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Propagation of *Dipterocarpus retusus* in Thai Nguyen Province, Vietnam

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Abstract

Dipterocarpus retusus is a large hardwood species that grows relatively fast. However, it is currently threatened with extinction due to the degradation of its habitat, which is primarily caused by agricultural conversion and logging. In Thai Nguyen province, only slightly over 200 individuals are remaining with a diameter of over 6 cm. Therefore, there is an urgent need for research on propagation techniques in order to conserve and develop the genetic resources of the *D. retusus*, which is a native species in Thai Nguyen Province. This study presents the results of rooting hormone treatments for *D. retusus* cuttings. The results indicate that *D. retusus* cuttings face difficulties in rooting. However, the use of NAA as a rooting hormone treatment at a concentration of 1500 ppm resulted in the highest rooting percentage, which reached 42.59%. Additionally, the survival rate was 37.41%. It was observed that the age of the cuttings has an impact on the rooting percentage, with semi-hardwood cuttings exhibiting the highest rooting percentage at 43.33%. Furthermore, an increase in shade cover tends to slow down diameter growth, while height growth increases. The substrate formula that combines 15% compost with 2% NPK shows the best growth performance. In the fact that the cuttings grown in this substrate reached a diameter of 0.51 cm and a height of 45.3 cm. These research findings have laid the foundation for establishing a propagation protocol for *D. retusus* using the cutting methods.

Keywords: *Dipterocarpus retusus*, propagation, cuttings, rooting hormone.

Introduction

Dipterocarpus retusus, has its local name in Vietnamese as "*Cho nau*," "*Trai dầu*," or "*Sao xoay*," is a large hardwood species that can be found at altitudes ranging from 300 to 1,500 meters above sea level. It is distributed in India, Laos, Cambodia, Vietnam, Indonesia, and China. Despite its wide natural distribution, the population of *D. retusus* is sparse and scattered, and it is currently under threat due to the degradation of its native habitat, which is linked to agricultural land conversion and timber exploitation (Singh et al., 2014; Xiwen et al., 2007).

In Vietnam, *D. retusus* is distributed in several provinces in the Northern Mountains, Central Vietnam, and the Central Highlands. The decline of this species has had a significant impact on its seed source and natural regeneration ability. Directive No. 19/2004/CT-TTg has proposed various solutions for developing the wood processing industry and exporting wood products. The Forestry Restructuring Project (Ministry of Agriculture and Rural Development, 2013; Forestry Law 2017), as well as the most recent Vietnam Forestry Development Strategy for the period 2021-2030, emphasize the importance of conserving biodiversity and preserving valuable native genetic resources by planting large timber forests with indigenous species, including *D. retusus*.

However, research on *D. retusus* is still limited, with only a few results from experimental planting in certain projects. There is currently no technical guideline available for the propagation of *D. retusus*. Therefore, this article presents the research results on the propagation technique for *D. retusus* in Thai Nguyen province as part of a conservation research project funded by the Thai Nguyen Department of Science and Technology. Our results have provided useful information for propagating *D. retusus* trees by the cuttings method.

Materials and methods

Materials

The cuttings of *D. retusus* tree (60 samples), which are naturally distributed in 5 districts (Dong Hy, Dinh Hoa, Phu Luong, Dai Tu, and Vo Nhai) in Thai Nguyen province, were collected for this research. Twelve tree samples were collected in each district were used in this study.

Methods

Method for studying the effects of rooting hormone on the rooting ability of cuttings

The experiment utilized three different rooting hormones (IAA, IBA, and NAA) with four concentration formulas (1000ppm, 1500ppm, 2000ppm, and a control group without any rooting stimulant). A total of 360 cuttings were involved in the experiment, with 30 cuttings per recipe and 3 repetitions, resulting in 90 cuttings per recipe and 4 recipes. Each recipe was treated with 3 different hormones, totaling 1,080 cuttings. The experiment commenced on December 10th, 2021. The measured parameters included: (1) Number of cuttings with callus formation; (2) Number of rooted cuttings; (3) Number of surviving cuttings after being transplanted into pots for fifteen days.

Methods for study on the influence of cutting age on the rooting ability of cuttings

In order to examine the impact of cutting age on the rooting ability of cuttings, branches from mother plants of different ages were selected for taking the cuttings. The cuttings were obtained from the middle of the canopy. Since accurately determining the age of the cuttings was not feasible, they were divided into three age groups: mature cuttings, semi-hardwood cuttings, and young cuttings. Mature cuttings were derived from mature branches, semi-hardwood cuttings from semi-hardwood branches, and young cuttings from young branches. The appropriate rooting hormones, identified through previous experiments, were used. Each treatment consisted of 30 cuttings, with 3 replications, resulting in 90 cuttings per treatment. There were 3 treatments in total, representing different cutting ages, resulting in a total of 270 cuttings. The observation parameters included the number of rooted cuttings, the average number of roots per cutting, and root length.

Method to examine the influence of seasons on the rooting ability of cuttings

The experiment consists of four batches of cuttings, each planted at different times. The schedule for each batch is as follows: Batch 2: Experiment conducted on June 10, 2022. Batch 3: Experiment conducted on September 10, 2022. Batch 4: Experiment conducted on December 10, 2022. The most suitable rooting hormone was determined based on previous experiments that investigated its effects. The experiment involves a total of thirty cuttings per formula, repeated three times, resulting in a total of ninety cuttings per formula. This is done across all four batches, resulting in a total of three hundred and sixty cuttings.

The following parameters are observed in this experiment: Percentage of callus formation (%): Indicates the proportion of cuttings that develop callus at the base or wound site. Percentage of rooting success (%): Measures the proportion of planted cuttings that successfully produce roots. Rate of survival of cuttings after transplanting (%): Evaluate how many rooted cuttings survived and established themselves as viable plants after being transplanted into pots for fifteen days.

Effect of lighting conditions on growth performance

The experiment was designed with three shading formulas: (1) 0.3, (2) 0.5, and (3) control without shading (expressed as a fraction of 10). The degree of shading for each formula was determined as follows: For formulas with shading levels of 0.3 and 0.5, split branches of the hom plant were used to create segments with a width of 1cm. These segments were then woven into a sheet measuring 1.5m x 2m. The distance between segments was adjusted according to the desired degree of shading. Specifically, for a shading level of 0.3, the horizontal and vertical distances between segments were set at 2.3cm. In contrast, for a shading level of 0.5, the vertical and horizontal distances were set at 1cm. A mixed core formula was used, consisting of 83% soil from layer B, 15% compost, and 2% NPK fertilizer (20-20-20). A total of 30 pots per formula were used in three replications for a total of 90 pots. The indicators monitored were root collar diameter and seedling height.

The method for studying the effects of the potting mix on seedlings

Potting mix: Soil from layer B with a pH of 4.8-5.5. The soil was finely ground and sieved through an iron mesh with small diameter holes ranging from 0.7-1cm. The potting mix should be loose and retain water well to allow for good root development. It must also have a certain level of cohesion to prevent the pot from breaking during transportation. Composition of the potting mix: Several formulas for the potting mix were tested: Formula 1: 100% soil from layer B, without any fertilizer. Formula 2: 80% soil from layer B + 20% composted manure. Formula 3: 83% soil from layer B + 15% composted manure + 2% NPK (20-20-20). Formula 4: 95% soil from layer B + 5% NPK (20-20-20). After germinating for one and a half months, seedlings grown on sand were transplanted into pots measuring 13x18 cm. At regular intervals of fifteen days, the soil was loosened once, weeds were completely removed, and the plants were irrigated with a diluted solution of NPK at a concentration of 1%.

The plants were watered adequately depending on the specific weather conditions. To prevent fungal diseases in young plants, a spray of Benlate solution at a concentration of 0.15%, or Benlate-C at a concentration of 0.3%, was applied every ten days. Growth indicators, such as root collar diameter and seedling height, were observed and measured.

Statistical analysis

All raw collected and the mean of data were statistically analyzed by Excel ver 2016.

Results

Effects of rooting hormone on the rooting rate of cuttings

Through cutting test results, it is found that *D. retusus* cuttings are very difficult to root, and the rate of callus formation was not high. In the same treatment, however, at different concentrations, the rate of callus formation was also different. Detailed data are summarized in Table 1.

Table 1. Rate of cuttings producing callus tissue

Hormone	Number of cuttings	concentration (ppm)	Rate of cuttings producing callus tissue						Average (%)
			1 st		2 nd		3 rd		
			Number	%	Number	%	Number	%	
IAA	90	1000	37	41.11	38	42.22	36	40.00	41.11
	90	1500	38	42.22	38	41.11	37	41.11	41.48
	90	2000	37	41.11	37	40.00	37	41.11	40.74
NAA	90	1000	37	41.11	38	42.22	37	41.11	41.48
	90	1500	40	44.44	42	45.55	41	45.56	45.18
	90	2000	38	42.22	39	44.44	40	44.44	43.70
IBA	90	1000	36	40.00	35	41.11	36	40.00	40.37
	90	1500	37	41.11	38	42.22	37	41.11	41.48
	90	2000	35	38.89	36	40.00	36	40.00	39.63
Control	90	0	30	33.33	31	32.22	31	34.44	33.33

As shown in Table 1, the rate of callus tissue formation showed variations among the formulas used by the different hormones and concentrations. Among the three experimental treatments, NAA had the highest rate of callus tissue formation, reached by 43.46%. This was followed by IAA and IBA, which both reached 40.11% and 40.49%, respectively. Similarly, within the same hormones but at different concentrations, the rate of callus tissue formation also varied. Both IAA and IBA hormones at a concentration of 1500 ppm showed the highest callus production rate, reaching 41.48%. The highest callus production rate for NAA hormone was achieved at a concentration of 1500 ppm, reaching 45.18%.

Table 2. Rooting rate of cuttings

Hormone	Number of cuttings	concentration (ppm)	Rooting rate of cuttings						Average (%)
			1 st		2 nd		3 rd		
			Number	%	Number	%	Number	%	
IAA	90	1000	34	37.78	33	36.67	33	36.67	37.04
	90	1500	35	38.89	35	38.89	34	37.78	38.52
	90	2000	33	36.67	32	35.56	33	36.67	36.30
NAA	90	1000	35	38.89	36	40.00	35	38.89	39.26
	90	1500	38	42.22	39	43.33	38	42.22	42.59
	90	2000	37	41.11	36	40.00	37	41.11	40.74
IBA	90	1000	35	38.89	34	37.78	34	37.78	38.15
	90	1500	36	40.00	36	40.00	35	38.89	39.63
	90	2000	34	37.78	33	36.67	34	37.78	37.41
Control	90	0	28	31.11	27	30.00	28	31.11	30.74

The ratio of rooted cuttings compared to the number of initial experimental cuttings varies depending on the hormone and concentration used. The IAA drug showed the highest rate of rooting cuttings, reaching 38.52%, at a concentration of 1500 ppm. The NAA treatment had the highest rate of rooting cuttings by 42.59%, at a concentration of 1500 ppm. Similarly, the IBA treatment achieved the highest rooting rate by reaching 39.63% at a concentration of 1500 ppm.



Figure 1. *Dipterocarpus retusus* cuttings rooting in the formula using NAA, concentration 1500 ppm

The results of evaluating the effects of growth hormones on the survival rate of cuttings after transplanting into pots are summarized in Table 3.

Table 3. Survival rate of cuttings after transplanting into pots

Hormone	N of cuttings	concentration (ppm)	Survival rate of cuttings						Average (%)
			1 st		2 nd		3 rd		
			N	%	N	%	N	%	
IAA	90	1000	32	35.56	31	34.44	31	34.44	34.81
	90	1500	31	34.44	32	35.56	32	35.56	35.19
	90	2000	31	34.44	32	35.56	31	34.44	34.81
NAA	90	1000	32	35.56	32	35.56	32	35.56	35.56
	90	1500	33	36.67	33	36.67	35	38.89	37.41
	90	2000	31	34.44	30	33.33	32	35.56	34.44
IBA	90	1000	31	34.44	30	33.33	31	34.44	34.07
	90	1500	33	36.67	33	36.67	31	34.44	35.93
	90	2000	32	35.56	31	34.44	30	33.33	34.44
Control	90	0	27	30.00	25	27.78	26	28.89	28.89

Note: N: Number

The percentage of survival of cuttings after being transplanted into pots for 15 days was compared to the number of initial experimental cuttings with different treatments and concentrations (Table 3). Specifically, the IAA drug exhibited the highest survival rate of cuttings at a concentration of 1500 ppm by 35.19%. The NAA treatment achieved the highest survival rate of cuttings at a concentration of 1500 ppm by 37.41%, respectively. The IBA treatment had the highest survival rate of cuttings at a concentration of 1500 ppm, which was equal to 35.93%.

Effect of cutting age on rooting rate of cuttings

The results of research on the influence of cutting age on rooting rate are summarized in Table 4.

Table 4. Results of cutting propagation of *Dipterocarpus retusus* at different cutting ages

Cutting age	N ^o of cuttings	N ^o of rooted cuttings	Rooting rate of cuttings (%)	Average number of roots/cutting	The average length of roots (cm)
Mature	90	35	38.88	2.2	2.9
Semi-hardwood	90	39	43.33	2.9	3.2
Young	90	52	36.66	1.9	2.4

Note: N^o: Number

As shown in Table 4, it is observed that the age of the cutting influences the rooting rate of *D. retusus*. Under the same conditions of growth hormone, concentration, and season, different rooting rates were recorded. Specifically, for mature cuttings, the rooting rate reached 38.88%, while for Ssemi-hardwood cuttings (43.33%) and young cuttings (36.66%, respectively. To confirm whether the age of the cuttings significantly affects the

rooting rate or not, a one-way analysis of variance was conducted. The results indicated that the age of the cuttings had a significant impact on the rooting rate of the *D. retusus*. Based on the analyses, an optimal formula for the rooting rate was determined to be using semi-hardwood cuttings.



Figure 2. Cuttings of *Dipterocarpus retusus* at the different ages

The age of the cutting directly impacts the number of roots it produces. Even when it is provided with the same concentration of growth hormone, under the same conditions and during the same season, the number of roots per cutting was varied. This variation can be explained as follows: In older cuttings, the cells in the cambium layer are aged and deteriorated, resulting in a reduced ability to divide and form callus tissue necessary for root formation. Consequently, the rooting rate of these cuttings was lower. Younger cuttings, on the other hand, experience more active cell division in the cambium layer, leading to the formation of callus tissue. However, their rooting rate was also lower due to their instability and less developed against defence mechanisms. In contrast, semi-hardwood cuttings exhibited strong and stable cell division in the cambium layer, resulting in a higher rooting rate compared to older or younger cuttings. Moreover, the age of cutting also affects root length. Older cuttings had an average root length of 2.9 cm, while younger ones attained an average root length of 2.4 cm. Semi-hardwood cuttings showed the longest average root length, which was 3.2 cm.

Effects of the cutting season

The influence of the cutting season is summarized in Table 5. Based on the obtained data, it provides evidence that the various experimental seasons resulted in differences in the ability of cutting propagation for *D. retusus*. These differences are observed in the formation of callus tissue, the ratio of root formation, and the survival ratio after transplantation into pots, all under the same type of growth stimulant and concentration

ratio. Therefore, it can be concluded that seasonal variation impacts the cutting propagation ability of *D. retusus*.

Table 5. Results of cutting propagation of *Dipterocarpus retusus* at the different seasons

Hormone	Rate of callus formation (%)				Rooting rate of cuttings (%)				Rate of survival cuttings after transplanting (%)			
	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
NAA	45.6	46.7	46.6	47.8	43.3	44.4	43.3	42.2	36.7	37.8	35.6	36.67
Control	33.3	32.2	32.2	33.3	30.0	30.0	27.8	28.9	28.9	28.9	27.8	28.9

All experimental formulas and control formulas (without using hormones) for cutting propagation in different seasons resulted in callus tissue formation ranging from 33.3% to 47.8%. Among these, experiments using growth stimulants showed variations from 45.6% to 47.8%. While the root formation ratios in formulas using growth stimulants ranged from 42.2% to 44.4%. The survival rate after root transplantation into pots, following 15 days of using growth regulators, varied from 35.6% to 37.8%, respectively.

Effect of lighting regime on growth of cuttings

The results of studying the effects of the lighting regime on cutting growth are summarized in Table 6.

Table 6. Effect of lighting regime on growth of cuttings

Formulas	Growth (after 6 months)	
	D ₀₀ (cm)	H _{vn} (m)
Shading 0.3	0.47	0.45
Shading 0.5	0.45	0.47
No shading	0.52	0.42

Notes: D₀₀: Root collar diameter; H_{vn}: Height from the base of the tree to the top of the seedling

Based on the results shown in Table 6, it is evident that as the shading intensity increases, the rate of diameter growth slows down, while the height growth tends to increase. This phenomenon can be explained as follows: When shade increases, seedlings instinctively grow taller in an attempt to compensate for the lack of light. Additionally, the increase in shading also causes a decrease in temperature. It is well-known that cambium activity, which directly affects diameter growth, is closely associated with temperature. When the temperature decreases, the activity of the cambium decreases as well, and vice versa.

Effect of potting mix on seedling growth

Table 7 summarizes the research results on the effect of potting mix on root diameter growth of cuttings during the nursery stage. Data for root diameter growth in all formulas tend to increase gradually with age; in formulas 1 and 2, the first and second months have the strongest diameter growth; after that the growth tends to slow down.

Table 7. Diameter growth of *Dipterocarpus retusus* cuttings

Age (months)	Root collar diameter (cm)			
	CT1	CT2	CT3	CT4
1	0.23	0.25	0.27	0.26
2	0.28	0.28	0.31	0.29
3	0.31	0.32	0.34	0.33
4	0.33	0.34	0.39	0.37
5	0.36	0.36	0.44	0.41
6	0.37	0.39	0.51	0.46

In formula 3, cuttings with ages of 5 and 6 months maximumly increased. There were clear differences between experimental formulas for diameter growth (calculated $F = 17.005 > F_{\alpha} = 4.0$, or $P \text{ value} = 0.00078 < 0.05$). In formula 3 (with manure and NPK fertilizer), diameter growth was the strongest, at the 6th measurement reaching 0.51cm, the worst in the control formula, diameter was 0.37cm, respectively.

Along with diameter growth, seedling height growth is also an important indicator of the growth capacity of seedlings during the nursery stage. The results of monitoring the height growth of *D.retusus* seedlings during the nursery period are summarized in Table 8.

Table 8. The height of growth of *Dipterocarpus retusus* plant cuttings

Age (months)	CT1	CT2	CT3	CT4
1	14.1	15.3	15.5	14.9
2	19.5	20.1	23.1	20.4
3	23.6	24.2	28.8	24.8
4	27.8	28.9	34.4	30.1
5	32.2	33.7	39.5	36.4
6	36.1	38.9	45.3	44.2

Table 8 shows that the height growth in the control formula was the slowest, reaching 36.1 cm after 6 months of age. The fastest growth was in formula 3, reaching 45.3cm, followed by formula 4 at 44.2cm and formula 2 at 39.9 cm.

Discussion

Due to the impact of continuous exploitation many years ago, many tree species including the *D. retusus* trees have been exhausted in Thai Nguyen province. Survey results in the entire province recorded only 244 *D. retusus* trees with a diameter of over 6 cm (Thao *et al.*, 2023). This is an alarming number. Results of phenological monitoring surveys also show that many trees flower and bear fruit but have a high sterility rate (Thao *et al.*, 2023), so research on propagation by cuttings is very necessary.

This study shows that *D.retusus* cuttings were very difficult to root, and the rate of callus formation was not high. However, NAA at a concentration of 1500 ppm gave the highest rate of rooting cuttings, which reached by 42.59%. While using NAA 1500 ppm

to grow *D. retusus* cuttings also gave the maximum survival rate of cuttings, reaching 37.41%. Experimental results of *Fernandoa brilletii* cuttings by Thao and Thong (2022), who indicated that using the growth hormone IBA, 1.5% concentration, gives the highest rate of rooting cuttings (74.6%).), highest number of roots (4.7 roots/cutting) and largest average root length (4.5 cm). Previous studies showed that *D. odorifera* cuttings root strongly when treated with IBA at a concentration of 1500 ppm (Nhung *et al.*, 2019). However, this study revealed that using IBA was not as effective as NAA. This rooting stimulant has been used to grow cuttings of some species of the genus *Dalbergia*. Puri and Verma (1996) treated *Dalbergia sissoo* (*D. sissoo*) cuttings with NAA or IBA at a concentration of 100 mg/l helped increase the rooting rate to over 90%. Another study showed that IBA-stimulated rooting was more effective than NAA when treating *D. sericea* cuttings (Amri *et al.*, 2010).

The addition of manure and NPK fertilizer and substrate showed that the cuttings grew the strongest, reaching 0.51cm in diameter and 45.3cm in height, respectively. The composition of the potting mix also has a clear effect on the rate of *Fernandoa brilletii* seedlings. Adding 15% manure + 2% NPK + 83% B-layer soil gives the highest survival rate and the ability to grow in diameter and height is strongest (Thao and Thong, 2022). Adding 15% of manure to the nursery medium gives outstanding results in the growth of *Bauhinia variegata* tree (Yen *et al.*, 2022). Experiments on sowing *Dialium cochinchinense* Pierre tree also showed that using a substrate consisting of 84% soil + 15% composted manure + 1% NPK fertilizer resulted in outstanding growth (Ngoc *et al.*, 2019). Previous studies have identified suitable growing media formulas to effectively serve the nursery of native tree species such as *Sandoricum indicum* (Cuong *et al.*, 2021), *Vatica mangachapoi* (Hoi *et al.*, 2022).

The age of *D. retusus* cuttings affects the rooting rate of cuttings, in which semi-hardwood cuttings have the highest rooting rate, reaching 43.33%. Previous research with *Fernandoa brilletii* and *D. odorifera* cuttings also showed similar results (Thao, 2022; Nhung *et al.*, 2019). These research results will contribute to the scientific basis for developing a technical guideline for propagating the *D.odorifera* tree using cuttings to serve the development of genetic resources for this rare tree species.

Conclusion

In conclusion, *D. retusus* cuttings were very difficult to root, and the rate of callus formation was not high. NAA at a concentration of 1500 ppm gave the highest rate of rooting cuttings, reaching 42.59%. Using NAA 1500ppm to grow *D. retusus* cuttings also gave the highest survival rate of cuttings (37.41%). The age of cuttings affects the rooting rate of *D. retusus* cuttings, in which semi-hardwood cuttings had the highest rooting rate (43.33%). As shade increases, diameter growth tends to slow down, while height growth gradually increases. The fertilizer formula of 15% manure + 2% NPK showed the strongest growth of cuttings, reaching 0.51cm in diameter and 45.3cm in height. Our study has provided the basis for developing a technical protocol for propagating *D. retusus* trees by the cuttings method.

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