New Forest (2006) 32:9–19
DOI 10.1007/s11056-005-3144-1

Seed development, maturation and storage behaviour of *Mimusops elengi* L.

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Received 3 February 2004; accepted in revised form 8 September 2005

Key words: Desiccation, Germination, Intermediate seed storage behaviour, Maturation, Viability

Abstract. Mass maturity (end of the seed-filling phase) occurred at about 72 days after flowering (DAF) in developing seeds of *Mimusops elengi*, at which time seed moisture content had declined to about 55%. The onset of ability to germinate was detected at 56 DAF and seeds showed 98% germination by 84 DAF. Tolerance of desiccation to 10% moisture content was first detected at 70 DAF and was maximal by 84 DAF. Delaying collection by a further 14 days to 98 DAF, when fruits began to be shed, reduced seed viability, particularly for seeds first dried to 10% moisture content. Hence the best time for seed collection appears to be about 14 days before fruits shed. In a separate investigation with six different seed lots, desiccation below about 8–12% moisture content reduced viability (considerably in some lots). The viability of dry seeds (below about 10% moisture content) stored hermetically was reduced at cool temperatures (5 °C and below), and none survived storage at sub-zero temperatures. The results suggest that *Mimusops elengi* shows intermediate seed storage behaviour and that the optimal hermetic seed storage environment is about 10% moisture content at 10 °C, while short-term, moist, aerated storage at high (40%) moisture content is also feasible.

Introduction

Reforestation in degraded areas is an urgent task throughout the tropics where deforestation is seriously damaging the environment, biodiversity and natural resources. Tree planting with endemic species has been advocated for biodiversity conservation and environmental protection in Vietnam (Sam and Nghia 2002). The success of endemic tree species planting programmes depends on the availability of good quality seeds from which to produce saplings. It is the role of seed research to provide the foundation of understanding for this aspect of biodiversity conservation.

Three categories of seed storage behaviour have been identified. Orthodox seeds can be dried without damage to low moisture contents and, over a wide range of environments, their longevity increases with decreases in seed storage moisture content and temperature in a quantifiable and predictable way

(Roberts 1973). Long-term seed storage is possible only for orthodox species. Recalcitrant seeds, in contrast, cannot be dried without damage (Roberts 1973). Consequently they can only be stored over the short-term (typically less than 1 year for species of tropical origin) since they can neither be stored at low moisture contents nor at sub-zero temperatures because of freezing damage due to ice formation (Roberts 1973). A third category intermediate between orthodox and recalcitrant has been recognized (Ellis et al. 1990). The main feature is that seeds are able to tolerate desiccation to moisture contents in equilibrium with about 40–50% relative humidity (r.h.), i.e., about 7–10% moisture content depending upon species, but further drying results in more rapid loss in viability of stored seeds and sometimes immediate damage occurs on further desiccation (Ellis et al. 1990; Hong and Ellis 1996). In addition, the dry intermediate seeds of species of tropical origin show more rapid loss in viability when seed storage temperature is reduced below about 10 °C (Ellis et al. 1990, 1991a, b; Hong and Ellis 1992). Hence, optimum air-dry storage environments for the maintenance of the viability of intermediate seeds of tropical lowland species comprise seed moisture contents in equilibrium with 40–50% r.h. and around 10 °C (Hong and Ellis 2002).

Mimusops elengi L. (Sapotaceae), an evergreen tree 10–20 m high, occurs naturally in evergreen or semi-deciduous forests of Central and Southern Vietnam and provides good timber (Hop and Ha 1997). In India it provides railway sleepers (Rajput et al. 1986). Research in seed germination and storage of *Mimusops* spp. and species within Sapotaceae has been comparatively meagre and ambiguous (Hong et al. 1996). For example, seeds of most species within Sapotaceae are reported to be short-lived in open storage at room temperature, and were hence thought to be recalcitrant (Hong et al. 1996). The objectives of this study was to investigate both seed development and subsequent survival in storage of *Mimusops elengi*, with the aim of providing advice to foresters wishing to collect seeds and produce saplings for reforestation purposes.

Methods and materials

In order to obtain a sufficient quantity of seeds, fruits were collected from three neighbouring five-year old trees (5 m high, trunk diameter 14 cm at 1.3 m height) in the important economic endemic tree collection garden on the campus of the University of Agriculture and Forestry (UAF), Ho Chi Minh (HCM) City (10°47′ North, 106°42′ East, 9 m above sea level), Vietnam, at two-weekly intervals between 17 August 2002, 14 days after full flowering (DAF), and 9 November 2002. Fruits were considered completely mature (dark red) and began to be shed at this last date. Full flowering began on 3 August 2002, on which day almost all flowers opened. The branches on which flowers opened on 3 August were tagged, and developing fruits were collected at random from these branches.

On each date, about 220 fruits (each containing one seed) were collected at random. The fresh weights of a random sample of each of 100 fruits and 100 seeds were determined. The moisture content of 10 fruits or 10 seeds were determined by the high-constant-temperature oven method, 130 °C for 2 h (ISTA 2004), and calculated on the fresh weight basis (w.b.). Immediately after collection, seeds were extracted by hand, cleaned and surface dried, a sample of 100 fresh seeds tested for ability to germinate, and another sample of 120 seeds dried to 10 ±2% moisture content over silica gel at room temperature (28–32 °C) in order to determine ability to tolerate rapid enforced desiccation to 10% moisture content. To establish the seed population response to different levels of desiccation, additional seed sub-samples collected on 29 October 2002 (Lot E) and seeds from greenish yellow to red fruits collected from other trees in UAF on 21 March 2003 (Lot F) (Table 1) were dried over newly-regenerated silica gel to various moisture contents between 40 and 6% and then viability estimated by determining ability to germinate. Fresh and dried seeds were tested for ability to germinate in rolled moist paper towels, four replicates of 25 seeds, at room temperature (28–32 °C) for 77 days at UAF, until all seeds had either germinated or rotted. In addition, seven sub-samples of seed lot F dried to six moisture contents between 40.5 and 6.0% were stored at −18 to +30 °C for 3–4 months. For each treatment combination, 100 seeds (55 ml volume) were placed in a closed polyethylene bag (12×20 cm), which, in turn, was put in a closed polyethylene bottle (500 ml). Except for samples stored dry (6–10% moisture content) at −18 °C, containers and polyethylene bags of wetter seeds stored at 15 and 30 °C were opened weekly in order to change the air. After storage, seeds were tested for ability to germinate as described above.

In another investigation, seed storage behaviour was determined according to the protocol of Hong and Ellis (1996). Seeds of lots A, B, C, and D (Table 1), collected from trees in various places of HCM City, were extracted at HCM City, surface-dried, and then brought by hand to Reading in perforated polyethylene bags within 10 days of collection or extraction from fruits (Table 1). Upon receipt at Reading, samples of seeds of each lot were drawn for the determination of moisture content, equilibrium relative humidity (e.r.h.) and initial germination. The remaining seeds were hermetically stored at 15 °C for up to 4 days before the investigations began. Seeds were placed in a forced-air drying cabinet maintained at 10–12% r.h. and 15 ± 2 °C and dried to various moisture contents between the initial value and 8%. Further desiccation to 4% moisture content was achieved using regularly-regenerated silica gel at 20 °C. The seeds dried to each moisture content were sealed in a laminated aluminium foil packet and held at 15 °C for 3 days to allow moisture to equilibrate among and within the seeds. Seed moisture content, equilibrium relative humidity and viability were then estimated.

Seeds of lot C were dried to 8.2% moisture content and subsequently stored hermetically in laminated aluminium foil packets at four constant temperatures (15, 10, 5 and −18 °C) for up to 550 days. Seeds of lot D were stored hermetically at 4 moisture contents between 14.4 and 5.5% factorially

Table 1. Information on six seed lots of *Mimusops elengi*.

Seed lot	Collection date	Date of seed extraction (in HCM City)	Date of receipt at Reading	Seed moisture content (%, w.b.) at receipt	1000 seed weight (g)	Germination test		Initial germination (%)
						No. of seeds test^{-1}	No. of replicates test^{-1}	
A	29 May 2001	30 May 2001	4 June 2001	32.7	759	50	2	93
B	20 June 2001	22 June 2001	2 July 2001	18.3	–	125	5	91
C	28 June 2001	28 June 2001	2 July 2001	22.0	–	60	2	86
D	25 January 2002	1 February 2002	4 February 2002	33.7	580	100	4	95
E	29 October 2002	29 October 2002	*	40.5	712	100	4	99
F	21 March 2003	21 March 2003	*	40.5	813	100	4	100

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combined with five constant temperatures (15, 10, 5, 0 and −18 °C) for up to 365 days.

Equilibrium relative humidity (e.r.h) of seeds was determined at 20 °C using a water activity meter AQUALAB CX-2 (Decagon, USA) previously calibrated at 14, 25, 50 and 76% r.h. with appropriate saturated salt solutions. Seed moisture contents were determined gravimetrically for samples of six seeds, after cutting into small pieces, by oven-drying at 103 ± 2 °C for 17 h and are expressed on the wet basis (ISTA 2004).

Viability was estimated by testing samples of 50–120 seeds, depending upon seed availability within each lot (Table 1), for ability to germinate. Each replicate of 25 seeds was tested for ability to germinate between moist rolled paper towels at 30 °C for 63 days. Seeds at moisture contents below 10% were first humidified above water at 20 °C for one day to raise the moisture content to 15–17% in order to avoid imbibition damage (Ellis et al. 1985). The criterion of germination in all tests was normal seedling development (ISTA 2004).

Results

Seed development and maturation

The seed production environment from 17 August to 9 November 2002 was warm (monthly average 28.0 °C, maximum 35.5 °C) and humid (average 80% relative humidity). Mean fruit and seed fresh weights increased rapidly to maximum values at 70 days after full flowering (DAF) (Figure 1a). Maximum mean dry weights were also achieved at 70 DAF (Figure 1b). To estimate the end of the seed-filling period, termed mass maturity (Ellis and Pieta Filho 1992), an iterative regression analysis procedure was used to determine the best "broken-stick" model of relations between mean seed dry weight and time from full flowering. This occurred when a positive relation was fitted to observations between 42 and 70 DAF and a horizontal relation among later observations, with mass maturity estimated as 72 DAF (Figure 1b).

Moisture contents of fruit and seed (Figure 1c) remained stable during early development until 56 DAF. Both declined thereafter to 48 and 38%, respectively, at maturity when the fruits were mature (dark red) and about to shed. Interpolation suggests that seed moisture content had declined naturally to about 55% at mass maturity.

Onset of ability to germinate was detected at 56 DAF (Figure 1d). Almost all seeds (99%) were able to germinate by 84 DAF, but viability then declined to 88% at 98 DAF.

Germination of dried seeds

Desiccation tolerance (to 10% moisture content) was first detected in a small fraction of the developing seed population at 70 DAF, 2 days before mass

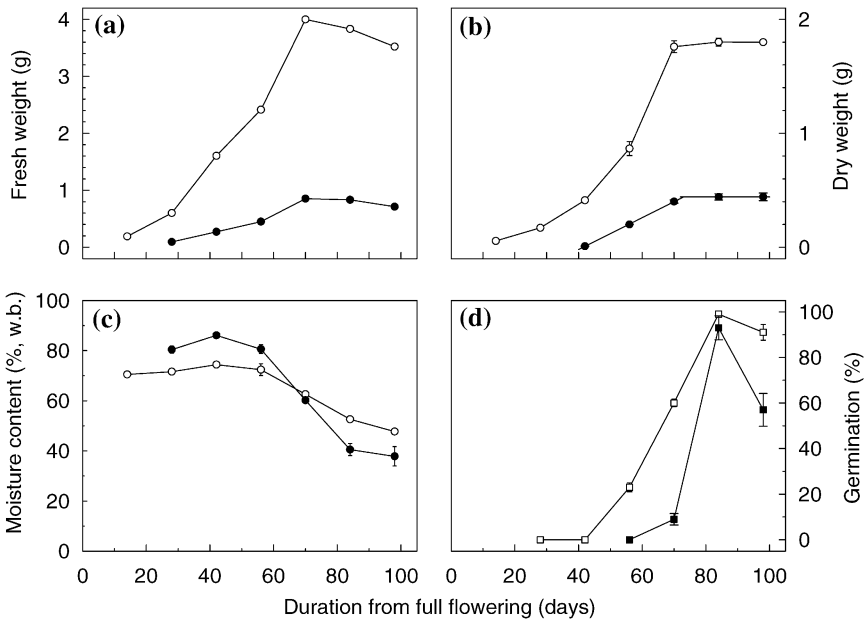

Figure 1. Changes in mean fresh weight (a), dry weight (b), and moisture content (c) of developing fruit (open circles) or seed (solid circles) of *Mimusops elengi.* Vertical bars indicate ±SE (where larger than symbols). The estimate of mass maturity is provided by the intersect of the two thick fitted lines in (b). Ability to germinate of fresh (open squares) and dried seeds (solid squares) is shown in (d).

maturity (Figure 1d). The ability of the whole population to survive desiccation to 10% moisture content was achieved at 84 DAF, 12 days after mass maturity, but desiccation tolerance then declined substantially in the following 14 days.

Seeds of the five lots A, B, D, E, F tolerated desiccation to about 10–12% moisture content, but further drying reduced viability considerably (Figure 2). Among the five lots, A, E and F were the more tolerant to desiccation.

Survival in moist storage

No loss in viability of seeds stored moist and aerated for 3 months at 40.5% moisture content was detected at either 30 or 15 °C, but the viability of seeds stored similarly for 4 months at the lower moisture content of 30.5% with 15 °C was reduced slightly (Table 2).

Survival in hermetic storage

Reducing seed moisture content from 14.4 to 10.2% increased longevity considerably at the warmer temperatures studied (10 and 15 °C) for lot D, but

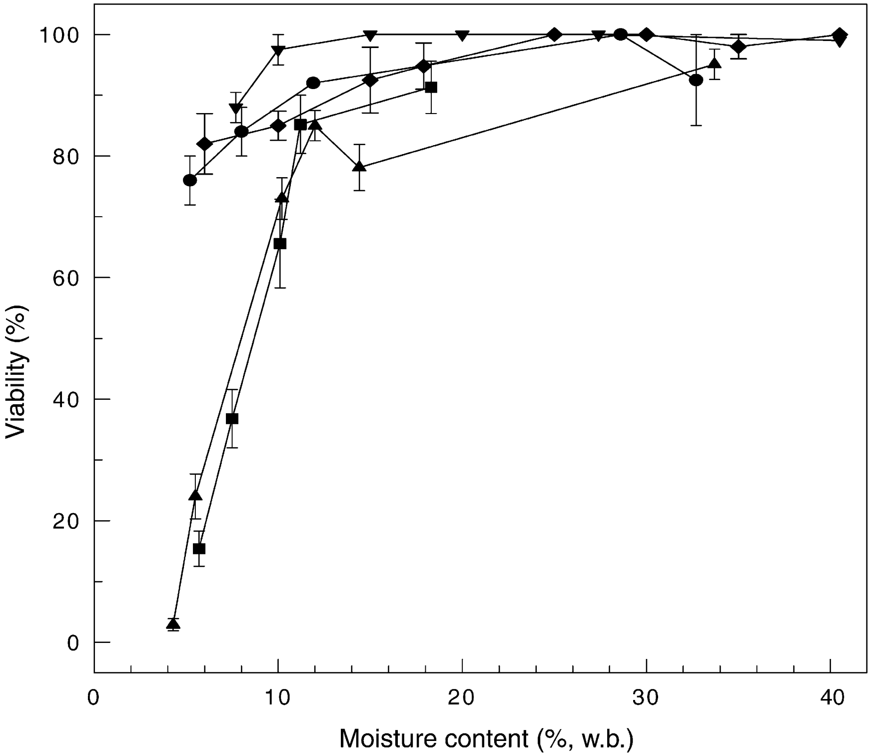

Figure 2. Effect of desiccation on subsequent ability to germinate (%, criterion normal germination) for seeds of five seed lots of *Mimusops elengi*: A (●), B (■), D (▲), E (▼), F (◆).

further desiccation to 5.5% moisture content reduced viability substantially (Figure 3). Among the five storage temperatures investigated, the optimal temperature was 10 °C, with 15 °C being superior to 5 °C. No seeds survived

Table 2. Germination of seed lot F of *Mimusops elengi* before or after 3–4 months' storage at different moisture contents and temperatures.

Moisture content (%, w.b.)	Temperature (°C)	Germination (%) (SE)		
		Duration of storage (months)		
		0 (before storage)	3	4
40.5	30	100	99 (1.0)	
40.5	15	100	98 (2.0)	
30.5	15	100		92 (1.9)
17.9	15	95 (3.8)		94 (4.8)
10.0	– 18	85 (2.4)	0	
7.7	– 18	85 (1.0)	0	
6.0	– 18	82 (5.0)	0	

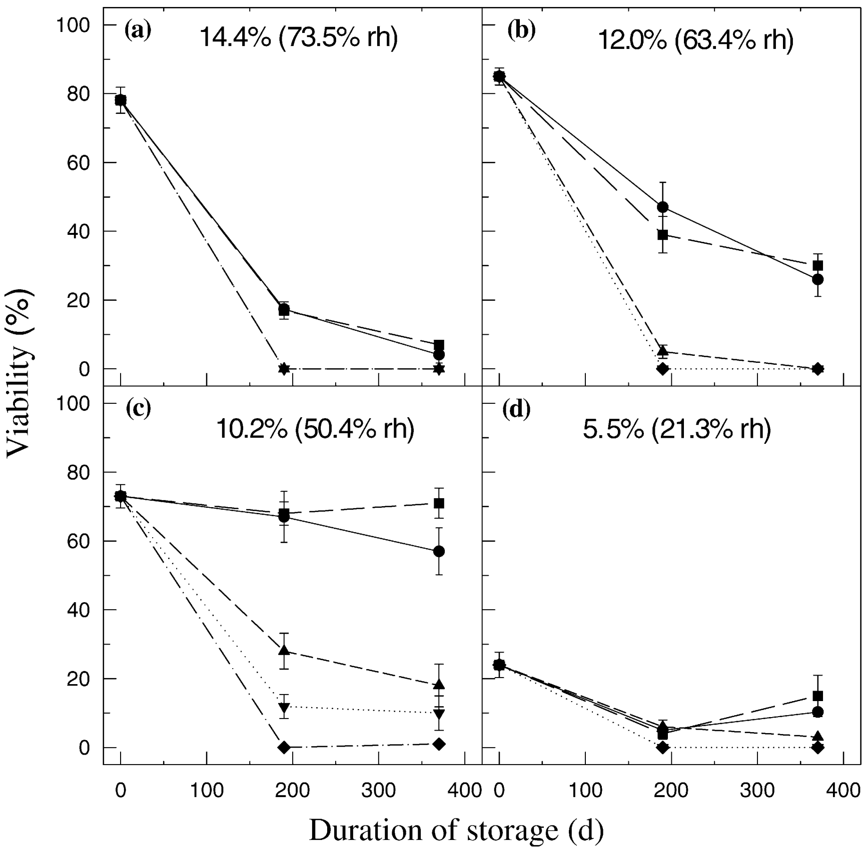

Figure 3. Survival of seeds of lot D of *Mimusops elengi* after desiccation to four different moisture contents and subsequent hermetic storage with 15°(●), 10°(■), 5°(▲), 0°(▼) or −18 °C (◆). Values in parenthesis after each moisture content are equilibrium relative humidities at 20 °C. Vertical bars represent means ± SE where larger than symbols.

exposure to −18 °C for 190 days whatever the moisture content (Figure 3). Seeds of lot D stored at 10.2% moisture content (50.4% e.r.h.) with 10 °C showed no loss in viability during 12 months' hermetic storage (Figure 3c). Similarly, seeds of lot C at 8.2% moisture content (41.1% e.r.h.) and 10 °C or 15 °C showed little loss in viability during 550 days' hermetic storage, but no seeds survived this duration at −18 °C Figure 4). Moreover, no seeds of lot F stored hermetically for 3 months at −18 °C with moisture contents between 6.0 and 10.0% survived (Table 2).

Discussion

Seeds of *Mimusops elengi* tolerated desiccation to around 8–12% moisture content, but further drying reduced viability (Figure 2). Furthermore, the

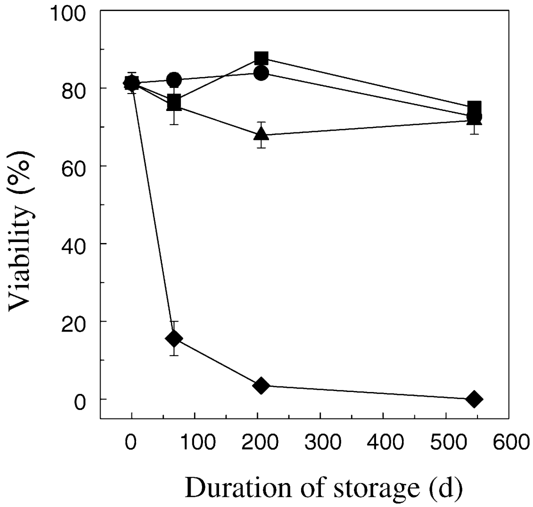

Figure 4. Survival of seeds of lot C of *Mimusops elengi* in hermetic storage at 8.2% moisture content (in equilibrium with 41.1% relative humidity at 20 °C) and 15°(●), 10° (■), 5° (▲) or − 18 °C (◆). Vertical bars represent means ± SE where larger than symbols.

viability of seeds stored dry was lost more rapidly at the cooler temperatures investigated (Figures 3 and 4). These observations are compatible with the characteristics of intermediate seed storage behaviour (Ellis et al. 1990; Hong and Ellis 2002). The optimal seed storage environment identified for *Mimusops elengi* appears to be between 8.2% (41.1% e.r.h.) and 10.2% moisture content (50.4% e.r.h.) at 10 °C (Figures 3 and 4). Thus, we conclude that *Mimusops elengi* shows intermediate seed storage behaviour. Comparison with results for other species with intermediate seed storage behaviour, such as *Coffea arabica* L. (Ellis et al. 1990, 1991c; Hong and Ellis 1992, 2002); *Azadirachta indica* A. Juss. (Hong and Ellis 1998), *Carica papaya* L. (Ellis et al. 1991a) and *Elaeis guineensis* Jacq. (Ellis et al. 1991b) confirms that hermetic storage at 10 °C with seed moisture contents in equilibrium with 40–50% r.h. (determined at 20 °C) is suitable for species adapted to tropical lowlands (Hong and Ellis 1996, 2002).

Seeds of *Mimusops elengi* can also be stored successfully over the short-term if maintained very moist (40.5%) and aerated (Table 2). Similarly in *Coffea arabica* the longevity of seeds stored at 15 °C was greater at 41% than at 21% moisture content in moist aerated storage, but also greater at 10% than at 21% seed moisture content in air-dry storage (Van Der Vossen 1979).

Mass maturity of developing seeds of *Mimusops elengi* occurred at 72 DAF, when seed moisture content had declined naturally on the mother plant to 55% (Figure 1). Whereas onset of ability to germinate was detected about 16 days before mass maturity, the whole population was not able to germinate until 28 days later, i.e., 12 days after mass maturity (Figure 1d). This pattern is broadly similar to that for the tree legume *Peltophorum pterocarpum* (DC) K. Heyne in similar environments (Mai-Hong et al. 2003).

Ability to tolerate desiccation to 10% moisture content was first detected just before mass maturity. The whole population (almost) was able to survive desiccation to 10% moisture content at 84 DAF, i.e., 12 days after mass maturity. However, in the subsequent 14 days desiccation tolerance declined substantially. Similarly, *Peltophorum pterocarpum* seeds (which show orthodox seed storage behaviour) began to acquire the ability to tolerate desiccation to 10% moisture content about 6 days before mass maturity, and full desiccation tolerance was achieved 22 days after mass maturity (Mai-Hong et al. 2003). The crucial difference between the (intermediate) *Mimusops elengi* and the (orthodox) *Peltophorum pterocarpum* (and indeed orthodox species in general) is that seeds of orthodox species which remain on the mother plant maintain high viability and high desiccation tolerance for some considerable period thereafter whereas *M. elengi* did not. In (orthodox) tomato (*Lycopersicon esculentum* Mill.), for example, the whole seed population remained desiccation tolerant for at least 40 days when fruit harvest was delayed (Demir and Ellis 1992). Hence, these results for *Mimusops elengi* run counter to the general expectation that desiccation tolerance reaches a maximum at or close to the point of natural seed dispersal. There were no signs whatsoever of visible germination of these seeds at 98 DAF. This does not rule out the possibility that germination *sensu strictu* might have occurred between 84 and 98 DAF, however, and could conceivably account for the reduction in desiccation tolerance.

The brief period during seed development and maturation when (most) seeds of *Mimusops elengi* were able to tolerate desiccation to 10% moisture content (as fruit colour began to change from greenish yellow to red and the fruit began to be edible) may explain the variation in desiccation sensitivity among seed lots (Figure 2). Presumably, the better seed lots (A, E and F) were from fruits collected closer to the most suitable time whereas the remainder were possibly collected a week or so later. Indeed, the best lots (E and F) were collected as fruits changed colour from greenish yellow to red, whereas the other fruits were already dark red. *Coffea arabica* also shows intermediate seed storage behaviour and in that species too seeds extracted from fruits just before they became red (and so ripe) showed greatest desiccation tolerance (Ellis et al. 1991a). Hence, we recommend fruit collection and seed extraction in *Mimusops elengi* just as fruits begin to change colour from greenish yellow to red, which is about 14 days before they are shed from the tree.

Acknowledgement

We thank the Darwin Initiative for the Survival of Species of the Department for Environment, Food and Rural Affairs, UK, for support.

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