

Germination Characteristics of Fresh and Dried *Hyophorbe lagenicaulis* Seeds

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Germination of two seed lots of the bottle palm (*Hyophorbe lagenicaulis*) was investigated before and after drying. Seed desiccation tolerance was observed in this endangered species, suggesting possibilities for *ex situ* conservation, which would complement current *in situ* programmes.

Hyophorbe lagenicaulis (L.H. Bailey) H.E. Moore, the bottle palm, is relatively easy to grow, generously fruiting and flowering within the glasshouse environment (Ellison & Ellison 2001). Cultivated specimens have been easily obtainable from commercial sources for about the last 20 years (Dransfield 1996). As a consequence, it is used extensively in civic areas, particularly for the aesthetic shape of its trunk. However, its cultivated abundance is in stark contrast to its restricted natural distribution in the Mascarene Islands (Moore & Guého 1984).

The Mascarenes contain five native species of *Hyophorbe*, all of which are either rare or on the verge of extinction (Dransfield 1996). The geographical spread of *H. lagenicaulis* is now limited to Round Island, off the north coast of Mauritius. In the 1980s only eight adult

individuals were known in the wild (Uhl & Dransfield 1987). However, since the eradication of exotic herbivores in 1986 the native population has been recovering. Furthermore, in 1998 about 300 young specimens were planted to further enhance this population (CPDU 2000). Nonetheless, much needs to be done to ensure the conservation of this and other endangered palm species.

More effective world-wide policies guiding the protection of palm species are highly desirable, particularly concerning the complementary use of *in situ* and *ex situ* approaches. Seeds are arguably the most convenient and practical part of the plant to store *ex situ*, consuming relatively small volumes of space compared to the parent plant (Smith et al. 1998). Moreover, the application of suitable collecting methods can result in an

appropriate representation of the genetic diversity found within the species.

A recent review of seed storage behaviour has revealed that the majority of higher plants produce seed which may be stored long-term in the dry state (Hong et al. 1998). Such seeds are described as 'orthodox' in their storage response (Roberts 1973). In contrast, 'recalcitrant' seeds are desiccation sensitive and tend not to be storable for more than a few months (King & Roberts 1979; Black & Pritchard 2002).

Detailed seed storage characteristics of the palms have been examined for only 102 species (ca. 5% of the family; Hong et al. 1998), and only about one quarter of these are thought to have seeds suitable for conventional storage (i.e. dry and cold). The seed storage response of *H. lagenicaulis* remains unknown. Numerous features of the seed biology of *H. lagenicaulis* are investigated here: the characteristics of seed germination, including the role of light, and the effects of desiccation and storage on seed viability and vigour.

Materials and Methods

Seed Procurement and Processing

Two lots of *H. lagenicaulis* fruits were received from the Sir Seewoosagar Ramgoolum Botanic Garden in Mauritius, arriving at Wakehurst Place on 31 March (seed lot 1) and 8 August 2000 (seed lot 2), respectively. Immediately after receipt, a description of the fruit and seed characteristics were recorded for five individuals per seed lot.

The fruit tissue was removed from the seed by gently rubbing between the thumb and forefinger under running tap-water. The seeds were then rinsed three times in distilled water and blotted between paper towels to remove any excess water.

For temporary storage, each cleaned seed lot was individually wrapped in a loosely tied black plastic bag and placed at 15°C. The bags were frequently opened (every 2 to 3 days) for ventilation and the fresh seeds mixed well to avoid fermentation. For experimentation, each seed lot was split into two. These served as duplicates for the desiccation and germination studies.

Seed Germination Assessment

In a preliminary experiment, seed germination was assessed at 25°C in the light (12 h/day). For germination, seeds were sown on 1% agar water in Perspex 'sandwich' boxes (175 × 115 × 60 mm). However, only ca. 5% of seeds germinated. Thereafter, seeds were incubated at 30°C in the light, which proved to be a more suitable temperature regime for germination. A separate comparison was made with dark germination, achieved by wrapping boxes in aluminium foil to

exclude light. All germination tests were based on 2 × 10 seeds per treatment. The effects of environment on germination were statistically tested by two-way ANOVA following arc-sine transformation of the data.

Seeds were scored as germinated when the operculum was lifted by a coleorhiza-like organ. Mean time to germinate (MTG), at 30°C, was assessed in days as: $\sum(Dn)/\sum n$ where n was the number of seeds that germinated on day D , and D was the number of days from the beginning of the germination test. The germination test lasted ca. 80 days. From the germination progress curves (see Figs 2A and B) a maximum rate of germination (%/day) was estimated, based on the linear increase in germination from 10 to 60%.

Seed weight for 10 individual seeds per seed lot was recorded weekly in the germination test. This estimate of water uptake by the seeds was used ultimately to determine the seed moisture content during imbibition and at the point of germination.

Each stage of germinative growth was recorded and photographed using a Nikon Coolpix 995 digital camera (Nikon Corporation, Tokyo, Japan).

Seed Drying and Moisture Content Determination

Moisture contents were determined gravimetrically before and after drying using a 103 ± 1°C oven for 17 ± 1 h (ISTA 1999). Determinations were made on seven individual embryos and endosperms per treatment sample.

For each batch of seeds, duplicate determinations of seed equilibrium relative humidity (eRH) were made on sub-lots of five seeds over a 1 h period at 20°C using a Rollog Agent-HT1 unit (Rotronic Ltd, Crawley, UK).

For drying, 50 seeds per batch (i.e. 2 × 25 per seed lot) were placed as a mono-layer, on a slatted-tray and left in a dry-room operating at ca. 15% RH and 15°C, until a constant weight (two decimal places) was reached over two consecutive days.

Storage Longevity Assessment

To assess storage longevity, dried seeds (at moisture contents of ca. 14% and ca. 8% embryo and rest of seed respectively) were hermetically sealed in aluminium foil laminate bags (to maintain their low levels of humidity), and held at 15°C for 18 months. After storage, the seeds were placed for germination as described above.

Results

Fruit and Seed Characteristics

Fruits had a smooth epicarp, thin mesocarp and a thin fleshy endocarp. The fruit was one-seeded and the seeds were generally black in colour,

indicating physiological maturity (Dransfield 1996). For the two seed lots, fruit length and width were ca. 22 mm and ca. 18 mm on average. The initial weight of the fruits (containing seed) varied from 4.8 ± 0.6 g (seed lot 1) to 3.7 ± 0.3 g (seed lot 2), which was significantly different ($P > 0.05$). Seeds were ellipsoid and varied in length between 15.5 and 15.7 mm and in width between 11.8 and 13.2 mm (Table 1). On dissection, the endosperm appeared homogeneous and the embryos were ca. 1.8 mm long and 1 mm wide (Table 1), lateral, but located close to the seed apex.

After cleaning, the initial moisture content for both seed lots was ca. 54% for the embryo and ca. 30% for the rest of seed material (Table 2). Seed equilibrium relative humidity of freshly cleaned seeds was 94.7% and 95.5% for seed lot 1 and 2, respectively.

Seed Imbibition and Germination of Fresh Seeds

For both lots, the mean moisture content of the seed increased from ca. 24% to 30% over the first 15 days of the germination test (Figs 2A and B). Thereafter, seed moisture content remained at 30% until the end of the test. Germination started ca. 25 days after sowing and reached ca. 75% after approximately 80 days (Figs 2A and B). The MTG was ca. 44 days. A maximum germination rate, of 1.5%/day, was observed over the period ca. 20–60 days (Figs 2A and B).

Germination Morphology

Germination occurred when the operculum was forced open (Fig. 1A). Five days after the initiation of germination (DAI), a coleorrhiza-like organ was clearly evident. At 8 DAI, the epiblast and primary root began to elongate (Fig. 1B). Secondary roots started to appear by 11 DAI as did the coleoptile and leaf tip (Fig. 1C), demonstrating an adjacent-

ligular growth pattern. The first leaf had emerged 20 DAI (Fig. 1D). The roots and the first leaf developed rapidly through 26 DAI (Fig. 1E) and 30 DAI (Fig. 1F).

Effects of Desiccation and Storage on Germination

Drying typically took 14 days and reduced the average tissue moisture contents across the seed lots to $14.3 \pm 2.0\%$ and $8.0 \pm 0.1\%$ for the embryo and rest of seed material respectively (Table 2). Seed equilibrium relative humidity of dried seeds was 35.2% and 32.7% for seed lot 1 and 2, respectively.

Drying, and drying plus storage, had no effect on the final level of germination, being about 75% in both treatments (Figs 2A and B). Moreover, post-imbibition, maximum germination rates were similar for freshly cleaned, dried and dry-stored seeds (1.5%/day). However, the starting point for this phase of growth was typically 20 days later for the dried material compared to the fresh material. In addition, dried and dry-stored seeds had an extended MTG of ca. 64 days (cf. un-dried seeds at ca. 44 days). The increase in moisture content of dried seeds was tri-phasic (Figs 2A and B). Initially, there was a sharp increase in moisture content from ca. 8 to 25% over the first 20 days, then a more gradual increase to ca. 30% by 40–45 days beyond which moisture content remained the same. The achievement of this seed moisture content coincided approximately with the onset of germination (Figs 2A and B).

The Effects of Light on Seed Germination

The effects of light on seed germination in both seed lots before and after drying are shown in Table 2. In the presence of light, germination reached 65–75% irrespective of treatment. In contrast, dark germination was $\leq 10\%$. Two-way ANOVA revealed neither an effect of drying on germination ($P = 0.753$) nor an interaction between light and drying ($P = 0.43$). The impact of light on germination was, however, statistically significant ($P = 0.002$).

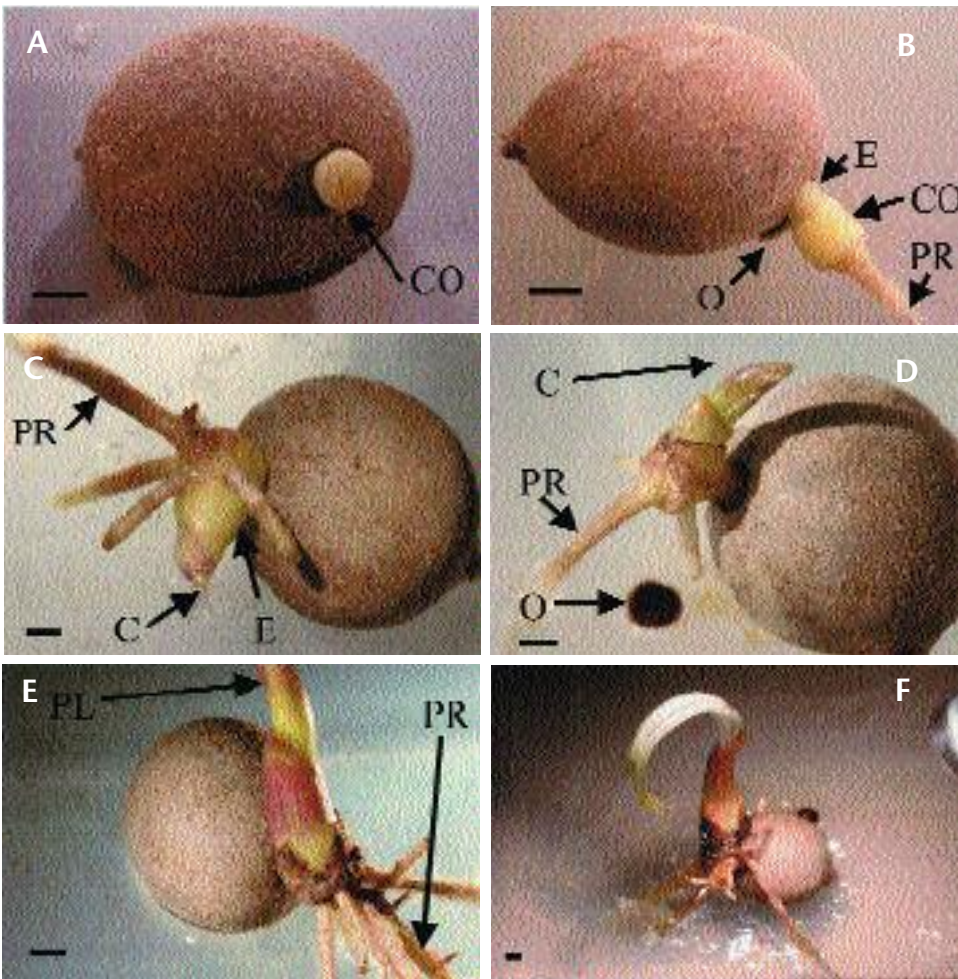
Discussion

The general appearance of the seeds following cleaning (Table 1) and the similar pattern of imbibition and germination (time and rate) for fresh seeds of both lots (Figs 1A and B) supports the suggestion that seeds of both harvests were of comparable maturity. Nonetheless, there were some differences in fruit and seed size between lots, although these were minor (Table 1).

Germinated *H. lagenicaulis* seeds have an adjacent-ligular growth pattern (Fig. 1), which is thought to be an adaptation for growth in moist environments (Uhl & Dransfield 1987). However,

Table 1. Seed lot characteristics of *H. lagenicaulis*. All measurements are means based on five individual seeds per batch. Data are significantly different (paired t-test; $P > 0.05$) when followed by different letters.

Seed characteristic	Seed lot 1	Seed lot 2
Fruit color	3 Black/ 2 Dark Green	5 Black
Fruit weight (g)	4.8 ± 0.6^a	3.7 ± 0.3^b
Embryo length (mm)	1.6 ± 0.5^a	2.0 ± 0.6^a
Embryo width (mm)	1.0 ± 0.0^a	1.0 ± 0.0^a
Seed length (mm)	15.5 ± 0.4^a	15.7 ± 0.9^a
Seed width (mm)	13.2 ± 1.1^a	11.8 ± 0.2^b



1. Stages of seed germination, rooting and shoot emergence. A. 5 days after the initiation of germination (DAI) i.e. after first signs of the operculum lifting, B. 8 DAI, C. 11 DAI, D. 20 DAI, E. 26 DAI, F. 30 DAI. (O) operculum, (CO) coleorhiza-like organ, (E) epiblast, (C) coleoptile, (PR) primary root and (PL) primary leaf. Scale bars represent 5 mm.

H. lagenicaulis is typically associated with open and drier habitats (Moore and Guého 1984). These two observations are not necessarily in conflict. Both the light requirement for germination (Table 2) and their tolerance of dehydration (Figs 2A and B) suggest adaptations for a relatively open environment. Nonetheless, selection pressures away from the adjacent ligular form of growth would not be strong if seed shedding and germination closely coincided with seasonal rains.

Seed imbibition was either bi- or tri-phasic depending on the initial seed moisture content. These patterns can be related to the physiology of the germinating seeds. For dried seeds, initial re-hydration is rapid up to ca. 25% moisture content (Figs 2A and B). This is driven by the low matric potential of the seed endosperm. The rate of imbibition then slows as the seed water potential nears that of its surroundings. In this phase, major

metabolic events are initiated prior to coleorhiza emergence / germination (Bewley & Black 1994). These events continue during the third phase of hydration when water uptake appears to stop (Figs 2A and B). This represents the point at which water saturation within the endosperm has been reached, similar to that observed in other endospermic seeds (Bewley & Black 1994). Moreover, the start of germination early in this hydration phase (i.e. a few days at most) suggests that the seeds are non-dormant. For fresh seeds, only two hydration phases were seen, as the seed moisture content was already close to that observed at full hydration. Thus, the seeds were already in the second phase of hydration. Overall, the observations support the proposition that chemical reactions associated with, and providing the driving forces for germination occur at discrete water thresholds (Bewley 1997; Obroucheva & Antipova 1997).

Table 2. The combined effects of desiccation and light on the seed germination of *H. lagenicaulis* seed lots. Germination results show the standard error of the mean based on duplicate sowings of 2 x 10 per treatment.

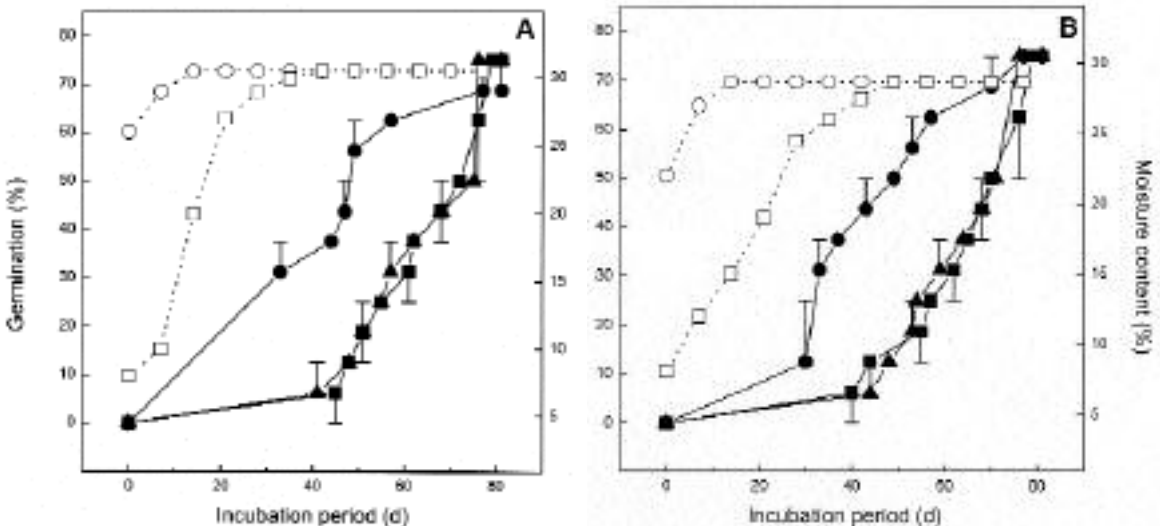
Seed lot	Treatment	Moisture content (%)	Light (+/-)	Germination (%)
1	Undried	56.3 ± 3.5 (Embryo)	+	75 ± 25
		30.5 ± 9.2 (Rest of Seed)	-	0 ± 0
1	Dried	12.3 ± 4.4 (Embryo)	+	70 ± 10
		8.0 ± 0.1 (Rest of Seed)	-	10 ± 10
2	Undried	51.4 ± 4.5 (Embryo)	+	70 ± 15
		28.7 ± 3.2 (Rest of Seed)	-	15 ± 5
2	Dried	16.2 ± 5.3 (Embryo)	+	65 ± 15
		8.1 ± 1.2 (Rest of Seed)	-	10 ± 10

The speed of germination (a measure of vigour), as determined by MTG, was lengthened by seed drying (Figs 2A and B). However, MTG was not affected by storage, post-desiccation. Consequently, it appears that drying *per se* simply extends the time taken to reach the critical seed moisture content for germination. Presumably, shortening the imbibition period, e.g. through pre-soaking, would enhance the germination speed of dried *H. lagenicaulis* seeds. This approach has worked previously for *Sabal palmetto* and *Serenoa repens* seeds, resulting in a 25% increase in germination rate (Carpenter 1987). Similarly, pre-soaking of *Hyphaene thebaica* seeds also resulted in enhanced germination rates (Karschon 1962). In

contrast, Davies and Pritchard (1998) germinated seeds of *Hyphaene thebaica*, *H. petersiana* and *Medemia argun* in water, with a reduction in mean emergence time from ca. 24–50 days to 17–22 days across the three species.

In addition to light and seed moisture content, germination in *H. lagenicaulis* is governed by temperature, 30°C being better than 25°C. Similarly, 30°C has also been shown to be optimal for germination in *Archontophoenix alexandrae*, *Butia capitata*, *Caryota mitis*, *Livistona chinensis*, *Phoenix canariensis*, *P. humilis*, *P. sylvestris* and *Washingtonia filifera* (Sento 1976; Chatty & Tissaoui 1999). However, a large degree of variation

2. Germination progress curves for seed lots 1 (A) and 2 (B): fresh/un-dried (solid circles), dried to 8% moisture content (solid squares) and dried seed stored for 18 months at 15°C (solid triangles). Increases in moisture contents are also shown for freshly isolated seeds (open circles) and dried seed (open squares). Mean time to germinate (MTG) was 43.8 ± 4.8, 64.3 ± 1.0 and 63.5 ± 0.7 respectively for treatments of seed lot 1 (A) and 44.5 ± 1.3, 64.3 ± 0.1 and 64.1 ± 0.8 respectively for treatments of seed lot 2 (B). Bars, where shown, are larger than the symbols and represent one standard error of the mean.



in optimal germination temperatures has also been demonstrated for seeds of the Arecaceae (Sento 1976).

Acknowledgments

Financial support for this work from The Millennium Commission, The Wellcome Trust, English Nature Species Recovery Programme and Orange plc. is gratefully acknowledged. The Royal Botanic Gardens, Kew is supported by grant-in-aid from Defra. We would also like to thank the Sir Seewoosagur Ramgoolam Botanic Garden, Ministry of Agriculture, Food Technologies and Natural Resources, Mauritius for the donation of seed and Dr Mike Maunder (formerly of the Royal Botanic Gardens, Kew) who facilitated seed receipt.

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