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ARTICLE

Dormancy, storability, and germination of seeds in *Magnolia punduana* (Magnoliaceae)

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Abstract: *Magnolia punduana* Hk. f. & Th. (syn. *Michelia punduana*) is a threatened tree species restricted to north-eastern India. The seeds remain dormant for 6 months after dispersal. Seed development and breaking of dormancy was investigated in the present study. Physical treatments such as water (cold, hot, and boiling) and acid and manual scarification proved ineffective in breaking dormancy. Matured seeds had linear and underdeveloped embryos at the time of dispersal with an embryo to seed length (E:S) ratio of 0.13. Germinating seeds showed a significant increase in the embryo length with an E:S ratio of 0.36. Cold stratification (CS) at 5 °C effectively broke dormancy. The embryo grew significantly after CS, indicating the presence of "non-deep simple" morphophysiological dormancy (MPD). Application of GA_3 had a significant effect on germination and the lowest germination time with the shortest T_{50} was observed in seeds treated with 3000 mg·L^{-1} GA_3. KNO_3 did not have an influence on breaking dormancy except in combination with GA_3, which increased the germination percentage significantly. CS for 90 days and GA_3 (3000 mg·L^{-1}) at 25 °C resulted in optimum germination. In the test for viability, seeds stored at 5 °C showed highest viability of 66.66% after 180 days.

Key words: dormancy, GA_3, germination, *Magnolia*, stratification, viability.

Résumé : *Magnolia punduana* Hk. f. & Th. (syn. *Michelia punduana*) est une espèce d'arbre menacée dont la distribution est restreinte au nord-est de l'Inde. Les semences demeurent dormantes pendant 6 mois après leur dispersion. Le développement des semences et le bris de la dormance ont été examinés dans l'étude présente. Des traitements physiques avec de l'eau (froide, chaude et bouillante) ou de l'acide et la scarification manuelle se sont montrés inefficaces pour briser la dormance. Les semences matures portaient des embryons linéaires et sous-développés au moment de la dispersion, le ratio de la longueur de l'embryon sur la longueur de la semence (E:S) étant de 0,13. Les semences en germination présentaient un accroissement significatif de la longueur de l'embryon, le ratio E:S étant de 0,36. La stratification à froid (SF) à 5 °C brisait efficacement la dormance. L'embryon croissait significativement à la suite de la SF, indiquant la présence d'une dormance morphophysiologique (DMP) simple et non profonde. L'application de GA_3 avait un effet significatif sur la germination et le temps de germination le plus faible, avec le T_{50} le plus court, était observé chez les graines traitées avec 3000 mg·L^{-1} de GA_3. Le KNO_3 n'avait pas d'influence sur le bris de la dormance, sauf lorsqu'il était combiné au GA_3 alors que le pourcentage de germination augmentait significativement. La stratification à froid pendant 90 jours et le GA_3 (3000 mg·L^{-1}) à 25 °C provoquaient une germination optimale. Lors d'un test de viabilité, les semences entreposées à 5 °C présentaient la viabilité la plus élevée à 66,66 % après 180 jours. [Traduit par la Rédaction]

Mots-clés : dormance, GA_3, germination, *Magnolia*, stratification, viabilité.

Introduction

Magnolia punduana Hk. f. & Th. (syn. *Michelia punduana*) of Magnoliaceae is an endemic tree species of Meghalaya, northeastern India (Haridasan and Rao 1985; Nayar and Sastry 1990; IUCN 2014). The subgenus *Michelia* comprises approximately 80 species of trees and shrubs distributed in Asian temperate, subtropical, and tropical zones (Zhang 2007). *Magnolia punduana* is a middle-sized tree having a straight trunk with an average height of about 15 m, often forming the canopy layer (Iralu and Upadhaya 2015). The species has a restricted distribution and grows in subtropical broadleaved forests at an elevation range of 800–1500 m a.s.l. The species was listed as "rare" by Walter and Gillett (1998) and Nayar and Sastry (1990) and is classified as "Data Deficient" by the IUCN Red List (Wheeler and Rivers 2015). The restricted distribution of

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the species coupled with exploitation for timber has made it imperative to conserve the species.

There are a number of studies on the class of dormancy in *Magnolia* seeds. Some studies have established that seeds of *Magnolia* have physiological dormancy (PD) evidenced by enhanced germination with application of GA_3 (Bahuguna et al. 1988), while others are of the view that the species has morphological dormancy (MD) due to underdeveloped embryo at the time of maturation (Baskin and Baskin 1998). There are studies that indicate that the species has both MD and morphophysiological dormancy (MPD) (Martin 1946; Zhou and Hu 1990). In addition to exhibiting seed dormancy, *Magnolia* seeds are short-lived. In *Magnolia champaca*, a species under the same subgenus, seeds have been classified as recalcitrant (Bahuguna et al. 1987; Robbins 1988; Bisht and Ahlawat 1999). Due to the complicated propagation of *Magnolia* seeds, an attempt to understand seed germination and dormancy-breaking techniques have been carried out for some species such as *Michelia yunnanensis* (Han et al. 2010), *Magnolia champaca* (Fernando et al. 2013), *Magnolia grandiflora* (Fetouh and Hassan 2014), and *Manglietiastrum sinicum* (Zheng and Sun 2009). However, there is no information on dormancy-breaking approaches and germination requirements of the lesser known *Magnolia punduana*, which is facing the threat of extinction (Iralu and Upadhaya 2015). Information on the type and class of prevailing dormancy, embryo development at maturity, dormancy-breaking techniques to speed up germination, and identifying a favourable temperature for growth will help in propagating this threatened taxon ex situ, thus, relieving threat pressures. Therefore, this study was undertaken with the following objectives:

(1) To characterize the class of dormancy present by studying seed morphology, germination, and embryo development.
(2) To test the effects of physical (cold, hot, and boiling water treatments, manual scarification), chemical (acid scarification, GA_3 and KNO_3 treatments), and stratification (cold and warm) on dormancy-breaking and germination.
(3) To understand the effect of storage on viability and germination of *Magnolia punduana* seeds.

Materials and methods

Seed source

Mature follicles of *Magnolia punduana* were collected during August 2015 from Jarain (25°19.016′N, 92°8.566′E) in the Jaintia hills of Meghalaya, northeastern India. The fruits were collected from 15 randomly distributed trees. About 2000 seeds were collected for the study. Healthy seeds were separated from those seeds that were predated and damaged by ants and insects on the forest floor in the laboratory by using the floatation method (Pipinis et al. 2011). Seeds were soaked in water for 24 h till the arils softened. The seeds were washed manually to remove the arillus and disinfected in 0.2% $KMnO_4$ solution for 2 h following Zuo (1994*a*). Seeds were stored under laboratory conditions (temperature: 24 ± 1 °C, relative humidity: 60%–75%) and subjected to different treatments within 5 days of seed collection to avoid loss of viability.

Moisture content

To determine the moisture content, three replicates of seeds weighing 10 ± 0.01 g each (approximately 137 seeds) were oven dried at 80 °C for 24 h and the final mass was measured. Three sets of seeds weighing 10 ± 0.01 g each were placed under laboratory conditions and the mass was measured after 30 days to observe changes in moisture. Moisture content was calculated following ISTA (2008).

Imbibition test

To understand the imbibing capacity of seeds of *Magnolia punduana*, 30 seeds were manually scarified using a razor blade and the initial mass of each seed was measured. Non-scarified seeds were also weighed in the same way. Seeds of both sets were placed on moist filter paper in Petri dishes and kept in the laboratory. Each seed was re-weighed after 2, 4, 6, 8, 10, 20, 30, and 40 h and the percentage change in mass was calculated.

Germination tests

Seeds were soaked in cold (20 °C), hot (seeds submerged in 80 ± 0.50 °C until the water cooled), and boiling water (2 min in 100 °C and immediately transferred to cold water). The seeds were then transferred to fresh water and kept for 48 h, changing the water every 12 h. The seeds were sown in plastic trays filled with a mixture of garden soil and sand in the ratio of 3:1 under laboratory conditions. A control was maintained by sowing seeds without prior soaking. The trays were kept under laboratory conditions at a temperature of 24 °C (±1 °C) with an average 8 h of light (>700 lx) and monitored at 2-day intervals for germination. For each experiment, three replicates of 50 seeds were maintained.

To test the effect of scarification, seeds were manually scarified using a sharp razor. Seeds were subjected to acid scarification by soaking in concentrated H_2SO_4 (95%) for 10 min and washed thoroughly in running water. Both manually scarified and acid-scarified seeds were placed in respective 150 mm Petri plates lined with moist filter paper (Whatman No. 1) and kept under laboratory conditions. For each experiment, three replicates of 30 seeds were maintained.

For cold stratification (CS), seeds were packed in sealed polybags containing moist sand and stored in the dark at a temperature of 5 °C (±1 °C). Similarly, seeds were packed in sealed polybags with moist sand and placed in an incubator at a constant temperature of 25 °C (±1 °C) for warm stratification (WS). Germination tests for the seeds were carried out after 0, 30, 60, 90, and 120 days of CS and WS. After each stratification period, the seeds were washed thoroughly in running water and transferred to growth chambers fitted with LED tubes (>4000 lx) with a

photoperiod of 8 h. To understand the suitable temperature for germination, seeds were germinated at a constant temperature of 25 °C (±1 °C) and 30 °C (±1 °C). For each experiment, three replicates of 30 seeds were maintained.

The effect of GA_3 on breaking dormancy and germination of *Magnolia punduana* was investigated following Han et al. (2010). Seeds were soaked in 0, 200, 500, 1000, 2000, 2500, and 3000 mg·L^{-1} GA_3 for 48 h at room temperature. The GA_3 hydrated seeds were transferred to the growth chamber at a constant temperature of 25 °C (±1 °C) for germination. Similarly, seeds were soaked in 0, 0.1, 0.5, 1.0, and 1.5% KNO_3 for 48 h and further transferred to the growth chamber. The combined effects of GA_3 and KNO_3 were tested where seeds were soaked separately in two concentrations of GA_3 (200 and 500 mg·L^{-1}) for 48 h and subsequently soaked in two concentrations of KNO_3 (0.1% and 0.5%) for another 48 h, respectively, before incubation. The germination of the seeds was monitored at an interval of every 2 days up to 60 days. For each treatment, three replicates of 30 seeds were maintained.

Embryo development

For seed and embryo morphology and dimensional characterizations, 30 seeds each at different stages of growth were examined, including fresh seeds, seeds cold stratified for 100 days, and germinated seeds. Fresh seeds were soaked for 24 h and the embryos were removed and measured. Similarly, for measuring embryo growth, embryos from seeds cold stratified for 100 days were excised. These embryos were soaked in TTZ (1%) solution for 3 h to assess viability (Wang et al. 2005). Length of the embryos was measured using a dissecting microscope and the ratio of embryo to seed length (E:S ratio) was calculated. To determine the critical E:S ratio, 30 seeds were kept in a growth chamber (25 ± 1 °C and 8 h photoperiod) until the seed coats ruptured. The seeds were then removed before radicle protrusion and the average ratio was calculated (Walck et al. 2000; Vandelook et al. 2007).

Viability and storage

To understand the viability characteristics of *Magnolia punduana* seeds and identify suitable storage conditions, four tests were conducted. In the first test, fresh, clean and disinfected seeds were packed in airtight containers and stored at room temperature (24 ± 2 °C and 65% humidity). In the second test, seeds were packed in airtight polybags and stored at 5 °C. The third and fourth tests followed a similar approach with the addition of a substrate (moist sand) and storage at constant temperature (25 °C and 5 °C, respectively). Seed viability was determined using the TTZ assay (Enescu 1991) and seeds were tested for viability after 15, 30, 45, 60, and 90 days. As seeds stored at 5 °C remained viable even after 90 days of storage, these seeds were further monitored up to 6 months. For each treatment, three replicates of 30 seeds were maintained.

Data analysis

For each replication in each treatment, the germination percentage was calculated using the formula:

$$G(\%) = n/N \times 100$$

where n is the number of germinated seeds and N is the total number of seeds.

Mean germination time was calculated using the formula: $\frac{n_1+n_2+n_3....}{N}$ where n_1, n_2, n_3.... = the number of days taken by individual seeds to germinate, and N = the total number of seeds.

The time to 50% of total germination (T_{50}) was calculated by following Farooq et al. (2006):

$$T_{50} = t_1 + [(N/2 - n_i)(t_j - t_i)]/n_j - n_i$$

where N is the final number of emergence and n_i and n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

To determine the effect of different treatments on germination, analysis of variance (ANOVA) was used with Tukey's least significant difference ($p < 0.05$). Assumptions of ANOVA were met through test for normality of variables (Kolmogorov–Smirnov test) and homogeneity of group variances (Levene's test) using SPSS (version 20). Means and standard errors of germination percentages, embryo lengths, and viability test were calculated.

Results

Imbibition

The result from the imbibition test revealed that both the non-scarified and scarified seeds imbibed water during the initial 6 h at different rates (Fig. 1). However, the increase in mass was not significant ($p > 0.05$) and the seeds did not show any difference in mass after 12 h. Increase in the mass of non-scarified and scarified seeds after 40 h was 3% and 6%, respectively.

Germination test

Seeds soaked in cold water 48 h prior to sowing germinated to 44%. The mean germination time was 213 ± 3.55 days (T_{50} = 40.28 days). Seeds did not respond to the hot and boiling water treatments. The seeds without any treatment (control) germinated to 78% with a mean germination of 212 ± 1.79 days (T_{50} = 38.04 days). There was no significant difference in the germination time with seeds that were subjected to soaking and the control ($p > 0.05$). Manual scarification of seeds reduced germination to 20% and seeds subjected to acid scarification did not germinate (Table 1).

CS effectively broke dormancy in seeds of *Magnolia punduana*. Seeds stratified for 30 days at 5 °C took a mean

Fig. 1. Imbibition of non-scarified and scarified seeds of *Magnolia punduana*.

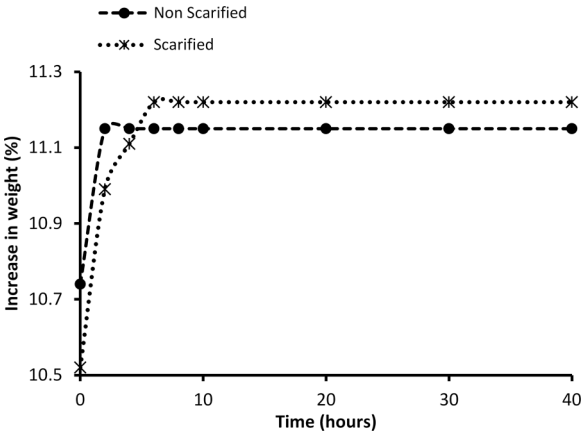

Table 1. Effects of physical treatments (water and scarification), GA_3, KNO_3, and combination of GA_3 and KNO_3 on number of days for mean (±SEM) germination, germination percentage, and time to 50% germination (T_{50}) of *Magnolia punduana* seeds.

Treatment	Germination (days)	Germination (%)	T_{50} (days)
Water			
No treatment	212.62±1.79a	78.00±1.15	31.77±3.39
Cold (20 °C)	213.32±3.25a	44.00±4.61	38.00±4.28
Hot (80 °C)	0.00	0.00	0.00
Boiling (100 °C)	0.00	0.00	0.00
Scarification			
Manual	207±1.58	20.00±0.00	38.88±3.89
Acid (95% H_2SO_4)	0.00	0.00	0.00
GA_3 (mg·L^{-1})			
0	0.00	6.66±3.33	—
200	24.78±1.69a	46.66±3.84	9.66±2.40
500	21.21±0.93ab	48.88±2.22	1.00±0.00
1000	19.06±0.92b	53.33±0.00	6.40±1.76
2000	17.91±0.81b	75.55±1.92	4.25±1.89
2500	17.90±0.87b	66.66±3.84	4.93±1.89
3000	14.17±0.58	76.66±5.77	1.00±0.00
KNO_3 (%)			
0	0.00	0.00	—
0.1	48.00±10.50a	11.10±2.22	21.00±16.00
0.5	29.20±1.74ab	16.66±1.92	9.37±1.93
1.0	32.50±3.40abc	13.33±3.85	20.50±4.35
1.5	23.40±4.25c	16.66±1.92	9.00±4.71
GA_3 (mg·L^{-1}) + KNO_3 (%)			
0	0.00	0.00	—
200 GA_3 + 0.1 KNO_3	20.05±1.13a	69.99±1.92	9.05±2.67
500 GA_3 + 0.5 KNO_3	20.65±1.35a	56.66±1.92	8.39±2.32

Note: For each treatment, means followed by the same letter do not differ significantly at $p < 0.05$ (Tukey's multiple range test).

of 18.71 ± 0.64 days and 29.29 ± 1.30 days to germinate at 25 °C and 30 °C, respectively. With the increase in stratification time, there was a steady decline in the mean number of days for germination. After 120 days of CS, the mean days for germination were reduced to 10.68 ± 0.36 days and 12.97 ± 0.50 days at 25 °C and 30 °C, respectively. Results of ANOVA reveal that mean germination days after the four stratification periods (30, 60, 90, and 120 days) at 25 °C and 30 °C, respectively, differed significantly ($p < 0.05$). However, there was no significant difference in germination of seeds stratified for 90 days and 120 days ($p > 0.05$) under both temperature regimes. A statistically significant difference was observed in the number of days for germination of seeds incubated at 25 °C and 30 °C after 30 days of CS ($p < 0.05$).

Germination increased with increasing period of CS and the highest percentage (93.33%) was observed after 90 days of CS for both temperature regimes. A decline in germination percentage was observed after 120 days of CS for both temperatures (Fig. 2). T_{50} decreased from 30 (6.7 ± 1.75 and 12.38 ± 2.72) to 60 (3.10 ± 0.76 and 4.61 ± 0.99) days of CS followed by an increase up to 90 days (6.25 ± 1.49 and 6.04 ± 1.47) for the seeds incubated at 25 °C and 30 °C, respectively. With prolonged stratification, T_{50} further declined at 120 days under both temperatures (Fig. 2). Therefore, CS broke dormancy in *Magnolia punduana* seeds, with more rapid germination observed in seeds stratified for 30 days (Fig. 2). WS did not have any effect in breaking dormancy.

Application of GA_3 had a marked effect on the germination of seeds. There was a steady rise in the germination percentage with the increase in concentration of GA_3. At higher concentration, the number of days required for germination also declined. The highest germination percentage (76.66% ± 5.77%) with T_{50} of 1 day was observed in seeds treated with 3000 mg·L^{-1} GA_3 (Table 1). The germination percentage of seeds under the control condition was lowest (6.66% ± 3.33%). Results from multiple comparison tests between the various concentrations of GA_3 on germination showed a significant difference between seeds treated in 200 mg·L^{-1} GA_3 with all higher concentrations of GA_3 ($p < 0.05$) except for 500 mg·L^{-1} (Table 1). Overall, KNO_3 had no influence in breaking seed dormancy with very low germination percentage (14%). When KNO_3 was combined with GA_3, the percent germination increased significantly, more so than the seeds treated in 200 mg·L^{-1} and 500 mg·L^{-1} GA_3 alone (Table 1). The results obtained from the application of plant growth regulators (gibberellic acid and potassium nitrate) on germination is given in Table 1.

Embryo development and growth

Seeds of *Magnolia punduana* had a mean mass of 109.00 ± 1.00 mg at maturity. The seeds were oval–ellipsoid or ellipsoid in shaped with a mean seed length of 7.13 ± 0.11 mm. There was a significant difference in the growth and development of the embryo from the time of dispersal to the time of germination ($p < 0.01$). The embryo was situated at the base of the seeds and was enclosed by the fleshy endosperm. The embryos in fresh seeds were small and measured 0.93 ± 0.03 mm with an E:S ratio of 0.13 ± 0.01. Embryo length after 100 days of CS measured 1.30 ± 0.04 mm, with an E:S ratio of 0.18 ± 0.01. Germinat-

Fig. 2. Mean (±SEM) germination percentage (*G*%) and time to 50% germination (T_{50}) under 25 °C and 30 °C after different periods of cold stratification.

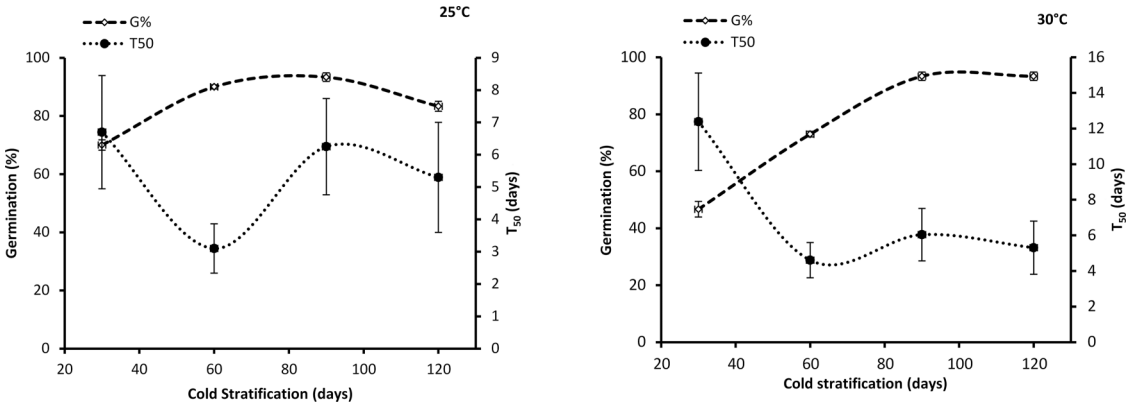

Table 2. Viability of *Magnolia punduana* seeds subjected to different storage conditions.

	Viability (%)					
Storage type	15 days	30 days	45 days	60 days	90 days	180 days
Airtight container at room temperature	43.33±3.33	—	—	—	—	—
5 °C in airtight polybags	70±5.77	46.66±3.33	23.33±3.33	—	—	—
Storage in moist sand (25 °C)	100	86.66±3.33	60±5.77	46.66±8.81	16.66±3.33	—
Storage in moist sand (5 °C)	100	100	96.66±3.33	93.33±6.66	86.66±3.33	66.66±6.66

Note: Values are the means ± SEM, $n = 150$.

ing seeds showed a significant increase in the embryo length (2.53 ± 0.18 mm) with an E:S ratio of 0.36 ± 0.03. There was a significant difference ($p < 0.01$) in the length of embryos of germinating seeds when compared with fresh seeds and stratified seeds.

Moisture content, viability, and storage

The moisture content of fresh seeds dried in the oven at 80 °C for 24 h was 26.43% ± 0.31%. Seeds kept at room temperature for 30 days showed a decline in the moisture content (17.82% ± 0.18%). Viability of seeds stored in airtight containers and kept at room temperature declined to 43.33% after 15 days with complete loss of viability after 30 days of storage. Seeds stored at 5 °C showed a viability percentage of 70% after 15 days that declined gradually to 46.66% and 23.33% after the 30th and 45th day, respectively. Seeds in moist sand stored at 25 °C remained 100% viable in the initial 15 days. A steady decline in viability was observed and after 90 days (16.66%). The highest viability was observed in seeds stored in moist sand at 5 °C after 6 months with 66.66% viability (Table 2).

Discussion

Seeds of *Magnolia punduana* did not show any sign of germination under laboratory conditions up to 6 months (212 days), indicating the prevalence of dormancy in the species. Seed coat scarification tests failed to overcome seed dormancy, thus, nullifying the presence of physical dormancy. Embryos in fresh seeds of *Magnolia punduana* were small and located at the tapering base of the seeds. The embryos were differentiated into cotyledons and radicle with an E:S ratio of 0.13 at the time of dispersal. The species exhibited a "basal type" embryo (Martin 1946), characteristic of embryos restricted to the lower half of the seeds with abundant endosperm. Basal type embryos are usually common in medium to large seeds with cotyledons that are usually rudimentary and obscure (Martin 1946). Several studies on seeds of Magnoliaceae members (*Magnolia accuminata*, *Liriodendron tulipfera*) have established that they have rudimentary embryos (Martin 1946; Baskin and Baskin 1998). The seeds of *Magnolia punduana* can be classified as having linear and underdeveloped embryos (Martin 1946; Baskin and Baskin 1998).

The dormancy in seeds of *Magnolia punduana* induced by underdeveloped embryos indicates that seeds have MD and PD or MPD. The embryos were small and differentiated into cotyledons and radicle that continued to grow inside the seeds after dispersal, indicating MD. According to Nikolaeva (1977) and Baskin and Baskin (2007), seeds of this species have non-deep PD as the species satisfies a wider range of conditions including GA_3-induced germination, response to CS, and fruits ripening in dry storage evidenced by the continued ripening of fruits within the follicles post collection. A similar case of CS-induced germination has been reported in *Michelia yunnanensis* (Han et al. 2010; Zuo 1994*a*, 1994*b*). Under natural conditions, the seeds of the species undergo a period of CS (soil temperature = 8 °C) during winter (November to February) and it may be considered as a crucial period for embryo growth and development. Therefore, the low viability and germination of seeds stored at 25 °C under laboratory conditions may be due

to absence of CS. *Magnolia punduana* seeds showed physiological changes in response to temperature. The seeds ripen in the months of August to September and germination under natural conditions occurs in the month of March the following year. Thus, during the progression from dormancy to non-dormancy, the temperature gradient shifts from cold (8 °C) to warm (17.9 °C). Germination that progresses from a lower to higher temperature has been classified under Type-1 Non Deep PD (Baskin and Baskin 2007). CS effectively broke dormancy in *Magnolia punduana* with up to 93.33% of seeds germinating after 90 days (Fig. 2). WS did not aid in overcoming dormancy.

Based on the rate of radicle protrusion and germination percentage (Fig. 2), a temperature of 25 °C can be considered as the suitable temperature for growth tested. Based on our results, embryo growth was found to be evident in seeds incubated at 25 °C after CS at 5 °C. Thus, MPD present in *Magnolia punduana* can be categorised as "non-deep simple" (Baskin and Baskin 1998). Similar CS induced dormancy breaking has been reported in many species of *Michelia* such as *M. yunnanensis* (Han et al. 2010), *M. maudiae* (Shi et al. 1986), and *M. platypetala* (Zhou and Hu 1990).

Application of GA_3 effectively broke seed dormancy in *Magnolia punduana* and this treatment can be used as a substitute to CS. There was an increase in germination percentage with the increase in the concentration of GA_3. The successful implementation of GA_3 in breaking seed dormancy has been reported in other species such as *Michelia chapensis* (Zhou 1990) and *Manglietia insignis* (Zhou and Hu 1990).

Though the stimulatory action of potassium nitrate has been reported by many researchers (Roberts and Smith 1977; Bewley and Black 1982; Hilhorst and Karssen 2000) in species like *Chenopodium album* (Saini et al. 1985), including ferns (Scheuerlein et al. 1985), the application of KNO_3, however, was not effective in overcoming the dormancy in seeds of *Magnolia punduana* unless GA_3 was used simultaneously The combined effect of KNO_3 and GA_3 significantly increased germination in seeds. This could be attributed to the fact that nitrate decreases the abscisic acid (ABA) content in seeds that is responsible for dormancy, whereas GA_3 promotes germination (Hilhorst and Karssen 1992).

A decline in moisture content over a short period of time reveals that the seed is of intermediate type (Hong and Ellis 1996). In the test for viability, it was observed that seeds stored in dry airtight containers and in polybags remained viable for the initial 15 days but lost viability within 45 days. However, the addition of moist sand prolonged the life of the seeds up to 90 days indicating moisture requirement. Seeds stored in moist sand at 5 °C survived for almost 6 months. Thus, based on the moisture content and the storage behaviour, seeds of *Magnolia punduana* can be best classified as intermediate or semi-recalcitrant.

Conclusion

From this study, it can be concluded that seeds of *Magnolia punduana* exhibit simple MPD. A pre-treatment of CS or application of GA_3 can effectively break dormancy. CS for 90 days and GA_3 (3000 $mg \cdot L^{-1}$) were the most effective period and concentration, respectively, for optimum germination at a temperature of 25 °C. GA_3 can be used as a substitute for breaking dormancy. CS, on the other hand, is the best option for storage as seeds stored at 5 °C showed the highest viability (66.66%) after 180 days. The information and results acquired from this study can be incorporated in making seed germination protocols for mass production and reintroduction of the species in its natural habitat, thus, relieving threat pressures from anthropogenic activities. The results can also contribute to ex situ conservation of the species.

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