treatments.

**Extraction of heat-stable proteins and Western blot analysis**

Heat-stable proteins were extracted from cotyledons and axes isolated from immature seeds (at mid-maturation), from mature seeds and from seedlings, as outlined in Close *et al.* (1993a). Briefly, total soluble proteins were extracted by grinding tissues in 30 mM N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer pH 8.0, containing 20 mM NaCl and 1 mM phenylmethylsulphonyl fluoride (PMSF) using a ground-glass homogenizer. This was followed by centrifugation at 14000 rpm in a microcentrifuge for 15 min at 4 °C. The supernatants were boiled at 100 °C for 10 min, kept on ice and then centrifuged as before. Protein concentrations were determined by the Bio-Rad protein assay (Bradford, 1976). The supernatants were then centrifuged at 95 °C, cooled and then fractionated by SDS–PAGE on 12% mini gels, according to the method of Laemmli (1970). For all Western blots, the same amount of heat-stable protein (64 μg in 32 μl sample buffer) was loaded on to each lane. After electrophoresis on to nitrocellulose (using a BioRad Trans-Blot Semi-Dry Transfer Cell), dehydrin proteins were detected with rabbit anti-dehydrin serum (diluted 1/6000 before use), followed by goat anti-rabbit IgG (Promega, Madison, WI) to which was conjugated alkaline phosphatase. The anti-dehydrin serum was produced from a synthetic polypeptide containing a highly conserved sequence (K1KEKLPG) (kindly provided by TJ Close, UC Riverside) (Close *et al.*, 1993a, b). The pre-immune serum was used as a control.

**Results**

Dehydrin-related proteins are differentially produced during seed development in *Castanospermum australe* and *Trichilia dregeana*.

Western blot analysis shows that dehydrins are present in the axes and cotyledons of mature and immature seeds of *C. austral* (Fig. 1, lanes 1–4). Two dehydrin-related polypeptides (40 and 37 kDa) were detected in the mature axes of *C. austral* seeds (lane 1); an additional 31 kDa polypeptide was also present in the cotyledons (lane 2). In contrast, no dehydrins were detectable in whole seeds of *T. dregeana* at maturity (data shown in Fig. 5A, MS).

It is noteworthy that new dehydrins were synthesized as seeds of *C. austral* matured. In immature seeds, 31 kDa and 85 kDa dehydrins were detected in axes (Fig. 1, lane 3); neither dehydrin was present in mature axes, in which polypeptides of 37 and 40 kDa accumulated (compare lane 3 with lane 1). A similar change in the
Dehydrins were present following the desiccation treatment (Fig. 2B). Thus it appears that dehydrins fail to accumulate under conditions of more extreme water loss. It is likely that the seeds did not survive the treatment and therefore were incapable of protein synthesis. Dehydrins were present in cotyledons of seeds subjected to a control water treatment (see Materials and methods) but not in the axes of these control seeds (Fig. 2A, B, H₂O). Thus, the detachment of immature seeds from the parent plant can induce dehydrin production. There was no 31 kDa dehydrin in ABA-treated cotyledons of immature seeds; instead a 37 kDa protein was detected along with the 40 kDa protein (Fig. 2B). The 37 kDa protein was also detected in ABA-treated axes (Fig. 2A). All concentrations of ABA (10⁻³–10⁻⁶ M) induced 40 kDa and 37 kDa dehydrin proteins in immature whole seeds (axis and cotyledons) (Fig. 2C).

**Dehydrins decline following ABA and water-deficit-related treatments in the cotyledons of C. australe seedlings and are undetectable in the roots of the seedlings**

Dehydrin-related proteins in cotyledons and roots of C. australe seedlings exposed to the different water-deficit-related stresses are shown in Fig. 3. In all the experiments, seedlings were subjected to 7 d treatments. Following imbibition of mature seed (i.e. in untreated seedlings), the dehydrins present in cotyledons of mature seeds persisted (compare Fig. 1 mature cotyledons, lane 2, with Fig. 3, Con [untreated]). Following the 7 d treatments, all dehydrins became less abundant in the cotyledons of the seedlings, with the exception of the 40 kDa protein in cold-treated seedlings (4°C).

Water-treated seedlings served as an additional control (Fig. 3, H₂O). These seedlings, which were maintained on water for the same length of time as that required to impose the stress treatments (7 d), showed a persistence of the 40 and 37 kDa dehydrins, although the 31 kDa protein declined. Dehydrins were not detectable in cotyledons of desiccation-treated seedlings (Fig. 3), which was similar to the response of the cotyledons of desiccation-treated immature seeds (Fig. 2B).

Interestingly, although dehydrin-related polypeptides were present in mature axes (Fig. 1, lane 1), no dehydrins were detectable in the roots of C. australe untreated seedlings (Fig. 3, Root, Con [untreated]) nor in the seedlings subjected to the different water-deficit-related treatments (Fig. 3). A lack of dehydrins in the roots may contribute to desiccation sensitivity in C. australe.

The presence of dehydrin-related proteins was examined in cotyledons and axes of C. australe seedlings following 7 d treatments in different concentrations of ABA (Fig. 4). All three dehydrin proteins (40 kDa, 37 kDa and 31 kDa) persisted in the cotyledons of seedlings, following treatment with 10⁻⁵ and 10⁻⁶ M ABA.
(A) axis

Immune

Pre-immune

B) coty

Immune

Pre-immune

(C) whole seed

Fig. 2. Western blot analysis of dehydrins in the axes and cotyledons of immature C. australe seeds subjected to ABA and various water-deficit-related stresses. (A) Axes of immature C. australe seeds subjected to 10^{-5} M ABA, various water-deficit-related stresses and a control water treatment (H₂O). (B) Cotyledons of immature C. australe seeds subjected to 10^{-5} M ABA, various water-deficit-related stresses and a control water treatment (H₂O). (C) Whole seeds of immature C. australe seeds subjected to different concentrations of ABA. See Fig. 1 for details. Left-most lanes = molecular mass markers. (D, desiccation; WS, water stress; ABA, abscisic acid at 10^{-5} M; Man, mannitol.)

(compare Fig. 4, Coty, 10^{-5} M ABA and 10^{-6} M ABA with Fig. 3 Coty, Con). However, the proteins were generally no more abundant than those found in the water-treated seedlings (Fig. 3, Coty, H₂O). In the seedlings treated with 10^{-4} M ABA, the 31 kDa dehydrin was undetectable in cotyledons, but 40 kDa and 37 kDa dehydrins persisted. Consistent with the lack of dehydrins in the roots following exposure of seedlings to the water-deficit-related stresses (Fig. 3, Root), no dehydrins were detected in roots when seedlings were subjected to all three concentrations of ABA (Fig. 4, Root).

Dehydrins are absent from immature seeds and seedlings of T. dregeana following ABA and water-deficit-related treatments

Dehydrin-related proteins were undetectable in whole seeds of T. dregeana at maturity (Fig. 5A, MS). Likewise,
Cotyledons and roots of *C. australis* seedlings subjected to various water-deficit-related stresses for 7 d. Untreated seedlings (Con [untreated]) and seedlings maintained on water for 7 d (H2O) served as controls. See Fig. 1 for details. Left-most lanes = molecular mass markers. (D, desiccation; WS, water stress; Man, mannitol.)

**Fig. 3.** Western blot analysis of dehydrins in cotyledons and roots of *C. australis* seedlings subjected to various water-deficit-related stresses for 7 d. Untreated seedlings (Con [untreated]) and seedlings maintained on water for 7 d (H2O) served as controls. See Fig. 1 for details. Left-most lanes = molecular mass markers. (D, desiccation; WS, water stress; Man, mannitol.)

Fig. 4. Western blot analysis of dehydrins in cotyledons and roots of *C. australis* seedlings subjected to different concentrations of ABA for 7 d. Dehydrin-related proteins were detected by a dehydrin antiserum and pre-immune blots of extracts from seedlings treated with 10^{-5} M ABA served as a control (pre-immune). See Fig. 1 for details. Left-most lanes = molecular mass markers.

Discussion

There are several hypotheses to explain how certain plant cells are able to cope with water stress or full desiccation (Bray, 1993; Ingram and Bartels, 1996; Kermode, 1997). Although an essential component of desiccation tolerance may involve the ability to effect repair upon subsequent rehydration, it is likely that desiccation tolerant tissues accumulate protective substances that limit the amount of damage which otherwise would be induced by water loss (Bewley and Oliver, 1992). Sugars (disaccharides such as sucrose and oligosaccharides such as raffinose and stachyose) may play a key protective role by accumulating under water deficit conditions and functioning to replace water and thus stabilizing membranes and other sensitive systems (Crowe *et al.*, 1992). However, in recal-
Dehydrins in recalcitrant seeds

A. Seeds

![Western blot analysis of Dehydrins in mature seeds of T. dregeana](image)

B. Seedlings

![Western blot analysis of Dehydrins in immature seeds and seedlings of T. dregeana](image)

Fig. 5. Western blot analysis to detect dehydrins in mature seeds of T. dregeana and in immature seeds and seedlings of T. dregeana subjected to various water-deficit-related stresses. (A) Untreated mature seeds (MS) and immature seeds subjected to water-deficit-related stresses or a control water treatment (H2O) for 7 d. (D, desiccation; WS, water stress; Man, mannitol). (B) Cotyledons and roots of seedlings of T. dregeana subjected to water-deficit-related stresses (desiccation, D and cold, 4 °C) or a control water treatment (H2O) for 2 d or 4 d as indicated. Also shown are the untreated seedlings (Con [untreated]). See Fig. 1 for details. Left-most lanes = molecular mass markers.

citrant seeds, viability is generally lost at water contents far higher than those at which water replacement (if it occurs in seeds at all) would take place. Another protective mechanism may involve the group of proteins induced in seeds and plant vegetative tissues undergoing mild water loss or full desiccation—namely dehydrins. Desiccation tolerance is acquired during development of orthodox seeds; tolerance to full desiccation is generally
lost after germination. Recalcitrant seeds, unlike orthodox seeds, are sensitive to desiccation when shed from the parent plant, and thus provide a system to study temporal and stress-induced changes in dehydrins.

In this study, dehydrin-related polypeptides were identified in recalcitrant seeds of Castanospermum australe. However, they were absent from mature seeds of Trichilia dregeana, another tropical species and none of the water-deficit-related treatments imposed at any stage were effective in eliciting dehydrin production in this species.

Given that dehydrins are produced by mature seeds of C. australe, but not by those of T. dregeana, a pertinent question is whether there are differences in the relative desiccation sensitivity of the seeds of these tropical species. However, no clear correlations are evident; following flash drying of mature isolated axes of C. australe, the critical moisture level at which the electrolyte conductivity of the leachate increases (indicative of membrane damage) is 0.98 g H₂O g⁻¹ dry mass. For T. dregeana, the critical moisture content for survival of mature axes in tissue culture is 0.2 g H₂O g⁻¹ dry mass (Pammenter and Berjak, unpublished results). (However, it may not be valid to directly compare desiccation sensitivity on the basis of the different indices of survival, i.e. electrolyte leakage and capacity to survive and grow in tissue culture.)

Although more extensive investigation is required, there is some evidence to suggest that while dehydrin-like proteins are consistently found in recalcitrant seeds of temperate species, they are often absent from recalcitrant seeds of tropical wetland species (Farrant et al., 1996). Finch-Savage et al. (1994) detected dehydrins in mature seeds of five desiccation sensitive (recalcitrant) trees (all temperate species), and Lea mRNA can also be induced in stored recalcitrant seeds of Quercus robur L. (English oak) by ABA and limited drying treatments. Dehydrins have also been detected in seeds of Zizania palustris, a cold temperate aquatic grass, the seeds of which exhibit characteristics intermediate between recalcitrance and orthodoxy (Bradford and Chandler, 1992).

In contrast to the consistent presence of dehydrins in a range of recalcitrant seeds of temperate species, dehydrins have been reported to be absent during the late stages of development of recalcitrant mangrove (Avicennia marina) seeds, a finding suggested to account for the lack of desiccation tolerance of this tropical species (Farrant et al., 1992, 1996). In addition to Avicennia marina, seeds of several other tropical wetland species do not contain dehydrins in amounts detectable by Western blotting (Farrant et al., 1996; reviewed in Kermode, 1997). Thus it is important to analyse species that inhabit a range of climatic zones and environments. Both seeds studied herein are of tropical origin, but in contrast to T. dregeana, seeds of C. australe underwent maturation in a temperate climate. C. australe is an Australian species; the material collected for these studies was from trees grown in the streets of Pietermaritzburg, South Africa, which is hotter in summer and cooler in winter than Durban (where seeds of T. dregeana were obtained) and has slightly lower rainfall.

There were striking changes in the nature of the dehydrins present during development of C. australe seeds. A distinct set of dehydrin-related polypeptides was present in the axes and cotyledons of immature seeds at mid-maturation; new dehydrins were present in mature seeds. Immature C. australe seeds subjected to ABA or various water-deficit-related treatments produced some of the dehydrins associated with late development, but some of these were also present in detached hydrated seeds, at least in the cotyledons. The general characteristics of dehydrin production in the recalcitrant C. australe seeds during development (i.e. new polypeptides induced during late development and following mild stress or detachment of immature seeds) are very similar to those exhibited by seeds of an orthodox tropical species that we have been studying, namely castor bean (Ricinus communis L.) (Han et al., 1995, 1997; Han and Kermode, 1996).

In relation to dehydrin production, the major differences between seeds of C. australe and R. communis appear to be exhibited following the transition to germination and seedling growth. In R. communis, production of dehydrin-related polypeptides exhibits some tissue specificity, is dependent upon the physiological stage of the seed and exhibits some qualitative and quantitative differences in response to different water-deficit-related stresses (Han and Kermode, 1996). In other orthodox species such as barley, three Lea mRNAs respond differently in seedlings subjected to salt, cold, mannitol and ABA (Espelund et al., 1992). These mRNAs also show different expression patterns during barley seed development. Similar to the endosperm of R. communis seeds, the dehydrin-related polypeptides persist in the cotyledons of C. australe seeds following imbibition. However, following ABA treatments or the imposition of all water-deficit-related stresses (except cold) these dehydrins became less abundant in the cotyledons of C. australe seedlings and no new polypeptides were detected. In contrast, R. communis seedlings exhibit a strong induction of dehydrin synthesis in the endosperm, cotyledons and radicle as a consequence of ABA or stress imposition (Han and Kermode, 1996). Furthermore, in the endosperm of R. communis seedlings the stress-inducible dehydrins are distinct from those that are induced during late seed development and which persist following germination/growth of mature seeds.

Perhaps the most significant finding is the lack of dehydrins in the axis and roots of C. australe following seed germination and the inability of high concentrations of ABA or stress imposition to induce their production. It seems that the axes of C. australe lose their ability to
produce dehydrins after germination, despite having this capacity during seed development. In contrast to C. australis, mature seeds of T. dregeana did not contain dehydrins and none were induced by stress or ABA treatments imposed either during seed development or after germination.

There is limited evidence that seeds of recalcitrant species are much less sensitive to applied ABA than are their orthodox counterparts; part of the underlying basis for the apparent insensitivity may be a very active ABA metabolism demonstrated by a rapid $[^3]H$-ABA turnover and high levels of endogenous conjugates (e.g., in recalcitrant Hopea oreada seeds) (Garello and Le Page-Degivry, 1995; see also Farrall et al., 1996).

A decline in the ability of the recalcitrant seeds studied herein to produce dehydrins following the transition to seedling growth may be more important to the viability of shed seed than a previous capacity for synthesis during seed development. It may be significant that the changes that occur on dehydration of recalcitrant seeds of the mangrove, Avicennia marina, are very similar to changes brought about by desiccation of orthodox seeds during the intolerant stage following germination (Farrall et al., 1986). Recalcitrant seeds initiate germination-related metabolism shortly after shedding (reviewed in Vertucci and Farrall, 1995), and in Avicennia marina, 10–15 d before shedding (Farrall et al., 1993). As germination events progress, the seeds become increasingly sensitive to drying and attempting to store these seeds is akin to air-dry storage of germinated, orthodox seeds (Farrall et al., 1986, 1988). There is no clear-cut event delineating the end of seed development and the start of germination; during both phases, recalcitrant seeds appear to remain metabolically active, although the axes may undergo a very brief period of relative quiescence. If indeed the seed recalcitrant seed responds in a manner similar to germinated orthodox seeds, it is also important to distinguish between dehydrin production in storage tissues (cotyledons or endosperm) and that occurring in the axis. For example, the axes of orthodox seeds such as soybean rapidly lose their tolerance to desiccation (brought about by air-drying to 10% water content) during the course of germination, while the cotyledons remain tolerant for a considerably longer period (Senaratna and McKersie, 1983).

Finch-Savage et al. (1994) showed an induction of Lea mRNA in axes of stored recalcitrant seeds of Quercus robur L. (English oak, a temperate species) following treatment with ABA or limited desiccation. However, in this temperate species, similar to the tropical species, C. australis, there was no true induction nor even enhanced synthesis of dehydrin-related polypeptides in response to ABA or limited desiccation, which is characteristic of orthodox seeds (reviewed by Bray, 1991, 1993; Thomas et al., 1991; Bewley and Oliver, 1992; Kermode, 1990, 1995, 1997; Chandler and Robertson, 1994; Bewley and Black, 1994). The very small amount of dehydrin-related polypeptides found in the untreated mature or stored Q. robur seeds declined upon application of ABA or imposition of stress (Finch-Savage et al., 1994).

The results of this study support the contention that desiccation-sensitivity of recalcitrant seeds is due, in part, to an inability to accumulate sufficient dehydrins and/or to the absence of specific LEA or dehydrin proteins, especially following the transition to a germination/growth programme under stress conditions. Future studies will determine whether there is a direct relationship between a loss of viability during storage (when seeds can be considered to be under stress) and a decline in the ability of the axes of recalcitrant species to synthesize dehydrins. The role of environment during seed maturation also needs further investigation. It is likely that the underlying basis of desiccation tolerance is diverse and is not simply restricted to the synthesis of specific proteins.

Acknowledgements

We are grateful to TJ Close, UC Riverside, for providing the dehydrin antibody. This research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Operating and Equipment Grants to ARK.

References


Farrant JM, Pammenter NW, Berjak P, Farnsworth EJ, Vertucci CW. 1996. Presence of dehydrin-like proteins and levels of abscisic acid in recalcitrant (desiccation sensitive) seeds may be related to habitat. Seed Science Research 6, 175–82.


Probert RJ, Brierley ER. 1989. Desiccation tolerance in seeds of Zizania palustris is not related to developmental age or the duration of post-harvest storage. Annals of Botany 64, 669–74.


