NOTES

EFFECTS OF DESICCATION AND TEMPERATURE ON THE STORAGE OF *AEGLE MARMELOS* SEEDS

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Aegle marmelos is a deciduous tree of the family Rutaceae, distributed in South-East Asia and tropical Africa. In India, this aromatic species is considered sacred as the leaves are being used in many religious rituals. Their edible fruit, root, bark and seed are of value in indigenous system of medicine (Anonymous 1948). Due to unsustainable harvesting, this species has become an endangered medicinal plant (Vibha Rao & Rajasekharan 2002). Propagation is mainly through seeds, although root cuttings and layering are also feasible (Singh & Roy 1984). Clonal multiplication from axillary meristem culture was reported by Ajith Kumar and Seeni (1998). Arumugam and Rao (2002) reported somatic embryogenesis, synseed encapsulation and germination techniques in *A. marmelos.*

Cryostorage of seed was initially developed for preservation of genetic resources of agriculturally important species (Ross & Stanwood 1981, Stanford 1985, Vertussi 1989), while several recent studies have demonstrated the potential of cryostorage for conservation of wild species (Pence 1991, Touchell & Dixon 1994, Gonzalez-Benito *et al.* 1995, Merritt *et al.* 2005).

Considering the economic value of this species and its threatened state, detailed studies on germination response of seeds to desiccation, temperature and storage under cryostorage were undertaken.

Fully ripened fruits of *A. marmelos* were hand harvested from eight selected trees growing at the Tropical Botanic Garden and Research Institute, at an altitude of 150 msl amongst the foothills of the Southern Western Ghats (latitude: 8° 45' and 8° 47' N; longitude: 77° 1' and 77° 4' E).

Fruiting of *A. marmelos* is from September till December and each tree bears ovoid to subglobose berries of woody hesperidium. Embedded in thick orange mucilaginous sweet pulp, the thousand exalbuminous seeds weigh 94 ± 4 g. Seeds were scooped out and de-pulped with sawdust. They were then washed with running tap water and 76.7% of the submerged sound seeds were collected. After rinsing in distilled water, the seeds were surface dried by exposure in the laboratory (28 ± 2 °C/65% RH).

To understand the pattern of desiccation as a viability-limiting factor, seeds were desiccated with silica gel in a desiccator (28 ± 2 °C/ 60% RH). Four seedlots, each with different moisture contents ranging from 18.9% (initial moisture content), 13.8%, 6.4% (critical moisture content value) and 4.5% were collected at an interval of 15 days of continuous drying. Moisture content on a fresh weight basis of five replicates of three seedlots at each stage was determined by drying for one hour at 130 °C. Viability of the stored seeds was assessed at pre-planned intervals (Tables 1 and 2).

Seeds kept open at laboratory conditions (28 ± 2 °C/60% RH) served as the control. Viability tests related to the effect of different temperatures (30, 20, 10, 0, –20 °C and in liquid nitrogen (LN), i.e. -196 °C) and moisture contents (18.9, 13.8, 6.3 and 4.47%) were conducted. These seed samples were stored for a week in polycarbonate bottles kept at different temperatures before being tested for germination and other viability tests. For cryostorage, seeds were thoroughly wrapped in aluminium foil, then directly plunged into LN for seven days, followed by rapid thawing in a water bath at 37 °C for 5 min.

To avoid imbibitional injury, dehydrated seeds were slowly hydrated for 24 hours by exposing to humid conditions before being tested for germination. Five replicates of 10 seeds each were placed on filter paper, wetted with distilled water in Petri dishes and kept in a seed germinator maintained at 30 °C / 80% RH in the dark. Simultaneous to each germination test, 10 seeds were selected randomly and sectioned longitudinally imbibing in 1% w/v of 2,3,5-triphenyl tetrazolium chloride solution for 24 hours in the dark for evaluation of the staining pattern (Moore 1985). Along with this, five replicates of 10 seeds each were soaked in 30 ml double distilled water at laboratory conditions for 24 hours and electrical conductivity of seed leachates was measured using a dip cell conductivity meter (Systronics 306). Data were

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Table 1 Effects of different storage temperatures on one-week-old hermetically-sealed fresh and desiccated *Aegle marmelos* seeds

Prestorage moisture content & germination (%)	Storage temperature (°C)	Germination (%)	Germination period (days)	Seed leachate conductance (mS)	Tetrazolium test results
18.9 ± 1.2	28 ± 2^c	92 ± 7.04^c	10.4 ± 0.5^c	0.201 ± 0.02^c	Emb.,cot. deep red
94 ± 5.9	30	92 ± 6.4*	10.8 ± 1.3*	0.200 ± 0.03*	Emb.,cot. deep red
	20	74 ± 15.2	14.4 ± 1.8*	0.305 ± 0.01	Emb., cot red.
	10	88 ± 10.95	12.8 ± 1.9*	0.333 ± 0.01	Emb., cot red.
	0	0	0	0.676 ± 0.95	Emb., cot. pale red.
	-20	0	0	0.662 ± 0.05	Not stained
13.8 ± 0.4	28 ± 2^c	86 ± 11.4^c	11 ± 1^c	0.334 ± 0.02^c	Emb.,cot.deep red.
90 ± 12.2	30	80 ± 14.14	13.6 ± 1.1	0.335 ± 0.06*	Emb.,cot. deep red
	20	78 ± 8.4	14 ± 0.7*	0.392 ± 0.07	Emb.,cot. deep red.
	10	82 ± 8.37	13.8 ± 0.8*	0.341 ± 0.12	Emb.,cot.deep red.
	0	0	0	0.654 ± 0.02	Emb., cot. pale red.
	-20	0	0	0.652 ± 0.02	Not stained.
6.35 ± 0.6	28 ± 2^c	70 ± 7.07^c	13.8 ± 2.2*	0.303 ± 0.01^c	Emb., red, cot. red
72 ± 4.5	30	68 ± 4.47*	12.8 ± 0.8*	0.317 ± 0.01*	Emb.,cot.red.
	20	66 ± 5.48	13.2 ± 1.3*	0.332 ± 0.02*	Emb., cot. red.
	10	66 ± 5.48	16 ± 1.2	0.342 ± 0.02*	Emb., cot. red.
	0	0	0	0.669 ± 0.1	Emb., cot. pale red.
	-20	36 ± 5.5	16.4 ± 0.5	0.612 ± 0.1	Emb., cot. pale red.
4.5 ± 0.2 36 ± 5.5	28 ± 2^c	36 ± 11.4^c	18.2 ± 1.6	0.507 ± 0.2^c	Emb., cot. pale red.

Emb. = embryo, cot. = cotyledons
* = Mean values ± standard error of a column of each moisture content level are not significantly different at p = 0.05 based on Duncan's multiple range test compared with the respective control values with superscript c. Control values were also not significantly different from the respective values before seed storage.

analysed by Analysis of Variance and the mean values were compared using Duncan's multiple range test at the level of p = 0.05.

Seeds hermetically stored at different temperatures with initial moisture content of 18.9% and desiccated to 13.8 and 6.35% recorded gradual reduction in germination percentage up to 10 °C but died at 0 °C which was statistically significant in all treatments except at 30 °C storage with 18.9 and 6.35% moisture levels (Table 1). While at -20 °C, the seeds were viable with 6.35% moisture content but died after seven days of storage at 0 and -20 °C (Table 1). Conductivity values of 0 and -20 °C stored seeds were more than 0.610 mS. Loss of viability was characterized by extended period of germination and reduced staining intensity. Irrespective of the difference in the level of desiccation and range of storage temperatures, all germination tests spread out over a period of 10 till 18 days (Table 1).

Seeds with 6.35% moisture content recorded 70% germination and a further reduction of moisture content to 4.5%, germination was reduced to 36% in seeds stored at room temperature (28 ± 2 °C) (Table 1). Seeds with initial moisture contents of 13.8 and 6.35%, conductivity values were approximately 0.300 mS, while the efflux of electrolyte of seeds with moisture content 4.5% was approximately 0.500 mS (Table 1).

After seven days of cryopreservation, fresh seeds with 18.9% moisture content recorded 18.0% germination, which took an extended period of 19 days (Table 2). Seeds with 13.8 and 6.35% moisture content recorded 80 and 68% germination respectively within 16 days and the conductivity values of cryostored seeds were found to be as low as 0.360 and 0.321 mS respectively (Table 2). Seeds with 4.5% moisture content recorded only 36% germination during storage at laboratory condition and in all other regime the seeds were dead (Table 1).

Normally, *A. marmelos* seeds remain viable up to between six and nine months and have the ability to survive at deciduous habitats. Nevertheless, the survival of this species is at stake due to biotic pressures primarily in the form of excessive harvest. As part of conservation measures initiated with seeds, when the moisture content was reduced from the initial level to one third, 70% viability was maintained which was lost upon further desiccation to 4.5% moisture content.

The electrical conductivity of leachates was found to be almost doubled in non-viable seeds compared with viable seeds and germination percentage and conductivity values were inversely proportional to each other (Table 1). In *Dalbergia sissoo* seeds, Thapliyal and Conner (1997) reported an increase in conductance due to enhanced permeability of seeds undergoing accelerated aging. Fresh as well as

Table 2 Effects of cryopreservation on viability and germination of *Aegle marmelos* seeds after seven days in liquid nitrogen at -196 °C

Pre-storage condition		Cryopreserved seeds			
Moisture content (%)	Germination (%)	Germination (%)	Germination period (days)	Seed leachate conductance (mS)	Tetrazolium test results
18.9 ± 1.2[c]	94 ± 8.9[c]	18 ± 8.4[c]	19.4 ± 1.8[c]	0.682 ± 0.04[c]	Emb., cot. pale red
13.8 ± 0.4	90 ± 12.2*	80 ± 10.0	16.2 ± 1.5	0.360 ± 0.06	Emb., cot. red
6.35 ± 0.6	72 ± 4.5	68 ± 8.4	16 ± 1.2	0.321 ± 0.03	Emb., cot. red
4.5 ± 0.2	36 ± 5.5	NIL		0.689 ± 0.1*	Not stained

Emb. = embryo, cot. = cotyledons
* = Mean values ± standard error of a column of each moisture content level are not significantly different at p = 0.05 based on Duncan's multiple range test compared with the respective control values with superscript c. Control values were also not significantly different from the respective values before seed storage.

desiccated *Aegle* seeds stored at 10 °C and cryostored seeds with 6–13% moisture content maintained almost stable electrical conductivity (0.320–0.360 mS) that indicated possible membrane stability as reported in the case of soybean seeds by Vieira *et al.* (2001). Tetrazolium test is also a reliable indicator of seed viability as in the case of staining pattern of *Myristica malabarica* seeds reported by Anilkumar *et al.* (2002). The staining intensity of *A. marmelos* embryo became pale red with unstained margins when viability was below 60% (Table 1).

Many earlier reports on desiccation injury were in fact due to the subsequent imbibition damage, which can be avoided if the dry seeds are first conditioned to high moisture contents in saturated air prior to germination (Ellis *et al.* 1990). In the present study, germination tests were conducted only after conditioning seeds with moisture content 10% and below in saturated air with 85% RH for 24 hours. Apart from cryostorage, irrespective of moisture content, storage of *A. marmelos* seeds at sub-zero temperatures was found to be detrimental (Table 1). Possibility of seed cryopreservation reported in the case of *Piper nigrum* (Chaudhury & Chandel 1994) and *Citrus suhuiensis* cv. limau langkat (Makeen *et al.* 2005) with narrow hydration range substantiates the need for standardization of desiccation techniques. The optimum moisture content for seed cryopreservation can vary from about 7 to 14%, depending on species and seed oil content (Pritchard 2007). With 9–10% moisture content, papaya seeds survived 24 hours of exposure to LN (Becwar *et al.* 1983, Chin & Krishnapillay 1989). In our experiments with *A. marmelos* seeds, parameters such as adaptation to dry habitats, occurrence of small and flat seeds and their sensitivity to sub-freezing temperatures and sudden desiccation sensitivity below 6% moisture content are agreeable with the intermediate type of storage behavior as described by Ellis *et al.* (1990). Regarding cryostorage, 80% of the *Aegle* seeds dried to 13% moisture content remained viable compared with the 68% of those dried to 6% critical moisture content, which may be substantiated by the report of *Anigozanthos manglesii* seeds by Merritt *et al.* (2005) that if the initial quality of seeds is maintained in due course, it may be advisable to cryostore such seeds with water content close to the maximum range of critical threshold.

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