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Apical shoot propagation of *Taxus wallichiana* Zucc. in Vietnam: a conservation-oriented approach to overcome the limitations of lateral cuttings

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Abstract

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Taxus wallichiana Zucc. is an endangered medicinal tree species valued for its production of paclitaxel, but its conservation is constrained by overexploitation and poor natural regeneration. This study evaluated the feasibility of propagating *T. wallichiana* by apical cuttings under nursery conditions as a conservation-oriented alternative to seed propagation and lateral cuttings. Two-year-old donor plants subjected to apical shoot removal produced a cumulative 25.93 ± 0.84 suitable shoots per plant after 12 months, providing a reliable source of propagules. Rooting performance was significantly affected by IBA concentration, rooting substrate, and cutting length. Among the tested treatments, 1.0% IBA was the most effective concentration for root induction. The best rooting performance was obtained in a substrate containing 25% sand and 75% coir dust, with a rooting percentage of 90.00 ± 5.00%, 8.13 ± 0.12 roots per cutting, and a mean root length of 4.83 ± 0.24 cm. Apical cuttings 10–15 cm in length rooted better than 15–20 cm cuttings. For nursery growth, potting media containing 75% soil + 25% coir dust or 50% soil + 50% coir dust gave the best performance, with 100% survival and plant heights of 30.93 ± 0.66 cm and 30.33 ± 0.56 cm, respectively. After 18 months, cutting-derived plants reached 81.43 ± 2.13 cm in height and 1.15 ± 0.05 cm in basal diameter, outperforming seed-derived seedlings. These findings indicate that apical cutting is a promising propagation approach for conservation and restoration of *T. wallichiana*.

Keywords

apical cutting, IBA, substrate, *Taxus wallichiana*, topophysis

Introduction

Taxus wallichiana Zucc., commonly known as Himalayan yew (English), Thông đỏ Nam or Thông đỏ lá dài in Vietnamese, and Xu mi hong doushan in Chinese, is a gymnosperm species belonging to the family Taxaceae. Its native distribution extends across Bhutan, China, India, Indonesia, Myanmar, Nepal, Pakistan, the Philippines, and Vietnam (Hiep et al., 2004; Thomas and Farjion, 2010). The species typically occurs at elevations ranging from 900 to 3,700 m asl, with the Himalayan region recognized as its principal center of distribution (Khan et al., 2006; Thomas and Farjon, 2010). In Vietnam, *T. wallichiana* is mainly found in secondary forests of Lam Dong Province at elevations of 900–1,600 m asl (Hiep et al., 2004). This is a large, evergreen tree that can reach 28–30 m in height and up to 150 cm in trunk diameter (Hiep et al., 2004; Thomas and Farjon, 2010).

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The leaves are spirally arranged, lanceolate, and 1.5–3.5 cm in long, with dark green adaxial surfaces and prominent stomatal bands on the abaxial side (Thomas and Farjon, 2010). The species is dioecious, with male cones borne singly in leaf axils as small spherical structures, whereas female cones are surrounded by numerous bracts that develop into a fleshy bright red aril enclosing a naked seed approximately 7–8 mm in size. These arils are vital for zoochorous seed dispersal (Nhut et al., 2007; Rikhari et al., 1998).

T. wallichiana has attracted considerable attention because of its ability to produce paclitaxel, a highly bioactive taxane compound primarily extracted from its bark and leaves. Paclitaxel is widely used as a chemotherapeutic agent in treatment of breast, ovarian, and lung cancers (Cragg et al., 1997). In addition, flavonoids and polysaccharides isolated from this species have been reported to possess antioxidant, anti-inflammatory, and immunomodulatory properties (Kang et al., 2010). Owing to its ecological value and medicinal importance, *T. wallichiana* is regarded as a species of both conservation and economic significance.

Despite its importance, *T. wallichiana* is experiencing rapid population declines as a result of overharvesting and habitat degradation, and it is currently listed as Endangered on the IUCN Red List (Thomas and Farjon, 2010). In Vietnam, the species is legally protected and classified as valuable species under national conservation regulations (Government of The Socialist Republic of Vietnam, 2021; Vietnam Academy of Science and Technology, 2024). However, natural regeneration remains limited because of poor seed germination, low seedling survival, and prolonged dormancy, which may last 1.5–2 years (Chee, 1994; Rikhari et al., 1998). These constraints make large-scale propagation and restoration of the species particularly difficult under natural and nursery conditions.

Vegetative propagation through cuttings therefore represents a promising alternative for producing genetically uniform planting materials when seed availability is limited (Saini, 2001). Among vegetative propagation approaches, successful adventitious root formation is particularly important because rooting ability, root number, and root length are critical determinants of subsequent plant establishment and growth (Aslam et al., 2017). However, in long-lived woody species such as *T. wallichiana*, vegetative propagation may be complicated by physiological constraints related to Topophysis, whereby tissues retain the positional identity and growth orientation of their original location on the donor plant (Hartmann and Kester, 1959; Olensen, 1978). As a result, cuttings derived from lateral branches often exhibit plagiotrophic growth, reduced vigor, and limited capacity to develop into an orthotropic (upright) tree (Hartmann and Kester, 1959). In Vietnam and elsewhere, previous vegetative propagation efforts in *T. wallichiana* have mainly focused on lateral cuttings for biomass production, particularly for paclitaxel extraction (Aslam et al., 2017; Iqbal et al., 2022). Although this approach may be suitable for pharmaceutical applications, it is less appropriate for forest restoration and long-term conservation, where upright growth form and normal tree architecture are required. In contrast, apical (terminal) cuttings derived from orthotropic shoots are more likely to retain vertical growth orientation and show greater potential to develop into mature trees. Nevertheless, information on the use of apical cuttings for propagation of *T. wallichiana* under nursery conditions remains limited. Therefore, the present study was conducted to evaluate the feasibility of propagating *T. wallichiana* through apical cuttings. Specifically, the study assessed shoot availability, rooting performance, and subsequent seedling development under nursery conditions. The findings are expected to contribute to conservation-oriented propagation practices and to support the sustainable management of this endangered species.

Materials and methods

The plant material used in this study consisted of two-year-old *T. wallichiana* seedlings. The seeds were originally collected from Bidoup – Nui Ba National Park, and the resulting seedlings were maintained under nursery conditions at the Forest Science Institute of Central Highlands and South of Central Vietnam.

Study site

The study was conducted at the Forest Science Institute of Central Highlands and South of Central Vietnam, located in Cam Ly – Da Lat Ward, Lam Dong Province, Vietnam (11°56′35″N, 108°24′23″E), at an elevation of 1,504 m asl. The site experiences a subtropical highland climate, with average annual temperatures ranging from 18 °C to 25 °C and average annual precipitation of approximately 2,200 mm, predominantly occurring during the wet season from May to November. The greenhouse was maintained under an automated misting system, activated for 20 seconds every 2 hours.

Plant growth regulator

β-Indole-3-butyric acid (β-IBA; Duchefa, Netherlands) was used as the rooting regulator. Five IBA concentrations were tested: 0.0%, 0.5%, 1.0%, 1.5%, and 2.0% (w w^{-1}). To prepare the powdered formulations, β-IBA was first dissolved in ethanol and then mixed thoroughly with talc powder (activated charcoal) as a carrier to obtain the desired concentrations. The mixture was gently heated to evaporate the solvent, then ground and sieved to produce homogeneous powder formulation for application to the cutting base.

Substrate materials

The rooting and potting substrates included river sand (thoroughly washed), coir dust (pre-treated to eliminate salinity and impurities), and topsoil collected from the 0–50 cm depth layer (decontaminated and sun-dried to reduce fungal pathogens). All materials were sun-dried before use.

Experimental design

This study was designed as a sequential six-experiment workflow. Experiment 1 was conducted to identify a stock-plant management method for producing suitable apical shoots. The shoots obtained from Experiment 1 were then used as propagules in Experiment 2 to determine the optimal IBA concentration for rooting. Using the optimal IBA concentration, Experiment 3 evaluated the influence of rooting substrate conditions. Subsequently, Experiment 4 tested the effect of cutting length using the optimal IBA concentration and rooting substrate determined in the previous experiments. Successfully rooted and fully acclimatized cuttings produced under these optimized conditions were then used in Experiment 5 to examine potting media for nursery growth. Finally, well-established cutting-derived plants produced through the optimized protocol were compared with seed-derived seedlings in Experiment 6.

Experiment 1. Effect of apical shoot removal on shoot multiplication

Experiment 1 evaluated the effect of apical shoot removal on the production of suitable apical shoots from stock plants. Uniform seed-derived seedlings aged 24 months, with heights of 60–65 cm and basal diameters of 0.8–0.9 cm, were used as stock plants. Two treatments were established: (1) apical shoot removal, and (2) apical shoot retention, in which the main shoot was left intact. In both treatments, lateral branches were pruned to promote the development of apical shoots.

The stock plants were grown in plastic pots (27 × 22 × 23 cm; top diameter × bottom diameter × height) filled with a potting medium composed of 75% topsoil (0–50 cm) and 25% coir dust and amended with decomposed cow manure. Each plant received 10 g of NPK fertilizer (16–16–8) every 15 days. Each treatment consisted of three replicates, with 10 plants per replicate. Data were collected at 2-month intervals over a 12-month period. Specifically, five batches of apical cuttings were harvested throughout the year. The initial apical pinching was performed two months after potting, and the first batch of cuttings was subsequently collected at month four. The number of suitable apical shoots per plant was recorded. The shoot multiplication coefficient was expressed as the mean number of suitable apical shoots produced per stock plant at each observation time. Suitable apical shoots were defined as shoots longer than 10 cm and partially lignified.

Experiment 2. Effect of IBA concentration on rooting of apical cuttings

Experiment 2 assessed the effect of IBA concentration on the rooting of apical cuttings derived from Experiment 1. Newly formed apical shoots that met the predefined selection criteria were harvested and prepared as cuttings 10–15 cm in length. The cuttings were treated with five IBA concentrations: 0.0% (DHST0), 0.5% (DHST1), 1.0% (DHST2), 1.5% (DHST3), and 2.0% (DHST4). Rooting was conducted in plastic trays (35 × 26 × 10 cm) using river sand as the substrate. Each formula included 20 cuttings and 3 replicates. After 105 days, survival rate (%), rooting rate (%), average root length (cm), average number of roots per cutting, and rooting index were recorded (Rooting index = average root length × average number of roots/ cuttings).

Experiment 3. Effect of substrate composition on rooting of apical cuttings

Using the optimal IBA concentration identified in Experiment 2, Experiment 3 evaluated the effect of rooting substrate composition on the rooting performance of apical cuttings. Cuttings of 10–15 cm in length were rooted in five substrate treatments: 100% sand (GT1), 75% sand + 25% coir dust (GT2), 50% sand + 50% coir dust (GT3), 25% sand + 75% coir dust (GT4), and 100% coir dust (GT5) with 3 replicates per treatment and 20 cutting per treatment. After 90 days, survival rate (%), rooting rate (%), average root length (cm), average number of roots per cutting, and rooting index were recorded.

Experiment 4. Effect of cutting length on rooting performance

Experiment 4 examined the effect of cutting length on rooting under the optimized IBA concentration and substrate composition identified in Experiments 2 and 3. Two cutting-length classes were tested: 10–15 cm (LH1) and 15–20 cm (LH2). The experiment was conducted in plastic trays (35 × 26 × 10 cm), with 20 cuttings per treatment and 3 replicates. After 90 days, survival rate (%), rooting rate (%), average root length (cm), average number of roots per cutting, and rooting index were recorded.

Experiment 5. Effect of potting mix composition on sapling growth

Rooted and acclimatized cuttings obtained under the optimized rooting conditions were used in Experiment 5 to evaluate the effect of potting medium on subsequent nursery growth. Five potting media treatments were tested: 100% topsoil (TPRB1), 75% soil + 25% coir dust (TPRB2), 50% soil + 50% coir dust (TPRB3), 25% soil + 75% coir dust (TPRB4), and 100% coir dust (TPRB5). Plantlets were grown in plastic pots (15 × 13 × 10 cm: top diameter × bottom diameter × height). Each mixture was amended with 10% well-decomposed cow manure, and 5 g of NPK fertilizer (16–16–8) was applied every 15 days. Twenty plantlets per formula were used, with 3 replicates. After 120 days, survival rate (%), plant height (cm), and basal diameter (cm) were recorded.

Experiment 6. Comparison of growth between cutting- and seed-derived seedlings

Experiment 6 compared the nursery growth of cutting-derived plants produced using the optimized propagation protocol with that of seed-derived seedlings. Only well-rooted, acclimatized, and uniform cutting-derived plants were included in this experiment. At the start of the experiment, both propagule types were standardized to an initial height of 25–30 cm.

Plants were grown in plastic pots (27 × 22 × 23 cm; top diameter × bottom diameter × height) filled with a mixture of 75% topsoil, 25% coir dust, and amended with decomposed cow manure. Each plant received 10 g of NPK fertilizer (16–16–8) every 15 days. Each treatment consisted of three replicates, with 10 plants per treatment. Plant height (cm) and basal diameter were measured after 6, 12, and 18 months.

Statistical analysis

All experimental data were analyzed using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) and independent-samples t-tests were performed to determine significant differences among formulas. Post hoc comparisons were conducted using Duncan's Multiple Range Test (DMRT) at a 5% significance level ($p < 0.05$).

Results and discussion

The apical shoot multiplication factor

A statistically significant difference in the number of suitable apical shoots produced per stock plant was observed between the apical-shoot-removal treatment and the control treatment (Table 1, Fig. 1). Across all observation times, the shoot multiplication coefficient was consistently higher in plants subjected to apical shoot removal than in those in the control treatment. In the control treatment, the shoot multiplication coefficient increased gradually from 0.46 ± 0.12 to 3.80 ± 0.37 over the 12-month experimental period. By contrast, in the apical-shoot-removal treatment, the coefficient increased from 1.36 ± 0.09 to 9.53 ± 0.38, representing an approximately two- to three-fold increase

Table 1. Shoot multiplication coefficient of long-leaved *T. wallichiana* in the nursery

Treatment	Number of cuttings obtained					Total cuttings per year
	Time 1	Time 2	Time 3	Time 4	Time 5	
No apical removal	0.46 ± 0.12	0.90 ± 0.08	1.30 ± 0.20	2.20 ± 0.32	3.80 ± 0.37	8.67 ± 0.84
Apical removal	1.36 ± 0.09	2.86 ± 0.12	5.03 ± 0.24	7.13 ± 0.58	9.53 ± 0.38	25.93 ± 0.84
p-value	0.004	0.000	0.000	0.002	0.000	0.000

Fig. 1. Bud sprouting and morphology of apical and lateral shoots of *T. wallichiana*. a, b. Apical bud sprouting after apical dominance removal; c, d. Apical bud sprouting without apical dominance removal; e. Morphology of the apical (left) and lateral (right) shoots; f. Morphology of seedlings propagated from apical shoot cuttings; g, h. Morphology of seedlings propagated from lateral shoot cuttings.

compared to the control. At the end of the first year, the cumulative number of shoots suitable for propagation reached 25.93 ± 0.84 shoots plant^{-1} in the treated group, compared with only 8.67 ± 0.84 shoots plant^{-1} in the control group. These results indicate the apical shoot removal markedly enhanced the production of propagable shoots in *T. wallichiana* under nursery conditions.

From a practical propagation perspective, the control treatment produced too few suitable orthotropic shoots for coefficient cutting collection, whereas the apical-shoot-removal treatment provided a substantially larger and more reliable source of propagules. However, despite the high shoot production potential of treated stock plants, only 12–15 shoots were harvested from each stock plant at each collection round after the initial year in order to maintain donor plant vigor and ensure uniform shoot quality for subsequent rooting experiments. This management strategy was therefore considered appropriate for balancing propagation output with the long-term maintenance of stock plants.

The superior performance of the apical-shoot-removal treatment is consistent with the established role of apical dominance in suppressing axillary bud outgrowth (Rameau et al., 2015). Apical shoot removal may have reduced the inhibitory influence of apex-derived auxin transport, thereby releasing axillary and subapical buds from hormonal suppression and promoting the formation of new shoots (Prusinkiewicz et al., 2009; Rameau et al., 2015). Similar responses have been reported in other species. Kool and Van De Pol (1993) found that topping stimulated shoot growth and canopy development in *Rosa hybrida*, while Rajeswari and Paliwal (2008) and Sujatha (2007) reported increased shoot regeneration following removal of apical tissues in *Albizia odoratissima* and *Pongamia pinnata*, respectively. Therefore, the present results suggest that apical shoot removal is a promising stock-plant management practice for increasing the availability of suitable apical shoots for vegetative propagation of *T. wallichiana*.

The effect of IBA concentration on apical cutting rooting ability

The application of powdered IBA in combination with activated charcoal had a statistically significant effect on the rooting performance of *T. wallichiana* cuttings ($p < 0.05$). Compared to the untreated control, all IBA-treated cuttings showed improved rooting performance, as reflected by significant improvements in key rooting parameters, including rooting percentage, mean number of roots per cutting, root length, and rooting index (Table 2).

Survival rates remained high (>90%) in formulas DHST1 and DHST2, comparable to the control group. However, survival declined in DHST3 (83.33%) and DHST4 (76.67%), indicating potential phytotoxicity at higher IBA concentrations. Despite this reduction in survival, DHST2 and DHST3 produced stronger rooting response than the other treatments across the measured rooting parameters. Among all treatments, DHST2 formula (1.0% IBA) gave the best overall performance, with a rooting percentage of 65.00%, 3.83 ± 0.24 roots per cutting, a mean root length of 3.13 ± 0.09 cm, and a rooting index of 10.65 ± 0.14, which was approximately ten times higher than that of the control treatment (1.04 ± 0.08). These findings indicate that 1.0% IBA was the most effective concentration among those tested for promoting adventitious rooting in *T. wallichiana* cuttings under the present formulation conditions.

The superior rooting response observed after IBA treatment is consistent with previous reports in related species. Iqbal et al. (2020) reported that IBA was more effective than NAA, IAA, and GA3, in inducing root formation in *Taxus baccata* with a maximum rooting percentage of 85.22%. Similarly, Averyanov et al. (2015) showed that powdered IBA effectively promoted rooting in lateral branch cuttings of *Taxus chinensis* with rooting percentages exceeding 80% and average root lengths of approximately 5 cm.

Beyond the genus *Taxus*, (Thomas, 2008; Chen et al., 2014) also highlighted the positive role of IBA in stimulating meristematic activity and promoting adventitious root initiation in difficult-to-root woody species such as *Cinnamomum micranthum* and *Camellia* spp. These reports support the interpretation that exogenous IBA served as an effective auxin source for root primordium initiation and early root development in *T. wallichiana*.

In the present study, activated charcoal was included as a carrier component in all powdered IBA formulations. Therefore, its independent effect was not evaluated separately. Nevertheless, activated charcoal may have contributed to improving the microenvironment at the cutting base by absorbing inhibitory compounds released after wounding and

Table 2. Effect of IBA concentration on apical cutting rooting ability

Treatment		Rooting parameters				
Code	IBA concentration (%)	Survival rate (%)	Rooting rate (%)	No. of roots	Root length (cm)	Rooting index
DHST0	0	$91.67 \pm 2.88^{a*}$	35.00 ± 5.00^{c}	1.50 ± 0.12^{c}	0.70 ± 0.05^{e}	1.04 ± 0.08^{d}
DHST1	0.5	93.33 ± 2.88^{a}	51.67 ± 5.77^{b}	3.20 ± 0.21^{b}	1.47 ± 0.14^{d}	4.75 ± 0.76^{c}
DHST2	1	93.33 ± 2.88^{a}	65.00 ± 5.00^{a}	3.83 ± 0.24^{a}	3.13 ± 0.09^{a}	10.65 ± 0.14^{a}
DHST3	1.5	83.33 ± 2.88^{b}	61.67 ± 2.88^{a}	3.60 ± 0.26^{ab}	2.83 ± 0.07^{b}	10.45 ± 0.65^{a}
DHST4	2	76.67 ± 5.77^{c}	53.33 ± 2.88^{c}	3.10 ± 0.10^{b}	2.47 ± 0.09^{c}	7.64 ± 0.26^{b}
p-value		0.001	0.000	0.000	0.000	0.000

*Different letters indicate statistically significant differences at α = 0.05 according to Duncan's test.

stabilizing local physicochemical conditions, as suggested Thomas (2008). Accordingly, the present results should be interpreted as the effect of IBA concentration within an activated-charcoal-based powdered formulation, rather than as a separate evaluation of activated charcoal itself.

The reduction in survival observed at the highest IBA concentration (2.0%; DHST4) suggests that excessive auxin levels may be detrimental to cutting establishment. High IBA concentrations have been reported to induce tissue necrosis at the cutting base, suppress normal root initiation, and reduce overall plantlet viability (Kordzadeh and Sarikhani, 2021). A similar response was described by Kaul (2008) in *T. wallichiana*, where elevated IBA concentrations reduced rooting success and increased mortality in cuttings derived from mature donor plants. Therefore, although auxin application was necessary to enhance rooting, increasing the concentration beyond the effective range was not beneficial and may have caused phytotoxic effects. Overall, the findings indicate that 1.0% IBA was the most suitable concentration among those tested for rooting apical cuttings of *T. wallichiana*.

The effect of substrate on apical cutting rooting ability

Substrate composition significantly affected the rooting performance of *T. wallichiana* cuttings, although survival

Table 3. Effect of substrate on apical cutting rooting ability

Treatment		Rooting parameters				
Code	Substrate	Survival rate (%)	Rooting rate (%)	No. of roots	Root length (cm)	Rooting index
GT1	100% sand	96.67 ± 2.88	48.33 ± 2.88^{c*}	2.83 ± 0.18^{c}	1.20 ± 0.11^{c}	3.39 ± 0.34^{c}
GT2	75% sand + 25 % coir dust	96.67 ± 2.88	68.33 ± 2.88^{b}	3.80 ± 0.46^{c}	1.40 ± 0.05^{c}	5.33 ± 0.70^{c}
GT3	50% sand + 50% coir dust	95.00 ± 0.00	75.00 ± 5.00^{b}	7.90 ± 0.17^{a}	3.43 ± 0.37^{b}	27.17 ± 3.39^{b}
GT4	25% sand + 75% coir dust	95.00 ± 5.00	90.00 ± 5.00^{a}	8.13 ± 0.12^{a}	4.83 ± 0.24^{a}	39.30 ± 2.28^{a}
GT5	100% coir dust	93.33 ± 2.88	91.67 ± 2.88^{a}	7.76 ± 0.14^{b}	4.17 ± 0.14^{a}	32.37 ± 1.74^{b}
p-value		0.682	0.000	0.000	0.000	0.000

* Different letters indicate statistically significant differences at α = 0.05 according to Duncan's test.

Fig. 2. Root formation from apical shoot cuttings of *T. wallichiana* in different rooting substrates. a. Apical shoot cuttings collected from the nursery mother plants for the rooting experiment; b. Apical shoot cuttings on sand substrate, treated with 1% IBA after 90 days; c. Apical shoot cuttings on substrate of 75% sand + 25% coir dust, treated with 1% IBA after 90 days; d. Apical shoot cuttings on substrate of 50% sand + 50% coir dust, treated with 1% IBA after 90 days; e. Apical shoot cuttings on substrate of 25% sand + 75% coir dust, treated with 1% IBA after 90 days; f. Apical shoot cuttings on substrate of 100% coir dust, treated with 1% IBA after 90 days.

Table 4. Effect of cutting length on rooting ability

Treatment		Rooting parameters				
Code	Cutting length	Survival rate (%)	Rooting rate (%)	No. of roots	Root length (cm)	Rooting index
LH1	10–15	93.33 ± 2.88	88.33 ± 7.64	8.00 ± 0.25	4.86 ± 0.12	38.98 ± 2.05
LH2	15–20	83.33 ± 2.88	71.67 ± 2.88	4.93 ± 0.14	1.07 ± 0.09	5.28 ± 0.52
p-value		0.130	0.024	0.000	0.000	0.000

Fig. 3. Root formation from apical shoot cuttings of *T. wallichiana* at different cutting lengths. a, b. Apical shoot cuttings on substrate of 25% sand + 75% coir dust, treated with 1% IBA after 90 days, with cutting length of 10–15 cm; c, d. Apical shoot cuttings on substrate of 25% sand + 75% coir dust, treated with 1% IBA after 90 days, with cutting length of 15–20 cm.

rate did not differ significantly among treatments (Table 3, Fig. 2). Survival remained high across all substrate treatments, ranging from 93.33% to 96.67%. However, significant differences were observed in rooting percentage, root number, root length, and rooting index. Among the tested substrates, GT4 (25% sand + 75% coir dust) produced the best overall rooting performance, with a rooting percentage of 90.00 ± 5.00%, 8.13 ± 0.12 roots per cutting, a mean root length of 4.83 ± 0.24 cm, and a rooting index of 39.30 ± 2.28. GT5 (100% coir dust) showed a similarly high rooting percentage (91.67 ± 2.88%), but lower values for root number and rooting index. In contrast, GT1 (100% sand) gave the poorest rooting performance, with only 2.83 ± 0.18 roots per cutting and a rooting index of 3.39 ± 0.34. These results suggest that the addition of coir dust substantially improved rooting quality compared with sand alone, while the combination of 75% coir dust and 25% sand provided the most favorable rooting conditions among the substrates tested.

These findings are consistent with earlier studies reporting the advantages of coir dust as a rooting medium. Coir dust is rich in lignin (approximately 45%) and hemicellulose (about 25%), which are associated with antimicrobial properties that suppress root pathogens such as *Fusarium* and *Pythium* (Gleena Mary and Mathew, 2016). Furthermore, the high porosity and water-holding capacity of coir dust improve substrate aeration and reduce water tension around root meristems – conditions favorable for root initiation (Reddy, 2019; Liyanage et al., 2021).

However, the use of 100% coir dust also presents limitations. According to Hartmann and Kester (1959), moisture levels above 85% can cause substrate compaction, reducing air-filled pore space to below 15% ($v\ v^{-1}$), which restricts oxygen diffusion and impairs meristematic activity at the cutting base. This may explain the slight reduction in root number and rooting index observed in GT5 despite its high rooting percentage. Incorporating sand (25%) into the coir matrix, as in GT4, appears to improve substrate structure, enhancing drainage and aeration, and thus optimizing rooting conditions.

Supporting evidence for this interpretation includes work by Le et al. (2024), who demonstrated that combining coir with sand mitigates compaction and waterlogging – two common constraints to adventitious rooting. Similarly, Sharma and Verma (2011) found that a 70% coir dust + 30% sand mixture significantly increased root length (4.2 cm) in *Pinus roxburghii*, compared to only 2.8 cm in 100% sand.

Overall, the GT4 substrate (75% coir dust + 25% sand) was found to offer an optimal balance between moisture retention, aeration, and pathogen suppression, making it a suitable medium for rooting *T. wallichiana* cuttings.

The effect of cutting length on rooting ability

Cutting length significantly affected the rooting performance of *T. wallichiana* cutting ($p < 0.05$) (Table 4, Fig. 3). The shorter cuttings (10–15 cm; LH1) consistently outperformed the longer cuttings (15–20 cm; LH2) across all evaluated parameters. In LH1, the survival rate reached 93.33 ± 2.88%, rooting percentage was 88.33 ± 7.64%, the average number of roots was 8.00 ± 0.25 per cutting, mean root length was 4.86 ± 0.12 cm, and the rooting index was 38.98 ± 2.05. In contrast, LH2 showed lower values for all measured traits, including survival rate (83.33 ± 2.88%), rooting percentage (71.67 ± 2.88%), mean root number (4.93 ± 0.14), root length (1.07 ± 0.09 cm), and rooting index (5.28 ± 0.52). These results indicate that shorter apical cuttings provided more favorable rooting performance than longer cuttings under the tested conditions.

The superior rooting performance of LH1 may be related to the physiological characteristics of shorter apical cuttings. Compared with longer cuttings, shorter cuttings generally have lower transpirational demand and may therefore maintain internal water balance more effectively during the early stages of root initiation. In addition, shorter apical cuttings likely retain a greater relative influence of juvenile apical tissue, which is often associated with better rooting capacity in woody species. Leakey (2004) emphasized the importance of juvenility in vegetative propagation, while Hartmann and Kester (1959) noted that excessive water loss can negatively affect root initiation and subsequent root development. Similar results were also reported by Denaxa et al. (2021), who found that 10 cm cuttings of *Olea europaea* rooted more successfully than 20 cm cuttings, with rooting percentages of 85% and 55%, respectively. Taken together, these observations suggest that a cutting length of 10–15 cm was the most suitable range among those tested for rooting apical cuttings of *T. wallichiana*.

The effect of potting mix composition on the growth of saplings from apical cuttings

Potting medium composition significantly affected the survival and growth of *T. wallichiana* saplings derived from apical cuttings ($p < 0.05$) (Table 5, Fig. 4). Among the tested media, TPRB2 (75% soil + 25% coir dust) and TPRB3 (50% soil + 50% coir dust) produced the best over-

Table 5. Effect of potting mix composition on the growth of saplings from apical cuttings

Treatment		Growth parameters		
Code	Potting mix composition	Survival rate (%)	Height (cm)	Basal diameter (cm)
TPRB1	100% soil	96.66 ± 2.88^{a*}	22.96 ± 0.73^{c}	0.34 ± 0.08^{c}
TPRB2	75% soil + 25 % coir dust	100^{a}	30.93 ± 0.66^{a}	0.41 ± 0.08^{a}
TPRB3	50% soil + 50% coir dust	100^{a}	30.33 ± 0.56^{a}	0.41 ± 0.08^{a}
TPRB4	25% soil + 75% coir dust	98.33 ± 2.88^{a}	27.03 ± 0.56^{b}	0.36 ± 0.08^{b}
TPRB5	100% coir dust	88.33 ± 2.88^{b}	21.90 ± 0.72^{c}	0.32 ± 0.10^{a}
p-value		0.000	0.000	0.000

*Different letters indicate statistically significant differences at α = 0.05 according to Duncan's test.

Fig. 4. Growth of *T. wallichiana* saplings in different potting media after 4 months of cultivation. a. Growth of *T. wallichiana* seedlings in 100% soil substrate; b, f. Growth of *T. wallichiana* seedlings in substrate composed of 75% soil + 25% coir dust; c. Growth of *T. wallichiana* seedlings in substrate composed of 50% soil + 50% coir dust; d. Growth of *T. wallichiana* seedlings in substrate composed of 25% soil + 75% coir dust; e. Growth of *T. wallichiana* seedlings in 100% coir dust substrate.

Table 6. Growth assessment of plants during the nursery stage

Growth parameter	Sampling time (months)	Growth of plants		
		Seedlings (ST1)	Saplings from Apical cuttings (ST2)	*p*-value
Height (cm)	Initial	29.77 ± 0.49	30.46 ± 0.43	0.345
	6 months	36.66 ± 0.84	45.83 ± 1.96	0.130
	12 months	50.20 ± 1.10	61.20 ± 0.98	0.002
	18 months	62.86 ± 2.46	81.43 ± 2.13	0.005
Diameter (cm)	Initial	0.31 ± 0.01	0.41 ± 0.01	0.001
	6 months	0.38 ± 0.01	0.45 ± 0.01	0.008
	12 months	0.58 ± 0.01	0.72 ± 0.02	0.008
	18 months	0.89 ± 0.02	1.15 ± 0.05	0.007

Fig. 5. Growth of seedlings and saplings from apical cuttings of *T. wallichiana* in the nursery. a. Seedlings grown used for growth monitoring; b. Saplings grown from apical cuttings used for growth monitoring; c. Seedlings after 18 months; d. Saplings from apical cuttings after 18 months; e. Comparison of growth between seedlings and saplings from apical cuttings after 18 months.

all performance. Saplings grown in these two media reached plant heights of 30.93 ± 0.66 cm and 30.33 ± 0.56 cm, respectively, with basal diameters of 0.41 ± 0.08 cm in both treatments and survival rates of 100%. In contrast, TPRB5 (100% coir dust) showed the poorest performance, with the lowest growth values and a survival rate of 88.33 ± 2.88%. These results indicate that partial substitution of soil with coir dust improved nursery growth, whereas the use of coir dust alone was less suitable under the present conditions.

The superior performance of TPRB2 and TPRB3 may be related to a more favorable balance between moisture retention and aeration in the root zone. Previous studies have shown that coir dust can improve substrate porosity and water-holding capacity (WHC: 4.5-5.5 g H_2O g^{-1} substrate), while soil contributes to structural stability, drainage, and regulation of water availability (Coutts, 1982; Fort et al., 1998; Green and Clothier, 1999; Rose and Haase, 2000), 2000). Biologically, the lignin and phenolic compounds in coir dust inhibit pathogenic fungi such as *Fusarium* and *Pythium*, reducing pathogenic microbial load by more than 40% compared with pure soil (Gleena Mary and Mathew, 2016). However, the use of 100% coir dust (TPRB5) can result in excessive moisture (>85%) leading to oxygen depletion in the root zone (O_2 < 5 mg L^{-1}) and reduced root absorption efficiency. Conversely, pure soil (TPRB1) has a high bulk density (>1.4 g cm^{-3}), which causes substrate compaction and restricts root system development (Allaire et al., 2008). Therefore, combining these two components at intermediate proportions may provide a more suitable physical environment for root development and nutrient uptake than either material used alone. Similar responses have been reported in other species. Liyanage et al. (2021) observed improved plant growth in substrates containing moderate proportions of coir dust, and Le et al. (2024) reported comparable findings in *Camellia piquetiana*.

Seedling and sapling growth evaluation during the nursery stage

The 18-month nursery evaluation showed that *T. wallichiana* plants propagated from apical cuttings (ST2) performed overall better than seed-derived seedlings (ST1) in both height and basal diameter (Table 6, Fig. 5).

At the beginning of the experiment, both propagule types had a similar initial height of approximately 30 cm, with no statistically significant differences between treatments. However, the basal diameter of ST2 plants was already greater than that ST1 plants (0.41 ± 0.01 cm compared to 0.31 ± 0.01 cm). After 6 months, the height of cutting-derived plants reached 45.83 ± 1.96 cm, compared with 36.66 ± 0.84 cm in seed-derived plants, while basal diameter was 0.45 ± 0.01 cm in ST2 and 0.38 ± 0.01 cm in ST1. After 12 months, ST2 reached 61.20 ± 0.98 cm in height and 0.72 ± 0.02 cm in basal diameter, whereas ST1 reached 50.20 ± 1.10 cm and 0.58 ± 0.01 cm, respectively. At the end of the 18-month period, ST2 attained 81.43 ± 2.13 cm in height and 1.15 ± 0.05 cm in basal diameter, exceeding ST1, which reached 62.86 ± 2.46 cm and 0.89 ± 0.02 cm, respectively. These results indicate that cutting-derived plants maintained superior nursery performance throughout the observation period and achieved greater final size than seed-derived seedlings under the tested conditions. The better nursery performance of cutting-derived plants may be related to both propagation pathway and plant uniformity. As clonal propagules, plants derived from apical cuttings retain the genotype of the donor plant, whereas seed-derived seedlings are expected to show greater genetic variation due to sexual recombination, which may result in less uniform population (Crawford and Whitney, 2010). Arnold et al. (2021) et al. (2021) similarly reported that cutting-propagated plants exhibited greater growth than seed-grown plants in serval ornamental woody species: *Lagerstroemia indica × L. fauriei*, *Chilopsis linearis*, *Vitex agnus-castus*, and *Nerium oleander*. In addition, vegetative propagules derived from actively growing apical tissues may establish more rapidly after transplanting, because they originate from physiologically developed shoot tissues and bypass the germination and early seedling establishment stages, which are often sensitive to environmental variation (Druege et al., 2019; Ghosh et al., 2018). A similar study was reported by Nahar (2025), who observed that Jatropha propagated from cuttings achieved nursery-ready growth more rapidly than seed-derived plants. Therefore, the present results suggest that propagation by apical cuttings is a promising approach for producing vigorous and more uniform nursery plants of *T. wallicchiana*.

Conclusions

This study established an effective nursery protocol for clonal propagation of *Taxus wallichiana* through apical cuttings. Apical shoot removal in 2-year-old donor plants markedly enhanced the production of suitable shoots for propagation. During the rooting stage, the best performance was obtained with 1.0% IBA, a rooting substrate composed of 25% sand and 75% coir dust, and cuttings 10–15 cm in length. For subsequent nursery growth, potting media containing 50–75% soil combined with 25–50% coir dust were the most suitable for sapling establishment. During the nursery evaluation period, plants propagated from apical cuttings showed superior growth compared with seed-derived seedlings. Overall, apical cutting represents a promising approach for the propagation, conservation, and restoration of *T. wallichiana*.

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