



Effect of Seed Treatments on Seed Germination and Seedling Growth of *Madhuca longifolia* var. *longifolia* ("Mee")

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ABSTRACT

Madhuca longifolia (Koenig) J.F. Macb. var. *longifolia* is a forest tree species with high reforestation potential in the dry zone of Sri Lanka. Seasonality in seed production and lack of plant production are the drawbacks of this species regenerating naturally. Seeds were treated with gibberellic acid (GA₃), potassium nitrate (KNO₃), distilled water (DW), and scarified in addition to control without any treatment. Data were evaluated for germination and seedling growth under *ex vitro* and *in vitro* conditions. The objective of this study is to determine the dormancy associated with *M. longifolia* seeds and the effect of pre-treatments on seedling growth. Mechanically scarified seeds, under *ex vitro* conditions, showed higher germination than all the other treatments after 28 days and a significantly higher percentage ($p < 0.05$) than the control under both light (90.00%) and dark (85.45%). Dark conditions stimulated germination significantly within the first seven days of sowing. The highest shoot growth was observed for GA₃, KNO₃, and DW (8.3, 8.6, and 8.5 cm, respectively) treatments while rooting was not significantly affected by the applied treatments. *In vitro* germination was tested for de-coated, surface sterilized seeds on half-strength liquid Murashige and Skoog (½ MS) media with different concentrations of benzylaminopurine (BAP) such as 0, 0.2, 0.5, and 1.0 mg/L. The germination percentage of ½ MS with 0.5 mg/L BAP (77.71%) was higher than all the other media compositions. Results revealed that *M. longifolia* seeds show a lower level of germination due to physical dormancy, and it can be released by mechanical scarification.

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INTRODUCTION

Madhuca longifolia (Mee/Mahua/Butternut tree) is a multipurpose tree belonging to the family Sapotaceae. Van Royen (1960) recorded 84 species of *Madhuca*, and in the Sri Lankan context, *M. longifolia* (Koenig) J.F. Macb. var. *longifolia* was identified as the most common variety, along with *Madhuca longifolia* (Koenig) J.F. Macb. var. *latifolia* (Roxb.) Cheval (Akshatha et al., 2013; Munasinghe and Wansapala, 2015).

Apart from its economic value as timber, every part of the plant, including leaves, flowers, roots, bark, and seeds, has enormous benefits for the industrial and medicinal sectors (Akshatha et al., 2013; Munasinghe and Wansapala, 2015). Arseculeratne et al. (1985) experimented on the traditional Ayurvedic pharmacopoeia of Sri Lanka and discovered the medicinal uses of *M. longifolia* for chronic ulcers, acute and chronic tonsils, and pharyngitis.

As a tree, *M. longifolia* has a wide distribution in dry tropical and subtropical regions, receiving around 550–1500 mm of annual rainfall, temperatures ranging from 2 to 46 °C, and elevations up to 1200 m (Abraham et al., 2010). In Sri Lanka, *M. longifolia* is commonly found in districts including Jaffna, Puttalam, Mannar, Vavunia, Anuradhapura, Trincomalee, Kurunegala, Matale, Polonnaruwa, Kandy, Badulla, Ampara, Moneragala, and Hambantota (Dassanayake et al., 1997).

Similar to some other woody plants, the seed production of *M. longifolia* is seasonal. Although it produces a large number of seeds in one season, the production of plantlets near the mother plant is low. The unique scent of its flowers and fruits attracts frugivorous animals. Seeds are mainly dispersed by fruit bats, and seed damage caused by them may reduce seed germination. The frugivorous bat species *Pteropus giganteus* and *Cynopterus sphinx* are common feeders of *M. longifolia* seeds (Raghuram et al., 2011; David et al., 2015). In dry tropical forests, the high seasonality of rainfall is one of the environmental factors directly influencing seed germination,

survival, and seedling growth of forest tree species (Khurana and Singh, 2001; McLaren and McDonald, 2003; Nasr et al., 2013). Due to its numerous benefits, there is high demand for the tree, and the lack of germination under natural conditions has led to the species being categorized as "Near Threatened (NT)" (Jayasuriya, 2019). Early attempts at vegetative propagation with cuttings and layering did not succeed (Senaratne et al., 1981), although grafting and plant tissue culture have been successful (Senaratne et al., 1981; Shirin et al., 2020).

Propagation using seeds is common for most woody plants; however, dormancy hinders its efficiency. Among the five dormancy classes explained by Baskin and Baskin (2004), physical dormancy (PY) caused by the impermeable seed coat is prominent among dry forest tree species. Physical dormancy can be overcome through scarification, acid treatments, moist or warm scarification, and the use of plant growth regulators (Baskin et al., 2004; Ghosh and Maiti, 2014; Koutouan-Kontchoi et al., 2020). Light intensity is another factor essential for germination, but its impact can be positive or negative depending on the species. The effect of light on seed dormancy has been previously studied by Penfield (2017). Therefore, in the present study, the effect of light during the initial stages of germination was also evaluated. Researchers have focused on different aspects of *M. longifolia* seeds in the literature, including seed morphology (Shashikumar et al., 2018), chemical composition, medicinal and industrial uses, and seed germination (Abraham et al., 2010; Munasinghe and Wansapala, 2015). Kundu et al. (2013) confirmed that the seeds are free of dormancy, while a contrasting finding by Dayananda and Jayasuriya (2019) from Sri Lanka suggests that the seeds exhibit physical dormancy. Therefore, the present study provides clues to either strengthen or weaken the available results on *M. longifolia* seeds, raising the question of why the results are contradictory.

Furthermore, in vitro seed germination is a novel approach recommended by many researchers to bypass the physical barrier and enhance germination artificially. *In vitro*

germination is popular among tissue culturists as a preliminary method for extracting explants and regenerating a high number of in vitro plants using a single seed (Navarro, 1987). Rout and Das (1993) applied this technique to the micropropagation of *M. longifolia*.

The present study aimed to examine the effect of various pre-treatments to understand the type of dormancy associated with *M. longifolia* seeds and identify favorable conditions for seedling growth, which has high reforestation potential in the dry zone of Sri Lanka. Additionally, finding the optimal conditions for *ex vitro* and in vitro germination offers opportunities for producing healthy plants in large quantities for use in reforestation programs.

METHODOLOGY

Collection and preparation of seeds

The ripe, yellowish-green, ovoid-shaped berry-type fruits, measuring 3–5 cm in length, with prominent distal beaks, were identified as the optimal stage for harvesting (Dassanayake et al., 1997; Kundu et al., 2013). Between June and September 2020, seeds were harvested from several *M. longifolia* trees grown in the Dambulla and Kurunegala areas. The seeds, which were brown, shiny, elliptical, and 3–4 cm long, with a flattened shape on one or two sides, were extracted by removing the outer fleshy mesocarp and exocarp. After extraction, the seeds were thoroughly washed and used directly for germination experiments without any storage period. The laboratory experiments were conducted at the Plant and Environmental Science Laboratory of the National Institute of Fundamental Studies in Kandy.

Experimental design for testing *ex vitro* germination of *M. longifolia*

For the experiments, clean, dried, uniform, and healthy intact seeds were selected. The five treatments included untreated seeds (control), seeds dipped in distilled water (DW), seeds treated with 100 ppm gibberellic acid (GA_3), and seeds treated with 2% potassium nitrate (KNO_3). The seeds were

also scarified by rubbing them against a rough surface of sandpaper (#80, wood sandpaper) on an area opposite the embryo until the cotyledon was exposed. Three replicates, each containing ten seeds, were placed on filter papers moistened with distilled water in 10 cm diameter petri dishes. They were then maintained for 16 hours under fluorescent light conditions or in full-time darkness at room temperature (25 ± 2 °C). Germination was determined by the presence of at least 1 mm radical protrusion (Athugala et al., 2021). After one week, the germinated seedlings were planted in polybags (10 × 20 cm) with a compost to sand ratio of 2:1 and transferred to a greenhouse. The germination percentage (number of seeds with protruded radicals per replicate) after 28 days and growth parameters (length of the longest root and shoot per replicate) after 56 days were measured.

Experimental design for testing in vitro germination of *M. longifolia*

The seed coats were removed under laminar flow and sterilized with 5% (v/v) NaOCl for 5 minutes, followed by 0.1% (w/v) $HgCl_2$ for another 3 minutes. After thorough rinsing in sterilized distilled water, the seeds were cultured on liquid half-strength Murashige and Skoog ($\frac{1}{2}$ MS) media, which was discovered by Murashige and Skoog in 1962. The media contained 2% (w/v) sucrose and four concentrations of BAP (0, 0.2, 0.5, and 1.0 mg/L), with $\frac{1}{2}$ MS without BAP serving as the control. Previous experiments on seed germination have used reduced concentrations of basal media since the seeds themselves contain necessary food reserves (Koné et al., 2015). Furthermore, liquid media have advantages over solid media in seed germination, such as an increased growth rate and enhanced rapid uptake of nutrients (Rathore et al., 2014). The pH of the media was adjusted to 5.8 ± 0.2 and then sterilized by autoclaving at 15 lbs $inch^{-2}$ and 121 °C for 20 minutes. Disinfected seeds were inoculated in 5 × 10 cm jam jars containing 30 mL of autoclaved media. It is advised to shake cultures within the range of 100–300 rpm (Wittmann et al., 2003). Considering the seed size and growth rate, *M. longifolia* seeds were shaken at 100 rpm to avoid damaging the

newly emerging radical. Two light intensities were used, including LED lights with an intensity of 1000 lx for 16 hours and complete darkness (0 lx), while maintaining a temperature of 25 ± 2 °C. Previous studies have shown that 1000 lx is highly effective for in vitro seed germination (Awal et al., 2007; Sangma et al., 2018).

The experiment was conducted with three replicates, with each replicate consisting of ten seeds. Seeds were subcultured on fresh media every seven days. After 14 days, the final germination percentage (number of seeds with protruded radicals per replicate) and root growth (length of the longest root in each replicate) were evaluated. Due to the rapid root growth of *M. longifolia* under in vitro conditions, maintaining cultures inside glass containers for 56 days (2 months) was problematic. Additionally, subculturing could cause damage to the delicate root system. Therefore, the experiment was limited to evaluating germination percentage and root growth, excluding shoot growth.

Statistical analysis

The experiments were arranged in a Completely Randomized Design (CRD). The data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics (Version 26) predictive analytic software. The Duncan multiple range test was performed to determine significant differences ($p < 0.05$) among the mean values of the treatments. Percentage data were subjected to arcsine transformation before making mean comparisons.

RESULTS AND DISCUSSION

Ex vitro germination of *M. longifolia*

A significant increase in seed germination was observed under dark conditions compared to light during the first seven days after sowing. In contrast, scarification influenced the earliest germination regardless of the light conditions. Under light conditions, the mean germination percentages for scarified and control treatments were recorded as 19.13% and 14.46%, respectively. In dark conditions, the

percentages were 32.82% for the scarified treatment and 23.46% for the control. Although seeds treated with KNO_3 exhibited a lower percentage of germination (2.71%), it can be considered negligible due to a higher standard deviation. Overall, the chart illustrates that dark conditions have accelerated the germination of *M. longifolia* seeds (Figure 1a).

Eventually, other treated seeds also started germination, but in smaller quantities compared to scarified seeds (Figure 1). The effect of dark conditions is not significant after 28 days. The most important finding is that even after 28 days, only scarified seeds indicated a significantly higher value than the control (Figure 1b). Under light conditions, germination was recorded as 90.00% for scarified treatment and 68.05% for the control, while under dark conditions, it was 85.45% for scarified treatment and 63.21% for the control. The analyzed data originated from the arcsine transformed values, with the minimum and maximum values ranging between 0–90%. Regarding the results, the maximum germination was recorded for scarified seeds under light (90.00%), whereas it was 85.45% under dark conditions.

Of the two characteristics investigated, treatments significantly affected shoot elongation. The three treatments where seeds were dipped in GA_3 , DW, and KNO_3 resulted in significantly higher shoot elongation (8.3 cm, 8.5 cm, and 8.6 cm, respectively) than the control (5.1 cm). Early germination through scarification influenced shoot elongation (6.0 cm); however, it was not significant (Figure 3a). In comparison to shoot elongation, root elongation was not significantly influenced by the applied pre-treatments. After 28 days, the highest root growth was recorded as 19.5 cm for the control, while other treatments showed insignificant growth: GA_3 - 15.5 cm, DW - 17.2 cm, KNO_3 - 17.4 cm, and scarified - 13.6 cm (Figure 3b). Proportionally, the elongation of roots occurs at a rate about two times higher than shoot elongation

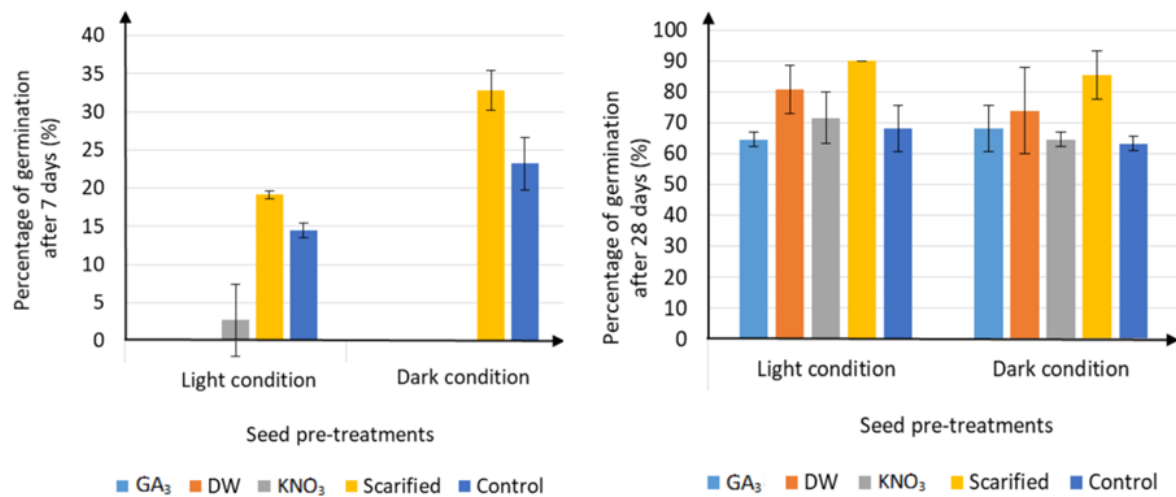


Figure 1: Percentage of germination (%) of *M. longifolia* seeds a. After seven days and b. After 28 days (data plotted under light and dark conditions). Standard deviation calculated at $p < 0.05$ significance level (Duncan multiple range test)



Figure 2: Seed germination of *M. longifolia* under dark condition ex vitro a. Seeds starting emerging radicles (after seven days) and b. Root growth continuing (after 14 days), bars 1.0 cm

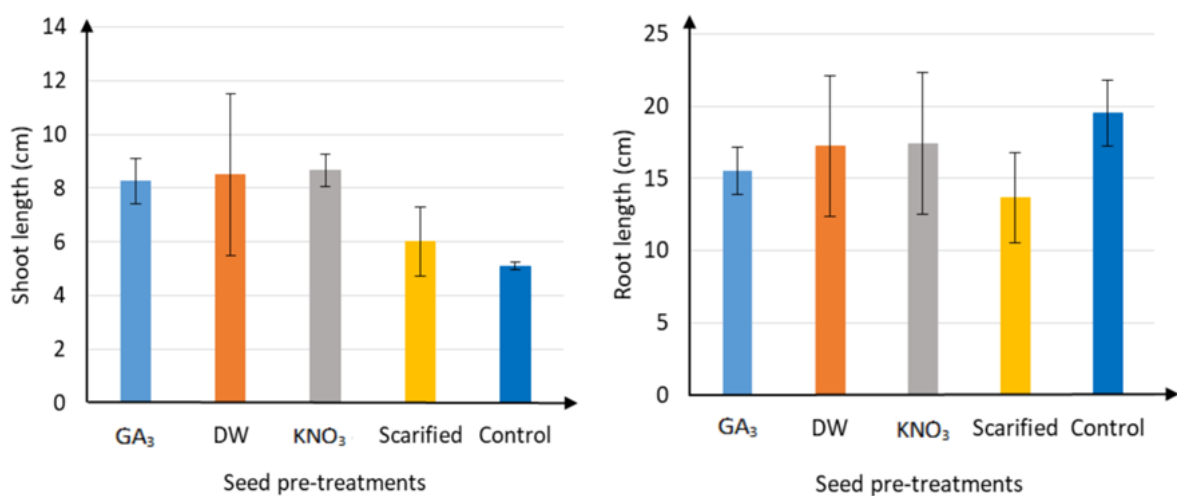


Figure 3: Seedling growth of *M. longifolia* after different pre-treatments a. Shoot length and b. Root length of ex vitro grown seedlings after 28 days. Standard deviation calculated at $p < 0.05$ significance level (Duncan multiple range test)

In-vitro germination of *M. longifolia*

Seed germination commenced within the first seven days, with browning observed in some cultures after two days. After the first subculture, browning was not observed in the media. However, the volume of the media decreased over time due to uptake by the seeds for their growth. The media incorporating BAP showed a higher germination percentage, indicating its positive influence. Among the four investigated concentrations (Table 1), higher amounts of BAP (0.5 and 1.0 mg/L) significantly accelerated germination compared to the two lower concentrations (0.0 and 0.2 mg/L). Therefore, considering hormone consumption, the treatment with the lowest concentration of BAP (0.5 mg/L) was identified as the most effective for stimulating the germination of *M. longifolia* seeds (Table 1). Results on rooting were recorded after 14 days without further subculturing. Additionally, shoot elongation was comparatively slower than root elongation. Consequently, the data on shoot elongation was not evaluated. Rooting of *M.*

longifolia was enhanced by lower levels of BAP (0-0.5 mg/L), while higher concentrations reduced the effect (1.0 mg/L). Table 1 demonstrates the significant effect of BAP on root growth. The highest elongation, measured as 0.7 ± 0.1 cm, was recorded in $\frac{1}{2}$ MS media without BAP, while $\frac{1}{2}$ MS with 0.2 mg/L and 0.5 mg/L indicated significantly higher results than the media with 1.0 mg/L BAP.

The germination of scarified *M. longifolia* seeds grown under 16 hours of light under *ex vitro* conditions resulted in a higher germination percentage (90.00%) compared to all other treatments; however, the difference was not statistically significant at $p < 0.05$. The findings of this study demonstrate that the seed coat can act as a barrier for the initiation of germination by preventing radical protrusion. Additionally, other treatments such as GA_3 , KNO_3 , and DW stimulated the germination of intact seeds regardless of the presence of the seed coat and the light conditions (Baskin and Baskin, 1998; Baskin et al., 2004).

Table 1. Effect of BAP with $\frac{1}{2}$ MS media on seed germination and mean root length of *M. longifolia*

BAP concentration (mg/L)	Mean % of germination	The mean length of roots after 14 days (cm)
0.0 (control)	55.07 ± 9.33^b	0.7 ± 0.1^a
0.2	48.84 ± 3.33^b	0.7 ± 0.0^a
0.5	77.71 ± 10.64^a	0.6 ± 0.1^a
1.0	83.85 ± 10.65^a	0.4 ± 0.0^b

Values represent the means $\pm$ SE of 3 replicates with 30 seeds. Values followed by the same letter are not significantly different at $p \leq 0.05$ (one-way ANOVA, Duncan's Multiple Range Test).



Figure 1: In vitro germination of *M. longifolia* seeds; a. Matured fruits, b. De-coated seeds, c. Seeds growing in liquid media on shaker and d. Seedling growth after 28 days, bars 1.0 cm

Breaking dormancy due to the seed coat

In the present study, *M. longifolia* seeds exhibited a preference for germination under dark conditions during the initial stages as opposed to light conditions. Kendrick (1976) explained that light is mandatory for germination in some species (e.g., *Tillaea aquatica* L.), while it can inhibit germination in others (e.g., *Acanthostachys strobilacea*) (Mclemore, 1967).

Furthermore, previous researchers have used pre-treatments to overcome the physical dormancy imposed by the seed coat. Athugala et al. (2021) conducted experiments on the dormancy of tropical montane forest species in Sri Lanka and highlighted the prominence of seed coat-imposed dormancy. For example, *Atylosia rugosa* and *Crotalaria walkeri* have seed coat dormancy that can be overcome by mechanical scarification followed by water imbibition. In the present study, *M. longifolia* seeds demonstrated early germination within the first seven days and reached a maximum germination of 90.00% after 28 days. The most notable results were observed in scarified seeds, indicating the release of the barrier effect of the seed coat on germination. KNO_3 is commonly used as a neutral solution and an oxidizer to break the physical dormancy of seeds. For instance, KNO_3 combined with GA_3 resulted in the highest germination of physically dormant *Cordia sinensis* Lam. seeds (Dev et al., 2020) and *Alstromeria ligtu* hybrid seeds (Nasri et al., 2014).

The present findings align with Dayananda and Jayasuriya (2019), who observed that the application of GA_3 , warm stratification, or manual scarification was necessary to enhance the germination of *M. longifolia* seeds, contradicting the results reported by Kundu et al. (2013), which stated that *M. longifolia* seeds do not exhibit any dormancy. Based on the evaluated results, *M. longifolia* seeds did not show a significant difference in germination under the two light conditions after 28 days. However, it confirmed the presence of imposed physical dormancy by showing a significant difference between the control and scarified seeds in the early and late stages of germination (Figures 1a and

1b). According to Hudson et al. (2015), seed recruitment can depend on the maternal environment and have ecological consequences. Therefore, contrasting results can be explained as a consequence of these factors.

According to Kundu et al. (2013), the seed viability of *M. longifolia* decreases over time. Therefore, long-term storage is not recommended due to their recalcitrant nature. Seeds lose viability when the moisture content is below 35%, and they are sensitive to chilling temperatures ($<15^\circ\text{C}$), which can damage the seeds. Under optimal conditions of 28°C and a moisture content of 40–41%, the seeds can maintain viability for five months (Kundu et al., 2013). However, due to their higher fat content (Munasinghe and Wansapala, 2015), the seeds are more susceptible to microbial attacks and deterioration. In field conditions, maintaining appropriate moisture levels and preventing contamination are major challenges in preserving seed viability. Furthermore, frugivorous animals can impact seed dispersal, sometimes breaking seed dormancy or damaging the seeds, which hinders germination (Hulme, 2002). The sweet nature of the burry attracts bats (*Pteropus giganteus* and *Cynopterus sphinx*), insects, and other microbes, affecting the longevity of seeds on the ground (Raghuram et al., 2011; David et al., 2015). Additionally, Karthiyayini (2017) explained that seed size in *M. longifolia* has an effect on seed germination, with medium-sized and large-sized seeds displaying a higher propensity to germinate.

The effect of pretreatments on plant growth parameters

The pretreatments significantly influenced the shoot growth of *ex vitro* germinated seedlings but not the root growth. Scarified seeds exhibited lower shoot length (6.02 cm), while rooting was superior compared to all other treatments. GA_3 , KNO_3 , and DW-treated seeds displayed the best shoot growth, reaching approximately 8.5 cm after 56 days. However, all experiments showed significantly higher results for root growth compared to the control. It is an inherent

characteristic of *M. longifolia* to develop a well-established, delicate, and fast-growing taproot (Kundu et al., 2013). Therefore, maintaining the plant inside a polybag for an extended period poses a challenge. Thus, it is crucial to determine the appropriate size of a compatible polybag.

***In vitro* germination of *M. longifolia* seeds**

In vitro germination has occasionally been employed for forest tree species to produce healthy explants for rapid *in vitro* regeneration programs. Explants from *in vitro*-grown plants are free from explant-derived contaminants and exhibit a higher rate of regeneration (Bramhanapalli et al., 2017). Furthermore, seeds of outcrossed plant species possess genetically heterogeneous characteristics compared to the mother plant, which is advantageous in regeneration programs such as reforestation and plantations where genetic variation is desired.

The incorporation of BAP along with the basal media ($\frac{1}{2}$ MS) enhanced the germination of *M. longifolia* seeds. It has been previously applied to several woody plant species for the production of plant materials through *in vitro* propagation. Pijut et al. (2012) reviewed the *in vitro* germination of tropical hardwood tree species and discussed the use of different plant growth regulators in seed germination as well as plant growth and development. The germination percentage of $\frac{1}{2}$ MS with 1.0 mg/L BAP (83.9%) and $\frac{1}{2}$ MS with 0.5 mg/L BAP (77.7%) was higher compared to media without any growth hormone. Contrary to previous studies, most species performed better in hormone-less media than with cytokines or auxins. For example, *Pterocarpus marsupium* (Tiwari et al., 2004) and *Swietenia macrophylla* (Collado et al., 2005) exhibited stimulated germination under $\frac{1}{2}$ MS media without plant growth hormones. In some species, BAP (0.2–0.5 mg/L) was beneficial for *in vitro* germination, such as *Citrus reticulata* (Hassanein and Azooz, 2003), *Carica papaya* L. (Bhattacharya and Khuspe, 2001), and *Santalum album* L. (Peeris and Senarath, 2015). The present results are consistent with previous experiments. Seeds with large food reserves do not require higher nutrient

content as they become autotrophic, and excessive nutrient concentrations can even be toxic to the plant. Therefore, low concentrations of BAP or reduced salt concentrations can be recommended for *in vitro* germination of *M. longifolia* seeds. It is worth noting that researchers have also utilized other growth hormones, such as gibberellins and auxins, in addition to cytokinins, to enhance germination (Pant et al., 2011; Mishra et al., 2018; Adhikari and Pant, 2019; Liyanage et al., 2020).

Constraints in *in vitro* germination of seeds

The strong inhibitory effect of the seed coat on seed germination of *M. longifolia* may be caused by several mechanisms, such as mechanical constraints that prevent water and mineral uptake or the retention of chemical inhibitors that could restrict germination (Fang et al., 2006). Liyanage et al. (2020) addressed this effect in some species belonging to the *Acronychia* genus in two ways: by de-coating or completely removing the seed coat (40–47% germination), and scarifying at the point where the radical emerges (>65% germination). This demonstrates that under *in vitro* conditions, the mechanical removal of the barrier should enhance the emergence of the radical.

It was evident that the seed coat of *M. longifolia* contained polyphenol compounds that could be toxic to the plant itself, as it showed browning in the liquid medium during the initial stages. The removal of the seed coat resulted in a slight brown color, which later recovered after subculturing. The success of *in vitro* germination experiments depends on selecting seeds at the correct maturity stage, practicing well-established sterilization techniques, and maintaining proper aseptic conditions with frequent subculturing (Murkute and Patil, 2003).

Breaking, softening, or completely removing the integument under *in vitro* conditions eliminates physical dormancy and helps eliminate toxic compounds derived from the seed coat (Fang et al., 2006). In fact, Tao et al. (2007), Ahmad et al. (2013), Jones and Saxena (2013), and several other researchers

have recommended pretreatments such as frequent subculturing, the use of polyvinylpyrrolidone (PVP), adsorbents (activated charcoal), or antioxidants such as ascorbic acid, cysteine, and silver nitrate to rescue the explants. Additionally, changing the light conditions to reduce the initial metabolic rate and frequent subculturing can alter the rate of browning (Soliva et al., 2000; Xu et al., 2011).

Avoiding contamination is crucial in in vitro culturing since it is necessary to maintain aseptic conditions throughout the entire process. To prevent seed destruction by the factors mentioned above, seeds should be harvested immediately at their ripe stage without any resting period on the ground.

Studies related to the germination of forest tree species mainly target reforestation programs (Koutouan-Kontchoi et al., 2020) and aim to enhance the production of species that face a risk of extinction in the wild due to poor germination and overexploitation. The present study confirms the presence of physical dormancy associated with *M. longifolia* seeds and the use of scarification as an initial step to overcome it. *In vitro* germination is less effective compared to *ex vitro* germination since it requires additional steps to acclimatize plants to the natural environment. Moreover, it is costly and challenging to grow under in vitro conditions due to the plant's higher growth rate and large seed size. However, it is beneficial when using these plants to obtain planting materials for plant tissue culture purposes.

CONCLUSIONS

In *M. longifolia*, pre-treatments significantly affect seed germination and subsequent seedling growth. The hard seed coat imposes mechanical restrictions, causing physical dormancy and hindering germination. At the initial stage, germination is favored in dark conditions. However, treating the seeds with solutions of GA₃, KNO₃, and DW enhances shoot growth, producing well-grown plants suitable for reforestation programs. The concentration of BAP also plays a role in seed germination under in vitro conditions. Among the tested concentrations, 0.5 mg/L BAP with

½ MS medium showed the highest germination rate. Although in vitro germination of *M. longifolia* seeds is less efficient compared to *ex vitro* germination, it can be considered a preliminary step before applying improved techniques in plant tissue culture. This approach allows for the production of healthy plants in larger quantities from a single seed, considering its cost-effectiveness and efficiency.

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