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8 Storage and Germination Response of Recalcitrant Seeds Subjected to Mild Dehydration

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Abstract

A problem associated with the storage of fully hydrated recalcitrant seeds is germination in storage, and it has been suggested that this problem could be overcome by partial dehydration, which is sufficient to prevent germination but high enough to avoid desiccation damage (i.e. 'sub-imbibed' storage). However, partial drying (pd) is shown to stimulate germination, and this process could reduce storage lifespan. Data are presented on recalcitrant seeds from a number of species, demonstrating the enhancement of germination by rapid mild dehydration, and the adverse effects of this mild dehydration on subsequent storage at a range of temperatures.

Introduction

Recalcitrant seeds, by definition, are desiccation-sensitive and hence cannot be stored by the conventional methods employed for orthodox seeds. Not only does this make the long-term conservation of their genetic resources difficult, but it also places limitations on normal seed-handling procedures. To date the only 'successful' way of storing recalcitrant seeds is in the hydrated condition, at their shedding water content, but storage lifespan varies from several months, at best, to a week or two, depending on the species and the physiological condition of the seeds (King and Roberts, 1980). There are two main problems that are associated with hydrated storage: (i) seeds will often germinate in storage (King and Roberts, 1980); and (ii) the effects of fungal contamination can be severe, as the conditions (i.e. high humidity and temperature) necessary for hydrated storage also favour fungal proliferation (Mycock and Berjak, 1990).

Recalcitrant seeds are metabolically active (Berjak *et al.*, 1984; Farrant *et al.*, 1997), undergo continued development after shedding that grades into germination-associated

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changes, and often germinate more rapidly after a short period of hydrated storage than when initially shed (Pammenter *et al.*, 1984; Farrant *et al.*, 1986). It was suggested that the ultimate loss of viability of recalcitrant seeds in hydrated storage is a consequence of this germinative metabolism, and that the longevity of stored seeds is inversely related to the rate at which germination-associated events occur (Berjak *et al.*, 1989; Pammenter *et al.*, 1994).

Attempts to increase storage lifespan of recalcitrant seeds have centred on reducing the rate of metabolism and germination-associated events, and hence the extent of germination in storage. For chilling-tolerant seeds this can be achieved by reducing storage temperature (King and Roberts, 1980; Pritchard *et al.*, 1995), although this must be above 0°C to prevent freezing damage to hydrated tissue. However, the recalcitrant seeds of a variety of species, particularly those of tropical origin, are chilling-sensitive (King and Roberts, 1980; Corbineau and Côme, 1988), placing limitations on storage temperatures. Attention has also been paid to manipulating the composition of the storage atmosphere. Tompsett (1983) showed that oxygen was necessary (presumably for respiration) for retention of viability in *Araucaria hunsteinii* K. Schum. seeds, suggesting that anaerobic storage to reduce metabolic rate is not a viable option. In contrast, Sowa *et al.* (1991) showed that treatment of *Litchi chinensis* Sonn. and *Dimocarpus longan* Lour. seeds with nitrous oxide, which reduced metabolic rate, increased their storage lifespan.

Lowering seed water content slightly is another possible approach that has been suggested to curtail the extent of germination in storage (King and Roberts, 1980). Reduction of water content below the fully hydrated state, but to the extent that viability is not compromised (a process termed 'sub-imbibed' storage), may lead to an extension of storage lifespan. However, Pammenter *et al.* (1994) suggested that the ongoing metabolism in recalcitrant seeds in storage, in the absence of exogenous water, actually imposed a mild but prolonged water stress, and it is possible that partial drying before storage could exacerbate this problem. This is in keeping with the findings of Corbineau and Côme (1986, 1988) that partial drying shortened the longevity of the recalcitrant seeds of four tropical species. Similarly, Drew *et al.* (2000) showed that partial drying of *Trichilia dregeana* Sond. seeds before storage severely reduced their longevity, but the seeds were in poor physiological condition initially, which could have confounded the results. Despite these findings, and the warning by King and Roberts (1980) that the rate of degeneration could increase with a decrease in seed water content, the suggestion of sub-imbibed storage providing an extension of seed lifespan has been made periodically since 1980, and recently by Hong and Ellis (1996). Thus, this possibility needs to be systematically investigated further.

The present study investigated the effect of partial drying on storage and germination of the recalcitrant seeds of four dicotyledonous tree species and one gymnosperm: *T. dregeana*, *T. emetica* Vahl. and *Ekebergia capensis* Sparrm. (all of the family Meliaceae), which are widespread in eastern and southern Africa; *Syzygium cumini* (L.) Skeels (*Myrtaceae*), which is native to India and tropical Asia, but has been introduced to southern and eastern Africa; and *Podocarpus henkelii* (Stapf.) (*Podocarpaceae*), which is a gymnosperm widely distributed in southern Africa.

Materials and Methods

Mature fruit of *T. dregeana*, *T. emetica* and *E. capensis* were harvested from trees locally, in and around Durban, South Africa. Seeds were manually removed from the fruit and surface-sterilized by soaking them in a fungicidal solution for a short period of time. Mature fruit of *S. cumini* were hand-harvested from trees in Tanzania, and seeds were extracted from the fruit and despatched to Durban. Fallen seeds of *P. henkelii* were collected from the ground below the trees in Pietermaritzburg, and transported 80 km to Durban within a few days of collection, where they were surface-sterilized.

For storage experiments, seeds were partially dried to a non-lethal water content (Table 8.1) by burying them in activated silica gel in sealed plastic bags. After partial drying, seeds were placed in pre-sterilized buckets, which were then filled with vermiculite. The contents were mixed, the buckets sealed and placed in storage at 6°C, 16°C or 25°C. Fully hydrated seeds were similarly treated. At appropriate intervals, samples were withdrawn for germination assessment. Germinating seeds were assessed daily to enable calculation of mean time to germinate (MTG):

$$\text{MTG} = \sum (t \cdot n) / \sum n$$

where t is the time in days from the start of the germination trial and n is the number of seeds completing germination on day t (Bewley and Black, 1994).

Water content was measured gravimetrically. The data presented here are for embryonic axes (whole embryos in the case of *P. henkelii*) and are expressed on a dry mass basis.

Results

Storage response

The initial water content (iwc) of the embryonic axes, the axis water content after partial drying to the levels at which the seeds were stored and the storage period for the five species are shown in Table 8.1. The extent of dehydration averaged a removal of 25% of the axis water initially present, but ranged from being fairly severe in *T. emetica* (60% loss) to very mild in *S. cumini* (2% loss). Irrespective of the extent of dehydration, all species were fully germinable following partial drying.

The effect of storage for 11 weeks at the initial water content, and storage after initial partial drying, on seeds of *P. henkelii* is shown in Fig. 8.1. Full germinability was retained in seeds stored at their initial water content at 6°C and 16°C (no data are available for seeds stored at 25°C because of severe fungal proliferation), but seeds stored after partial drying showed a loss of viability. This treatment appears to have imposed some chilling sensitivity on seeds of *P. henkelii* because the extent of viability loss increases with decreasing storage temperature. The results of the storage experiment for all five species are summarized in Table 8.2. The seeds of all species except *P. henkelii* showed some chilling sensitivity, and seeds stored at 6°C either did not survive the storage period or showed very poor germination. Partial drying before storage reduced viability in all species except *T. dregeana*. The extent

Table 8.1. The water content (± one standard deviation) of the embryonic axes (n = 10) of seeds at collection and after partial drying of the seeds for storage, and the time for which the seeds were stored.

Species	Initial water content (g/g)	Drying time (h)	Water content after partial drying (g/g)	Storage time (weeks)
Podocarpus henkelii	1.82 ± 0.17	163	1.60 ± 0.14	11
Trichilia emetica	2.13 ± 0.13	18	0.80 ± 0.18	3
T. dregeana	2.92 ± 0.16	3.5	2.16 ± 0.30	22
Ekebergia capensis	1.28 ± 0.40	6	0.94 ± 0.21	8
Syzygium cumini	1.66 ± 0.31	23	1.64 ± 0.36	4

of viability loss caused by partial drying was generally worse after storage at 25°C than at 16°C. *T. emetica* seeds were more susceptible to partial drying before storage than the other species. It might be suggested that this was a consequence of the fairly severe drying before storage (60% axis water removal). However, seeds of *S. cumini*, which had had only 2% of axis water removed, suffered similar viability loss, and even showed some loss when stored at the initial water content. Although *T. dregeana* seeds did not show viability loss during storage after partial drying, there were indications of loss of vigour (i.e. MTG increased from 10.1 days in fresh seeds to 12.6 and 14 days in seeds stored partially dry at 16°C and 25°C, respectively).

Response of germination rate

The influence of drying *T. emetica* seeds, to different extents, on the germination time course is illustrated in Fig. 8.2. Short-term drying (i.e. 2.5 h, sufficient to remove 15% of axis water in this instance) actually enhanced the rate of germination. The rate of germination can be conveniently expressed as the MTG, and the responses of the five species to short-term dehydration are summarized in Table 8.3. In all cases, except *T. dregeana*, there was a reduction in the MTG (i.e.

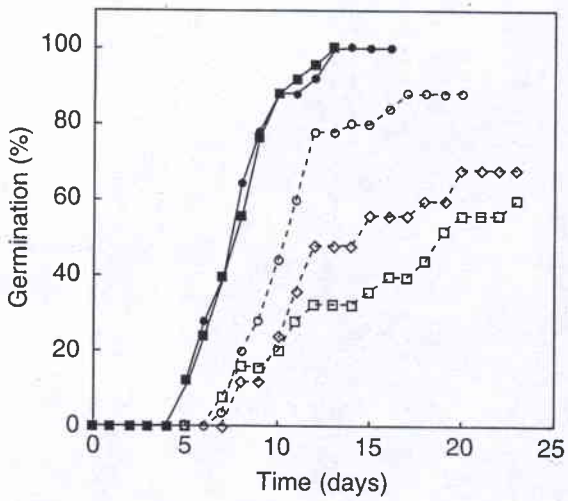

Fig. 8.1. Germination time course of seeds (n = 25) of *Podocarpus henkelii* stored at different temperatures at their initial water content (iwc) or after partial drying (pd) as described in Table 8.1. Solid lines and filled symbols = stored at iwc; broken lines and open symbols = stored after pd. Seeds were stored at 6°C (squares), 16°C (diamonds) or 25°C (circles).

Table 8.2. Final germination percentage of seeds (n = 25) stored at their initial water content (iwc), and after partial drying (pd) and storage as indicated in Table 8.1. Those species for which no data are presented for storage at 6°C had chilling-sensitive seeds that did not survive storage at that temperature. No data are available for *Podocarpus henkelii* seeds stored at their iwc at 25°C because of severe fungal infection.

		Final germination (%)					
		6°C		16°C		25°C	
Species	Fresh germination (%)	iwc	pd	iwc	pd	iwc	pd
Podocarpus henkelii	100	100	60	100	68		88
Trichilia emetica	100			100	20	100	0
T. dregeana	100	12	12	100	100	100	100
Ekebergia capensis	100			90	60	75	40
Syzygium cumini	100			60	15	100	25

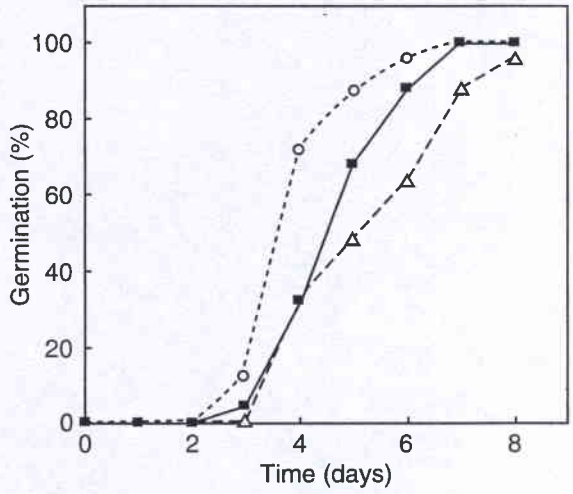

Fig. 8.2. The effect of drying time on the germination time course of seeds (n = 25) of *Trichilia emetica*. Solid line and filled squares = fresh undried seeds; broken lines = seeds partially dried for 2.5 h (open circles) or 18 h (open triangles). Corresponding water contents are given in Table 8.3.

Table 8.3. Axis water content (wc; g/g ± one standard deviation) and mean time to germinate (MTG; in days) of fresh seeds, seeds after mild dehydration and seeds after more severe partial dehydration. Drying times (dt; in hours) are indicated. For species where data for partial dehydration are not shown, drying beyond the level described as mild dehydration led to loss of germination capacity.

	Fresh		Mild dehydration			Partial drying		
Species	wc[a]	MTG	dt	wc	MTG	dt	wc	MTG
Podocarpus henkelii	1.82	11.5	22	1.80 ± 0.13	9.0	163	1.61 ± 0.13	14.6
Trichilia emetica	2.13	5.1	2.5	1.77 ± 0.23	4.3	18	0.80 ± 0.18	5.4
T. dregeana	2.92	8.9	3.5	2.16 ± 0.30	10.8			
Ekebergia capensis	1.28	3.3	6	0.94 ± 0.21	5.9			
Syzygium cumini	1.66	6.2	23	1.64 ± 0.36	5.9			

[a]Standard deviations for axes from fresh seeds are as in Table 8.1.

an increase in the germination rate) after mild dehydration. Further drying led to either an increase in the MTG (*P. henkelii* and *T. emetica*) or a loss of germinability, under which conditions the MTG becomes less meaningful.

Discussion

Recalcitrant seeds are metabolically active (Farrant *et al.*, 1997), initiate germination-associated developmental events (Berjak *et al.*, 1984; Farrant *et al.*, 1986, 1989) and often germinate in storage. Successful extension of medium-term storage would require a reduction in the rate of this metabolism and development. One possible approach is to reduce the water content to levels inhibiting germination (King and Roberts, 1980; Hong and Ellis, 1996). However, previous work has indicated that this is not a successful approach (Corbineau and Côme, 1986, 1988; Drew *et al.*, 2000). The data presented in this chapter confirm this. In every case, partial drying before storage led to a decline in storage lifespan, or a loss of vigour in the case of *T. dregeana*, relative to seeds stored at their initial water contents. The effect of temperature on the seeds was complex: partial drying before storage induced a chilling sensitivity in *P. henkelii*; *S. cumini* seeds survived marginally better at 25°C; but partially dried seeds of other species survived better at 16°C than at 25°C (Table 8.2). Although the greatest loss of viability occurred in the species that had been most severely dehydrated (i.e. *T. emetica*), the species that was least dehydrated before storage (i.e. *S. cumini*) showed the next greatest loss of viability. Generally, there were no apparent relationships between the extent to which axis water content was reduced by pre-storage partial drying, the rate of germination of fresh seeds and the performance in storage. Seeds were not stored at their initial water content until viability was lost, so the suggested relationship between storage lifespan and germination rate (Berjak *et al.*, 1989; Pammenter *et al.*, 1994) could not be assessed.

When the effect of drying on immediate post-dehydration germination was studied, it was observed that mild dehydration actually enhanced the rate of germination (Table 8.3). This observation has been reported before (Fu *et al.*, 1994; Pammenter *et al.*, 1998; Kioko *et al.*, 1999; Rodríguez *et al.*, 2000), although we suspect that it is an observation more often made than reported. Thus, seeds that are partially dried before storage could lose viability through one of the two-related causes: overdrying could lead to the accumulation of desiccation damage; or mild drying could stimulate germination events, which would likely lead to loss of viability in the absence of exogenous water.

The underlying processes leading to the enhancement of germination by mild dehydration are not yet understood. However, this phenomenon may have ecological implications. Partial dehydration is a risk to which most recalcitrant seeds on the soil surface are likely to be exposed. If this enhances germination, it could lead to the extending root accessing water deeper in the soil, thereby increasing the probability of survival of seeds exposed to a dry atmosphere at the soil surface. Many recalcitrant seeds are large (Daws *et al.*, 2005), providing them with the resources necessary for rapid germination.

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