

RESEARCH ARTICLE

## Identifying dormancy class and storage behaviour of champak (*Magnolia champaca*) seeds, an important tropical timber tree

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**Abstract:** *Magnolia champaca* is a culturally important tree in the tropics with a high commercial value. Its natural regeneration is rare. Hence, the species is usually propagated from seeds but with great difficulties. The class of dormancy in *M. champaca* seeds has been reported to be physical, physiological, and morphological. Their storage behaviour as orthodox, intermediate, and recalcitrant has been reported by various authors. During the present research, seeds of this species in Sri Lanka were collected to determine their dormancy class and storage behaviour. Both scarified and non-scarified seeds imbibed water; however, high germination occurred only on gibberellic acid (GA<sub>3</sub>) showing the presence of physiological dormancy. Embryos elongated inside of seeds at warm temperatures before radicle emergence indicating morphological dormancy. Thus, seeds have morphophysiological dormancy (MPD) and probably the non-deep simple level. The moisture content of fresh mature seeds was 25 %. Low germination occurred on GA<sub>3</sub> for seeds dried to 10 % moisture content, and the seeds stored dry at 4 and -1 °C for 1 month did not germinate. Thus, the storage behaviour may be best classified as intermediate. Knowing that the seeds of this species have MPD allows future studies to partition the factors driving embryo growth, unblocking physiological dormancy, and stimulating germination in nature and in propagation. But the storage behaviour may limit seed usage in propagation programmes relying on stored seeds.

**Keywords:** Embryo growth, intermediate storage, *Magnolia champaca*, *Michelia champaca*, moisture content, morphophysiological dormancy.

### INTRODUCTION

*Magnolia champaca* (L.) Baill. ex Pierre (= *Michelia champaca* L.), commonly known as champak, grows naturally in primary lowlands to montane rainforests

of the Eastern sub-Himalayan region as well as the Western Ghats (Troup, 1921; Hossain & Nizam, 2002). It is extensively planted in other parts of tropical Asia (Sri Lanka to Indonesia, Vietnam and China) and cultivated in tropical Central and South America and Africa (Candiani *et al.*, 2004; Orwa *et al.*, 2009; Tropicos®, 2012). *Magnolia champaca* is well known for its sweetly scented flowers (Armiyanti *et al.*, 2010), and various parts of the plant provide fodder, timber, fuel, medicines, and antifungal compounds (Orwa *et al.*, 2009). The species is often planted around Hindu and Jain temples. In addition, the plant has been identified as a well performing primary tree species in reforestation and restoration projects throughout South and South Eastern Asia (Hoque *et al.*, 2004; Raman *et al.*, 2009; Bhatt *et al.*, 2010).

The natural regeneration of the species is rare, and hence, it is often grown in nurseries from seeds with great difficulty before out-planting (Bahuguna *et al.*, 1987; Armiyanti, 2010). Four to twelve *M. champaca* seeds are enclosed in a follicle, which naturally split open at maturity (Norman, 1971). Propagation from seeds is complicated due to numerous factors (Norman, 1971; Campbell, 1980; Bahuguna *et al.*, 1988; Beniwal & Singh, 1989; Beniwal & Lal, 1993; Lal *et al.*, 1997; Murali, 1997; Bisht & Ahlawat, 1999; Hossain & Nizam, 2002; Candiani *et al.*, 2004): highly variable viability, 'hard' seed/fruit coat, very low (2 – 3 %) to low (23 – 39 %) germination, and slow germination (1 week to 4 months). However, moderate (50 – 53 %) to high (68 – 86 %) germination in seeds has also been reported (Norman, 1971; Campbell, 1980; Bahuguna *et al.*, 1987; Robbins, 1988; Lal *et al.*, 1997; Bisht & Ahlawat, 1999).

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Various treatments have been tried to enhance the germination. The 'hard' seed/fruit coat has been suggested to be responsible for delayed germination implying the presence of physical dormancy. However, seed coat softening treatments (even complete removal of the seed coat) have failed to improve germination (Bahuguna *et al.*, 1988), and in some instances mechanical scarification decreased germination (Norman, 1971). Bahuguna *et al.* (1988) classified seeds as having physiological dormancy based upon enhanced germination (up to 80 %) with gibberellic acid ( $GA_3$ ). In contrast, Baskin and Baskin (1998) classified seeds of the species as morphologically dormant based on: (1) the species being in Magnoliaceae, which is known to have underdeveloped embryos that must grow inside the seed before the radicle emerges and (2) germination (*albeit* low percentage) occurring without any treatments indicating no physiological component to dormancy (Beniwal & Singh, 1989). Supporting this latter contention is the fact that simply removing the aril enhances germination from 0 – 4 % to 74 – 86 % (Robbins, 1988; Candiani *et al.*, 2004).

Seeds of *M. champaca* are also short-lived (about 2 – 3 weeks), but the loss of viability can be minimized by moist storage especially at low temperatures (5 °C) for up to 9 months (Troup, 1921; Campbell, 1980; Bahuguna *et al.*, 1987; Beniwal & Singh, 1989; Murali, 1997; Bisht & Ahlawat, 1999). The species has been reported to be recalcitrant or 'short-lived orthodox' due to its relatively short life span and the requirement of high moisture to maintain viability (Bahuguna *et al.*, 1987; Robbins, 1988; Bisht & Ahlawat, 1999). In a graph of seed weight vs seed moisture content, *M. champaca* was plotted between the orthodox and recalcitrant species but closer to orthodox (Hong & Ellis, 1996). The moisture content in fresh seeds varies from 9 % (Candiani *et al.*, 2004) to 21 % (Bahuguna *et al.*, 1987) to 30 % (SID, 2008) and the seeds can tolerate desiccation up to 11 % (Robbins, 1988). Although these values fall mostly within the range of orthodox species (Hong & Ellis, 1996), SID (2008) suggested that further investigations with low moisture contents and with sub-zero temperatures are needed to better understand the storage behaviour.

The main purpose of the present study was to determine the class of dormancy (Baskin & Baskin, 2004) in *M. champaca* seeds. Imbibition tests, germination tests under laboratory conditions and with  $GA_3$ , and embryo growth studies were used to examine nondormancy and physical, physiological, combinational (physical + physiological), morphological, and morphophysiological (morphological + physiological) dormancies. The study was also aimed at better understanding of the storage behaviour of *M. champaca* seeds by investigating (1) the moisture content of fresh seeds and (2) the

viability of dried seeds and of the seeds under various storage conditions.

## METHODS AND MATERIALS

### General procedures

Seeds were collected from (freshly) fallen dehisced fruits of *M. champaca* trees along roadsides at the University of Peradeniya, the Royal Botanic Gardens in Peradeniya and a home garden in Matale, Sri Lanka, during the late fruiting season from July to September 2010. They were placed in polythene bags and taken to the laboratory where the aril surrounding the seeds was removed manually and the seeds were washed with water. Studies were carried out within 1 wk of collection of the seeds at 27 °C with fluorescent ceiling lights and diffuse sunlight from windows for ca. 10 h per day. Seeds were placed on Whatman No. 1 filter papers moistened either with water or 500 ppm  $GA_3$  in 9 cm diameter plastic petri dishes during the experiments.

### Imbibition test

Fifteen seeds were manually scarified by removing a small portion of the seed coat using a razor blade and the initial weight of each seed was measured; 15 non-scarified seeds also were weighed. Seeds of both sets were placed on moist filter papers in petri dishes and kept in the laboratory. Each seed was re-weighed after 3, 5, 7, 24 and 48 h. The percentage change in mass was calculated.

### Seed germination

Two experiments were conducted to examine the germination (i.e. appearance of the radicle). In the first experiment, germination was tested under four conditions: (1) non-scarified and moistened with water, (2) manually scarified (see above for method) and moistened with water, (3) non-scarified and moistened with  $GA_3$ , and (4) scarified and moistened with  $GA_3$ . Each treatment was conducted with three samples, each having 15 seeds, and on filter papers in petri dishes. Seeds were kept moist with water or  $GA_3$  in the laboratory and checked for germination at 2 d intervals for 3 months. For the second experiment, three sets of 15 seeds each were kept moist with water on filter papers in petri dishes in the laboratory. Seeds in the first, second, and third set were transferred to filter papers moistened with  $GA_3$  after 15, 30, and 50 days, respectively. Seed germination was recorded throughout the experimental period (before and after being transferred to  $GA_3$ ) at 5 d intervals for 105 days.

## Embryo and seed length measurements

A set of seeds was placed on a filter paper moistened either with water or with GA<sub>3</sub> in a petri dish and kept in the laboratory. Embryos were removed from seeds with a scalpel, and the length of the embryos and the seeds were measured using a compound microscope with a micrometer. The length of embryos and seeds were recorded at 2 – 9 d intervals starting on day 2 for seeds on water and on day 4 for seeds on GA<sub>3</sub>. Measurements were taken over a 75 d period or until all the seeds had rotted (on GA<sub>3</sub>). Five seeds in each treatment were used at each measurement, with the exception of three seeds on GA<sub>3</sub> in the last two measurements. To determine the critical length at which the embryo maximally grows inside the seed before radicle emergence, a set of seeds was treated with GA<sub>3</sub>, and the embryo and seed lengths were determined for germinated seeds. For seeds that had germinated, the length of the embryos were measured up to the point where the radicle exited from the seed. From the measurements, the ratio of embryo length to seed length was calculated.

## Moisture content

The initial weight of the six samples, each with six fresh seeds, was measured. The samples were oven dried for 3 h at 120 °C and the final weight of each sample was measured (ISTA, 2008). Another six samples, each with six seeds, were kept on dry filter papers in petri dishes in the laboratory, which had the same relative humidity and temperature as outside, for 30 days in order to observe the moisture content changes under simulated natural conditions. The samples were then oven dried (as before) and the final weight of each sample was measured. Moisture content for both sets of samples was calculated based on initial mass.

## Viability and storage

Two experiments were set-up to examine the viability under various storage conditions. The first experiment included three samples, each with 15 seeds that were dried up to 10% moisture content (fresh mass basis) in the laboratory. For the second experiment, two samples with three replicates containing six seeds each were wrapped with aluminium foil and then placed in petri dishes that were sealed in polythene bags. One sample was kept dry and stored for 1 month in a refrigerator at 4 °C while the other sample was stored at -1 °C. Following the specified condition in each experiment, seeds were placed on filter papers moistened with GA<sub>3</sub> and tested for viability (*via* germination test) with scoring done at 2 d intervals for 50 days.

## Statistical analysis

Means were compared using *t*-test or one-way analysis of variance (ANOVA) followed by protected least significant difference tests (PLSDs,  $p = 0.05$ ) (SPSS, 2010). Germination percentages were calculated based on the number of seeds placed in a petri dish, and they were arcsine square-root transformed for statistical analysis.

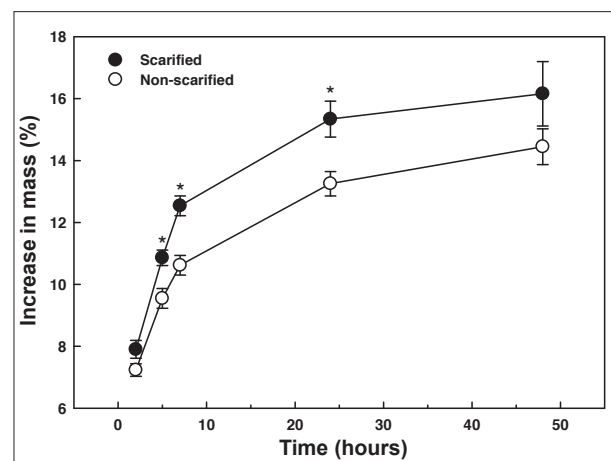
## RESULTS

### Imbibition test

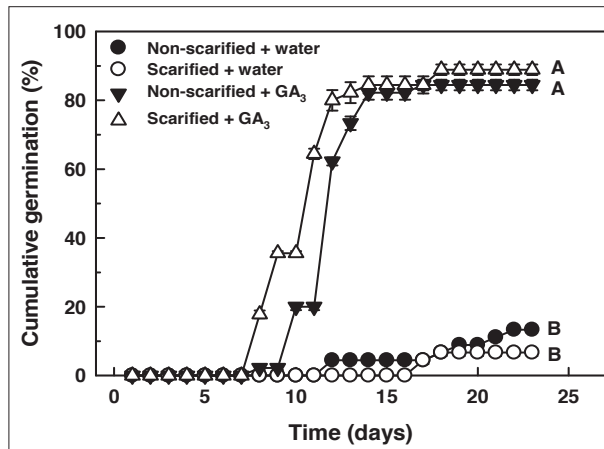
Both scarified and non-scarified seeds absorbed water (Figure 1), and the differences at 48 hours were not significant (*t*-test,  $p = 0.163$ ). Increase in the mass of seeds from both treatments was 14 – 16 % at 48 hours.

### Seed germination

In the first experiment, germination of both scarified and non-scarified seeds was significantly higher (1-way ANOVA,  $p < 0.0001$ ) on GA<sub>3</sub> than on water (Figure 2). No additional germination occurred after day 23. Non-germinated seeds on GA<sub>3</sub> eventually rotted but the seeds that were kept on water for up to 3 months never germinated or rotted. For the second experiment, 0 – 20 % of the seeds in sets 1, 2, and 3 germinated prior to GA<sub>3</sub> addition on day 15, 30, and 50, respectively (Figure 3). Germination increased to 67 – 80 % among the sets following GA<sub>3</sub> treatment.



**Figure 1:** Imbibition of manually scarified and of non-scarified seeds of *Magnolia champaca*. Error bars are  $\pm 1$  SE. An asterisk indicates the significant difference between scarified and non-scarified conditions (*t*-test,  $p \leq 0.05$ ).



**Figure 2:** Mean ( $\pm$  SE) germination of manually scarified and non-scarified seeds of *Magnolia champaca* on gibberellic acid (GA<sub>3</sub>) or water under laboratory conditions. Means at day 23 with dissimilar letters are significantly different (PLSD,  $p = 0.05$ ).

### Embryo length and seed length measurements

Seeds on GA<sub>3</sub> had a significantly higher ( $p = 0.016$ ) embryo : seed length ratio (0.461) at day 23, than those on water (0.211) at day 21 (Figure 4). No additional measurements were made on GA<sub>3</sub> treated seeds after day 23 since they were all rotted. For seeds on water, the ratio remained low from day 21 until the end of the experiment on day 75. However, seeds on water were judged to be viable since their embryos were white and firm (as opposed to brown and soft). The mean ( $\pm$  SE) ratio in the germinated seeds was  $0.608 \pm 0.028$ .

### Moisture content and viability

The mean ( $\pm$  SE) moisture content of fresh seeds was  $25.2 \pm 1.6\%$  and it was significantly reduced during 30 days of storage in the laboratory ( $5.7 \pm 0.9\%$ ) ( $p < 0.0001$ ). In the first experiment, a mean ( $\pm$  SE) germination of  $24 \pm 0\%$  occurred within 14 days after the GA<sub>3</sub> treatment for seeds dried to 10% moisture content; no additional germination took place after 14 days and all the non-germinating seeds eventually rotted. For dry seeds placed at 4 and  $-1^\circ\text{C}$  in the second experiment, none germinated and were ultimately rotted.

## DISCUSSION AND CONCLUSION

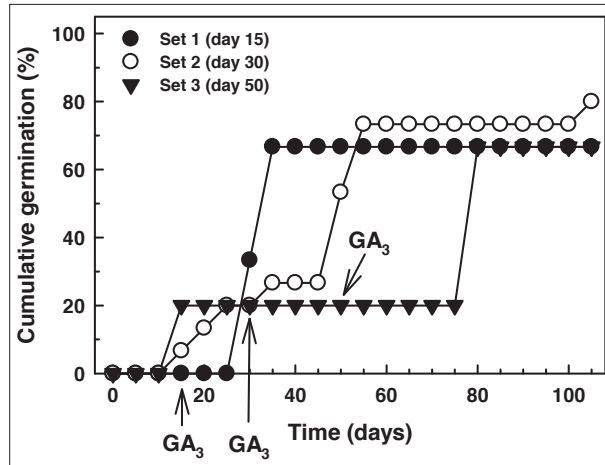
Seeds of *M. champaca* remained viable and a few seeds germinated on water at laboratory temperatures over 3 months (Figure 2) or ca. 1.5 months (Figure 3).

Baskin and Baskin (2004) recommended that the seeds be considered dormant if the seeds are placed under appropriate conditions and they do not germinate (or low percentages do so) within about 1 month. Since most *M. champaca* seeds did not germinate without treatment, it was concluded that many of the seeds in the present study as well as in other studies that reported low germination (Bahuguna *et al.*, 1988; Beniwal & Singh, 1989; Beniwal & Lal, 1993; Murali, 1997; Candiani *et al.*, 2004) were dormant at maturity. Although some studies have reported moderate to high germination (Norman, 1971; Campbell, 1980; Bahuguna *et al.*, 1987; Robbins, 1988; Lal *et al.*, 1997; Bisht & Ahlawat, 1999), the possible influence of pre-sowing storage conditions or the length is uncertain. We cannot rule out the possibility that seeds in some populations may have only a slight degree of dormancy.

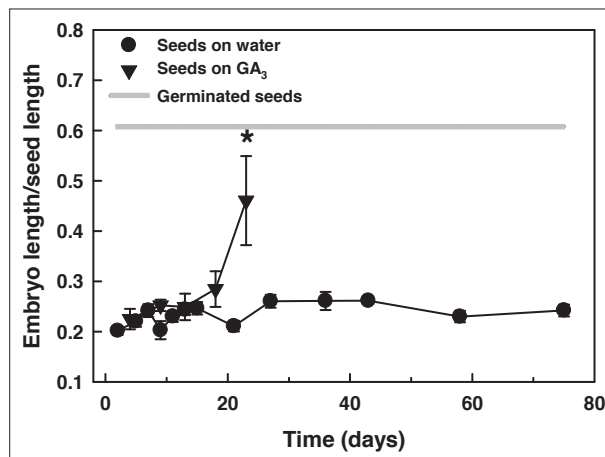
Physically dormant seeds are characterized by a water impermeable seed and/or fruit coat, and thus, can imbibe water only if specialized structures on the seed is opened or if the seed is damaged allowing water uptake (Baskin & Baskin, 1998). Although *M. champaca* seeds are described as very hard and impermeable to water (Norman, 1971; Bahuguna *et al.*, 1988; SID, 2008), both scarified and non-scarified seeds imbibed water (Figure 1) and germinated in the presence of high percentages on GA<sub>3</sub> (Figure 2). Thus, seeds of this species are neither physically dormant nor conditionally (physical + physiological) dormant.

Embryos inside of *M. champaca* seeds elongated about 190% (from ca. 0.21 to 0.61 mm) before radicle emergence. This aspect showed that the seeds have a morphological component to their dormancy. Due to embryo growth, an immediate radicle emergence would not be expected from the seeds and the radicles did not begin to emerge from the seeds for 8 days (or longer) after the start of GA<sub>3</sub> treatment (Figures 2 – 4). In addition to embryo growth, seeds treated with GA<sub>3</sub> germinated at high percentages but those not treated germinated at low percentages (Figure 2). Moreover, seeds that remained moist for up to 50 days did not germinate at high percentages unless they were treated with GA<sub>3</sub> (Figure 3). Our results support the conclusion of Bahuguna *et al.* (1988) that *M. champaca* seeds have a physiological component to dormancy. Seeds like *M. champaca*, that have both a morphological component (embryo growth) as well as a physiological block are defined as having morphophysiological dormancy (MPD).

The levels of MPD are first divided into two categories based on temperatures at the time of embryo growth: warm temperatures ( $\geq 15^\circ\text{C}$ ) are needed for embryo growth in



**Figure 3:** Germination for three sets of *Magnolia champaca* seeds on filter paper moistened with water under laboratory conditions (27 °C with light during day) and then transferred to GA<sub>3</sub> on day 15, 30, and 50, respectively.



**Figure 4:** Mean ( $\pm$  SE) embryo length:seed length ratio for *Magnolia champaca* seeds on GA<sub>3</sub> or water under laboratory conditions. The ratio in germinated seeds was 0.608 (grey line). An asterisk indicates significant difference between treatments (*t*-test,  $p \leq 0.05$ ).

seeds with simple levels of MPD and cold temperatures (0 – 10 °C) for those with complex levels of MPD (Baskin & Baskin, 2004). Considering that (1) embryos in GA<sub>3</sub>-treated seeds grew in the laboratory at 27 °C (Figure 4), (2) *M. champaca* grows primarily in (lowland) tropical regions where temperatures average > 20 °C (Smith & Smith, 2009), and (3) cold stratification at 5 °C did not overcome dormancy (Bahuguna *et al.*, 1988), it was concluded that the seeds have a simple type of MPD.

Simple MPD is divided into several categories depending on the requirements to break dormancy and whether GA<sub>3</sub> could overcome it. Intermediate and deep levels of simple MPD require warm followed by cold stratification (warm + cold) to break dormancy, but non-deep simple MPD needs only warm or cold stratification (Baskin & Baskin, 2004). GA<sub>3</sub> overcomes dormancy in seeds with non-deep and intermediate simple MPD; it substitutes for warm but not cold stratification in seeds with deep simple MPD. The level of MPD in seeds of *M. champaca* apparently is non-deep simple, since (1) cold stratification did not alleviate dormancy (Bahuguna *et al.*, 1988) and (2) GA<sub>3</sub> effectively broke it (Figures 2 – 4).

The moisture content of fresh *M. champaca* seeds in this study (25 %) was within the range (9 – 30 %) reported in other studies (Bahuguna *et al.*, 1987; Candiani *et al.*, 2004; SID, 2008). Orthodox, intermediate, and recalcitrant seeds generally have < 20 – 50, 23 – 55, and 36 – 90 %, moisture contents at maturity, respectively (Hong & Ellis, 1996). No generalization is possible when the moisture content is between 25 and 55 %, but if it is < 35 % then the species is unlikely to show recalcitrant behaviour. Only some of the seeds remained viable and germinated when dried to 10 % moisture content, confirming the findings reported by Robbins (1988) where the seeds were dried to 11 % moisture content. The seeds also remained viable for up to 2.5 months at warm temperatures if kept moist (Figure 4). However, the seeds died when stored dry at temperatures reduced to -1 °C for 1 month. Seeds that can survive drying to 10 – 12 % moisture content but die when dried to 5 % or when stored hermetically at -20 °C for 3 months are classified as intermediate (Hong & Ellis, 1996). Although the storage was not hermetic or subfreezing, the data suggest that seeds in the present study would not have survived in these conditions. Based on the moisture content in fresh seeds as well as the storage behaviour, the seeds of *M. champaca* would be best classified as intermediate.

The next step for understanding the dormancy and germination in *M. champaca* seeds is to resolve the temperature and moisture regimes under which morphological and physiological dormancy break occurs in nature and in out-of-door propagation. Knowing that seeds of this species have MPD allows ecologists and nurseries to study the partitioning of environmental factors driving embryo growth, unblocking physiological dormancy, and stimulating germination. Delays in emergence following sowing are expected due to MPD, and this aspect may limit the possibility of *M. champaca* seeds being ready-on-demand. Nurseries may need to consider the possibility of treating seeds with GA<sub>3</sub> before

sowing to achieve high emergence. In addition, the storage behaviour of *M. champaca* seeds may restrict its use in propagation and land rehabilitation programmes that rely on stored seeds.

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