

Chapter **39**

Exploring Conventional Seed Storage in Some Tropical Species



**Jalli Radhamani, Anurudh K. Singh and
Abha Sharma**

Germplasm Conservation Division, NBPGR, Pusa
Campus, New Delhi-110 012, India

Summary

The seeds of mangosteen (*Garcinia mangostana*), acacia (*Acacia nilotica*), date palm (*Phoenix dactylifera*), soapnut (*Sapindus trifoliatus*), Indian gooseberry (*Emblca officinalis*), mahuwa (*Madhuca indica*), rubber (*Hevea brasiliensis*), jackfruit (*Artocarpus heterophyllus*), jamun (*Syzygium cumini*), kusum (*Schleichera oleosa*) and sandalwood (*Santalum album*) were investigated for their storage behaviour. In freshly extracted seeds, the moisture content varied from 6 to 69% (fresh weight basis) and viability from 90 to 100%. Based on their response to desiccation and chilling, the seeds were classified as orthodox (acacia, date palm, sandalwood), intermediate (soapnut, Indian gooseberry, kusum) and recalcitrant (mangosteen, mahuwa, rubber, jamun, jackfruit). The seeds of both orthodox and intermediate categories tolerated desiccation to 5–6% moisture, whereas the critical moisture content for the recalcitrant seeds varied from 23 to 40%. Nonetheless, moist storage of recalcitrant seed was possible for 18–30 months at 20°C when various chemical treatments were applied.

Introduction

Although information on seed storage behaviour has been collated for about 7,000 species from 251 families of flowering plants (Hong *et. al.*, 1996), there is a poor understanding of reproductive biology, seed structure and storage behaviour of tropical tree species. This has been restricting their *ex situ* conservation and sustainable use. In addition, such information is essential for their sustained economic exploitation and use in meeting various environmental challenges, such as afforestation, restoration of degraded habitat and genetic improvement. A number of tropical species produce recalcitrant (i.e., desiccation sensitive) seeds making *ex situ* seed conservation of these species difficult. Therefore, there is a need to improve understanding of the seed storage behaviour of these species and to develop cost-effective medium- or short-term storage methods for their conservation. The current study investigated the seed storage responses of 11 trees of importance in India.

Materials and Methods

In the present study, mangosteen [*Garcinia mangostana* L.], acacia [*Acacia nilotica* (L.) Delile], date palm [*Phoenix dactylifera* (L.)], soapnut [*Sapindus trifoliatus* L.], Indian gooseberry [*Emblica officinalis* Gaertn.], mahuwa [*Madhuca indica* J.F.Gmel.], rubber [*Hevea brasiliensis* (Willd. ex A.D. Juss.) Muell. Arg.], jackfruit [*Artocarpus heterophyllus* Lam.], jamun [*Syzygium cumini* (L.) Skeels], kusum [*Schleichera oleosa* (Lour.) Oken] and sandalwood [*Santalum album* L.] were investigated.

Seeds were extracted from the fresh fruits, thoroughly washed 3-4 times with distilled water, coated with 0.5% fungicide (Captan) and shade dried for 1 d in an open room at 20°C and 45% relative humidity. The seeds were then stored at 15°C in perforated aluminium foil pouches until use.

Seed moisture content was determined gravimetrically using two replicates each of 25 seeds and results were expressed on a fresh weight basis (% FW). Measurements were taken when the seeds were fresh (i.e., before washing) and during the drying treatments. Morphological measurements were also made on fresh seed (3 × 25 or 50 seeds, for the recalcitrant and the other species, respectively). A 100-seed weight was determined using 2 × 100 seeds, except for *Hevea brasiliensis* where two replicates of 50 seeds were used.

Seeds were sown for germination on paper (TP = top of paper) in replicates of 3 × 50 at the temperatures specified in Table 39.1. Light was applied for 16 h d⁻¹, except for *Garcinia mangostana* which was illuminated continuously. Germination was assessed as normal seedling emergence. For some species, it was necessary to stimulate germination with 0.2% KNO₃ or GA₃ at 100 or 500 ppm (see Table 39.1). When seeds were soaked in solution, this lasted for 10–12 h in the respective solution; thereafter, the seeds were plated on the same medium, with the solution being replenished every week. When seeds were soaked in water as part of the germination test, soaking lasted overnight. A quick test of seed viability (%) was performed using a triphenyl tetrazolium chloride (TTC) test (0.5 to 1.0%) for 6 to 8 h, depending on the seed species. The red staining pattern was assessed in relation to the percentage area of the embryonic axis and cotyledons covered. The axis stained a deeper red than the cotyledons, which turned pink.

For desiccation, seeds tied in muslin cloth bags were kept over freshly-charged silica gel in a desiccator jar at 15°C. These seeds were taken out at intervals to determine their moisture content and viability. Dried seeds were re-humidified over water in a desiccator jar at 25°C for 24 h before conducting the germination test, in order to avoid imbibitional injury. Cold sensitivity was tested by putting the desiccated seed (minimum moisture content with maximum percentage survival) in aluminium foil packets, storing them at -20°C for 3 months and then evaluating them for germinability.

Processed intermediate and recalcitrant seeds were conditioned to different moisture levels by various methods (see Table 39.4) and stored at $20 \pm 2^\circ\text{C}$ in perforated plastic Petri dishes. For the drying/wetting cycle treatment, seeds were initially plated on moist germination paper and uniformly sprayed with water (10 ml) every fortnight to re-wet the paper; this cycle was repeated for the duration of the experiment. Seeds of some species were also coated with charcoal, sub-imbibed at about -12 MPa in polythene glycol (PEG) 6000 or treated with abscisic acid (10^{-4} M). All such treatments were continuously applied. During storage, the seeds were tested at regular intervals for their germinability.

Results and Discussion

There was a wide variation in seed dimensions among the species studied. *Artocarpus heterophyllus* seeds were found to be the largest and the heaviest (4.7×5.7 cm, 387 mg) followed by *Madhuca indica* (4.3×5.6 cm, 382 mg) (Table 39.1). Fresh seeds of *Artocarpus heterophyllus* showed the highest moisture content (68.9%) followed by *Garcinia mangostana* (68.4%). Most of the recalcitrant seeds (see Tables 39.2 and 39.3) germinated easily, i.e., without the need for external treatment. The exception to this was *Garcinia mangostana*, which required soaking, GA_3 treatment and continuous light for germination (Table 39.1). In contrast, desiccation tolerant (orthodox and intermediate) types (see Tables 39.2 and 39.3) had some constraints in germination due to seed coat dormancy. Treatment of seeds with light, chemicals (GA_3 , KNO_3), scarification, or soaking in water, improved their germination. In the case of dormant seeds, however, seed germinability after the appropriate treatments correlated well with the seed-staining pattern with triphenyl tetrazolium chloride (Table 39.1).

The five recalcitrant seeded species (see Table 39.2) exhibited greater variation in moisture content among the seed replicates compared to the desiccation tolerant seeds (data not shown). The relationship between moisture content and germination was investigated in detail for these species (Table 39.2). In *Garcinia mangostana*, *Madhuca indica*, *Hevea brasiliensis*, *Artocarpus heterophyllus*, and *Syzygium cumini*, the moisture contents after which a significant loss in seed germinability was observed varied from 23 to 40% (Table 39.2), below which the seed viability reduced drastically. This is consistent with recalcitrant (desiccation sensitive) seed storage behaviour. The variation in critical moisture content between these recalcitrant seeds is relatively higher (23 to 40%) than reported earlier (12 to 31%) for a range of other species (Roberts, 1973). More recent data have also revealed a wide range of critical moisture contents in recalcitrant seeds (see Hong *et al.*, 1996; Black and Pritchard, 2002).

Table 39.1 Seed characteristics and germination protocols for various species									
Species	Length (cm) ± S.D.	Diameter (cm) ± S.D.	100 seed weight (g) ± S.D.	MC (%) ± S.D.	Initial viability (germination %)	Viability with TTC (%)	Germination constraint	Standardised protocol	Germination after treatment (%)
<i>Acacia nilotica</i>	1.30 ± 0.08	1.15 ± 0.04	5.49 ± 0.06	9.8 ± 0.08	88	100	None	Pre-soaking overnight; TP, 20/30°C; 20°C	88
<i>Phoenix dactylifera</i>	2.54 ± 0.06	2.81 ± 0.02	6.87 ± 0.08	13.0 ± 0.09	10	100	Dormancy	Soaking in H ₂ O or GA ₃ (100ppm) or (KNO ₃ 0.2%) overnight + plating in the same medium, 27+2°C	96
<i>Santalum album</i>	1.24 ± 0.02	2.38 ± 0.02	6.41 ± 0.04	7.42 ± 0.05	30	98	Dormancy, seed coat	Breaking of seed coat + soaking in GA ₃ (500ppm) or KNO ₃ (0.2%) + plating (in the same medium; 30°C	90
<i>Emblia officinalis</i>	0.425 ± 0.04	0.66 ± 0.04	1.28 ± 0.04	11.0 ± 0.06	28	90	Dormancy	TP, 27+2°C, KNO ₃ (0.2%)	88
<i>Sapindus trifoliatus</i>	1.68 ± 0.06	3.50 ± 0.02	6.58 ± 0.10	9.37 ± 0.10	30	100	Seed coat	Removal of seed coat increases both % and speed of germination; TP, 27+2°C	86
<i>Schleichera oleosa</i>	1.75 ± 0.04	3.29 ± 0.06	22.04 ± 0.18	13.4 ± 0.12	25	100	Seed coat, Dormancy	Breaking of seed coat + soaking overnight in water followed by GA ₃ (500ppm) TP, 25°C	80
<i>Artocarpus heterophyllus</i>	4.72 ± 0.28	5.72 ± 1.28	38.70 ± 1.40	68.9 ± 1.14	95	100	None	TP 27+2°C	95
<i>Garcinia mangostana</i>	4.03 ± 0.30	3.31 ± 1.00	7.57 ± 1.20	68.4 ± 0.91	0	98.5	Dormancy, seed coat, photoperiod	Soaking + GA ₃ (500ppm) + continuous light, 27 + 2°C	98
<i>Hevea brasiliensis</i>	3.77 ± 0.24	6.35 ± 1.30	22.77 ± 2.10	43.4 ± 0.86	50	90	Seed coat	TP 27+2°C, removal of seed coat	80
<i>Madhuca indica</i>	4.28 ± 0.40	5.56 ± 1.60	38.22 ± 2.40	61.35 ± 2.12	98	100	None	TP 27+2°C	98
<i>Syzygium cumini</i>	2.88 ± 0.20	3.32 ± 1.20	9.11 ± 1.60	58.6 ± 1.16	98	100	None	TP 27+2°C	100

Table 39.2 Effect of desiccation on seed viability of presumed recalcitrant seeds

Species	Moisture content (%)	Germinability (%)
<i>Artocarpus heterophyllus</i>	68.90	98
	49.60	95
	41.30	95
	33.40	18
	30.00	3
<i>Garcenia mangostana</i>	68.40	96
	43.71	94
	30.40	80
	25.24	74
	22.80	28
<i>Hevea brasiliensis</i>	43.40	86
	34.10	83
	28.00	81
	23.00	10
	18.60	0
<i>Madhuca indica</i>	61.35	98
	45.20	91
	40.00	87
	39.80	18
	20.00	0
<i>Syzygium cumini</i>	58.60	98
	38.16	98
	30.80	92
	27.60	22
	18.90	12

Roberts and King (1980) suggested a direct association between plant ecology and seed storage behaviour. Orthodox seeds originate from environments subjected to occasional or seasonal drought and develop desiccation tolerance for survival and continued perpetuation of the species. In contrast, recalcitrant seeds tend to originate from moist ecosystems, where the seeds are adapted to high humidity during development. Nonetheless, all the recalcitrant seeds investigated here tolerated partial drying (Table 39.2). When the seeds were then transferred to -20°C for 3 months, a few seeds of *Garcinia mangostana*, *Artocarpus heterophyllus* and *Syzygium cumini* survived (Tables 39.3 and 39.4). Presumably, these seeds had insufficient moisture for ice formation or freezing damage was limited. In the case of *Syzygium cumini*, 15% of the seeds showed a positive response to TTC after drying and cold

Table 39.3 Effects of conventional storage practices on seed survival in various species

Species	Initial moisture content (%)	Critical moisture content (%)	Cold sensitivity ¹	Seed type ²
<i>Acacia nilotica</i>	09.8	≤5	None	O
<i>Phoenix dactylifera</i>	13.1	≤5	None	O
<i>Santalum album</i>	7.4	≤5	None	O
<i>Emblica officinalis</i>	11.0	≤5	+	I
<i>Sapindus trifoliatus</i>	8.9	≤5	+	I
<i>Schleichera oleosa</i>	13.0	≤5	++	I
<i>Artocarpus heterophyllus</i>	68.9	33.4	++	R
<i>Garcinia mangostana</i>	65.0	22.8	++	R
<i>Hevea brasiliensis</i>	43.4	23.0	+++	R
<i>Madhuca indica</i>	61.4	39.8	+++	R
<i>Syzygium cumini</i>	58.6	27.6	++	R

¹ three months storage at -20°C with 95–100 % (+++), > 70% (++) and > 40% (+) dying.

² Orthodox (O), Intermediate (I) and Recalcitrant (R).

storage. On germination though, all seedlings formed were found to be abnormal. Nonetheless, these preliminary results on three recalcitrant species suggest opportunities for the development of *ex situ* storage methods using partial drying and cold temperatures.

For the remaining species investigated, the seeds could be desiccated to very low moisture levels (5–6%) without significant loss of viability (data not shown). To assess whether these desiccation tolerant seeds were orthodox or intermediate in their storage response, the seeds were subjected to -20°C for 3 months. *Acacia nilotica*, *Phoenix dactylifera* and *Santalum album* maintained high viability after -20°C storage, suggesting a typical orthodox response (Table 39.3). In contrast, some seeds of *Emblica officinalis*, *Sapindus trifoliatus* and *Schleichera oleosa* either died or showed abnormal seedlings following low temperature dry storage, thereby suggesting their intermediate seed storage behaviour. The causes of this stress are not clear, but could relate to alterations in protein and lipid structure of the membrane leading to changes in membrane fluidity, thickness and permeability which might impact, *inter alia*, on bound enzyme activity (Wolfe, 1978; see Black and Pritchard, 2002).

Seed-lots of recalcitrant species only survived ambient temperature for a few days to 3 weeks (data not shown). However, seed storage lifespan at 20 ± 2°C was increased after subjecting them to various treatments, such as sub-imbibing in PEG 6000, treating with ABA (10⁻⁴M), equilibrating over saturated salt solutions of KNO₃, going through a drying/wetting cycle, and coating with

Table 39.4 Effects of longer-term storage on viability of seeds of different species

Species	MC for storage	Viability before storage (%)	Viability after storage (%) ¹	Conventional storage practice ²	Storage time (months)
<i>Acacia nilotica</i>	≤5	88	98	Long-term storage at -20°C	12
<i>Phoenix dactylifera</i>	≤5	96	100	Long-term storage at -20°C	12
<i>Santalum album</i>	≤5	90	98	Long-term storage at -20°C	16
<i>Embllica officinalis</i>	≤5	80	76	Storage at 10°C and 15°C	15
<i>Sapindus trifoliatus</i>	≤5	80	72	Desiccated seeds at 10°C	18
<i>Schleichera oleosa</i>	≤5	76	70	Desiccated seeds at 10°C	18
<i>Artocarpus heterophyllus</i>	41.3	94	90	Drying/wetting, PEG 6000 soaked	26
<i>Garcinia mangostana</i>	25.2	74	92	Sub-imbibed in PEG 6000, coated with charcoal	26
<i>Hevea brasiliensis</i>	28.0	80	80	Equilibrated with KNO ₃ ≈ 94% RH	18
<i>Madhuca indica</i>	40.0	86	98	Drying/wetting every 15 days, PEG soaked	27
<i>Syzygium cumini</i>	31.0	92	100	Over saturated salts of KNO ₃ , sub imbibed in PEG 6000, ABA (10 ⁻⁴ M), coated with charcoal	30

¹ viability was assessed based on the percentage viability of seeds before storage

² gibberellic acid (GA₃), potassium nitrate (KNO₃), polyethylene glycol (PEG), abscisic acid (ABA).

charcoal (Table 39.4). These treatments, which enabled storage times of 18 to 30 months to be achieved, probably switched off germination processes and/or induced 'dormancy' in the seeds (Kermode, 1990), thereby extending their longevity in hydrated storage.

In conclusion, the present investigation corroborates the recalcitrant-seeded nature of jackfruit (*Artocarpus heterophyllus*), mangosteen (*Garcinia mangostana*), rubber (*Hevea brasiliensis*), mahuwa (*Madhuca indica*) and jamun (*Syzygium cumini*). The seeds of Indian gooseberry (*Emblica officinalis*), soapnut (*Sapindus trifoliatus*) and kusum (*Schleichera oleosa*) are intermediate in nature, while those of acacia (*Acacia nilotica*), date palm (*Phoenix dactylifera*), and sandalwood (*Santalum album*) are orthodox. Although most orthodox seeds remain viable for long periods under dry (3–8% moisture content, or lower) conditions (Roberts *et al.*, 1984; Hong *et al.*, 1996), only three of the 11 tropical species investigated here survived conventional seed bank conditions (i.e., dry and cold). This indicates the importance of further research on storage protocols for tropical seeds that avoid chilling injury, desiccation damage and genetic instability.

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