



## POST HARVEST STORAGE PHYSIOLOGY OF *GMELENA ARBOREA* ROXB. SEEDS

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### SUMMARY

Germination and storability of undried and dried (slow and rapid) seeds of *Gmelina arborea* were evaluated at various storage temperatures. The freshly mature seeds (IMC 27.3±1.58%) showed 100% germination (excised seeds). Loss of germination was discernible if seeds were slow dried below 11.3±0.4% mc. In contrast rapid drying safely allowed dehydrating the seeds up to 3.4±0.14%mc with still exhibiting 100% germination. The seed viability was reduced to minimal (5%) when slow dried for a period of 350 days to 5.6±0.6%mc. Storage experiments conducted with undried (27.3±1.58%) and rapidly dried seeds (moisture contents: 18.5±0.5, 12.4±1.5, 5.9±0.6, 3.9±0.1, 1.9±0.2%) at range of temperatures (-196, -20, 0, 15 and 25°C) exhibited maximum survival (72-95%) after rapid drying to 3.9%mc at -20, 0 and 15°C after 270 days of storage. Undried and dried seeds failed to survive at cryostorage. *G. arborea* seeds when rapidly dried are desiccation and chilling tolerant albeit to limited period but are extremely sensitive to LN<sub>2</sub> temperature. High phenolic content in the funicular tissue of undried seeds inhibited germination. It is suggested that the functional unit of seed (that is commonly employed for sowing purpose in case of *G. arborea*) be rapidly dried to 7.1%mc in order to nullify the inhibitory effect of phenolics and enhance the germination per cent.

**Key words:** Germination, *Gmelina arborea*, post harvest storage, rapid drying

### INTRODUCTION

*Gmelina arborea* Roxb. a member of the Verbenaceae family, is indigenous to Southeast Asia, and originated in India, where it is commonly known as Gamahar or Khamar. In English it is known by several common names including the paper tree, snapdragon tree and white teak. It is also cultivated in many tropical countries in Africa, south and Central America. It is a moderate to large deciduous tree (30m high and 1.2-4.5m in girth) with a clear bole of 9-15m. It is found throughout the greater part of India, and is best developed in moist, fertile, well-drained valleys in moist

deciduous forests (Naithani *et al.* 2004). *G. arborea* is economically important species in many countries such as U.S., Japan, Thailand, Taiwan and India. The timber is extensively used for making furniture, musical instruments, paper, plywood, shipbuilding, and matchsticks. In India, tribals and villagers use the extract of bark, root, fruit, flowers and leaves of *G. arborea* in curing blood diseases, ulcers and fever (Duke 1983). In recent years, the cultivation of *Gmelina* is being used more and more in forest plantations in many countries as it is a fast growing species capable of producing a crop in just 5 to 6 years rotation, under intensive irrigation and use of fertilizers.

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*G. arborea* can easily be propagated by direct sowing, planting out nursery raised seedlings or stump plantation. However, the availability of seeds for raising adequate quantities of seedlings for large-scale plantation programmes is a major limitation. The storage of seeds from harvest to propagation is problematic as seeds lose their viability over a short period of one year (Prakash 1991). Little is known about the seed 'biology', handling and storage behaviour of these seeds. Studies on storage conditions for maintenance of initial or high viability are highly desirable. The knowledge of storage physiology and germination behaviour is beneficial for their short-term storage, transport for plantation purposes, breeding programs and also for long-term conservation.

The aim of the present study was to unravel the storage behaviour of *G. arborea* seeds by identifying the relationship of seed viability with seed moisture content and temperature during storage. Preliminary experiments carried out on germination of *G. arborea* seed and excised-seed showed variation due to the presence of phenolics present in the funicular tissue of stony endocarp. Therefore phenolic content and its inhibition on the germination of seed and excised-seed were evaluated.

## MATERIALS AND METHODS

Mature and ripe yellow fruits of *G. arborea* were plucked from 50 plus trees growing in village Kusumi, Durg, Chhattisgarh, India during May 2004. The collected fruits were transported to the laboratory in jute bags within 5 hours of its collection. Infected, mechanically damaged and off-sized fruits were removed from the harvested lot. To determine the Initial Moisture Content (IMC), one hundred seeds (5 replicates of 20 seeds each) extracted from fruits by manually removing the pulp were used (Varghese and Naithani 2000). Traces of pulp adhering on the seeds were removed forcibly by rubbing the seeds gently with sand and then washing thoroughly under running water. These seeds were gently surface dried using blotting papers. Hundred individual seeds were then weighed to determine the average weight of seeds (DFSC/IPGRI, 1999). The seed size including length and breadth was measured for fifty individual seeds. The seeds were soaked in 1% sodium hypochlorite solution for 10 minutes and then rinsed and blotted dry. They

were coated with 1 g Thiram per kg seed material so as to prevent any fungal attack and kept under lab conditions ( $25\pm 2^{\circ}\text{C}$  and  $38\pm 3\%$  RH) until further use. The seed samples were ready for various analyses within 10 hours of their harvest from the trees.

*Germination:* Seeds were surface sterilized with 1% sodium hypochlorite solution for 15 minutes, thoroughly washed in distilled water (DW) 5 times and germinated in dark ( $25\pm 2^{\circ}\text{C}$ ) in moist vermiculite. Germination was scored after every 24 hours as the radicle emerged to 5 mm (Varghese and Naithani 2000). Germination test was terminated when seeds did not show germination for a week or the seeds blackened and/or showed fungal manifestations. Germination tests were performed with 5 replicates of 30 seeds each. All experiments related to germination test during desiccation and storage trials were performed employing "excised-seeds" (without endocarp) unless otherwise mentioned. Excised-seeds were surface sterilized with  $\text{HgCl}_2$  (0.1%) for 2 minutes, thoroughly washed with DW 4-5 times, allowed to imbibe DW and germinated in dark ( $26-28^{\circ}\text{C}$ ) on DW saturated filter paper towels in petri dishes (Chaitanya and Naithani 1994). Germination was scored after every 24 h as the radicle emerged to 5mm.

*Moisture content:* Seed moisture content (mc) was determined by hot air oven method following International Seed Testing Association (ISTA) (1985). Five replicates of 10 seeds each were used for the test. Seeds, each cut out into 4 pieces, were weighed before and after drying at  $103^{\circ}\text{C}$  for 17 hours and mc was calculated as percentages water of fresh weight. Similar methodology was undertaken to determine the mc of the whole fruit, embryo and the endocarp.

*Desiccation trials:* For natural drying (slow drying), the seeds were kept in perforated plastic trays at ambient conditions ( $25\pm 2^{\circ}\text{C}$  and  $38\pm 3\%$  RH). Rapid drying was done using silica gel following the DFSC/IPGRI protocol (1999). The seeds were desiccated by mixing equal amount (w/w) of fresh blue coloured silica gel in glass desiccators at ambient conditions ( $25\pm 2^{\circ}\text{C}$ ). Separate desiccators were used for seeds with six different target moisture contents. The silica gel was changed (as the blue colour started fading) as and when required in all six containers at the same time. Moreover, the seeds

were mixed at least twice daily for proper aeration and avoiding anoxia. A batch of 25 fresh seeds was weighed and corresponded with initial moisture content estimated for fresh seeds. The corresponding target weight, for each target moisture content, was calculated by using the following formula:

$$\text{Weight of seeds (g) at TMC} = \frac{(100 - \text{IMC})}{(100 - \text{TMC})} \times \text{Initial seed weight}$$

where, TMC is the target moisture content and IMC is the initial moisture content.

The water loss in the desiccating seeds was monitored by weighing the seeds in electronic balance (0.1 mg least count) and simultaneously recording the duration of drying. The target weight for the seed batch from each of the containers was calculated and when the target weight was attained, a sample of seeds from the particular desiccator was used for the determination of the germination and moisture content.

*Storage trials:* The extracted seeds were desiccated to range of moisture contents (18.5, 12.4, 5.9, 3.9 and 1.9% mc) over silica gel and used for the storage trials. The seeds with different moisture regimes were then stored at four different temperatures, viz. 25°C, 15°C, 0°C and -20°C. The seeds were retrieved at 5, 20, 60, 90, 150, 200 and 270 days to determine the survival (germination %) and seed moisture content.

*Cryopreservation trials:* The freshly harvested seeds at initial moisture content and those desiccated to different moisture contents were tested for storage in liquid nitrogen temperature (-196°C). The seeds were plunged into liquid nitrogen containers (IBP cryocan, BA35) after being enclosed in polypropylene cryovials. These seeds were retrieved after 24 h and then immediately thawed by rapidly immersing the seeds in a water bath maintained at 37–38°C. Recording the percentage germination using the method previously described viability of thawed seeds was tested (Varghese and Naithani 1999).

#### *Total free phenols*

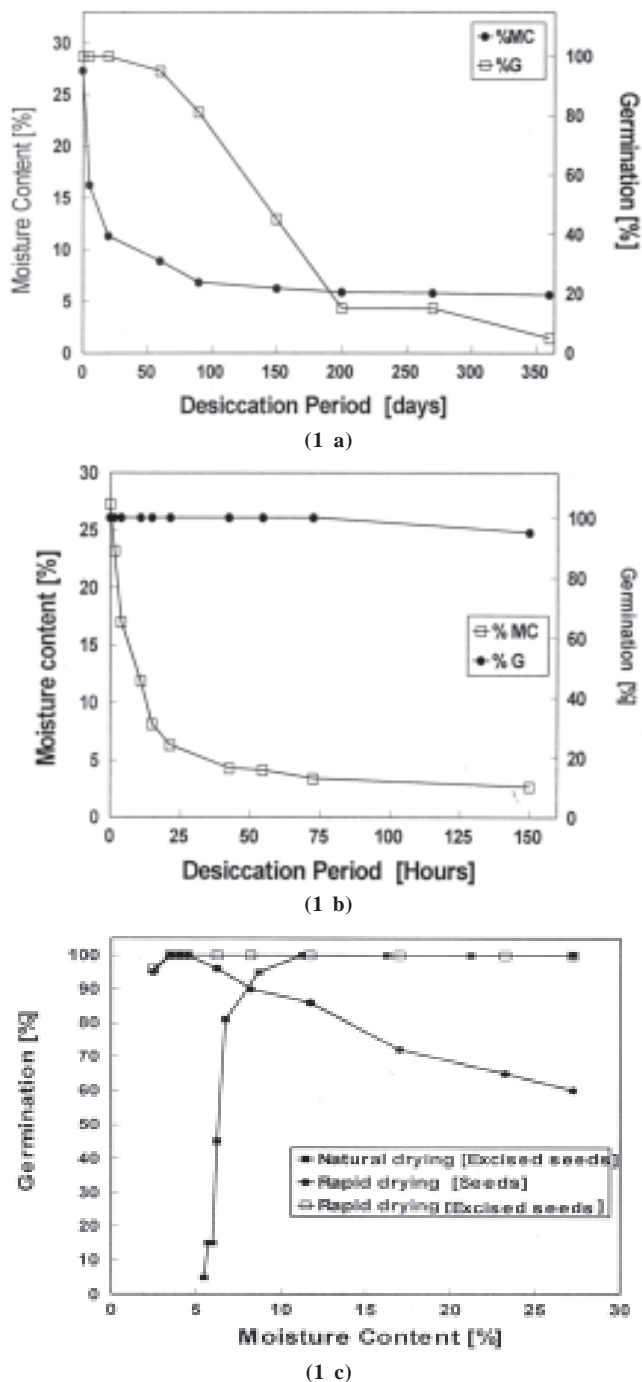
Total free phenol content was estimated by the method of Swain and Hills (1959). Funicular tissue

(500mg) of *G. arborea* was homogenized in extraction buffer (80% ethyl alcohol in 0.2M borate buffer, pH 7.6) with silica. The homogenate was centrifuged at 8000g for 5min and the supernatant was collected. The residue was washed twice with extraction buffer. Finally, all these ethanolic supernatants were mixed and evaporated in vacuum at 4°C to yield concentrated aqueous layers. This aqueous layer was used as the source of phenolic compounds. Standard curve was prepared using different concentrations of chlorogenic acids.

Effect of total free phenol on germination was studied by treating excised-seeds with phenolics extracted from funicular tissue of undried and dried seeds. Weighed (1gm) funicular tissue was extracted overnight with chilled 80% alcohol at 5°C and washed twice. All the alcoholic extract was pooled together and vacuum evaporated in dark at 25°C to yield concentrated water layer (semi-dry). The semi-dry residue was dissolved in 20ml of distilled water and was used as a phenolic extract in germination studies. The germination test using excised-seeds (extracted from hydrated seeds) was performed using 5ml of phenolic extract or 5ml of distilled water (control) in Petri plates.

## RESULTS AND DISCUSSION

Mature seeds of *G. arborea* shed at high moisture content of 27.82% (Table 1), were relatively desiccation tolerant if rapidly dried, as compared to the naturally dried seeds (Fig. 1a & 1b). Absolute germination (100%) was observed in seeds dried rapidly to even as low as 3.4%mc (Fig. 1b & 1c). Further reduction in moisture content (2.7%) resulted in slight loss of viability (95% viability), probably due to the removal of bound-water essential for maintaining integrity of cellular structures (Dasgupta *et al.* 1982). Damage occurring at such low water contents is defined as desiccation damage, *sensu stricto* and coincides with the perturbation of the non-freezable water. In contrast, the seeds when dried at a slow rate (naturally dried), showed loss of viability at relatively higher moisture contents i.e., below 11.3%mc (Fig. 1b & 1c). Significant drop in germination from 81 to 45% was observed in these seeds when dried from 6.8 to 6.2%mc at a slow rate. Seeds of *Azadirachta indica* (Varghese and Naithani 2000), coffee (Ellis *et al.* 1990, 1991a), papaya (Ellis *et al.* 1991b) and *Elaeis*



**Fig. 1.** The decline in germination (%) and moisture content (%) during storage of *Gmelina arborea* seeds (natural drying) (Fig. 1a) and silica gel drying (rapid drying) (Fig. 1b) at ambient conditions ( $25\pm 2^\circ\text{C}$  and  $38\pm 3\%$  RH). Germination test was performed using excised seeds. Each value of germination is a mean of 50 observations. Fig. 1c exhibits comparison between germination (%) of naturally dried and silica gel dried seeds as a function of moisture content.

**Table 1.** Some physical characteristics of fruits and seeds of *Gmelina arborea*

Fruit weight	$09.76 \pm 2.52\text{g}$
Seed weight	$0.97 \pm 0.17\text{g}$
Seed size (length/thickness)	$1.69\pm 0.19\text{cm}/1.14\pm 0.14\text{cm}$
Seed shape	ovoid
Initial seed MC (manually extracted)	$27.82 \pm 1.76$
Seed MC after processing (whole seed)	$26.07 \pm 2.33$
MC of whole fruit	$75.03\pm 1.0\%$
MC of seed coat (Stony endocarp)	$27.87\pm 1.29\%$
MC of excised seed (without endocarp)	$24.45 \pm 1.82\%$
MC of cotyledons	$18.48 \pm 2.97\%$
MC of axis	$28.79\pm 0.81\%$
MC of seed coat (papery)	$11.77\pm 1.67\%$

*guineensis* (Ellis *et al.* 1991c) etc., which exhibit intermediate storage physiology, have been reported to survive 9-13% moisture content and showed substantial loss of germination when desiccated below this water contents. Generally, these are non-orthodox seeds that are metabolically active on shedding from the mother plant and demonstrate initiation of germination related events well before they are shed (Farrant *et al.* 1986). Natural drying of such seeds render them sufficient time to undergo substantial germination-associated changes before the dehydration level becomes limiting (Farrant *et al.* 1986, Berjak *et al.* 1993). Therefore, the slow dried seeds withstand less water loss before losing viability than the rapidly dried seeds per se (Berjak *et al.* 1993, Farrant *et al.* 1993). Pammenter *et al.* (1991) suggested that rapid drying of seeds permit survival to lower water contents because moisture is removed rapidly enough to prevent aqueous based deterioration. Our data clearly indicates that the rate of removal of water in natural-drying seeds is extremely slow compared to silica gel dried seeds (Fig. 1a & 1b). For example *G. arborea* seeds dehydrated quickly from 27.3 to 4.3%mc in 42 hrs and to 2.6% in 150 hrs over silica gel (Fig. 1b), whereas,

it took 360 days by natural-drying to reduce the moisture content to 5.6%. The slow drying of seeds to 5.8% in 200 days and 5.59%mc after 360 days showed 15 and 5% germination respectively. On the contrary, the seeds desiccated by rapid-drying to as low as 3.4%mc exhibited 100% viability. The results indicate that *G. arborea* seeds may be categorized as intermediate.

The germination pattern was slightly different in seeds with endocarp compared to excised-seeds without endocarp (Fig. 1c). The endocarped seeds at 27.3%mc showed 60% germination that was enhanced to 100% when the moisture content reduced to 4.3%mc. In contrast, the excised seeds extracted from mature seeds germinated 100% and maintained such viability when desiccated up to 3.4%mc. High levels of free phenols estimated in the funicular tissue attached within the cavity of endocarp of freshly harvested seeds have been found to inhibit the germination of relatively hydrated seeds. Nearly 38% inhibition in germination was recorded when excised seeds were treated with alcoholic extract (containing free phenols) of funicular tissue of hydrated seeds (Table 2). The free phenolic content in the funicular tissue was initially 7.2mg/g fresh weight (fw) (Table 2). It reduced gradually to 6.18, 1.9 and 0.08mg/

g fw as the seeds desiccated to 15.5, 7.1 and 4%mc respectively. The phenolic compounds are considered potent inhibitors of germination and are well documented for their role in inducing dormancy in seeds (Bewley and Black 1994). Inhibitory effect of phenols on germination can be easily avoided by either using the excised-seeds or drying the seeds at least to 7.1%mc before sowing in the nursery beds; thus enhancing the germination.

The storage trials of undried and dried seeds of *G. arborea* at various temperatures further confirmed the intermediate storage physiology of these seeds. The best survival (72-95%) was obtained when dried seeds ( $4.12 \pm 0.1\%$ mc) were stored at  $-20$ ,  $0$  and  $15^\circ\text{C}$  for 270 days (Fig. 2e). The undried seeds were killed when exposed to freezing ( $0^\circ\text{C}$ ) and sub-freezing ( $-20^\circ\text{C}$ ) temperatures within 5 days of storage (Fig. 2a). The failure of high moisture content seeds to survive sub-freezing temperature reported here is in agreement with the results obtained for desiccation-sensitive (Wesley-Smith *et al.* 1992) and desiccation-tolerant (Hong and Ellis 1992) seeds. The seeds showed increased tolerance to freezing and sub-freezing temperatures ( $-20$  &  $0^\circ\text{C}$ ) when dried below 12.4% mc (Fig. 2c-2f). Drying of seeds to 4–6 % mc showed higher viability with longer

**Table 2.** Total free phenols from funicular tissue and its effect on germination of excised seeds of *Gmelina arborea* and survival of *Gmelina arborea* seeds after 24 hours of cryo storage

MC of whole seeds (%)	Total free phenols (mg phenol/g fw)	% Germination of excised seeds treated with free phenol extract	Cryostorage of excised seeds for 24 hrs.	
			Control	Cryostored
34.3±1.23	—	—	100	0
28.0±0.9	7.2±0.82	62	—	—
22.8±1.6	—	—	100	0
16.4±0.8	—	100	—	—
15.5±0.9	6.2±0.63	78	—	—
10.0±1.1	—	—	100	0
7.1±0.6	1.9±0.29	100	—	—
5.5±0.9	—	—	100	0
4.0±0.2	0.08±0.03	100	—	—
2.4±0.4	—	—	80	0

SEED STORAGE OF *GMELINA ARBOREA*

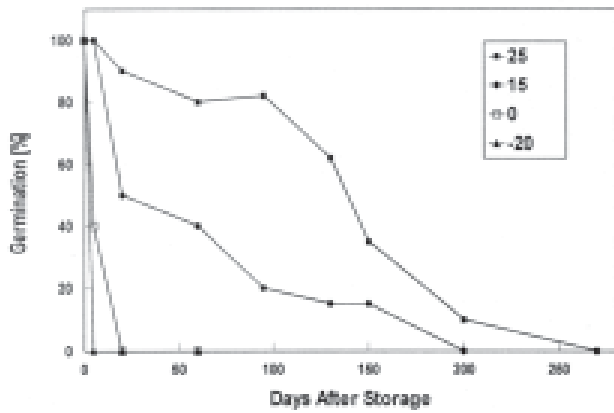


Fig. 2a

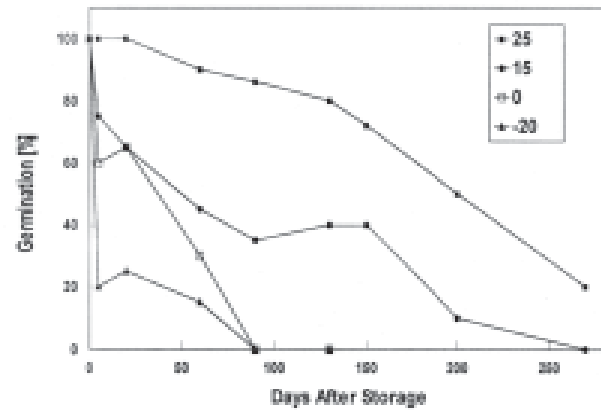


Fig. 2b

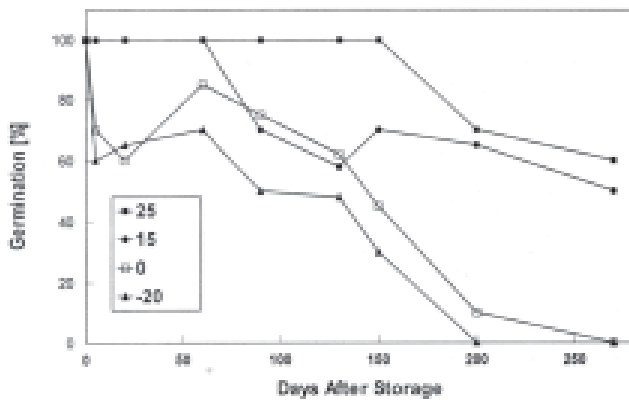


Fig. 2c

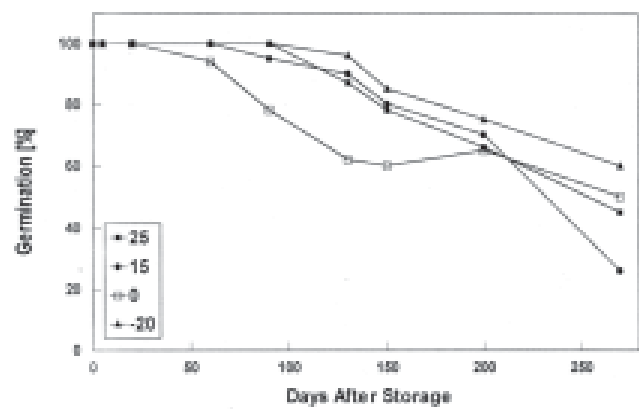


Fig. 2d

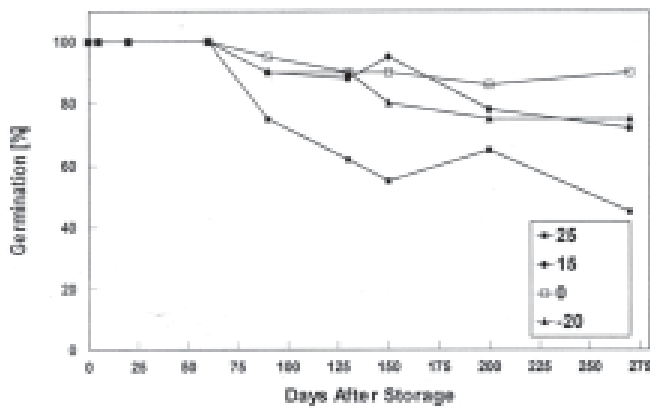


Fig. 2e

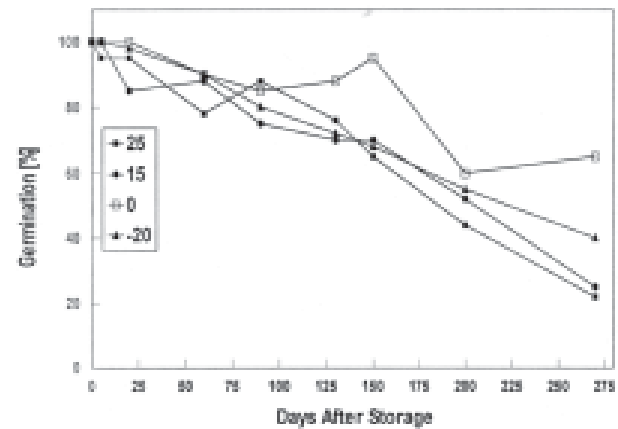


Fig. 2f

Fig. 2. Effect of storage temperature on germination (%) of undried (26.0%MC) (Fig. 2a), and rapidly dried (by silica gel) seeds to moisture contents 18.5% (Fig. 2b), 12.4% (Fig. 2c), 5.9% (Fig. 2d), 4.12% (Fig. 2e) and 3.9% (Fig. 2f) at -20, 0, 15 and 25°C. Germination test was performed using excised seeds. Each value of germination represents mean of 50 observations (Fig. 2a & 2c), 40 observations (Fig. 2b & 2f) and 35 observations (Fig. 2d & 2e).

period (270-280 days) of storability at freezing and sub-freezing temperatures (Fig. 2d & 2e).

Thus, *G. arborea* seeds may be classified as intermediate, as gradual loss of viability was detected during natural drying below 11.3%mc. The loss of viability was also evident in rapidly dried seeds (although considered relatively desiccation tolerant), at all storage temperatures, after 90 days of storage. However, substantial enhancement in desiccation and chilling tolerance attributes after rapid drying of these seeds, places it closer to orthodox storage behaviour as well. Our results corroborate the “concept of continuum” proposed by Berjak and Pammenter (1997), which states that continuum grades from extreme desiccation sensitivity through the minimally recalcitrant types to intermediate seed species that are chilling sensitive, to orthodox seeds that can tolerate extreme desiccation and ultra low temperatures.

Despite desiccation and chilling tolerance, though for limited period, *G. arborea* seeds were extremely sensitive to cryostorage (Table 2). The seeds of various moisture contents became non-viable when stored in LN2 for 24 hrs. The intolerance of desiccation and chilling tolerant *G. arborea* seeds to cryo temperature remains to be explained and is indicative of yet another unexplored aspect of intermediate/non-orthodox storage physiology.

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## SEED STORAGE OF *GMELINA ARBOREA*

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