Conservation of four tropical forest tree seeds from India

Subhash C. Naithani, Ranjana Naithani, Boby Varghese, J.K. Godheja and K.K Sahu

Seed Biology Laboratory, School of Life Sciences, Pt. Ravishankar Shukla University, Raipur- 492010 (Chhattisgarh), India

Abstract

Seed storage physiology of *Buchanania lanzan, Diospyros melanoxylon, Gmelina arborea* and *Madhuca indica*, four tropical tree species, were evaluated following the DFSC/IPGRI protocol. All the seeds were harvested with relatively high moisture contents between 17 and 63 % and showed 100 % initial germination. Drying the seeds over silica gel revealed desiccation-tolerance in *B. lanzan*, *D. melanoxylon* and *G. arborea*, and desiccation-sensitivity in *M. indica.* Seeds of *M. indica* lost viability when dried below 18.3 % MC, but could be stored for 55 days at 25°C, during which time 25 % germinated. Desiccation-tolerant seeds of *B. lanzan*, *D. melanoxylon* and *G. arborea* were also tolerant of low temperature (-20 and 0 °C) storage, retaining their initial viability over 60 to 150 days. Germinability was gradually lost with further storage (c. 280 days). Rapid-drying of *B. lanzan*, *D. melanoxylon* and *G. arborea* seeds improved their storage, maintaining higher viability over longer periods.

Introduction

India with over 45,000 plants and 81,000 animal species, is one of the world's top 12 "mega-biodiversity" nations. The recorded forest area of India is 76.52 million ha which constitutes almost 20 % of the total geographic area. Of these forest areas, 11 % are considered closed with more than 40 % of canopy coverage, while about 8% of the geographical area are open forests with 10-40 % canopy coverage (Tewari 1981). Forests in India have declined by nearly half in the twentieth century, and the rate of deforestation is still increasing. Further, serious threat from timber smuggling, overexploitation by industry, deteriorating law enforcement, ravaging forest fires, uncontrolled grazing, agricultural encroachment, urbanization, unmanaged exploitation for

firewood and other basic needs has put an immense pressure on the Indian forests (Hocking 1993). The estimated annual deficit in tropical forestry plantations in the country has increased to 36.4 million ha/year in the new millennium.

In recent years, tree planting has become an intensive activity for soil conservation and to meet the multifarious demands of timber, fuel, fodder and non-wood forest produce. This reinforces a series of *in-situ* and *ex-situ* measures and the legal and policy reforms needed to implement and enforce the National Biodiversity Action Plan (MoEF 2000). Industrial and commercial plantations today constitute 45 % of all plantations, with *Tectona grandis, Gmelina arborea, Dalbergia sissoo, Acacia nilotica, Populus, Eucalyptus* and *Pinus* sp. the main species used. However, apart from the few timber species largely preferred for plantations, there are other numerous tree species which have promising national and international markets for fruits (Report of Committee of Forests and Tribals of India 1982), medicine (Jain 1968) and lumber products (Murty *et al*. 1989). These species have not yet gained enough attention in afforestation programmes and remained under-utilised. If appropriate cultivation techniques and markets were developed, these species could greatly assist in the economic development of the country.

There is little information available on the seed biology of Indian forest trees, and availability of quality seed limits the management of forests (afforestation and plantation activities). Seed still remains the primary source of planting material in tropical countries and the annual demand for forest tree seeds in India is about 10,000 metric tones. However, seed is often in short supply, of low quality and variable maturity and has limited storage life. Plantings have been limited to species where basic knowledge of seed collection, processing and seed storage physiology is available. Desiccation-sensitivity and non-storability of many of the tropical forest tree seeds adds to these problems (Gunn 1991). Thus there is an urgent need to establish and strengthen seed programmes in India to ensure continuous and adequate supplies of well-adapted indigenous seeds. The use of indigenous species in afforestation and plantation programmes also has the advantage of using readily available reservoirs of genetically diverse stock. We have chosen to investigate the desiccation and storage behaviour of four important indigenous species from India.

Buchanania lanzan Spreng., also referred as *B. latifolia* Roxb., is a member of Anacardiaceae family and is locally known as ‘chironji’, ‘cuddapah almond’ or almondette tree. It is a common tree in the

deciduous forests of India, widely distributed in dry forests from the Sutlej and all through the central to south India (Prakash 1991). It is extensively found in moist and dry deciduous forests of south and north India. The tree plays an outstanding role in the rural and tribal economy of India, especially for its edible fruits and seeds (Hocking 1993). The kernels, which are nutritious, are eaten raw or roasted and serve as a substitute for almonds. The dry fruit is expensive. The wood provides poor quality timber and is used for firewood, boxes, cheap furniture and doors, etc. (Prakash 1991). The leaves and bark of the tree are of high medicinal value for the treatment of bleeding wounds, jaundice, throat ulcer and tooth ache, etc. (Hocking 1993). It is a small to moderate-sized tree with a straight trunk, often of a considerable height up to 18 m and girth up to 7.5 m. The seeds have been shown to lose viability significantly within the first year of storage (Prakash 1991).

Diospyros melanoxylon Roxb. also known as the Coromandel Ebony or locally as 'tendu', is a member of the Ebenaceae family. A small to moderate size tree usually not exceeding 12 m in height, it is widely distributed in the dry deciduous forests of India. Black heartwood from the tree is considered to be a valuable substitute for true ebony wood, which is heavy and durable. The leaves are a major source of income for the rural and tribal populace, being used for the wrapping of tobacco to prepare local cigarettes or bidis. Superior quality leaves of large size, papery texture, and inconspicuous veins fetch up to 5 times the price of inferior quality leaves. Around 300,000 tons of bidi leaves are produced annually in India, valued at 4,515 million Rupees. There is, however, vast opportunity for propagating better clones or strains. The fruit is edible and the dried fruit powder has medicinal properties. The dried flowers are used for the treatment of urinary, skin, blood diseases and dyspepsia. The species has very high potential national market for various non-wood forest products (NWFP). It is highly suitable for afforestation in dry and barren hill slopes.

Gmelina arborea Roxb., belongs to Verbenaceae family and is extensively used for mass plantations, locally referred as 'Khamar or Gamhar'. This medium-sized tree (30 m / 1.2-4.5 m) with clear bole of 9-15 m is generally used for pulp production for the paper industry. It occurs in many parts of India and develops best in moist, fertile, well-drained valleys and in moist deciduous forests. It is highly recommended for plantings in skeletal soils in medium rainfall areas, field boundaries and for agro- and social forestry plantations. The timber is extensively used for furniture, musical instruments, plywood,

ship building and paper making, etc. The bark, root, fruit, flowers and leaves are used by local people as medicine in blood diseases, ulcers and fever. Seeds are known to lose their viability within a year (Prakash 1991).

Madhuca indica J.F. Gmel. is part of the Sapotaceae family and is native to southern Asia, especially India. It is commonly known as 'mahua' in India and also referred to as *M. longifolia* (Koeing.) Macbride and *Bassia latifolia* Roxb. (Hocking 1993; Sastry and Kavathekar 1994). It is the lifeline of the tribal belt in central India, and culturally the tree most identified with Indian life in the plains. The tree has been used extensively for its multifarious properties, especially by various tribal communities and therefore hardly cut down though it is a good wood. It is large, much branched deciduous tree with a short bole and rounded crown, and is found throughout the greater part of India, up to 1200 m. The tree is valuable for its timber, flowers and seeds (Hocking 1993). The fleshy edible corollas have high economic value and are a rich source of sugars, vitamins and calcium and are used in the manufacture of country liquor and vinegar (Sastry and Kavathekar 1994; Bhanja 2000). The kernel contains a high percentage (20-43 %) of fatty oil, known as mahua oil or butter. It is used in commerce as a non-traditional oil (Sastry and Kavathekar 1994). The seeds of *M. indica* are generally referred to as short-lived (Prakash 1991; Hocking 1993; Sastry and Kavathekar 1994).

The DFSC/IPGRI protocol (1999, 2000) is a systematic and scientific approach to improving the post-harvest handling and storage of tropical tree seeds. The application of the protocol for these selected four tree species is important in designing the *ex-situ* storage methods for short and long term conservation. Hence, an attempt was made to (a) establish the storage physiology of *M. indica, B. lanzan, G. arborea* and *D. melanoxylon* seeds and (b) to maximise their storage potential by determining their lowest safe moisture content and most appropriate storage conditions. Additionally, we analysed lipid and phenol contents in *B. lanzan* and *G. arborea* seeds and presented the findings in this report. The *B. lanzan* manifest rancidity thus tastes pungent almost within a period of one year after harvest. Looking to the commercial and food value of the seeds an attempt was made to estimate the changes in the lipid content and FFA in response to slow and fast drying. The contents of phenols, known an inhibitors to germination, were also estimated in view to uncover their effects on fresh seeds of *G. arborea*.

Materials and methods

Seed collection and extraction

Mature fruits of *Madhuca indica, Buchanania lanzan, Diospyros melanoxylon* and *Gmelina arborea* were plucked from 20–25 marked plus trees. The protocol developed in the IPGRI/DFSC project (DFSC/IPGRI 1999; 2000) was followed to determine the seed desiccation tolerance. The collected fruits were transported in jute bags to the laboratory within 1 to 4 h of collection. The fruits were sorted and infected, mechanically damaged and very small fruits were discarded. The total weight of the harvest and the weight of 100 fruits were determined and the average weight of all these fruits was calculated.

Moisture contents were determined after manually extracting seeds. The fleshy pulp was removed and traces of pulp adhering to the extracted seeds were also removed by rubbing them with sand. The seeds were then washed under running water for 2-3 minutes and gently surface dried. One hundred individual seeds were then weighed to determine the average weight of seeds (DFSC/IPGRI 1999). The seed length and breadth was also measured for fifty individuals.

Desiccation and storage trials were performed following fungicidal treatment of extracted seeds. The seeds were first soaked in a 1 % solution of sodium hypochlorite for 10 minutes and then rinsed and blotted dry. They were coated with 1g Thiram per kg seed and were kept under ambient conditions (25-27 °C and 35-40 % RH) until further use. The seed samples were ready for various analyses within 6-10 h of their harvest from the trees.

Germination

Seeds were surface sterilized with 1 % sodium hypochlorite solution for 15 minutes, thoroughly washed with distilled water 4-5 times. Germination tests were performed with 5 replicates of 10 seeds each. *M. indica* seeds were allowed to imbibe distilled water and to germinate in the dark at 26-28 °C on filter paper towels rolled up inside two plastic sheets. The seeds of other three species, i.e. *B. lanzan, D. melanoxylon* and *G. arborea,* were germinated in vermiculite inside plastic boxes. During incubation, seeds were supplied with distilled water as necessary. Germination was scored after every 24 h, as radicle emergence to 5 mm (Varghese and Naithani 2002). The germination test

was terminated when no seed had germinated for a week or when seeds blackened and/or showed fungal manifestations.

Moisture content and desiccation trials

Moisture contents (MC) of whole fruit, seed coat, embryo, embryonic axes and the cotyledons were determined following the methods of the International Seed Testing Association (ISTA 1985).

Self-indicating silica gel was used for the desiccation trials following the DFSC/IPGRI (2000) protocol. Seed samples at the desired MC (using the target MC as a reference) were retrieved at various intervals and were tested for actual MC and germination. Another lot of seeds was allowed to dry at ambient conditions of 25-27 °C and 35-40 % relative humidity (natural drying) by placing them on perforated plastic trays.

Storage trials

Seeds of all species at different moisture contents were stored at four different temperatures of 25, 15, 0 and –20 °C. The seeds were regularly retrieved to determine their survival and moisture content. Seeds of *G. arborea* were also tested for survival at liquid nitrogen (LN_2) temperature (–196 °C) after rapid drying to various moisture contents. The seeds were packed in polypropylene cryovials and plunged into LN_2. After 24 h these seeds were thawed rapidly by immersing in a water bath maintained at 37 °C. Survival was assessed by germination testing (Varghese and Naithani 2001).

Total lipid & free fatty acid contents and total phenol determinations

The total lipid content of seeds of *B. lanzan* was estimated using the method of Raheja *et al.* (1973). Seeds were weighed and ground with LN_2 and total lipid content was calculated gravimetrically after distillation of the seed powder one hour in petroleum benzene at its boiling point (40-60 °C). The oil content was expressed as mg lipids per g fresh weight of the seed.

The free fatty acid (FFA) content in the lipid was estimated following the method described by Itaya and Ui (1965). The total lipid extracted in petroleum benzene from *B. lanzan* seeds was mixed with 0.66 M phosphate buffer (pH 6.2) (v/v) and incubated at

room temperature to separate the aqueous and chloroform layers. Copper triethanolamine solution was added to the chloroform layer, the mixture shaken in a cyclomixer and then incubated for 15-30 minutes to complete the reaction. Finally, to 3 ml of the chloroform layer pipetted out was added 11 mM diethyl dithio carbamate, and the absorbance was read at 440 nm. The standard curve was plotted using 0.2 mg/ml stearic acid.

The phenol content was estimated by the method of Swain and Hills (1959). Funicular tissue of 500 mg of *G. arborea* were homogenized in extraction buffer (made of 80 % ethyl alcohol in 0.2 M borate buffer, pH 7.6) with silica. The homogenate was centrifuged at 8,000 g for 5 minutes and the supernatant was collected. The residue was washed twice with extraction buffer. Finally, all the ethanolic supernatants were mixed and evaporated in vacuum to yield concentrated aqueous layers at 4 °C. The aqueous layer was used as the source of phenolic compounds. A standard curve was prepared using different concentrations of chlorogenic acid.

Results

Initial tests

Fruit and a seed of *B. lanzan* weighed on average 0.71 g and 0.21 g, respectively (Table 1). The seed was globular with a mean diameter of 0.95 cm and thickness of 0.69 cm. The initial moisture contents of both manually extracted, and processed seeds were similar at c. 17 % MC, showing that the processing did not affect initial seed moisture content. The fruits in contrast, had very high moisture contents.

About 6.8 kg of seeds were extracted out of 31.5 kg of *D. melanoxylon* fruits. The mean seed dimensions were 2.0 cm long by 1.1 cm breadth and the average seed weight was 1.3 g (Table 1). The fresh seed embryonic axes had 53.6 % MC and the cotyledons 36.3 % MC, while the whole seed moisture content was 38.4 %.

Botanically after removing the fleshy mesocarp from *G. arborea* fruits, the remaining stony entity is the pyrene. However, the pyrene has been used as ‘seed’ in this study, although extracted seeds (without endocarp) were used in some of the germination tests for comparison. The mean fruit and seed weights were 9.76g and 0.97 g, respectively (Table 1). The initial moisture contents were 26.1 % for the whole ‘seed’ (the pyrene) and 24.5 % for the excised seeds.

The berry fruits of *M. indica* weighed 16 g each (Table 1). The mean weight of an entire seed was 3.5 g with average length and thickness of 3.1 and 1.5 cm. The initial moisture content of manually extracted seeds was 63.2 %, with embryonic axes and cotyledons having respectively 66.6 and 47.6 % MC.

Table 1. Initial characteristics of fruits and seeds of *B. lanzan*, *D. melanoxylon*,
G.arborea and M. indica from India.

	B. lanzan	*D. melanoxylon*	*G.arborea*	*M. indica*
Fruit weight (g)	0.71 ± 0.68	28 ± 3.1	***09.76 ± 2.52***	16.0 ± 2.9
No. of fruits / kg	-	40 ± 5	-	-
Seed weight (g)	0.21 ± 0.02	1.30 ± 2.6	0.97 ± 0.17	3.5 ± 0.8
No. of seeds / kg	-	625 ± 5	-	-
Seed diameter (cm)	0.95 ± 0.07	-	-	-
Seed thickness (cm)	0.69 ± 0.03	-	1.14 ± 0.14	-
Seed length (cm)	-	2.0 ± 0.8	1.69 ± 0.19	3.1 ± 0.35
Seed breadth (cm)	-	1.1 ± 0.36	-	1.5 ± 0.18
Seed shape	globular	ellipsoidal, flat	Ovoid	ellipsoidal
Initial MC % (manually extracted)	17.1 ± 1.16	38.4 ± 1.0	27.82 ± 1.76	63.2 ± 1.0
Seed MC after processing	16.3 ± 0.42	38.0 ± 1.0	26.07 ± 2.33	-
MC whole fruit (%)	62.2 ± 3.3	58.0 ± 2.7	75.03 ± 1.0	73.4 ± 3.6
MC seed coat (%)	not separable	not separable	27.9 ± 1.29	63.4 ± 3.1
MC embryos (%)	15.4 ± 0.6	-	24.5 ± 1.8	57.8 ± 0.8
MC cotyledons (%)	14.0 ± 0.55	36.3 ± 0.5	18.48 ± 2.97	47.6 ± 0.4
MC axes (%)	16.7 ± 0.56	53.6 ± 2.4	28.79 ± 0.81	66.6 ± 3.5
MC endocarp (%)	10.3 ± 0.46	-	-	-

Desiccation trials

B. lanzan seeds were dehydrated from 16 % MC to 4 % MC over 48 h using silica gel. Germinated remained at 93-100 %, indicating that there was no adverse effect of drying on germination (Table 2).

Total lipids of *B. lanzan* declined gradually from 66.4 % to 23.6 % (fresh weight) in seeds slowly dried from 18.9 to 5.4 % MC over 310 days at ambient conditions of 25 °C and 35-40 % relative humidity (Table 3). This decrease occurred with simultaneous enhancement in free fatty acid (FFA) levels from 12 t o 219 µmol/g fw (Table 3). In contrast, silica gel dried seeds exhibited comparatively higher levels of lipids with very low levels of FFA. The loss of total lipids in slow dried seeds was accompanied by a fall in germination from 100 to 35 %. In

contrast, rapid drying of *B. lanzan* seeds to 4.8 % MC did not lead to a significant reduction in germination.

Table 2. Effect of desiccation using silica gel on germination (G %) of *B. lanzan* seeds.

Control			Dried		
Drying time (h)	MC (%)	G (%)	Target MC (%)	Actual MC (%)	G (%)
-	16.3± 0.4	100	Initial	-	-
20 min	17.8± 2.1	100	15	15.6 ± 0.9	100
40 min	18.1± 2.2	100	12	13.4 ± 0.7	100
1 h 50 min	18.1± 2.2	100	10	10.6 ± 0.2	100
2 h 20 min	17.0± 1.8	100	9	10.0 ± 0.3	100
3 h 20 min	17.0± 1.6	100	8	9.2 ± 0.3	100
3 h 50 min	17.3± 1.6	100	7	8.3 ± 0.7	100
4 h 50 min	17.2± 1.1	100	6	7.1 ± 0.5	100
24 h	18.3± 0.4	100	3	4.1 ± 0.3	100
48 h	23.4± 0.7	100	<3	3.8 ± 0.2	93

Table 3. Total lipid and free fatty acid (FFA) contents in *B. lanzan* seeds when
dried using silica gel or at ambient conditions, and their viability (G %).

Ambient drying				Silica gel drying			
MC (%)	G (%)	Total lipids **(%)**	FFA (µmol/g fw)	MC (%)	G (%)	Total lipids **(%)**	FFA (µmol/g fw)
18.9± 1.2	100	**66.4**	12	18.9 ± 1.2	100	**66.4**	10
14.5± 0.9	100	**61.0**	28	12.0 ± 0.6	100	**67.2**	9
8.8 ± 0.5	88	**52.4**	63	7.9 ± 0.4	100	**64.0**	17
5.4 ± 0.8	35	**23.6**	219	4.8 ± 0.3	90	**59.0**	54

The seeds of D. melanoxylon were desiccated to 4.2% MC without reduction in viability. (Table-4).

Table 4. Desiccation and germination of *D.melanoxylon* seeds.

Control			Dried		
Drying time (h)	MC (%)	G (%)	Target MC (%)	Actual MC (%)	G (%)
0	37.2 ± 0.63	97	Initial	37.2 ± 0.63	97
3	38.4 ± 0.26	90	30	30.7 ± 0.51	97
6	37.3 ± 0.35	90	25	21.8 ± 1.37	97
12	38.5 ± 0.87	86	20	17.8 ± 0.45	97
18	39.6 ± 0.62	90	15	15.3 ± 0.45	93
30	38.7 ± 0.29	93	10	10.6 ± 0.20	97
67	40.5 ± 0.72	90	5	6.9 ± 0.75	100
95	41.3 ± 0.75	93	3	4.4 ± 0.15	100
119	41.4 ± 0.69	90	2	4.2 ± 0.50	100

G. arborea whole seeds (pyrene) with 27 % MC showed 60 % germination (Table 5). Drying these seeds (with endocarp) from 23 to 3 % MC over 150 h improved the germination capacity from 60 to 96 %. Control seeds maintained in the vermiculite, showed a slight loss of moisture content but improved germination from 60 to 95 % after the same period (Table 5).

Total phenol extracted from funicular tissue, which remained tenaciously attached within the cavity of the stone or endocarp after depulping, showed a gradual decline from 7.21 to 0.08 mg/g fw when the pyrenes were dried from 28 to 4 % MC (Table 6). Excised seeds of *G. arborea* at 28 % MC treated with phenol-extracted (5 ml) from funicular tissue of the pyrene exhibited germination of only 62 %. As the phenol content of the extract fell, any inhibition effect on excised seed germination was lost.

Table 5. Desiccation and germination of *G.arborea* seeds with endocarp (pyrene) and without endocarp (excised seeds).

Control			Dried			
MC (%)	Germination (%)		Target MC (%)	MC (%)	Germination (%)	
	With endocarp	Excised seeds			With endocarp	Excised seeds
27.3 ± 1.6	60	100	Initial	27.3± 1.6	60	100
28.19± 1.2	60	100	22	23.0± 0.5	65	100
26.87± 1.1	62	100	17	17.0± 0.8	72	100
27.12± 1.2	60	100	12	11.9± 1.3	86	100
29.22± 0.8	64	100	8	8.1 ± 0.2	90	100
26.87± 1.1	65	100	6	6.3 ± 0.4	96	100
27.48± 1.7	85	100	5	4.3 ± 0.1	100	100
24.19± 1.8	85	100	4	4.1 ± 0.4	100	100
23.13± 1.3	94	94	3	3.4 ± 0.1	100	100
20.65± 2.0	95	95	2	2.7 ± 0.3	96	95

M. indica seeds desiccated with silica gel over a period of 200 h at 25 °C reached a moisture content of 9.4 % (Table 7). Initially seeds with 63.2 % MC germinated 100 %. However, germinability declined gradually to 10 % with desiccation and no seed germinated below 18 % MC (Table 7). Seeds kept as the control in vermiculite showed 100 % over the same treatment time

Table 6. Total phenol (80 % alcoholic extract) from funicular tissue attached within the cavity of endocarp and germination of *G. arborea* excised seeds treated with 5 ml of extracted phenol.

MC whole seeds (%)	Total phenol (mg phenol/g fw)	Germination (%)
28 ± 0.9	7.2	62
15.5 ± 0.9	6.2	78
7.1 ± 0.6	1.9	100
4.0 ± 0.2	0.08	100
4.0 ± 0.2	0	100

Table 7. Desiccation and germination (G %) of *M. indica* seeds.

Control			Dried		
Drying time (h)	MC (%)	G (%)	Target MC (%)	MC (%)	G (%)
0 h	63.2 ± 1.0	100	Initial	63.2 ± 1.0	100
20 h	59.6 ± 1.5	100	55	53.3 ± 2.9	100
30 h	60.0 ± 2.7	100	50	51.7 ± 0.9	100
46 h	60.9 ± 2.2	100	45	43.6 ± 2.2	95
80 h	60.1 ± 1.8	100	40	37.2 ± 2.6	80
105 h	59.7 ± 1.7	100	35	31.3 ± 3.0	75
130 h	60.2 ± 2.2	100	30	30.5 ± 2.5	60
150 h	-	-	25	26.2 ± 1.8	40
174 h	-	-	20	18.3 ± 0.9	10
200 h	58.1 ± 1.6	100	10	9.4 ± 2.9	0

Storage trial

Hydrated seeds of *B. lanzan* with ≥13 % MC could not tolerate low temperatures of –20, 0 and 15 °C, but these seeds germinated 35-68 % after storage at 25 °C for 280 days (Fig. 1). Further drying of seeds to 7-10 % MC improved their chilling tolerance and they better survived during storage. These seeds exhibited 95-100 % survival after 90 days at all storage temperatures and gradual loss in germinability (58-88 %) after 280 days of storage. *B. lanzan* seeds dried to c. 4 % MC showed 95-100 % germination after 180 days of storage at all tested temperatures. Further storage for 280 days led to a slight reduction in seed viability (85-92 % germination).

Initially all hydrated and dehydrated seeds of *D. melanoxylon* germinated 100 % (Fig. 1). Seeds with high moisture contents of 27-37 % could not tolerate storage at –20 and 0 °C, but further

desiccation to 18-22 % MC resulted in a gradual increase in survival, 7-23 % germination after 20 days at these temperatures (Fig. 1). However, seeds with 15-37 % MC were storable at 15 °C for 150 days and at 25 °C for 240 days, though with a gradual loss in viability. Seeds desiccated to 10.5, 6.9, and 4.5 % MC survived storage at all tested temperatures, germinating in the range of 80-100% after 150 days (Fig. 1). Dried seeds with 6.9 and 4.5 % MC germinated highly (85-95 %) after 250 days of storage at all tested temperatures (-20, 0, 15 and 25 °C).

Although *G. arborea* seeds with endocarp (pyrene) of various moisture contents were stored, germination tests were performed using excised seeds (after removing the endocarp) in order to avoid the inhibitory effect of the endocarp. Seeds with 18-26 % MC could not survive storage at –20 and 0 °C and became non-viable within 90 days (Fig. 1). Further drying to 12.4 % MC improved longevity as the seeds germinated 30-45 % after 150 days of storage at –20 and 0 °C. Seeds with ≥12.4 % MC showed similar survival after storage at 15 and 25 °C (Fig. 1). Seeds dehydrated to 3.9 % MC exhibited highest survival (72-95 %) at –20, 0 and 15 °C after 270 days of storage.

In a separate cryo-storage trial, all seeds with endocarp (silica gel) dried from 34.3 to 2.4 % MC could not survive cryo-temperature when stored for 24 h (data not shown).

M. indica seeds lost viability within 10 days when stored at zero and sub-zero temperatures (Table 6). However, best results were obtained at 25 °C in seeds with 43.6 % MC, which showed 25 % germination after 55 days of storage. Seeds with higher or lower than >43.6 % MC lost germinability within 40 days (Table 8). The seeds stored at 15 °C also lost viability within 40 days. It has been observed that these seeds at high moisture contents required a lot of care during storage to avoid fungal infestation and suffocation.

Discussion

Initially, mature seeds of all the four tree species studied exhibited almost 100% germination. Unlike some desiccation tolerant (or orthodox) seeds, the seeds of *M. indica*, *D. melanoxylon*, *G. arborea* and *B. lanzan* are shed at high (63.2 %) or relatively high (17.1-38.4 %) moisture contents (Table 1).

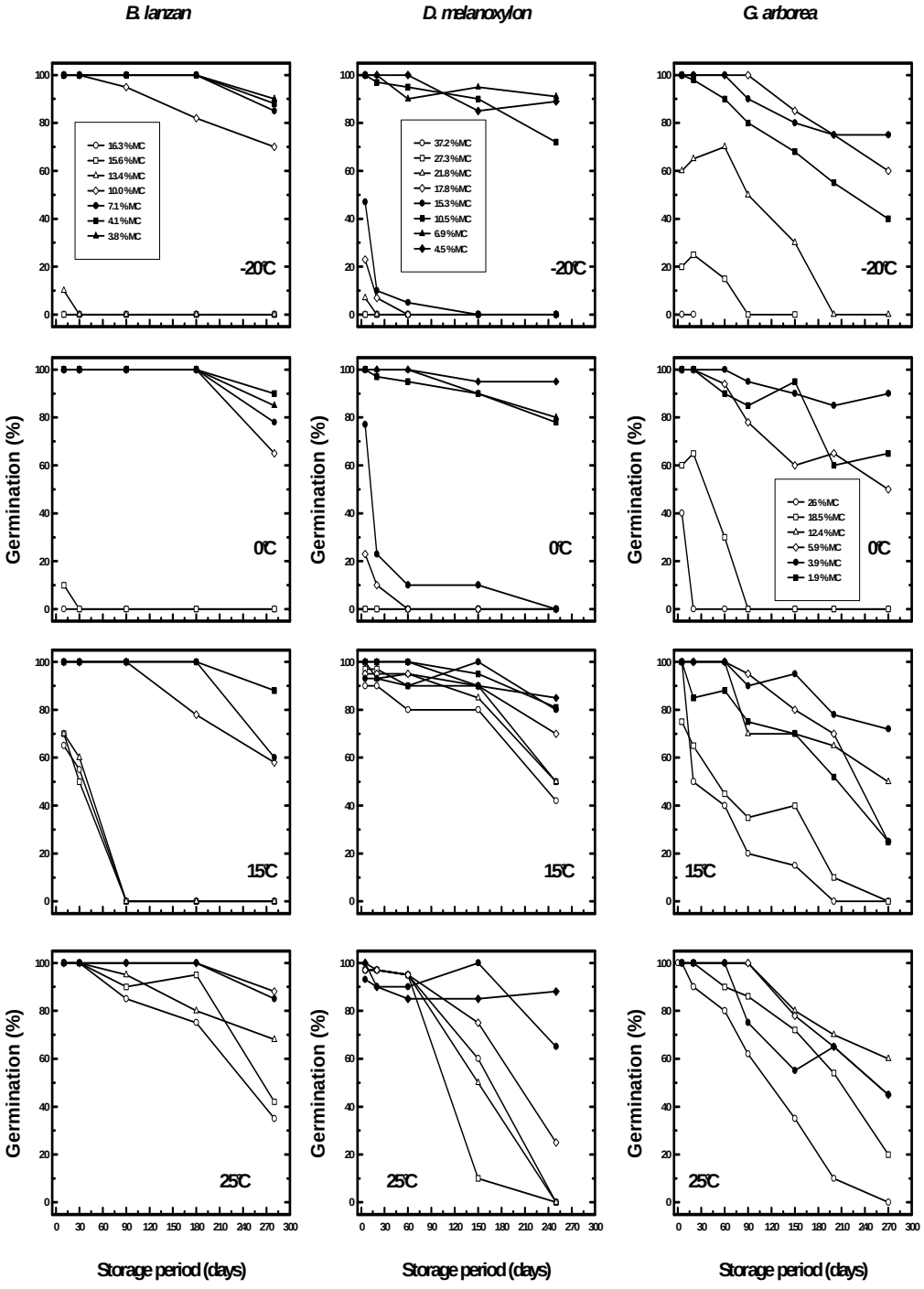

Figure 1. Germination of *B. lanzan*, *D. melanoxylon* and *G. melina* seeds at different moisture contents after storage at four temperature of –20, 0, 15 and 25 °C for about 9 months.

Table 8. Germination after days (d) of storage of *M. indica* seeds at 15 and 25 °C.

MC (%)	Storage temperature (°C)	Germination (%) after storage					
		0 d	10 d	20 d	30 d	40 d	55 d
63.2 ± 1.0	15		55	15	0	-	-
	25	100	75	40	10	0	-
53.3 ± 2.9	15		50	20	0	-	-
	25	100	50	20	10	0	-
51.7 ± 1.0	15		65	35	10	0	-
	25	100	70	40	20	0	0
43.6 ± 2.2	15		80	50	25	0	-
	25	95	90	75	55	35	25
37.2 ± 2.6	15		40	0	-	-	-
	25	80	65	40	20	5	0
31.3 ± 3.0	15		35	0	-	-	-
	25	75	45	20	0	-	-
30.5 ± 2.5	15		35	0	-	-	-
	25	60	30	0	-	-	-
26.2 ± 1.8	15		0	0	-	-	-
	25	40	10	0	-	-	-
18.3 ± 0.9	15		0	0	-	-	-
	25	10	0	0	-	-	-

Seeds of *B. lanzan*, *D. melanoxylon* and *G.arborea* tolerated desiccation over silica gel to as low as 4 % MC (Tables 2, 4 and 5), maintaining their initial viability. On the other hand, *M. indica* seeds showed a rapid loss of viability when dried to 43.6 % MC and did not tolerate dehydration below 18 % MC (Table 5). This result corroborates another study on this species showing a decline in seed viability with a reduction in moisture content (Varghese *et al.* 2002). *M.indica* seeds, like other desiccation-sensitive seeds of *Shorea robusta* (Chaitany and Naithani 1994), *Avicienia marina* (Berjak *et al.* 1989) and *Quercus robur* (Finch-Savage 1992), showed high critical moisture content (43.6 % MC) and can be categorized as true recalcitrant.

The seeds of *B. lanzan* exhibited acute rancidity when dried to 5.4 % MC at ambient temperature of 25 °C (Table 3). The seeds with 19 % MC were rich in oil content (66.4 % fw total lipids) with less FFA (12 μmol/g fw). However, they registered a gradual loss of total lipids (23.6 % fw total lipids) with a simultaneous increase in FFA and decrease in viability when dried to 5.4 % MC (Table 3). Our results corroborate findings on *L. kirki* and *S. membranaceous* (Farrant *et al.* 1989) which concluded that desiccation-sensitive seeds retain higher viability to a lower moisture content, if dried rapidly as compared to the seeds desiccated slowly in ambient conditions. Pammenter *et al.* (1991) suggested that rapid drying permits survival to l ower water contents

because the water is removed sufficiently rapidly to prevent aqueous-based deleterious reactionsoccurring. It is suggested that the rapidly dried seeds of *B. lanzan* maintained higher percentage of viability by not allowing the peroxidation of lipids and production of FFA. The lipid peroxidation has been shown to be a prime cause of membrane perturbations leading to loss of viability during desiccation-induced damage or accelerated ageing in seeds (McKersie *et al*. 1990). The peroxidized products of lipids and FFA are cytotoxic by nature (Varghese and Naithani 2002).

The germination pattern was slightly different in *G. arborea* seeds, when seeds with endocarp were compared to excised seeds, i.e. those without endocarp (Table 5). Initially the hydrated seeds (27.3 % MC) showed 60 % germination, which was enhanced to 100 % when the moisture content was reduced to 3-4 %. In contrast, the excised seeds extracted from hydrated seeds (27.3 % MC) germinated 100 % and maintained such viability when dried to 3 % MC. High levels of total phenols estimated in the funicular tissue attached within the cavity of stone or endocarp of hydrated seeds have been found to inhibit the germination of hydrated seeds (Table 6). Nearly 38 % inhibition in germination was recorded when excised seeds were treated with phenols extracted from funicular tissue of hydrated seeds. The phenolic compounds are known as potent inhibitors of germination and are well documented for their role in inducing dormancy in seeds (Bewley and Black 1994).

The seeds of all the four species with >15 % MC were extremely sensitive to low temperatures and were killed when exposed to -20 and 0 °C during storage (Fig. 1 and table 8). The failure of high moisture content seeds to survive freezing temperatures is in close agreement with the results obtained for both desiccation sensitive (Wesley-Smith *et al*. 1992) and desiccation tolerant (Hong and Ellis 1992) seeds. Seeds of *D. melanoxylon*, *G. arborea* and *B. lanzan* tolerated both desiccation and low temperatures (-20, 0 °C) when dried below 11 % MC (Fig. 1). *B. lanzan* and *G. arborea* seeds may be classified as intermediate as gradual loss of viability was distinct after 180 and 90 days of storage respectively, at all storage temperatures. Further experiments are necessary to define the intermediate nature of these seeds as the definition for desiccation-sensitivity remains elusive with ill defined boundaries. *D. melanoxylon* seeds also survived for 250 days with 85-95 % when dried to 6.9 and 4.5 % MC. These seeds are also not strictly following the orthodox storage physiology *per se*. It was apparent that although these seeds demonstrated strong desiccation tolerance

when rapidly dried to sufficiently low moisture contents, like orthodox seeds, they were not storable at freezing or other temperatures (15 and 25 °C) without loss of viability within a short period of (90-180 days) storage. Dried seeds of *G. arborea*, for example, were desiccation and chilling tolerant, but showed extreme sensitivity during cryo-storage for 24 hrs (data not shown). *M. indica* seeds also showed best survival of 25 % at 43.6 % MC, but further desiccation resulted in reduction of viability and longevity, as other desiccation-sensitive seeds that are damaged if desiccated below a critical moisture content (Chaitanya and Naithani 1994; Chaitanya and Naithani 1998; Chaitanya *et al*. 2000).

Conclusions

Buchanania lanzan and *Diospyros melanoxylon* seeds were desiccation and chilling temperature tolerant, and showed no significant loss of viability when stored for 250 days. Thus these seeds can be classified as having orthodox storage behaviour. Rapid drying of *B. lanzan* seeds with silica gel before storage can be recommended. Seeds of *Gmelina arborea* were both desiccation and low temperature tolerant at least up to 150 days, although they were extremely sensitive to cryo-temperature, indicating that long-term storage may be difficult. Because total phenols inhibited germination of viable *G. arborea* seeds, reduced germination in the nursery beds can be avoided by using excised seeds. *Madhuca indica* seeds were both desiccation and chilling temperature sensitive. The critical moisture content for *M. indica* seeds was 43.6 %. The seeds demonstrate true recalcitrant storage behaviour.

Achievements of the DFSC/IPGRI Project

The successful completion of the first year of the DFSC/IPGRI project paved the way for another research project on ‘Seed technology of Forest tree: Postharvest handling and ex-situ storage’. The project goal is to generate a DATABASE for *ex-situ* conservation for large number (nearly 25) of socio-economically important forest tree species, using the protocol developed by DFSC/IPGRI (1999, 2000), which are so far not commercially exploited due to the lack of sufficient knowledge on their handling and storage. The Ministry of Environment and Forest, Delhi,

India funded this project, in 2001. Data obtained on storage of *Madhuca indica* has already been published (Varghese *et al.* 2002). Two other papers ('Seed storage behaviour in *Buchanania lanzan* Spreng.' and 'Seed storage behaviour in *Gmelia arborea* Roxb.') are under preparation.

The Indian partners benefited from working in the DFSC/IPGRI project and the use of a systematically well-developed scientific protocol. This allowed the evaluation of storage physiology of four important tree species of tropical forest of India. Secondly attending the workshop organized by DFSC/IPGRI in 2001 at ASEAN Tree Seed Centre was an opportunity to learn and understand various difficulties faced in the execution of the protocol. The application of unified protocol by various partners associated in the project offered a unique platform forcomparing the data produced in different parts of the world. Further, four research scholars were trained in the execution of the protocol during 2000-2002.

References

Berjak, P., Farrant, J.M. and N.W. Pammenter. 1989. Homoiohydrous (recalcitrant) seeds : the enigma of their desiccation-sensitivity and the state of water in axes of *Landolphia kirkii* Dyer. Planta 186 : 249-261.

Bewley, J.D. and M. Black. 1994. Seeds: Physiology of Development and Germination. Plenum Press, New York, USA, 210 p.

Bhanja, M. 2000. Polyembryony in *Madhuca indica* J.F. Gmel. (Sapotaceae). The Indian Forester 126: 91-92.

Chaitanya, K.S.K. and S.C. Naithani. 1998. Kinetin-mediated prolongation of viability in recalcitrant sal (Shorea robusta Gaertn.f.) seeds at low temperature : Role of kinetin in delaying membrane deterioration during desiccation-induced injury. Journal of Plant Growth Regulation 17: 63-69.

Chaitanya K.S.K., Keshavkant, S. and S.C. Naithani. 2000. Changes in total protein and protease activity in dehydrating recalcitrant sal (*Shorea robusta*) seeds. Silva Fennica 34: 71-77.

DFSC/IPGRI. 1999. Danida Forest Seed Centre Newsletter : The Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds, Humlebaek, Denmark 5: 23-40.

DFSC/IPGRI. 2000. Danida Forest Seed Centre Newsletter : The Project on Handling and Storage of Recalcitrant and Intermediate Tropical Forest Tree Seeds, Humlebaek, Denmark 7: 23-26.

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

Finch-Savage W.E. 1992. Embryo water status and survival in the recalcitrant species Quercus robur L. : Evidence for a critical moisture content. Journal of Experimental Botany 43 : 663-669.

Gunn, S. 1991. Banking on the future. Kew Spring. United Kingdom. pp. 16-21.

Hocking, D. 1993. Trees for Dryland. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp. 238-241.

Hong, T.D. and R.H. Ellis. 1992. The survival of germinating orthodox seeds after desiccation and hermetic storage. Journal of Experimental Botany 43 : 239 - 247.

ISTA. 1985. Determination of moisture content. Seed Science & Technology 13 : 338-341.

Itaya, K. and M. Ui. 1965. Colorimetric determination of free fatty acids in biological fluids. Journal of Lipid Research 6 : 16-20.

Jain, S.K.. 1968. Medicinal plants. National Book Trust, New Delhi, India.

Ministry of Environment and Forests (MoEF). 2000. National Biodiversity Strategy and Action Plan India : Guidelines and Concept Papers., Ministry of Environment and Forests, New Delhi, India

Murty, A.V.S. and N.S. Subramanyan. 1989. A Text Book of Botany. Wiley Eastern Ltd. New Delhi, India.

Pammenter, N.W., Vertucci, C.W. and P. Berjak. 1991. Homoiohydrous (recalcitrant) seeds : Dehydration the state of water and viability characteristics in Landolphia kirki Plant Physiology 96 : 1093 - 1098.

Prakash, R. 1991. *Madhuca longifolia* In : Propagation Practices of Important Indian Trees. International Book Distributors, Dehradun, India.

Raheja, R.S., Kaur, C. Singh, A. and I. Bhatia. 1973. New colorimetric method for the quantitative estimation of phospholipids without acid digesion. Journal of Lipid Research 14: 695-697.

Report of Committee of Forests and Tribals of India, Tribal Development Division, M.H.A., Govt. of India, New Delhi, India.

Sastry, T.C.S. and K.Y. Kavathekar. 1994. *Madhuca* In : Plants for Reclamation of Wastelands. CSIR, New Delhi, India, pp. 300-304.

Swain, T. and W.E. Hills. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. Journal of Science Food and Agriculture 10: 63-68.

Tewari, D.N. 1981. State Trading of Forest Produce in India. Jugal Kishore & Co., Dehradun, India.

Varghese, B and S.C. Naithani. 2001. Desiccation stress in neem seeds : Physiological and biochemical considerations. Danida Forest Seed Centre Newsletter 8: 16-19.

Varghese, B. and S.C. Naithani. 2002. Desiccation-induced changes in lipid peroxidation, superoxide level and antioxidant enzymes capacity in neem (*Azadirachta indica*A. Juss) seeds. Acta Physiologiae Plantarum 24: 79-87.

Varghese, B., Naithani, R., Dulloo, M.E. and S.C. Naithani. 2002. Seed storage behaviour in *Madhuca indica* J.F. Gmel. Seed Science and Technolgy 30:107-117

Wesley-Smith, J., Vertucci, C.W., Berjak, P., Pammenter, N.W. and J. Crane. 1992. Cryopreservation of desiccation-sensitive axes of Camellia sinensis in relation to dehydration, freezing rate and thermal properties of tissue water. Journal of Plant Physiology 140 : 596 – 604.

India's environmental science and conservation news.Â The India State of Forest Report 2019 released recently shows an increase of 5,188 square kilometres of forest and tree cover across the country compared to the ISFR 2017. However, the report highlights that northeast India continues to lose forests when compared to ISFR 2017 and previous reports.Â While the overall forest and tree cover marked an increase on a national level, the report highlighted a decrease in the forest area in the countryâ€™s northeast region. This decline in forest area in the northeast has been an ongoing trend with the region witnessing a loss of about 3,199 sq. km. of forest area sine 2009. ISFR 2019 is a biennial report published by the Forest Survey of India (FSI) and is the 16th such report published. To assess the tropical forest productivity, phenology, and turnover of biomass, litterfall collection is a standard non-destructive technique (Newbould, 1967 ; Lowman, 1988). The amount of leaf material falling reflects a forest's productivity and represents a major flux of carbon from vegetation to soil in the forest.Â In the recent past, approaches such as the ex situ conservation , in situ conservation , creating biosphere reserves, protected areas, etc. have been extended to address the conservation and restoration of tropical forest resources (Shands, 1991 ; Uma Shannker et al., 2001b , c ; Nageswara Rao et al., 2007 , 2011). In particular tropical dry evergreen forests of southern India.Â A 24% increment occurred in the number of families during this period by an addition of four families (Table 1). Overall, tree density increased just by eight individuals. The basal area of stems decreased from 37.7 to 34.5 m2 ha 1 during the 10-year interval (Ta- ble 1). This was largely because of the mortality of the big trees.Â Plant biodiversity inventory and conservation of two tropical dry evergreen forests on the Coromandel coast, south India. Biodiversity and Conser- vation 6: 1063â€“1083. Parthasarathy N. and Sethi P. 1997. Tree and liana species diversity and population structure in a tropical dry evergreen forest in south India. Tropical Ecology 38: 19â€“30. Pennington R.T., Prado D.A. and Pendry C. 2000. The tropical forests of north-east India harbour a high diversity of little-studied terrestrial murid and hystricid rodents. We examined the role played by these rodents in determining the seed fates of tropical evergreen tree species in a forest site in north-east India.Â Seeds of all tree species were handled by at least one rodent taxon. Overall rates of seed removal (44.5%) were much higher than direct on-site seed predation (9.9%), but seed-handling behavior differed between the terrestrial rodent groups: two species of murid rodents removed and cached seeds, and two species of porcupines were on-site seed predators.Â Affiliation Eastern Himalaya Programme, Nature Conservation Foundation, Mysore, Karnataka, India. â¯. *Tropical rainforests have a lot of biological diversity due to their climate being very appropriate for a wide variety of species. *Factors that Decrease Biodiversity.Â Selective cutting: Intermediate-aged or mature trees in an uneven-aged forest are cut singly or in small groups. Selective cutting reduces crowding, encourages growth of younger trees, maintains an uneven-aged stand of tress of different species, and allows natural regeneration from surrounding trees.Â Seed-tree cutting: When loggers harvest nearly all of a stand's trees in one cutting but leave a few uniformly distributed seed-producing trees to regenerate the stand. Clear-cutting: Removes all trees from an area in a single cutting.