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Research Note

Germination requirements and storage behavior of *Myristica dactyloides* Gaertn. seeds

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Summary

A series of experiments was conducted to determine the storage behavior and germination requirements of *Myristica dactyloides* seeds. The trials involved desiccation of fresh mature seeds to different mc levels, low temperature tolerance, effects of growth promoters and germination substrates. The results show that germination was significantly reduced upon drying and non-desiccated seeds did not tolerate low temperature storage; indicating that seeds of *M. dactyloides* are recalcitrant. Exogenous application of GA_3 and IBA (1000 ppm for 24 hours) resulted in significantly higher percentage germination than untreated seeds that were failed to germinate at all. This indicates that seeds of *M. dactyloides* are not only recalcitrant but also exhibit dormancy. As a whole, non-desiccated seeds (34% mc) pretreated with IBA maintained 100% viability after three months of storage at 20°C. Percentage germination was significantly higher for seeds sown on germination paper, cotton towel or sponge sheet than other substrates. It can be concluded that the critical moisture content below which *M. dactyloides* seeds should not be dried is 34%. Seeds can be stored at 20°C for three months without deleterious effect on viability. For better germination, exogenous applications of growth promoters prior to sowing and good aeration conditions are recommended.

Experimental and discussion

Myristica dactyloides Gaertn. (Myristicaceae), commonly known as wild nutmeg, is an evergreen tree that occurs in evergreen forests of Western Ghats in Southern India and Sri Lanka (Nair and Nayyar, 1987). It is one of the valuable medicinal plants in this region and plays an important role in the rural economy (Rana and Chatterjee, 2000). Seeds and arils are the most commonly used parts of the plant as traditional medicine for treating intestinal worms and bowel disorders and often as an alternative to *Myristica fragrans* (common nutmeg). Its natural population is declining drastically due to constant harvest for medicine and over-exploitation in trade (Utkarsh and Bhat, 1999).

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Therefore, there is an urgent need for *ex situ* conservation of this species through collection and preservation of seeds in seed banks and simultaneous establishment of new plantation in its natural habitat. Seed banking has gained a wider recognition as a relatively new and under-exploited tool in combating the loss of global plant diversity (van Slageren, 2003). For successful ex-situ conservation, knowledge of the storage behavior of any given species is a prerequisite. Up to now, no documented data exist about the storage behavior of *M. dactyloides* seeds. In addition, the germination performance of *M. dactyloides* seeds under laboratory conditions has been unsatisfactory.

Four experiments were conducted to examine the storage behavior and germination requirements of *M. dactyloides* seeds. These trials were effects of desiccation, low temperature storage, application of growth promoters and germination substrate on viability. Mature fruits were collected from Silent Valley in Kerala and Kolli Hills, Tamil Nadu, India during the peak fruiting season. Seeds were manually extracted from the external pulp. To determine the moisture content of the seed lot, four replicates of six seeds were weighed before and after drying at 103°C for 16 hours. Moisture content was determined immediately upon arrival of seeds to the laboratory.

Seeds were desiccated from 34% initial moisture content to 30%, 20% and 10% mc by sun drying at 30-36°C. The water loss during desiccation was monitored by weighing the seeds at intervals. Fresh non-desiccated seeds (34% mc) were stored at six different temperatures: ambient (25-30°C), 20°C, 15°C, 10°C, 0°C and -5°C in closed containers for two weeks. To further examine the longevity of seeds in storage and enhancement of germination by growth regulators, non-desiccated seeds were hermitically stored at ambient temperature, 20°C, 15°C and 10°C for three months and then soaked in 1000 ppm GA_3 or 1000 ppm Indole butric acid (IBA) solutions for 24 hours prior to sowing while seeds soaked in water for 24 hours served as control. The germination of non-desiccated seeds stored at 20°C for three months was also tested using germination paper, moist vermiculite, mineral soil, sand, sponge sheet and cotton towel. Mineral soil (red soil) was collected from the vicinity of Institute of Forest Genetics and Tree Breeding, Coimbatore, India. Seeds were sown at 0.5 cm depth in sand, mineral soil and vermiculite. Equal amount of water was given for all substrates twice a day to maintain uniformity.

Germination tests were conducted in a germination room maintained at 25 ± 2°C. Three replications of 20 seeds each was used for studying the effects of desiccation and storage temperature on seed germination. For studying the effect of growth regulator and substrate, four replications of 25 seeds each were used. In desiccation, storage temperature and substrate effect studies, seeds were soaked in 1000 ppm GA_3 for 24 hours prior to sowing to overcome dormancy. Germination tests were conducted on cotton towel for all studies except the experiment on substrate effect, as the germination performance was good on this substrate. Germination initiated after 8 days of sowing and the final count was taken on the 30^{th} day. Percentage germination was computed for each trial, arcsine transformed and subjected to One-Way Anova. The significant effects of growth regulators and storage temperature as well as their interaction were determined with Two-Way Anova. Means that exhibited significant differences were compared by Tukey's honestly significant test ($\alpha = 0.05$).

Desiccation of *M. dactyloides* seeds from 34% (initial mc) to 30% and 20% mc significantly reduced germination ($p<0.001$) by 13% and 20%, respectively and seeds

became non-viable when desiccated to 10% moisture content (figure 1). This indicates that seeds of *M. dactyloides* are desiccation-sensitive or recalcitrant. It is a well-established fact that recalcitrant seeds rapidly lose viability upon drying. However, the critical moisture content below which viability is lost varies significantly between species, but is generally in the range between 12 and 31%, (Ellis, 1991; Tompsett, 1992). The loss of viability upon drying is associated with direct physical stress due to loss of water and/or physicochemical damage of tissues as a result of metabolic aberrations during drying (Pammenter *et al.*, 1994; Conner and Sowa, 2003). For *M. dactyloides* seeds, it seems that the critical moisture content is around 34% and drying below this level will have a detrimental effect on viability.

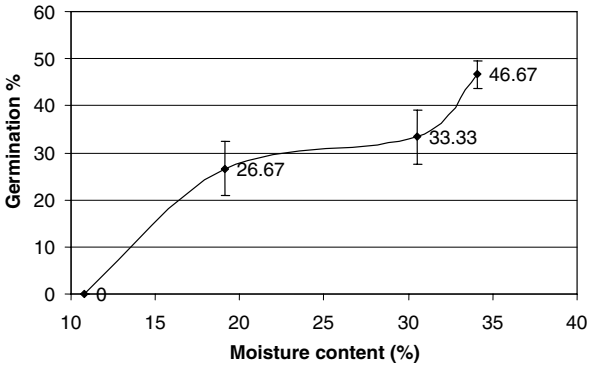

Figure 1. Effect of desiccation on germination of *M. dactyloides* seeds. Values are mean ± SE. Note that seeds were treated with GA_3 prior to sowing.

The germination of non-desiccated seeds stored for two weeks at different temperature regimes varied significantly ($p<0.001$). Seeds stored at 20 or 15°C had a significantly higher germination compared with those stored at ambient or low temperatures (figure 2). Seeds stored at low temperature (0 and -5°C) failed to germinate while viability declined to 20% for seeds stored at ambient temperature. The reduction in viability at freezing and sub-freezing temperatures could be associated with cell damage due to the formation of ice crystal within cells (Bewley and Black, 1994). The comparably low percentage germination of seeds stored at ambient temperature could be related to rapid deterioration by the action of microflora (Calistru *et al.*, 2000) and/or heating effect that eventually has killed the seeds (Bewley and Black, 1994).

The effect of growth regulators, storage temperature and their interaction were significant ($p<0.001$). Exogenous application of growth promoters enhanced the germination of non-desiccated seeds of *M. dactyloides* stored at 15 or 20°C for three months compared with untreated seeds that did not germinate at all (table 1). This suggests that seeds of *M. dactyloides* are not only recalcitrant but also possess physiological dormancy. Exogenous application of growth regulators have been shown to release dormancy and enhance germination in seeds exhibiting physiological dormancy (Baskin and Baskin, 1998). A combination of recalcitrance and dormancy is a rare phenomenon (Pritchard and Tompsett, 1995) and our finding contributes to the knowledge of such a rare case from tropical plants.

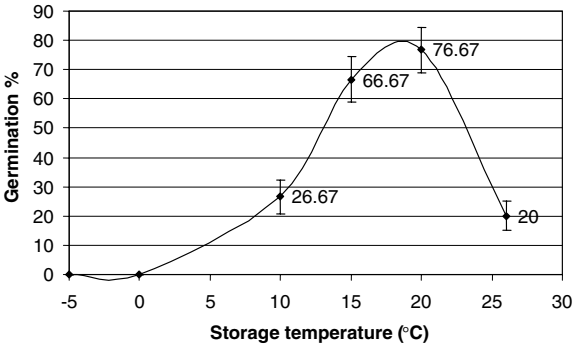

Figure 2. Germination of non-desiccated *M. dactyloides* seeds stored for two weeks at different storage temperatures. Values are mean ± SE. Note that seeds were treated with GA_3 prior to sowing.

Table 1. Germination (%) of non-desiccated *M. dactyloides* seeds stored at different temperature regimes for three months and pre-treated with growth regulators (mean ± SE).

	Storage temperature (°C)			
Growth regulators	26	20	15	10
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0
GA_3, 1000 ppm	0 ± 0	60 ± 2.8	67 ± 1.9	0 ± 0
IBA, 1000 ppm	0 ± 0	100 ± 0	89 ± 1.9	0 ± 0

Germination substrates significantly influenced the germination response of *M. dactyloides* seeds ($p<0.001$). Seeds sown on germination paper, cotton towel or sponge sheet had a significantly higher germination than those sown on mineral soil, sand or vermiculite (table 2). The available air space could be entirely replenished by water in these substrates which in turn reduced the availability of oxygen for the embryo, thus causing reduction or inhibition of germination. Substrate effects have also been observed in seeds of other species (Baskin and Baskin, 1990; Hidayati *et al.*, 2000). We conclude that the critical moisture content below which *M. dactyloides* seeds should not be dried is 34%. Seeds can be hermitically stored at 20°C for three months without deleterious effect on viability. For better germination, exogenous applications of growth promoters prior to sowing and good aeration conditions, perhaps by reducing the watering frequency, are recommended.

Table 2. Effect of substrate on germination of non desiccated *M. dactyloides* seeds stored at 20°C for three months and pretreated with GA_3 (Mean ± SE). Means followed by the same letter are not significantly different at 5% level using Tukey's test.

Substrate	Germination (%)
Mineral soil	0 ± 0a
Sand	3 ± 1b
Moist vermiculite	10 ± 1.2c
Sponge sheet	82 ± 1.2d
Cotton towel	86 ± 1.2d
Germination paper	88 ± 1.6d

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