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Conventional Vegetative Propagation Studies in Madhuca Diplostemon (C.B.Clarke) P. Royen (Sapotaceae): A Critically Endangered and Endemic Iconic Tree of Kerala, Sw. India

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Abstract

Preliminary vegetative propagation trials were carried out as part of the ex-situ conservation of Madhuca diplostemon, a critically endangered and endemic tree of Kerala (south-west India). Being a lone species seen only in a single fragile locality, four conventional propagation techniques viz. stem cuttings, air layering, softwood grafting, and approach grafting with nursery-raised seedlings were undertaken to save the species from extinction. Stem cuttings treated with the gel of Aloe vera achieved 100% rooting, compared to 70% in controls, and rooted earlier. Air layering was a complete success, yielding well-developed roots within 100 days in control. Both softwood and approach grafting into Madhuca neriifolia rootstocks yielded complete success. The study highlights the potential of vegetative propagation as an effective means for ex-situ conservation and mass multiplication of this imperilled species.

Keywords: Vegetative Propagation, Stem Cuttings, Air Layering, Softwood Grafting, Approach Grafting

INTRODUCTION

Madhuca diplostemon (C.B. Clarke) P. Royen (Sapotaceae) is a critically endangered and endemic tree of Kerala (S.W. India). The species was first described by Robert Wight in 1848, but since then no authentic information was available regarding its precise locality or existence in the wild until its rediscovery by Shailajakumari et al. (2020), after a lapse of 184 years. At present, the species is represented by a single mature tree located in front of the Ayiravilli Siva Temple near Paravur, Kollam district, Kerala. This tree is revered as sacred by the temple authorities, with two subsidiary deities enshrined beneath it, making it an integral part of local belief and culture. As part of it, the collection of seeds or other propagules directly from the tree were not permitted for a long time.

However, a few seedlings were successfully raised from fallen fruits collected from the vicinity of the tree during 2022–2024 and are now conserved at the Threatened Species Park of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram. Preliminary studies revealed that the seeds of M. diplostemon are recalcitrant in nature, and the germination trials showed 70% success in untreated controls and 80% success with hot-water treatment (Sreekala et al., 2023). In

addition, a few 1–2-year-old seedlings were collected from the vicinity of the parent tree. Currently, 25 different aged seedlings are being maintained, but this number is insufficient to support ex-situ conservation and restoration programmes. In this context, conventional vegetative propagation studies were undertaken using these nursery-raised seedlings to facilitate large-scale multiplication.

Materials and methods

Different conventional vegetative propagation experiments such as stem cuttings, air layering and grafting were conducted during 2022–2024 using nursery-raised seedlings of Madhuca diplostemon conserved at the Threatened Species Park, JNTBGRI, Palode, Thiruvananthapuram.

Experiment 1.1 Stem cutting propagation

The seedlings of M. diplostemon are slow-growing and are usually producing small sucker shoots from the base of the seedlings, after 2–3 years of growth. These basal shoots were selected for the present study. Lateral shoots measuring 8–10 cm in length and 0.2–0.3 cm in diameter were treated with fresh *Aloe vera* gel and planted in pots containing a potting mixture of river sand, topsoil, and cow dung manure in a 2:1:1 ratio. The treated cuttings were maintained in a mist house equipped with an intermittent misting system, where the temperature ranged between 23–28 °C and the relative humidity of 80–90%. A total of seven cuttings were used in this trial, along with a control set. The experiment was conducted during June 2023, and data on rooting parameters such as percentage of rooting, number and length of roots, and number of secondary roots were recorded after 70 days of planting.

Experiment 1. 2. Air layering

Air layering was carried out on four vigorously growing seedlings raised in the nursery during the onset of the southwest monsoon in June 2023. A ring of bark measuring 1.5–2.0 cm in width was carefully removed with a sharp knife, about 20 cm below the shoot tip. The girdled portions were then enclosed in white polythene sleeves filled with a potting mixture consisting of coco peat, river sand, topsoil, and dried cow dung manure in a 2:1:1:1 ratio. The ends of the sleeves were tightly secured with thread to prevent desiccation of the rooting medium. The layered shoots were regularly monitored for root initiation without disturbing the plastic covering.

Experiment 1. 3. Softwood grafting

Softwood grafting of M.diplostemon was carried out onto 10–15-month-old rootstocks of M. neriifolia (Moon) H. J. Lam, a widely distributed riparian species in India and Sri Lanka. Seedlings of M. neriifolia were collected from the vicinity of naturally grown mother trees within the Jawaharlal Nehru Tropical Botanic Garden and Research Institute campus, transferred to polythene bags, and maintained under mist house conditions for proper establishment. Once the seedlings reached 10–12 months of age, they were selected as rootstocks for grafting. A total of 10 seedlings were used in the grafting trial.

Scions were collected from three-year-old nursery-raised seedlings of M. diplostemon. These scions were 10–15 cm long and 0.5–1.0 cm in diameter. Leaves were removed from the scions, and a wedge-shaped cut of about 5 cm was made at their basal portion. The rootstocks were headed back at 12–15 cm above the polythene bag, and their leaves were removed, leaving only 2–3 leaves on the lower side. A downward vertical slit (3–5 cm) was then made at the central portion of the rootstock with a sharp knife.

The prepared scions were carefully inserted into the slit of the rootstock, and the graft union was tied firmly using a 1–1.5 cm wide polythene strip (100 gauge). The scions were covered with a white polythene bag and tied with thread to maintain freshness until union formation. The cover was removed after sprouting. Grafting was carried out on five rootstocks of M. neriifolia, and scion sprouting was monitored.

Experiment 1.4. Approach grafting

Approach grafting of M. diplostemon was carried out onto the rootstocks of M. neriifolia, measuring 30–35 cm in height and about 0.8 cm in diameter, during February 2023. Scion branches from 2–3-year-old nursery-raised M. diplostemon seedlings were used for grafting. A longitudinal cut (20–25 cm below the shoot tip) was made on one side of each scion branch with a sharp knife to expose the cambium. A corresponding cut, 5–6 cm in length and about 0.5 cm in width, was made on one side of the M. neriifolia rootstocks, about 8–10 cm above the soil level, to expose the matching cambial region. The cut surfaces were then carefully joined and bound tightly with 1.0–1.5 cm wide plastic strips, ensuring close contact between the cambial layers and avoiding gaps at the union.

The grafting trial was conducted on only five seedlings of M. neriifolia owing to the limited availability of branches. The grafted seedlings were watered daily except on rainy days. About one month after grafting, a light "V"-shaped cut was made below the graft union on the scion and above the graft union on the stock at monthly intervals to promote vascular connection. Final detachment of the graft was carried out 100 days after grafting by completely severing the top of the stock above the graft union and the scion below the graft union. The detached grafted plants were kept under shade for a few weeks to facilitate establishment. Any new outgrowths arising from the base of the rootstocks were also removed periodically.

Results and discussion

Conventional clonal propagation techniques in M. diplostemon yielded positive results, confirming that clonal saplings of this critically endangered species can be successfully and efficiently produced from juvenile plants through stem cutting, air layering, and grafting. The results of which are discussed in detail below.

Stem cuttings planted in potting medium were evaluated for the rooting performance after 70 days of planting in polybags by recording the number, length, and diameter of primary roots, along with the number of lateral roots (Table 1). Analysis revealed that stem cuttings treated with *Aloe vera* gel achieved 100% rooting success (Figure 1), whereas the control set achieved only 70% success (Figure 2). The remaining 30% of the control cuttings also developed callus tissue without root formation.

Table 1. Rooting Performance of M. diplostemon Stem Cuttings After 70 Days of Planting

	Cuttings in control (mean)	**Cuttings treated with Aloe vera gel (mean)**
Percentage of success	70	100
Number of primary roots	2.5 ± 1.37	4.57 ± 2.63
Length of primary roots	2.88 ± 1.94 cm	4.21 ± 1.84 cm
Diameter of primary roots	1.45 ± 0.44 cm	1.62 ± 0.61cm
Number of lateral roots	10.83 ± 7.57	11.27 ± 8.41
Survival percentage	100	100

Figure 1. Stem Cuttings Treated with Aloe vera Gel

Figure 2. Stem Cuttings in Control

Stem cuttings treated with *Aloe vera* gel consistently produced higher percentage in the number, length, and diameter of primary roots, as well as lateral root formation, compared to that of the control experiment. Rooted cuttings were successfully transplanted into polythene bags containing a sand, soil, and dried cow dung manure mixture (1:2:1) and maintained in a mist house for further establishment.

Interestingly, 30% of control cuttings that initially developed only callus also produced roots when replanted in the same medium and maintained for an additional 50 days. Thus, *Aloe vera* gel-treated cuttings required an average of 70 days for sufficient rooting, whereas control cuttings required 70–120 days for complete root formation. These results confirm the effectiveness of *Aloe vera* gel as a natural rooting enhancer for M. diplostemon (Figure 1A & B).

Aloe vera leaf gel is increasingly recognized as a natural and eco-friendly alternative to synthetic plant growth regulators. It is cost-effective and contains phytohormones such as auxins and gibberellins, along with root-promoting compounds like salicylic acid (Surjushe et al., 2008). Its efficacy in enhancing root induction has been demonstrated in *Citrus aurantifolia* (Mirihagalla & Fernando, 2020), *Lippia javanica* (Zhakata & Abduel, 2021) and Camellia sinensis (Vidanapathirana et al., 2023). The present success with M. diplostemon is consistent with these earlier findings and further reinforces the potential of *Aloe vera* gel as a natural rooting enhancer in woody plant cuttings.

Air layering in M. diplostemon was found to be highly effective, with all layers successfully producing roots without the application of root-promoting agents. Root proliferation beyond the potting mixture was observed 70–80 days after layering and the layers were detached from the mother plant after 100 days, by which time the potting mixture ball was completely covered with adventitious roots that had turned brown

in colour (Figure 3). The polythene film was carefully removed without damaging the roots and recorded the average in number of roots (5-6), length (7.8±3.2 cm) and its diameter (1.54±o.52 cm). The rooted layers were then planted in containers and were kept under shade for a few weeks to facilitate proper establishment of the root system. These results clearly indicate that the application of external rooting promoters is not required for producing layered plants of M. diplostemon.

Softwood grafting of M. diplostemon onto rootstocks of M. neriifolia proved effective. Scion sprouting occurred within 20–25 days of grafting. The polythene covers were removed one week after sprouting, and grafts were maintained under partial shade for two weeks. Out of the 10 grafted seedlings, 8 scions sprouted, and 6 survived successfully (Figure 4).

Figure 3. Madhuca diplostemon Air layers

Figure 4. Graft plants raised through softwood grafting

Approach grafts of M. diplostemon were detached from the parent plant 110 days after grafting and subsequently maintained in partial shade for two weeks. 100% success was achieved in approach grafting, and the independent grafts produced new leaves during the following season (Figure 4).

The success of grafting largely depends on the establishment of a compatible union between scion and rootstock. Grafting is generally feasible among closely related varieties of the same species, species within a genus, or even occasionally between species of different genera. However, compatibility is not universal within a genus. The successful softwood and approach grafting of M. diplostemon onto M. neriifolia rootstocks demonstrates effective interspecific compatibility within the genus *Madhuca*.

The interspecific grafting of M. diplostemon onto M. neriifolia rootstocks demonstrates strong compatibility between the two species, resulting in a stable vascular union and long-term survival. This finding is consistent with earlier reports, such as the grafting of *Garcinia indica* onto G. tinctoria, G. hombroniana, and G. cowa (Mathew et al., 2004), as well as G. mangostana onto G. hombroniana (John et al., 2008). Comparable grafting successes have also been documented in other genera, including Manilkara zapota onto M. hexandra and M. kauki (Sulladmath & Reddy, 1985) and *Coffea arabica* onto C. canephora (Koutouleas et al., 2004). Conservation-focused interspecific grafting experiments include Garcinia wightii onto G. hombroniana (Shareef, 2012) and Ixora polyantha onto I. finlaysoniana (Shareef & Mathew, 2011). Similarly, successful grafting has been reported in Sophora toromiro (Phil.) Skottsb. onto S. cassioides (Phil.) Sparre (Espejo et al., 2013). More recently, interspecific grafting success has been reported in Pinus, including P. rzedowskii onto P. pinceana (Solorio-Barragán et al., 2021) and P. patula onto P. teocote (González-Jiménez et al., 2024), further highlighting the potential of this technique for species conservation.

Figure 5. Approach graft of Madhuca diplostemon

Conclusion

M. diplostemon is represented globally by a single surviving mature tree, making it an iconic yet highly imperilled species of Kerala. This lone individual continues to face severe threats from both natural and anthropogenic pressures, while religious sentiments associated with the tree have further restricted detailed phenological studies, thereby limiting the development of effective conservation strategies. Recognizing this urgency, JNTBGRI incorporated M. diplostemon into its species recovery programme. Seeds were collected from the type locality, and conventional vegetative propagation methods were initiated using seedlings maintained in the field conservatory. The present study demonstrates that vegetative propagation offers a practical and effective strategy for the multiplication of M. diplostemon, thereby supporting ex situ conservation and restoration programmes. Among the methods tested, air layering, softwood grafting, and approach grafting were highly successful, though comparatively time-consuming and labour-intensive. In contrast, stem cuttings treated with *Aloe vera* gel proved to be the most efficient and reliable method for large-scale multiplication, offering a cost-effective and eco-friendly alternative for propagation.

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