Seed Science Research (2000) **10**, 355–363

'Sub-imbibed' storage is not an option for extending longevity of recalcitrant seeds of the tropical species, *Trichilia dregeana* Sond.

Penelope J. Drew, N.W. Pammenter and Patricia Berjak*

Plant Cell Biology Research Unit, School of Life and Environmental Sciences, University of Natal, Durban, 4041 South Africa

Abstract

Recalcitrant seeds of *Trichilia dregeana* were stored at 16 or 25°C, either at the water content at which they were shed or partially dried. Although having been exposed to a short period (approx. 6 h) at temperatures up to 30°C prior to storage, seeds at the original water content maintained viability for several weeks at 16°C. However, storage of undried seeds at 25°C was deleterious within 8 d, indicating a chemical basis for degeneration of hydrated recalcitrant seeds. Seeds that had been mildly dehydrated to the relatively high axis level of 1.68 g H_2O g^{-1} dry mass, while maintaining full germinability immediately after drying, exhibited only 4% viability after 8 d in storage at 16°C and had completely lost viability after the same storage period at 25°C. Ultrastructural features characterizing hydrated seeds included indications of enhanced activity associated with initial exposure to the elevated temperature as well as some signs of stress. However, over an effective 15 d storage period at 16°C, ultrastructural features showed the cells to have retained little damage and to have been in an enhanced state of activity commensurate with ongoing development towards germination. After a longer storage period, however, signs of damage, including indirect evidence for disarray of the cytoskeleton in some axis cells, became apparent in line with the declining seed viability. Immediately following dehydration from an average axis water content of 1.97 to 1.68 H_2O g g^{-1} (the sub-imbibed condition), some ultrastructural abnormalities were apparent, but the seeds remained 100% germinable. However, within the 8 d storage period in this sub-imbibed condition, a spectrum of severe ultrastructural degeneration, including indirect evidence of the collapse of the nucleoskeleton and extensive cell lysis, accompanied viability decline of the seeds to 4%.

Keywords: Seed hydration, non-orthodox, recalcitrant, seeds, storage, sub-imbibed, *Trichilia dregeana*

*Corrrespondence
Fax: +27–31–260–1195
Email: berjak@biology.und.ac.za

Introduction

If one defines orthodox seeds as those that will tolerate relatively extreme dehydration and will survive in the desiccated state for predictable periods under defined storage conditions, then any seeds that do not behave in this way should be considered to be non-orthodox (Berjak *et al*., 1989). The latter grouping includes the desiccation-sensitive, recalcitrant seeds and those described as intermediate (Hong and Ellis, 1996), but considers these and the orthodox category to be open-ended: in this way, seed behaviour is viewed as constituting a continuum, subtended by the most desiccation-tolerant of the orthodox types on the one end and those of the most desiccation-sensitive recalcitrant species on the other (Pammenter and Berjak, 1999). This notwithstanding, the options for germplasm conservation of species producing non-orthodox seeds are very limited, with cryopreservation seemingly being the only possibility for long-term storage (e.g. Berjak *et al*., 1996; Engelmann, 1997; Pammenter and Berjak, 1999). However, biotechnological manipulations for cryo-preservation are complex and presently need to be

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

tailored to individual species and, additionally, require sophisticated *in vitro* procedures and good glasshouse facilities for ultimate plant production. Hence, it is desirable that other means be developed for short- to medium-term storage, especially to facilitate germplasm exchange and planting programmes.

A prevailing problem, in the storage of recalcitrant types that are desiccation-sensitive, is that the water content cannot be lowered to levels that would permit 'conventional' (low temperature/low relative humidity [RH]) seed banking methods to be used. The desiccation-sensitivity of such seeds has led to their being wet-stored (at the water content at which they are shed). However, hydrated, recalcitrant seeds die in storage, some more rapidly and others more slowly, depending on the species (e.g. Chin and Roberts, 1980). While seeds categorized as intermediate may withstand the requisite degree of dehydration, many of these – particularly of tropical species – appear to be chilling-sensitive (Hong and Ellis, 1996), thus precluding use of low temperature storage. Although it has not been systematically documented, many of the tropical recalcitrant seed species are also reputedly chilling-sensitive, some (e.g. *Theobroma cacao* [King and Roberts, 1980]) apparently being damaged at storage temperatures lower than 15°C. The seemingly-invariable presence, and proliferation in hydrated storage, of seed-borne micro-organisms, especially fungi, compounds the problems (Berjak, 1996).

The pivotal endogenous problem that curtails the storage lifespan of recalcitrant seeds is the initiation of germination in storage at the shedding water content (Farrant *et al*., 1986; Berjak *et al*., 1989), unless unusually, this is too low (as recorded for *Quercus robur* in an isolated season [Finch-Savage and Clay, 1994]). Desiccation-sensitivity of hydrated recalcitrant seeds increases during storage (Farrant *et al*., 1986; Tompsett and Pritchard, 1998), in parallel with the progression of germination-related events, particularly the phase of extensive vacuolation following the onset of mitosis (Farrant *et al*., 1986). If additional water is not made available (as is the case in storage), the seeds deteriorate. Some 20 years ago, germination in storage was documented as a nuisance in that such seeds could no longer contribute to the stored stock (King and Roberts, 1980), but we now view the phenomenon as being a 'killer event', principally in terms of metabolism-related damage occurring as a consequence of the requirement for additional water (Pammenter *et al*., 1994, 1998).

Not surprisingly, the suggestion was made relatively early, that the post-harvest lifespan of recalcitrant seeds might be extended if the water content could be lowered to levels that would preclude the initiation of germination (King and Roberts, 1980). Those authors recorded a number of instances where such 'sub-imbibed' storage had been used to facilitate transport of recalcitrant seeds (which would effectively constitute short-term storage) or in attempts to extend longevity between growing seasons (medium-term storage). However, King and Roberts (1980) reported that substantial viability decline occurred even in the short term following endeavours to store non-orthodox seeds in the sub-imbibed condition and mentioned specifically that long-term storage had not been achieved. Corbineau and Côme (1986a,b, 1988), working with recalcitrant seeds of four species stored under defined RH conditions, showed that viability declined after only the loss of a small proportion of water, suggesting that sub-imbibed storage was not beneficial. Nevertheless, the possible efficacy of sub-imbibed storage in prolonging the post-harvest lifespan of recalcitrant seeds has been suggested periodically since 1980. For example, Hong and Ellis (1996) suggested that there may be advantages of sub-imbibed storage, particularly to reduce micro-organism proliferation and germination in storage. However, Pammenter *et al*. (1994) suggested that germinative metabolism of recalcitrant seeds in storage actually imposes a water stress. If this is the case, some limited drying before storage to achieve the sub-imbibed condition is likely to exacerbate the situation and increase damage consequent upon water stress, as, irrespective of the fact that germinative events might be reduced or precluded, the seeds necessarily must remain actively metabolic or die.

The present investigation was designed to ascertain whether sub-imbibed conditions affect seed storage lifespan – either positively or negatively – of the recalcitrant tropical species, *Trichilia dregeana* Sond. (Meliaceae).

Materials and methods

Materials

Trichilia dregeana is an evergreen tropical/sub-tropical forest species, naturally occurring in South Africa in relatively-high-rainfall, eastern areas of KwaZulu-Natal and the Eastern Cape Provinces to a latitude of about 33°S. The species is not endemic to these areas and occurs in much of east Africa. Capsules ripen from March to mid-year, opening on the tree to reveal up to six large seeds, each of which is almost completely enclosed by an orange to scarlet aril (Pooley, 1993). The seeds of *T. dregeana* have previously been confirmed as being recalcitrant (Choinski, 1990; Kioko *et al*., 1998). The entire capsule is then abscised, when generally, the tenuous connection with the fruit wall is broken, resulting in a localized scattering of the seeds. *Trichilia dregeana* has

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

been extensively planted in Durban as a street tree, seeds thus being easily accessible. Seed dimensions are approx. 18 ¥ 10 ¥ 10 mm.

Preliminary investigations, chiefly to ascertain experimental parameters, were carried out on early-season, newly-shed seeds harvested in the early morning (Batch 1), whereas the main investigation utilized later-season seeds, collected within a few hours of being shed, but in the early afternoon (Batch 2). (While this may seem trivial, it has an important bearing on the interpretation of the results, as is discussed below.)

Upon arrival in the laboratory, seeds were immediately assessed for axis and cotyledonary water contents, and germination, and axes were sampled and immediately processed for electron microscopy (the control material).

Methods

Preparation for, and temporary storage (Batch 2)

Seeds were soaked in tap water for several hours to facilitate aril removal (which is vital, as many fungal propagules are harboured in this waxy covering). The testa was also removed, after which the seeds were surface-sterilized in 1% sodium hypochlorite for 20 min, rinsed in sterile, distilled water, and the surface water was removed by blotting followed by air-drying. The seeds were then re-sampled for water content determinations, and the remainder dusted with a benomyl fungicide (Benlate® [DuPont, USA]), placed within polythene bags under conditions that precluded water loss and stored for 1 week at 16°C before use. (Such storage conditions have previously been used successfully for several weeks for high-vigour *T. dregeana* seeds).

Germination assessment

Whether subjected to dehydration or not, seeds were suspended above water in closed containers overnight, after which they were briefly surface-sterilized with 1% NaOCl, and set to germinate at a depth of 10 mm in moistened vermiculite at 25°C with a 14 h photoperiod. Germination (n=25) was scored as the production of a radicle ⩾5 mm and was assessed daily to monitor rate.

Dehydration and storage

Seeds (testa removed) were dehydrated by shallow burial in a fixed volume of activated silica gel for 8 h at 25–28°C, while control material was similarly maintained in dry vermiculite (unpublished IPGRI/Danida protocol). After the drying treatment, partially dried and undried seeds were stored at 16 or 25°C, enclosed in plastic bags. All material was sampled after 8, 14 and 22 d storage.

Water content determination

Water content was determined gravimetrically on individual, separated embryonic axes and cotyledon segments for 10 seeds per sample and expressed as g H_2O g^{-1} dry mass (g g^{-1}).

Processing for microscopy

The distal portion of the hypocotyl was excised from each of five embryonic axes per sample into 2.5% 0.1 M phosphate-buffered glutaraldehyde containing 0.5% caffeine (pH 7.2). After fixation (min. 2 h), samples were buffer-rinsed, processed with 1% aqueous osmium tetroxide (1 h), water-rinsed, then dehydrated through an acetone series, and embedded in a low-viscosity epoxy resin. Sections cut on a Reichert Ultracut E and collected on copper grids were post-stained with lead citrate and viewed and photographed with a Jeol 1010 transmission electron microscope.

Results and discussion

There was a trend towards lower axis (and cotyledon) water contents as the season progressed. Whereas axes from Batch 1 seeds averaged 2.73 ± 0.62 g g^{-1}, the water content of those from seeds harvested later (Batch 2) was 1.99 ± 0.25 g g^{-1}. A similar trend has been noted in previous years and is considered typical for *T. dregeana* seeds (Kioko, pers. comm.[1]). Irrespective of the difference in water content, fresh seeds (aril and testa removed) of both batches readily achieved 100% germination. Using seeds from Batch 1, it was found that removal of the seed outer coverings by soaking increased axis and cotyledon water content by 18.7% and 10.3%, respectively; thus, the water contents at harvest were lower than the figures given above. Preliminary experiments with material from Batch 1 showed that axes (and cotyledons) from seeds with intact arils lost little water over the first 24 h, even when buried in activated silica gel. Thus, although the seeds from Batch 2 were collected in the middle of the day and consequently many may have been shed several hours earlier, no significant degree of dehydration would have occurred.

During storage (Batch 2 seeds) at both 16 and 25°C, there was a significant (P<0.05) increase in water content of the axes of undried seeds, from 1.99 ± 0.25 to 2.55 ± 0.22 g g^{-1} over 22 d at 16°C, and to 2.35 ± 0.17 g g^{-1} after 8 d at 25°C. This increase was probably a consequence of metabolism of the seeds themselves or of associated fungi (not isolated). Partial drying reduced axis water content to 1.68

[1]J.I. Kioko, School of Life and Environmental Sciences, University of Natal, Durban, 4041 South Africa.

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

$\pm$ 0.18 g g^{-1}, and during 8 d of storage of the partially dehydrated seeds there was a significant ($P<0.05$), but slight, further decrease in axis water content to 1.47 $\pm$ 0.14 g g^{-1} at 16°C and to 1.52 $\pm$ 0.13 g g^{-1} at 25°C. Partial drying reduced cotyledonary water content from 0.63 $\pm$ 0.14 to 0.41 $\pm$ 0.07 g g^{-1}. There was no significant change in cotyledon water content with storage, and as the cotyledons constitute 98% of the seed mass, this implies no change in whole seed water content. Changes in axis water content during storage of partially dried seeds of *Aesculus hippocastanum* have been shown by Tompsett and Pritchard (1998), although in that instance there was an increase in axis water content. Whatever the trend, redistribution of water between tissues after partial drying complicates the interpretation of storage experiments, and further, measurements of whole seed water contents may be misleading as they may mask changes in the more metabolically active embryonic axis.

Germination percentages of *T. dregeana* seeds stored in the hydrated condition at 16 or 25°C, or partially dehydrated before storage at the same temperatures, are shown in Fig. 1. All seeds survived dehydration for 8 h to an average axis water content of 1.68 g g^{-1}. Seeds that had not been dehydrated after collection survived well (96%) in storage at 16°C for 8 d, but declined markedly in viability in the same time period at 25°C. However, those seeds that had been dried to an axis water content of 1.68 g g^{-1}, which might be viewed as a sub-imbibed condition, retained only 4% viability after 8 d at 16°C, and none remained germinable when stored for the same time at 25°C. Therefore, despite the postulated stressed condition of the seeds at the outset, and even in the short timeframe of the present storage experiments, partial dehydration to a relatively high axis water content reduced seed viability in 8 d by almost 96% relative to that of the undried seeds.

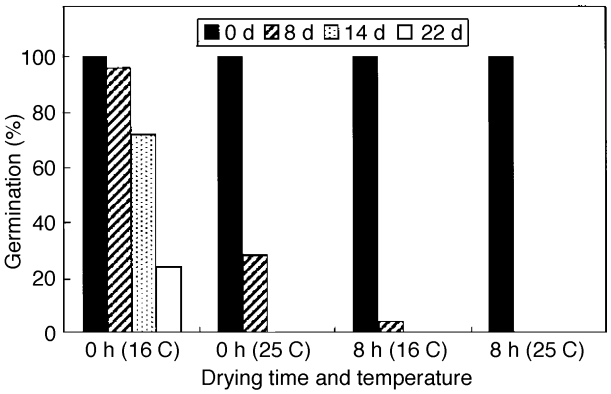


Figure 1. Viability (assessed as germination percentage) of *Trichilia dregeana* seeds dried for 0 or 8 h and stored for 0, 8, 14 or 22 d at 16 or 25°C (n=25).

Among the undried seeds in storage at 16°C, 72% remained viable after 14 d, but there was an abrupt decline to 24% after 22 d. It is interesting that for undried material, viability declined more rapidly at the higher storage temperature (Fig. 1). This argues that chemically-based degradation prevailed during storage, which is in agreement with the concept of unbalanced metabolism with associated damage occurring at relatively high water contents during hydrated storage of recalcitrant seeds (Pammenter *et al.*, 1994). While the nature of such damage has not been unequivocally demonstrated for hydrated, recalcitrant seeds during storage, those authors discussed the possibility of the uncontrolled consequences of free radical generation, which is also supported as a major cause of damage during dehydration of desiccation-sensitive seeds (reviewed by Côme and Corbineau, 1996). The nature of the biochemical lesions in *T. dregeana* seeds stored at water contents reduced from the level at shedding remains to be ascertained. However, in the present case exacerbation of deterioration by associated fungi must be borne in mind, especially in the context of the

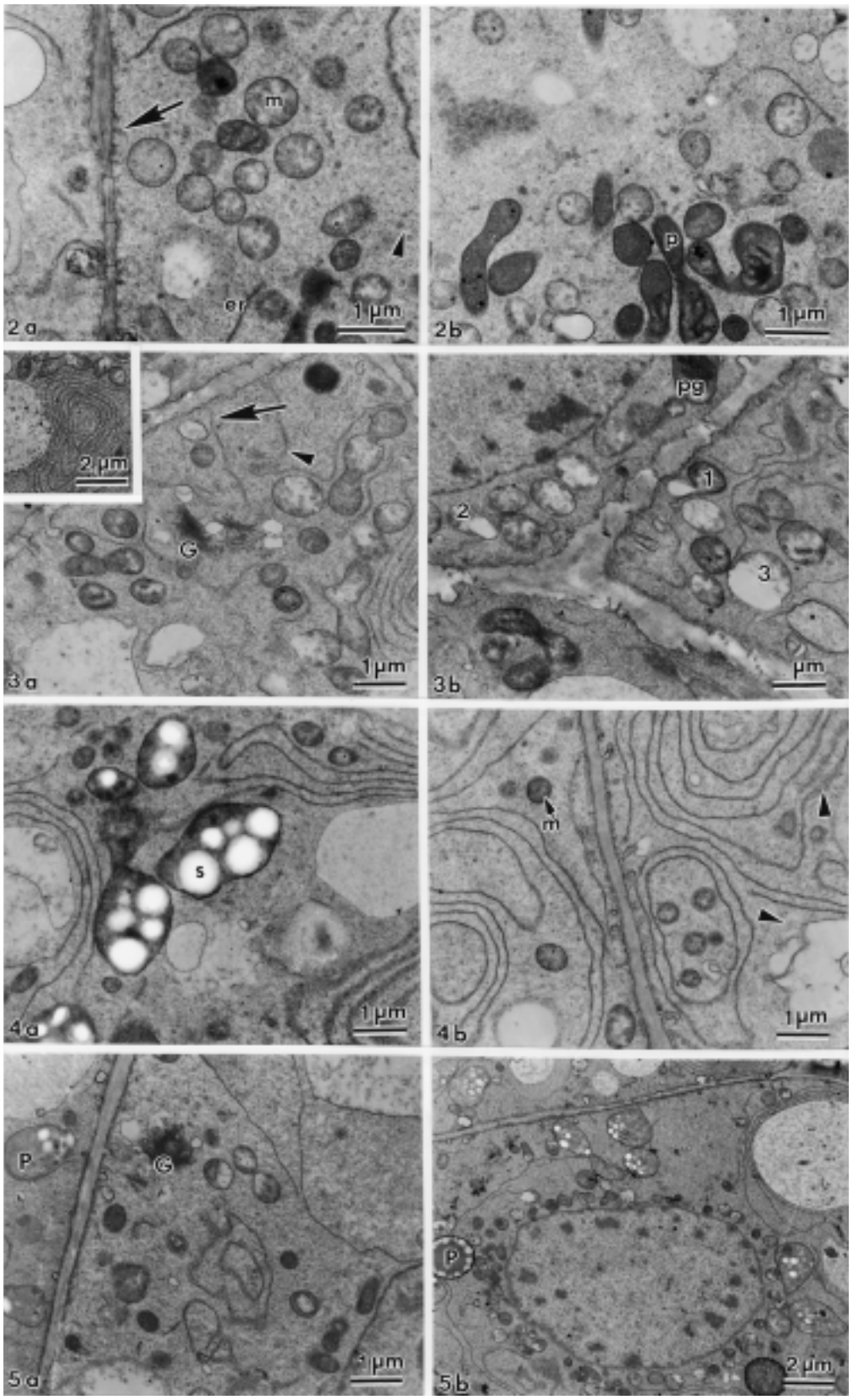
Figure 2. Ultrastructural features of root meristematic cells of axes from newly-shed seeds include: (a) predominantly spherical mitochondria (m) with relatively dense matrices and some crista development, scattered ER profiles (er), many polysomes (arrowhead) and very small inward protrusions of the plasmalemma (arrowed), and (b) uniformly-dense plastids (p) showing a measure of internal membrane development, but no starch.

Figure 3. Axes from seeds exposed to elevated air temperatures for approx. 6 h after being shed showed the following features. (a) There was marked enhancement of mitochondrial development, strong ER development, cytolysome formation (inset), many polysomes (arrowhead) and frequent Golgi bodies (G). Also, plasmalemma infoldings were more noticeable, and some showed an ER association (arrow). (b) A degree of abnormality, possibly associated with heat stress, is apparent in the regression of the internal membranes of some of the plastids, with associated formation of plastoglobuli (pg), and examples of vesiculating (blebbing) mitochondria, numbered 1–3 in order of severity of the damage.

Figure 4. After 8 (15 [see text]) d storage at 16°C of seeds at the original water content, changes indicative of ongoing metabolism included: (a) ER proliferation and starch (s) accumulation within plastids, and (b) uniformly dense mitochondria (m), cytomatrical and membrane-associated polysomes (arrowheads) and more prominent infoldings of the plasmalemma.

Figure 5. Hydrated seeds stored for 14 (21 [see text]) d at 16°C maintained 72% viability. (a) Axis cells from presumably viable seeds showed abnormal features including regression of the internal structure of plastids (p) and strongly recurved Golgi bodies (G). (b) In other axes, cells showed considerably more severe plastid (p) damage and a peri-nuclear orientation of organelles and nuclear appearance suggestive of cyto- and nucleoskeleton disorganization.

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press



https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

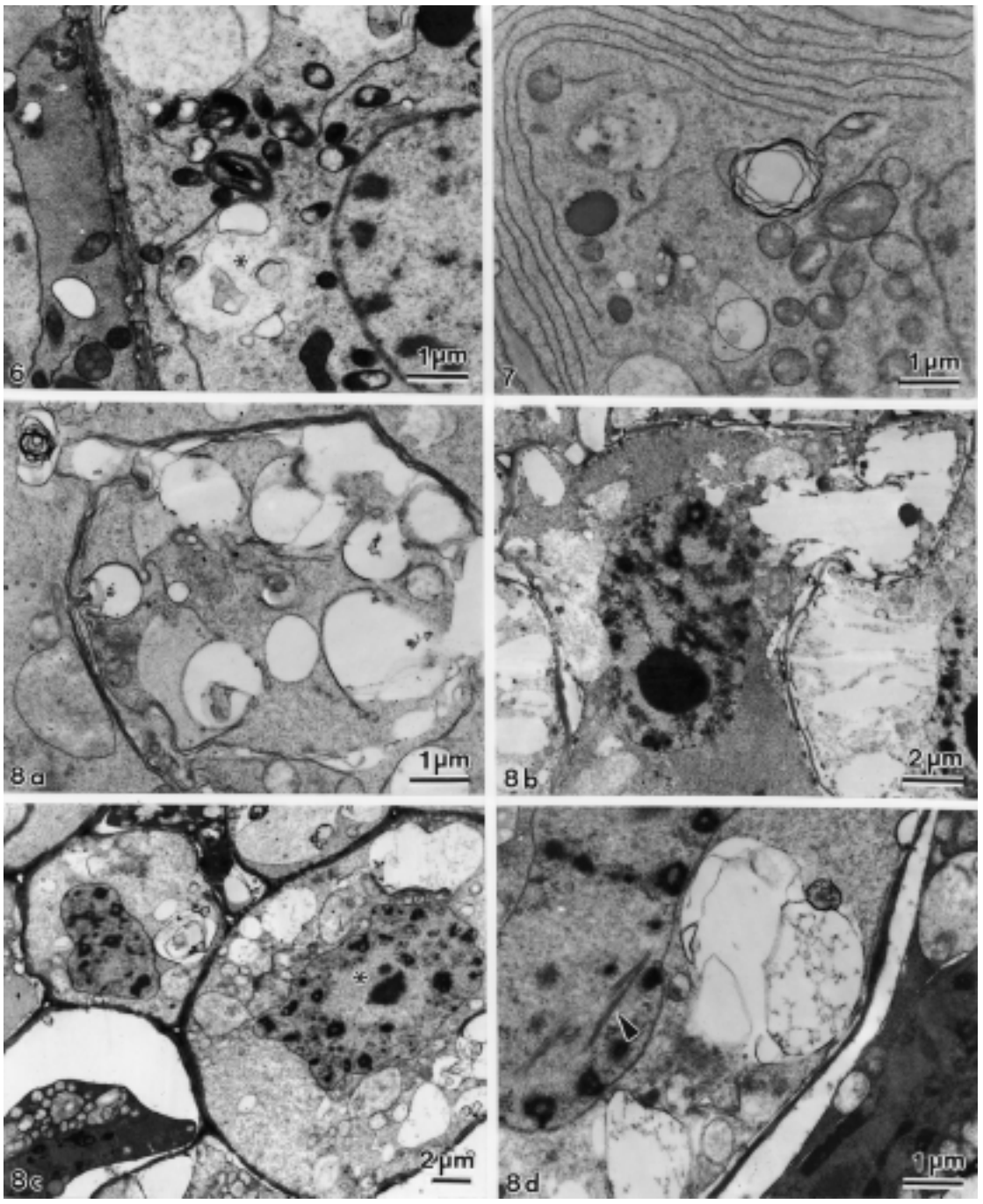


Figure 6. After hydrated seeds were placed in storage at 25°C for 8 d, most axis cells were visibly damaged to a lesser or greater extent. Both cells have severely damaged mitochondria and plastids, while a region of cytoplasmic lysis (*) is apparent.
Figure 7. Typical ultrastructure of axis cells immediately after seeds had been dehydrated for 8 h to an average water content of 1.68 g g^{-1}. A measure of regression in organelle development and recurving of Golgi bodies can be seen; however, the seeds maintained 100% germinability despite these dehydration-associated features.
Figure 8. Degenerative changes in axis cells from seeds that had been stored for 8 d at 16°C in the sub-imbibed condition (axis water content 1.68 g g^{-1}). (a) Sequestration of various organelles by compressed layers of endomembrane, possibly a compensatory response that occurred during storage. (b) Cell remains in an entirely degenerated area. (c and d) Aspects of nuclei suggesting collapse of the nucleoskeleton in terms of the cleared region around the nucleolus (*, c) and bundles of intranuclear filaments (arrowhead, d).

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

slightly elevated water contents that were recorded during storage for the undried seeds.

The subcellular situation

There were subtle differences in the ultrastructure of root apical meristem cells between seeds from Batches 1 and 2, indicative of both an enhanced metabolic status and the suggested heat-stress-imposed damage in the latter (cf. Figs 2 and 3). The subcellular situation of these cells from axes of seeds collected as soon as they were shed, is illustrated in Figs 2a and b. There were many, predominantly spherical, mitochondria, showing a measure of crista development and having relatively dense matrices (Fig. 2a): these features are indicative of the respiratory activity known to characterize newly-shed, recalcitrant seeds (Côme and Corbineau, 1996). Single profiles of rough endoplasmic reticulum (rER) occurred and cytomatrical polysomes abounded (Fig. 2a), suggesting a measure of endomembrane activity and considerable protein synthesis, respectively. Plastids were characterized by having a uniform stromal density in which internal membranes could be discerned, but starch was notably absent (Fig. 2b). Typically, for axis root apex cells in *T. dregeana*, there were frequent, small protrusions of the plasmalemma into the cytomatrix (Fig. 2a).

The ultrastructure of similar cells from seeds of Batch 2 reflected a higher degree of activity, where many mitochondria, some showing well-developed cristae, had assumed a more cylindrical form (Fig. 3a). In this material, polysomes were frequent and the ER had proliferated considerably; not unusually, ER-derived membranes had formed the concentric whorls typical of vacuolation by cytolysome formation (Fig. 3a, inset). This process typifies a developmental event by which new vacuoles are formed rapidly, occurring commonly in the axes of hydrated non-orthodox seeds (Berjak *et al*., 1992), often as a response to stress (Berjak *et al*., 1995). A further feature, indicative of the enhanced metabolic status of axis cells from seeds of Batch 2, was the frequent occurrence of active Golgi bodies (Fig. 3a). These component organelles of the endomembrane system are normally rarely encountered in embryos from newly-shed seeds but form rapidly once further axis development occurs. The plasmalemma protrusions mentioned for axis cells of Batch 1 seeds had become more evident, and in places, could be seen as being narrowly cylindrical and often closely associated with an ER profile (Fig. 3a). While it cannot be unequivocally stated from non-serial micrographs whether or not such plasmalemma-associated structures are derived by endo- or exocytosis, according to Staehelin (1997), secretory (exocytotic) vesicles fusing with the plasmalemma form infoldings that become 'capped' by appositional contacts of the ER in the process of membrane lipid recycling. Thus, it might be suggested that in these axis cells, an aspect of the apparently heightened metabolic activity involved the exocytotic fusion of Golgi-derived secretory vesicles, the membrane components of which are recycled. All these features suggest that some factor has activated axis cells from Batch 2 seeds, and it is presently suggested that this may have been a temperature stimulus. However, in addition to cytolysome formation which might indicate a stress response, there were also some signs of marked irregularity in some mitochondria and plastids. Figure 3b illustrates three mitochondria suggested to show successive stages of severe bleb formation, a phenomenon also seen in the occasional plastid. Such irregular lobing has previously been noted for temperature-stressed, non-orthodox neem seeds in response to chilling (Berjak *et al*., 1995). A further plastid abnormality can be seen in the occurrence of several plastoglobuli (Fig. 3b), thought to represent lipid accumulations derived from degenerated inner membranes of these organelles.

One environmental variable between early morning and afternoon that was consistent during the season was air temperature. Whereas those seeds collected early in the morning (Batch 1) would not have been subjected to temperatures above approx. 24°C, and if so, only for a short time, many of the seeds collected in the early afternoon (Batch 2) had been exposed to air temperatures in the range 24–30°C for several hours. This is considered to have imposed a heat-stress, which might be equated with a period of accelerated ageing (*sensu* orthodox seeds), that is borne out by comparison of aspects of the ultrastructure of control material from the two batches. A further manifestation of the proposed heat-stress imposed upon Batch 2 seeds was their curtailed lifespan in storage at the original water content (i.e. the control material, in relation to that which had been dehydrated). While hydrated storage lifespan of seeds of any species can be markedly variable (in line with the original condition of the seeds), that of *T. dregeana* can be as long as 6 months, if they are in optimal condition on collection and virtually free of fungal contaminants (Kioko, pers. comm.). While this is not often achieved, the stressed condition of the Batch 2 seeds curtailed the storage period to a few weeks. Although viability was lost relatively quickly irrespective of the water content at the start of storage, there was a clear indication that storage in the sub-imbibed condition was deleterious (Fig. 1).

Batch 2 seeds were maintained hydrated at 16°C for a week before experimentation started; therefore, those hydrated seeds sampled after 8, 14 or 22 d storage at 16°C had been in this condition for 15, 21 and 29 d, respectively. After 8 (15) d at 16°C, when 96% of the seeds had retained viability (Fig. 1), the

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

ultrastructure revealed that marked metabolic activity had occurred. Figure 4a shows considerable ER proliferation, with much of the resultant membrane seemingly directed to cytolysome formation. Starch had accumulated in the plastids, with most of these organelles showing several grains in any one section. There were many cytomatrical polysomes, mitochondria had uniformly dense matrices and the tube-like plasmalemma projections were relatively more prominent (Fig. 4b). These developments are consistent with ongoing metabolism directed towards germination during hydrated storage, which is a phenomenon reported for many recalcitrant seed species (e.g. Farrant *et al*., 1989). It is the process of vacuolation, however, that demands that additional water be available for the completion of germination; this was not provided during storage of these *T. dregeana* seeds, perhaps accounting for the increasing viability loss as the 16°C storage period progressed. After 14 (21) d storage at this temperature, there were changes in axis cells, some of which were not degenerative, suggesting abnormality, *e.g.* the formation of strongly recurved Golgi bodies (Fig. 5a). However, in the contiguous cell shown in Fig. 5a, degenerative changes such as regression of plastid structure could be seen. The most seriously damaged cells of axes from seeds stored for 14 (21) d showed not only features such as some markedly degenerated plastids, but also spatial disorientation of organelles to the peri-nuclear region (Fig. 5b). This figure also shows that the only observable heterochromatin was generally found at the periphery of the nucleus. These features suggest that there had been a breakdown in the orderly disposition of both cytoskeletal and nucleoskeletal elements that normally maintain the orientation of the organelles and chromatin, respectively (*e.g.* Staiger *et al*., 1997 and Moreno Díaz de la Espina, 1995, respectively). In contrast to the hydrated material stored at 16°C, that maintained at 25°C for 8 d (preceded by 7 d at 16°C) showed evidence of marked deterioration. These axes were characterized by a mosaic of less or more deteriorated cells which were frequently contiguous (Fig. 6). Viability of the seeds stored at 25°C had dropped to 28% over the few days, arguing strongly for temperature-accelerated deteriorative reactions associated with disorganized metabolic activity.

In keeping with the full viability retention of seeds dehydrated for 8 h to 1.68 g g^{-1} (Fig. 1), there were few signs of ultrastructural abnormality. However, there seemed generally to have been some regression of the internal structure of mitochondria and plastids (Fig. 7, cf. Fig. 3a). After 8 d storage at 16°C in this sub-imbibed condition, only 4% of the seeds remained germinable. The ultrastructure revealed extensive intracellular abnormality, the least extreme being sequestration of a variety of organelles including vacuoles where the tonoplast was incomplete, and by closely-packed layers of ER-derived membrane (Fig. 8a), which might be interpreted in the context of 'attempted' subcellular compensation. At the other extreme, there was almost total lysis of the cell contents, with only disorganized nuclei remaining recognisable, and collapsed walls (Fig. 8b). Other cells exhibited various degrees of degradation that had occurred in only 8 d of storage at 16°C in the sub-imbibed condition (Figs 8c and d). A particularly interesting feature was the disruption of nuclear morphology; while none had remained spherical, marked clearing of the nuclear ground substance in the vicinity of the nucleolus (Fig. 8c) was a common feature, apparently followed by the appearance of bundles of filaments (Fig. 8d) in the more extensively cleared nuclear interior. Taken together, these degradative changes are symptomatic of the breakdown of the nucleoskeleton, in which the lamin (intermediate) filaments are normally disposed as the lamina, underlying and connected to the inner surface of the nuclear envelope, and the matrix in the nuclear interior, which is exceptionally dense in the vicinity of the nucleolus (Moreno Díaz de la Espina, 1995).

Thus, despite the immediate retention of full viability and undiminished vigour (data not shown) of seeds dehydrated to around 1.68 g g^{-1}, which would have appeared to be an entirely adequate water content for their continued survival, there was rapid and complete degeneration associated with virtually total viability loss in only 8 d at the moderate temperature of 16°C. The degeneration (not shown) when such sub-imbibed seeds were stored at 25°C was markedly accelerated. Hence, despite the fact that these *T. dregeana* seeds are considered to have undergone the equivalent of a short period (no more than 6 h) of accelerated ageing before collection, these results indicated that sub-imbibed storage is not an option, and certainly not when other constraints are operating to curtail seed longevity even at the shedding water content.

References

Berjak, P. (1996) The role of micro-organisms in deterioration during storage of recalcitrant and intermediate seeds. pp. 121–126 *in* Ouédraogo, A.-S.; Poulsen, K.; Stubsgaard, I. (Eds) *Intermediate/recalcitrant tropical forest seeds*. Rome, IPGRI.

Berjak, P., Farrant, J.M. and Pammenter, N.W. (1989) The basis of recalcitrant seed behaviour. pp. 89–108 *in* Taylorson, R.B. (Ed.) *Recent advances in the development and germination of seeds*. New York, Plenum Press.

Berjak, P., Pammenter, N.W. and Vertucci, C. (1992) Homoiohydrous (recalcitrant) seeds: Developmental status, desiccation sensitivity and the state of water in axes of *Landolphia kirkii* Dyer. *Planta* **186**, 249–261.

https://doi.org/10.1017/S0960258500000398 Published online by Cambridge University Press

Berjak, P., Campbell, G.K., Farrant, J.M., Omondi-Oloo, W. and Pammenter, N.W. (1995) Responses of seeds of *Azadirachta indica* (neem) to short-term storage under ambient or chilled conditions. *Seed Science and Technology* **23**, 779–792.

Berjak, P., Mycock, D.J., Wesley-Smith, J., Dumet, D. and Watt, M.P. (1996) Strategies for *in vitro* conservation of hydrated germplasm. pp. 19–52 *in* Normah, M.N.; Narimah, M.K.; Clyde, M.M. (Eds) In vitro *conservation of plant genetic resources*. Kuala Lumpur, Malaysia, Percetakan Watan SDN.BHD.

Chin, H.F. and Roberts, E.H. (1980) *Recalcitrant crop seeds*. Kuala Lumpur, Malaysia, Tropical Press SDN.BDH.

Choinski, J.S. Jr. (1990) Aspects of viability and post-germinative growth in seeds of the tropical tree, *Trichilia dregeana* Sonder. *Annals of Botany* **66**, 437–442.

Côme, D. and Corbineau, F. (1996) Metabolic damage related to desiccation sensitivity. pp. 107–120 *in* Ouédraogo, A.-S.; Poulsen, K.; Stubsgaard, I. (Eds) *Intermediate/recalcitrant tropical forest seeds*. Rome, IPGRI.

Corbineau, F. and Côme, D. (1986a) Experiments on the storage of seeds and seedlings of *Symphonia globulifera* L.f. (Guttiferae). *Seed Science and Technology* **14**, 585–591.

Corbineau, F. and Côme, D. (1986b) Experiments on germination and storage of the seeds of two dipterocarp: *Shorea roxburghii* and *Hopea odorata*. *The Malaysian Forester* **49**, 371–381.

Corbineau, F. and Côme, D. (1988) Storage of recalcitrant seeds of four tropical species. *Seed Science and Technology* **16**, 97–103.

Engelmann, F. (1997) *In vitro* conservation methods. pp. 119–161 *in* Callow, J.A.; Ford-Lloyd, B.V.; Newbury, H.J. (Eds) *Biotechnology and plant genetic resources*. New York, CAB International.

Farrant, J.M., Pammenter, N.W. and Berjak, P. (1986) The increasing desiccation sensitivity of recalcitrant *Avicennia marina* seeds with storage time. *Physiologia Plantarum* **67**, 291–298.

Farrant, J.M., Pammenter, N.W. and Berjak, P. (1989) Germination-associated events and the desiccation sensitivity of recalcitrant seeds–a study on three unrelated species. *Planta* **178**, 189–198.

Finch-Savage, W.E. and Clay, H.A. (1994) Water relations of germination in the recalcitrant seeds of *Quercus robur* L. *Seed Science Research* **4**, 315–322.

Hong, T.D. and Ellis, R.H. (1996) *A protocol to determine seed storage behaviour*. Rome, IPGRI.

King, M.W. and Roberts, E.H. (1980) Maintenance of recalcitrant seeds in storage. pp. 53–89 *in* Chin, H.F.; Roberts, E.H. (Eds) *Recalcitrant crop seeds*. Kuala Lumpur, Malaysia, Tropical Press SDN.BHD.

Kioko, J., Berjak, P., Pammenter, N.W., Watt, M.P. and Wesley-Smith, J. (1998) Desiccation and cryo-preservation of embryonic axes of *Trichilia dregeana* Sond. *CryoLetters* **19**, 5–14.

Moreno Díaz de la Espina, S. (1995) Nuclear matrix isolated from plant cells. *International Review of Cytology* **162B**, 75–139.

Pammenter, N.W. and Berjak, P. (1999) A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* **9**, 13–37.

Pammenter, N.W., Berjak, P., Farrant, J.M., Smith, M.T. and Ross, G. (1994) Why do stored, hydrated recalcitrant seeds die? *Seed Science Research* **4**, 187–191.

Pammenter, N.W., Greggains, V., Kioko, J.I., Wesley-Smith, J., Berjak, P. and Finch-Savage, W.E. (1998) Effects of differential drying rates on viability retention of recalcitrant seeds of *Ekebergia capensis*. *Seed Science Research* **8**, 463–471.

Pooley, E. (1993) *The complete field guide to trees of Natal, Zululand and Transkei*. Durban, South Africa, Natal Flora Publications Trust.

Staehelin, L.A. (1997) The plant ER: a dynamic organelle composed of a large number of discrete functional domains. *The Plant Journal* **11**, 1151–1165.

Staiger, C.J., Gibbon, B.C., Kovar, D.R. and Zonia, L.E. (1997) Profilin and actin-polymerizing factor: modulators of actin organization in plants. *Trends in Plant Science* **2**, 275–281.

Tompsett, P.B. and Pritchard, H.W. (1998) The effect of chilling and moisture status on the germination, desiccation and longevity of *Aesculus hippocastanum* L. seed. *Annals of Botany* **82**, 249–261.

Received 30 December 1999
accepted after revision 20 April 2000