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# Propagation and seedling establishment of selected woody species of southern Western Ghats, India

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**Abstract**
Cultivation of threatened woody species with the goal of conserving them is hampered by little or no, information on how such species can be propagated or their seedlings established. We investigated in Rapinat Herbarium Conservatory propagation and seedling establishment of 10 endemic, threatened medicinal and multipurpose species much valued for traditional medicine in Western Ghats, India. The experiments included sexual and vegetative propagation, as well as seedling growth experiment. The multipurpose species *Hydnocarpus pentandrus, Hydnocarpus alpinus, Michelia champaca, Syzigium cumini* were easily propagated from seeds, and *Toddalia asiatica* from cuttings. The medicinal species *Rhododendron arboretum* did not germinate at all. *Elaeocarpus recurvatus, Hydnocarpus mcrocarpus and Toddalia asiatica* had high dormancy and low synchrony of seed emergence. *Syzigium* spp. seedlings established readily with addition of fertilizer (NPK) and moderate watering (three times a week). We conclude that the conservation efforts of the conservatory Rapinat Herbarium Trichy (RHT) one aiming at those taxa with imminent depletion of exploitation could be effectively target to other agencies of conservation.

**Keywords:** *Hydnocarpus* and *Syzigium* species, germination, seedling performance and re-afforestation

## Introduction

The Western Ghats is one of the renowned mega diversity centers of India. Being on the threshold of development and with increased anthropogenic pressure leading to depletion of much of its prime forests and unique habitats, the whole area has already been listed as one of the world's 'hottest hotspot' areas (Myers, 1988, Myers *et al.*, 2000) [10]. Nearly 40 % of natural forest vegetation in the Western Ghats has disappeared during the past 8-10 decades (Menon & Bawa, 1997) [11].

Conservation adopts propagation technique employing both by sexual or vegetative methods; sexual propagation by seed maintains genetic diversity and is also cheaper than asexual techniques (Hartmann *et al.* 2011) [2]. However, dependence on seed may not be the best solution from the farmer's point of view as seed progeny can be highly variable and also could have long emergence periods causing time delay. Vegetative propagation, on the other hand, may lead to low genetic variability but yields offspring that are highly precocious and thereby enable farmers to access products within a short timescale (Akinnifesi *et al.*, 2008; Hartmann *et al.*, 2011; Asaah *et al.*, 2012) [1, 2].

The Rapinat Herbarium in Trichy is an internationally recognized herbarium in the state of Tamilnadu with a holding of 250,000 herbarium specimens which also has a botanical garden in the palni hills The RHT also has established two conservatories with 296 species for rearing the rare, endangered and endemic plants.

On plants which are economically valuable and hence face increased threat from anthropogenic impact. This study was conducted to determine the propagation and seedling establishment of selected, highly valued medicinal and woody species. The results and protocols evolved could benefit other conservation agencies.

## Materials and Methods

Two propagation experiments and one seedling growth experiment were carried out at the conservatory in RHT, Trichy. The study comprised the following experiments: sexual propagation using seeds, vegetative propagation using leafy stem cuttings and seedling growth using results from seed propagation. Details of the 10 species investigated are given below in Table 1

**Table 1:** Woody medicinal species included in the experiments are (a) seed germplasm and (b) leafy stem cuttings. The dates of collection and areas of origin of the germplasm are shown in

| Species with voucher no | Local name | Date of collection | Locality |
|---|---|---|---|
| Seeds | | | . |
| *Hydnocarpus pentandrus* RHT68298 | Maravetti, Maravattai, Marotti | 10.11.2020 | Guruvayoor and Mala, Thrissur district, Kerala |
| *Hydnocarpus macrocarpus* RHT68237 & RHT68300 | Maravattai, Maravetti, Neeradimuthu Yetti. | 03.04.2020 | Idukki district, Kerala. |
| *Hydnocarpus alpinus* RHT68299 | Maravetti Attuchankalai,Koranguthalai, | 22.12.2020 | Halakkarai,Kotagiri road, Coonoor, Nilgiris district. |
| *Syzigium cumini* BSTB 151 | kottai-nakam, naval | late May, June and July 2020 | Palni hills, Blackburne Forest |
| *Syzigium densiflorum* | Naval | May and June 2020 | Bombay Shola; Common in most |

| | | | |
|---|---|---|---|
| RHT68132 | | | shola, Palni hills. |
| *Rauvolfia densiflora* RHT68876 | Sarppaganti | Winter months 2020 | Shembaganur College (arboretum) Pambar Shola |
| *Elaeocarpus recurvatus* BSTB 21& 121* | Rudraksham, | August-October.2020 | Bombay Shola, Palni hills. |
| Cutting | | | |
| *Michelia champaca* BSTB 124* | Sambagan | September to October 2021 | Velagevi. Old planted trees in Cardamum Estates |
| *Rhododendron arboretum* nilagiricum BSTB 54 | Puvaracu, Billi | April to May 2021 | Gundarvally & ridge paths, Palni hills. |
| *Toddali aasiatica, Floribundra* BSTB 50* | kattu-milaku | December-February. 2021 | Pambar Shola (common in all Sholas), Palni hills. |

**Sexual propagation experiment**

Seeds were collected from the upper ranges of Palni hills beyond Kodaikanal Township and in the State of Kerala with the help of traditional practitioners. Some seeds with a tough outer coating proved hard for a growing root to break through. For instance, *Hydnocarpus* seed being hard, had to be scarified to turn seed coat into a soft and penetrable area for root development. Scraping the outer shell was also dealt with the use of sand paper or a nail file; some seeds were perforated slightly with a sharp knife.

*Toddalia asiatica* was dipped in hot water at 90°C prior to soaking for 24 hours. An equal number of seeds of each species were treated as a control, with no pre-sowing treatment. The number of seeds sown for each species depended on the number of seeds available at the time of sowing.

Seeds were sown in a mixture of sand and black soil in a ratio of 1:1 in a raised bed (10 ×1 m) under a shade net (73% screening factor). Although germination is defined as the emergence of the embryonic root (the radicle), in germination trials, the emergence of radicle through seed coverings is difficult to observe for buried seeds and the emergence of the embryonic shoot (plumule) is usually taken to indicate germination (Forest Restoration Research Unit 2008). Therefore, in this experiment a seed was considered to have germinated upon the appearance of the plumule.

Seedlings were taken out of the seedbed 3 weeks after germination, and transplanted into polythene pots (3 inch diameter) containing a medium of a mixture of black soil and sand in a ratio of 3:1 and observed for growth for 97 days, still within the shade net. Seedlings were watered twice daily, in the morning and in the evening. Later, they were transferred from the shade net, hardened by exposing to gradually increasing sunlight and watered once a day for 14 days. Seed germination was recorded on a daily basis until no further germination was observed. The germination experiment was stopped when no new seeds had germinated for a consecutive 20-day period (Eyog-Matig *et al.,* 2007) [7].

**Vegetative propagation experiment**

The potential for asexual propagation was investigated by conducting a vegetative propagation experiment with stem cuttings of *Hydnocarpus pentandrus, Toddalia asiatica* and *Rhododendron arboretum*. Cuttings were propagated in trays (100 × 50 × 15 cm), raised 10 cm from the ground. The sowing medium comprised a base layer of sieved lake sand, a middle layer of gravel and a top layer of sieved lake sand. *T. asiatica* and *H. pentandrus were* sown in a top layer of a mixture of lake sand and black soil in a ratio of 1:1. The experiment was conducted in a humidity tunnel (120 × 90 × 230 cm), placed under a shade net with 73% screening factor. A 1000-gauge transparent polythene sheet was used for the tunnel cover. Cuttings (8–18 cm) were collected from stock plants in the morning (6.00 am) and kept in a cooling box to conserve moisture. They were planted the next day in the evening. Two or three leaves were cut half way and left on the cuttings. Twenty stem cuttings each for *T. asiatica* and *H. pentandrus* were planted in each treatment. Bases were squared using secateurs to avoid one sided rooting. Bases of half of the cuttings were treated by dipping in the rooting hormone (Seradix‘2′ 0.8% IBA) for 5 seconds before setting in the planting medium to facilitate adventitious root formation. The remaining half acted as a control was not treated with hormone. Allocation of the cuttings to treatment was random. Excess of the rooting hormone was tapped off before planting in pre-wetted rooting substrate to a depth of 2 cm and at a spacing of 15 cm. The substrate was compacted around the base of the cutting for support. The fungicide (Agro-laxyl MZ 63.5 WP; Asiatic Agricultural Industries, Singapore) was used to control fungal infection. An inspection was carried out every third day to remove fallen and infected leaves. Every $15^{th}$ day the number of calls used cuttings, rooted cuttings, roots per cutting, shoots, root length, dormant cuttings and degree of fungal infection was recorded. The experiment was terminated after 45 days from the time of planting.

**Seedling growth experiment**

The seedling growth experiment was carried out on *H. pentandrus* only. The seedlings were 8 weeks old at the time of the experiment and 80 seedlings of similar height were selected for the experiment. Half of the seedlings were treated with NPK (nitrogen, phosphorous, potassium mix) and the second half acted as a control and was not treated with NPK. Equal numbers of seedlings (n = 60) were subjected to either of three watering treatments: watering daily, three times a week or twice a week. Collar diameter and height (cm) of all seedlings were measured at the beginning of the experiment and every 20 days thereafter for 80 days (i.e. four times). Data analysis the propagation potential from seed of each species was evaluated by determining its germination percentage (GP), mean length of seedling emergence time (MLE) the number of days when 50% of the seeds have germinated–taken to be the measure of dormancy – and synchrony of germination. GP is considered high when 60% or more of the seeds germinate and low if less than 20% of the seeds germinate. The MLE is considered short if 50% of the seeds germinate within 21 days or less and long if they take 84 days or more. Germination is synchronous if all seedlings of a species emerge within 21 days, and highly asynchronous if they take more than 84 days (Blakesley *et al.,* 2002) [4]. The

Mann–Whitney U test was used to test for difference between GPs and mean length between treated and untreated seeds; the ANOVA test was used for differences between means of attributes of *H. pentandrus* seedlings; and the t-test was used for differences between means of lengths of roots developed on *R. arboretum*. All statistical tests were conducted using SPSS software 16 (SPSS Inc., Chicago, IL, USA).

## Results

### Germination experiment

Germination for all species in the study occurred between 2 and 48 days, except for *R. densiflora* and *R. arboretum* which failed to germinate in all treatments. Treatment by dipping seeds in either cool or hot water had no significant effect on the GP of all the species except *T. asiatica* (Mann–Whitney U; p > 0.05; Table 2). *T. asiatica* pre-treated with hot water failed to germinate and only those treated with cool water were germinated. Species with the highest percentage of germination in control and treated seeds, having low dormancy and with synchronous or intermediate synchronous germination were *H. macrocarpus, M. champaca, E. recurvatus* and *R. arboretum* (Table 2).

Stored seeds of *H. macrocarpus* had lower GP and longer dormancy compared to freshly harvested seeds. All other species had low GPs and were asynchronous. *T. asiatica* was highly asynchronous. *T. asiatica* and of *H. pentandrus* was still germinating one year after sowing. Vegetative propagation experiment *M. champaca and R. arboretum* cuttings did not sprout. *H. pentandrus* formed roots15 days after setting. More roots were observed on *H. pentandrus* and *T. asiatica* cuttings treated with the growth hormone than in untreated cuttings, but the mean root length was similar between treated and untreated cuttings and very shoots were formed. *T. asiatica* cuttings were prone to fungal attack and measurers were taken towards off infection. Almost all *T. asiatica* and *H. pentandrus* cuttings formed roots and shoots. Roots were significantly longer in untreated cuttingsthan in cuttings treated with rooting hormone (t-test0.144, p < 0.05). Shoots in treated cuttings were longer than those which were not treated, but the difference was not statistically significant. Overall, it would appear that treatment with growth hormone does not confer any advantage for rooting and sprouting in *T. asiatica* (Table 3). *H. pentandrus* seedling growth experiment treatment with NPK led to a significant increase both in the diameter and height of *H. pentandrus* (p < 0.05). Watering thrice a week was more effective at increasing diameter (p < 0.05), but had no effect on height (Figure 1).

**Table 2:** Germination trends in selected species. For details of the treatment see foot note of in table 1.

| Species | GP% | | MLE Days | | Synchrony of germination | | Germination and synchrony category | | Treatments |
|---|---|---|---|---|---|---|---|---|---|
| | T | C | T | C | T | C | T | C | |
| *H. pentandrus* | 30 | 35 | 20 | 21 | 32 | 28 | RG/LD/IS | RG/LD/IS | T3 |
| *H. alpinus* | 13 | 14 | 15 | 18 | 40 | 43 | LG/ID/IS | RG/ID/IS | T2 |
| *H. macrocarpus* | 7 | 10 | | | 15 | 35 | IG/LD/S | IG/LD/IS | T2 |
| *S. cumini* | 15 | 13 | 13 | 15 | 40 | 45 | RG/ID/AS | IG/ID/IS | T3 |
| *E. recurvatus* | 5 | | | | | | | | T1 |
| *M.champaca* | 35 | 36 | 18 | 20 | | 17 | | LG/LD/IS | |
| *S. densiflorum* | 15 | 17 | | | | 7 | | LG/LD/S | |
| *T. asiatica* | 13 | 12 | | | 30 | 44 | IG/LD/AS | LG/LD/S | T3 T5 |
| *R. arboretum* | 15 | 16 | 10 | 20 | 15 | 22 | LG/ID/IS | LG/ID/IS | T2 |
| *R. densiflora* | 2 | | | | | | | | T1 |

Key: Germination percentage (GP), mean length of seedling emergence (MLE), RG = rapid germination, IG = intermediate germination, LG = low Germination; LD = low dormancy, ID = intermediate dormancy, HD = high dormancy; S = synchronous, IS = intermediate synchrony, AS = asynchronous; T = treated, C = Control T1 = Soaked in water at room temperature for 12 hours, T2 = Soaked in water at room temperature for 24 hours, T3 = Soaked in water at room temperature. For 32 hours, T5 = Dipped in hot water (90°C) and left to soak for 24 hours.

**Table 3:** Growth responses in cuttings of *H. pentandrus, T. asiatica* and *S. cumini*

| | *H. pentandrus* | | *T. asiatica* | | *S. cumini* | |
|---|---|---|---|---|---|---|
| | Treated | untreated | Treated | untreated | Treated | untreated |
| Number of cutting with roots | 10 | 3 | 12 | 4 | 6 | 1 |
| Mean root length, cm, (range) | 3.5 (0.3–17) | 3.5 (1.3–3.6) | 3 (0.2–79) | 4 (0.2–56) | 3 | 3.7(1.8-65) |
| Number of cutting with shoots(range) | 5 | 2 | 7 | 2 | 7 | 1 |
| Mean shoot length, cm, (range) | 7.6 (0.2–31.5) | 6.6 (0.1–25.8) | - | - | - | - |

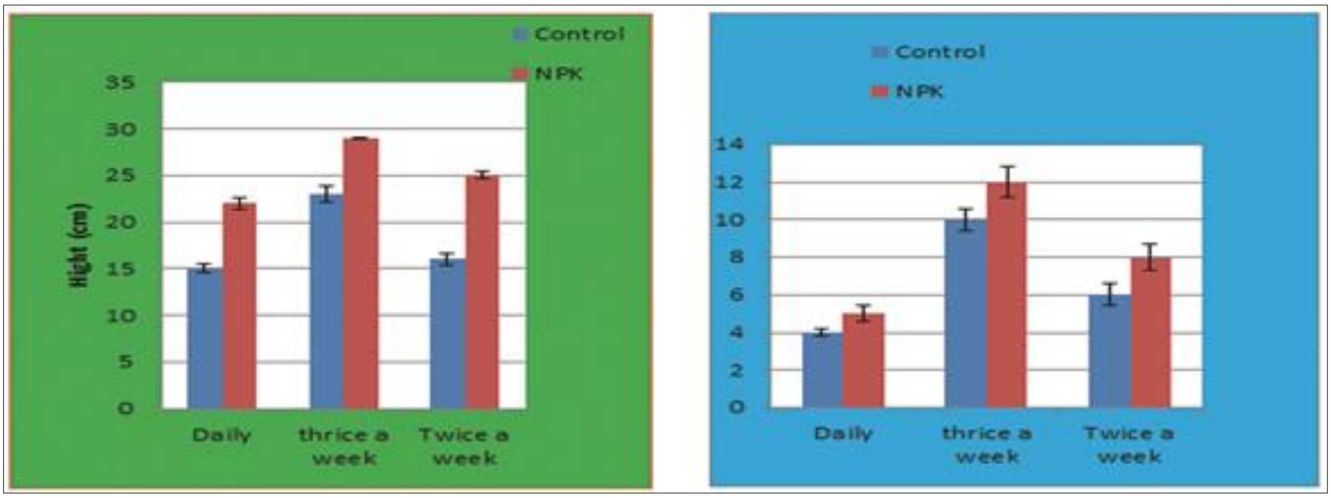


**Fig 1:** Influence of NPK and watering on height (a) and collar diameter (b) of *H. pentandrus*

**Discussion**

The aim of this study was to improve the conservation of important medicinal and woody species by providing information on propagation potential under different treatment regimes. The species, *H. pentandrus* and *S. cumini* propagates readily from seed and can be easily managed by farmers using simple treatments and technology. The other species studied here have low GPs and are fairly asynchronous in their germination. *T. asiatica* is hard to propagate because it has very low propagation potential as indicated by their low GPs, high dormancy and asynchronous germination. Asynchrony is an attribute that is not appreciated by farmers as they require seedlings that emerge at roughly the same time to facilitate efficient harvesting (Todd-Bockarie & Duryea 1993) [5]. It is recommended that further research be carried out on ways to shorten asynchrony in the seed species in this study. *R. densiflora* did not germinate at all, suggesting that the species is difficult to propagate from seed. The related species *H. macrocarpus* also had little success in RHT nursery garden. The pretreatment used in this experiment by soaking in cool or hot water, had no significant effect on the germination of seeds of the selected species. Species that failed to germinate or those that had low scores on the germination parameters, suggesting low synchrony, probably require alternate techniques like immersion in sulphuric acid, to improve their germination (Todd-Bockarie & Duryea 1993) [5]. *H. macrocarpus* and *E. recurvatus* failure to propagate may partly explain why these species are scarce in the wild and underscore the need for human intervention for their successful regeneration (Scarification). Recommendations for further studies should include experimentation with different treatments such as nicking and immersion in sulphuric acid, to find out if these treatments improve propagation rates. Experimentation using tissue culture, especially for important but difficult to regenerate species like *H. macrocarpus* would also benefit from further investigation. Intermediate watering to *H. macrocarpus* seedlings three times a week increases the growth by creating an increase in the girth without affecting height.

**Conclusions**

The aim of this study was to determine the success of propagation and seedling establishment of selected medicinal and woody tree species under different treatment regimes. The results suggest that the woody species *H. pentandrus*, *S. cumini* and *H. alpines* could be propagated easily by farmers with simple technology, minimal water and no addition of fertilizer. Conversely, the medicinal species *R. densiflora R. arboretum* and *H. macrocarpus* could not be propagated in this study. *H. pentandru* established readily with addition of fertilizer (NPK) and moderate watering (three times a week). This work has led to a greater understanding of the requirements for successful propagation and domestication of important medicinal and multipurpose plants of Sothern Western Ghats of the selected species.

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