

Research Article

CONSERVATION STATUS AND PROPAGATION OF *Camellia dalatensis* AND *Camellia capitata* BY USING CUTTINGS

Le Hong En^{1*}, Do Van Duong¹, Nguyen Pham Doan¹, Nguyen Van Phuc¹, Ngo Van Cam¹, Le Thi Thuy Hoa¹, Nguyen Ba Trung¹, Ho Si Hung¹, Ngo Giang Phi¹, Nguyen Thanh Nguyen¹, Hoang Thanh Truong¹, Truong Quang Cuong²

¹Forest Science Institute of Central Highlands and South of Central Vietnam, Da Lat City 66000, Lam Dong Province, Vietnam.
²Bidoup Nui Ba National Park, Da Lat City 66000, Lam Dong Province, Vietnam

ARTICLE HIGHLIGHTS

- *Camellia dalatensis* and *Camellia capitata* are critically endangered species.
- Habitat loss and deforestation threaten the survival of these *Camellia* species.
- Vegetative propagation aids conservation of critically endangered *Camellia* species.
- Rooting success depends on cutting types, growth regulators, and substrate mix.
- Research aids conservation efforts for Vietnam's rare and vulnerable flora.

ABSTRACT

Camellia dalatensis (V. D. Luong, Ninh & Hakoda) and *Camellia capitata* (Orel, Curry & Luu) are classified as critically endangered (CR) by the IUCN. Conservation solutions for these two species have not yet been fully implemented, while wild populations are declining. This research on vegetative propagation examined the efficacy of indole-3-butyric acid (IBA in solution or in powdered activated charcoal), cutting types (terminal shoot or stem cuttings), and substrates for the growth of cuttings (sand-coir dust mixes) on rooting efficiency. The highest rooting of *C. dalatensis* was observed in terminal shoot cuttings at 1,000 ppm IBA, and 25% sand and 75% coir dust substrate. Maximum values obtained were survival 95.6%, rooting percentage 88.9%, number of roots per cutting 6.9, root length 6.2 cm, and rooting index 42.9. For *C. capitata*, superior rooting was obtained with semi-hardwood cuttings, at 1,500 ppm IBA, and equal parts of sand and coir dust as substrate. *Camellia capitata* was more difficult to root, with maximum values of 65.6% survival, 52.2% rooting, 2.8 roots per cutting, 2.9 cm root length, and rooting index of 8.1. The results of this study can be used to support further propagation and conservation of these two endangered species.

Keywords:

Camellia capitata, *Camellia dalatensis*, endangered species, IBA, rooting, vegetative propagation

Article Information

Received 20 June 2024
Revised 16 August 2024
Accepted 10 September 2024

Reviewer:
Eucharia Chizoba Ezigbo & anonymous

*Corresponding author, e-mail:
lehongen@gmail.com

INTRODUCTION

Camellia L., the largest genus in the Theaceae family, is widely distributed from Bhutan, Northeast India, China, and Japan to Indonesia and the Philippines (Chang & Bartholomew 1984; Ming & Bartholomew 2007). Vietnam has a high diversity of *Camellia* and many species have been recently recorded, such as *C. hiepii*, *C. hoaana*, *C. hoabinhensis*, *C. maianhii*, *C. pyriformis*, and *C. vanlangensis* (Nguyen *et al.*

2023; Trinh *et al.* 2023; Yang *et al.* 2024; Trinh & Hoang 2024; Quach *et al.* 2024). *Camellia dalatensis* was described in 2012 (Tran & Luong 2012) and *C. capitata* in 2014 (Orel *et al.* 2014). These two species are classified as critically endangered (CR) by the IUCN, with low regeneration potential due to habitat changes under the impacts of climate change and human impacts, such as deforestation from shifting cultivation and overexploitation. Therefore, more quantitative information on the status and

conservation strategies of these threatened *Camellia* is needed (Rivers & Luu 2018; Orel *et al.* 2014).

Propagation is one of the important components to provide seedlings for species conservation and development programs. Each propagation method has its advantages and disadvantages. Propagation from seeds is easy to implement. However, these two species have disadvantages in implementing seed propagation, i.e., these two species have a small number of individuals, poor fruiting ability, and seasonal seed availability. Propagation via tissue culture has the potential to produce a large number of plants, but this technique requires high technology, high costs, and a long time to conduct. A cheaper alternative approach is to use cuttings for propagating these two *Camellia* species, in which this technique has the advantage of preserving the genetic characteristics of the mother trees.

Propagation by using cuttings has been successful for other indigenous *Camellia* species in Vietnam, including *C. chrysantha*, *C. flava*, *C. petelotii*, and *C. tamdaoensis* (Nguyen *et al.* 2017; Nguyen *et al.* 2021). The aims of this study were: 1) to assess the conservation status of *C. dalatensis* and *C. capitata* in the field and 2) to determine the effects of auxin, cutting types, and substrates on root formation of the cuttings. The research results will contribute to supporting the propagation and conservation of these two threatened species of *Camellia*.

MATERIALS AND METHODS

Assessment of Conservation Status

A survey of *C. dalatensis* and *C. capitata* was undertaken using the snowball sampling approach with 15 respondents (5 forest managers, 5 ethnic people living near the forest boundary, and 5 forest planters). Before completing the interview, all respondents were freely consented.

The interview questionnaire had three questions: (1) *Have you ever seen these two plant species?*; (2) *Where did you see these two plant species?*; and (3) *How many people know about these two species at this location?* Following the interviews, we established 5 survey transects per species, each ranging in length from 2 to 3 km, to locate mature trees.

The program <http://geocat.kew.org/editor> (Royal Botanic Garden 2024) and the IUCN Red List Criteria and Classification Guidelines (IUCN 2022) (<http://www.iucnredlist.org/documents/RedListGuidelines.pdf>) were used to calculate the Extent of Occurrence (EOO) and Area of Occupancy (AOO).

Cutting Experiments

Source of Mother Trees

C. dalatensis was obtained from natural forest in Tram Hanh Commune, Da Lat City, Lam Dong Province, Vietnam. *C. capitata* was gathered from natural forests in Phuoc Cat Commune, Cat Tien District, Lam Dong Province, Vietnam.

Sample Collection and Processing

The branches of *C. capitata* and *C. dalatensis* were gathered in January and May of 2022, respectively. Branches with a diameter of 0.5-0.8 cm were chopped into 50-60 cm long sections, placed in foam crates, moistened with wet towels, and then transported to Da Lat City. The stems were rinsed multiple times in clean water before being cut around 1 cm from the node into 10-15 cm long portions for the research trials.

Location of Propagation Experiments

The propagation experiments were set up in a greenhouse at the Forest Science Institute of Central Highlands and South of Central Vietnam (FSIH), Da Lat City, Lam Dong Province, Vietnam (11°56'35" N, 108°24'23" E). The altitude of the nursery was 1,504 m, the average annual temperature was 18-25 °C, the average annual rainfall was about 2,200 mm, and the wet season happened from May to November. The misting mode in the greenhouse was set up at 20 seconds every 2 hours.

Experimental Design

Experiment 1 - Effect of IBA on Root Formation

The experiment was undertaken with 2 types of indole-3-butyric acid (IBA) in solution and IBA powder combined with activated charcoal. Five concentrations of IBA solution were prepared (0; 500; 1,000; 1,500; and 2,000 ppm) in water. The cuttings were submerged in the IBA solution for 15

minutes. IBA-activated charcoal treatments were 0, 0.5, 1.0, 1.5, and 2.0%. The IBA was dissolved in a small volume of alcohol and then mixed with activated charcoal to form a slurry. The slurries were dried to evaporate the alcohol and then were ground and passed through a sieve (0.35 mm mesh size). The cuttings were briefly dipped into the powder. Experiments were carried out using washed river sand in rectangular plastic drainage baskets with dimensions of 35 cm (length) x 26 cm (width) x 10 cm (height). Rooting development was assessed on day 120.

Experiment 2 - Effect of Cutting Types on Root Formation

The experiment compared two treatments of cutting types, i.e., terminal shoot and semi-hardwood stem cuttings. Sand substrate was used in combination with the optimal result of IBA in experiment 1. Rooting development was assessed on day 120.

Experiment 3 - Effect of Substrates on Root Formation

Four treatments of substrates were applied, i.e., 75% sand and 25% coir dust, 50% sand and 50% coir dust, 25% sand and 75% coir dust, and 100% coir dust. Rooting development was assessed on day 90.

Data Analysis

The experiments were conducted in a randomized block design with three replications. The data measured were survival percentage (%), rooting percentage (%), number of roots, root length (cm), and rooting index (number of roots multiplied by root length). The data were analyzed using the Duncan test in SPSS 26.0.0 software (Statistical Package for Social Sciences version 26).

RESULTS AND DISCUSSION

Conservation Status

Camellia dalatensis has a narrow distribution in Phat Chi Village, Tram Hanh Commune, Da Lat City, Lam Dong Province. The number of mature individuals in the natural habitat was 205 plants. The Extent of Occurrence (EOO: 0.464 km²) and Area of Occupancy (AOO: 8,000 km²) for Critically Endangered (CR) *C. dalatensis* are

illustrated in Figure 1 (upper panel). *Camellia capitata* occurs in Cat Tien National Park, an area managed by Lam Dong Province (Cat Tien District). There were 5 mature plants in the natural habitat. *C. capitata* is categorized as Critically Endangered (CR) with EOO of 0.050 km² and AOO of 4,000 km² (Figure 1; lower panel). Both *C. dalatensis* and *C. capitata* were shown to have a very small distribution area (Fig.2), which were threatened by logging, deforestation, planting of industrial crops, such as cashew, rubber and coffee, and the harvesting of non-timber forest products (Beech *et al.* 2017; Orel *et al.* 2014). Recently, the forests are being exploited for medicinal herbs and ornamental plants with high polyphenol contents and beautiful flowers, including these two *Camellia* species (Fig. 3) (Trinh 2022).

Effect of IBA on Root Formation of *C. dalatensis* and *C. capitata*

Asexual propagation by using cuttings is the optimal solution for preserving genetic characteristics from mature plants when propagation by using seeds is limited by low fecundity. There are many factors affecting rooting, including the environment, the types of cuttings used for propagation, and the use of plant growth regulators (PGRs) (Bhupathireddy *et al.* 2022; Dewi & Sabhara 2022; Eed & Burgoyne 2014; Gautam *et al.* 2022; Griffin *et al.* 1998; Ray & Ali 2017). Cutting propagation efficiency is often increased by using exogenous PGRs, including IBA treatments (Bhupathireddy *et al.* 2022; Gautam *et al.* 2022). However, the optimum concentrations and PGR forms (solution or solid form) can differ markedly between species. For example, 1,000 ppm IBA in solution gave the best results in *Lycium barbarum* L., but 1% IBA was optimal for *Cornus mas* L. (Çelik & Çetin 2021). In this study, 1,000 ppm IBA was suitable for *C. dalatensis*, but *C. capitata* required a higher concentration of 1,500 ppm IBA for obtaining the highest rooting percentage.

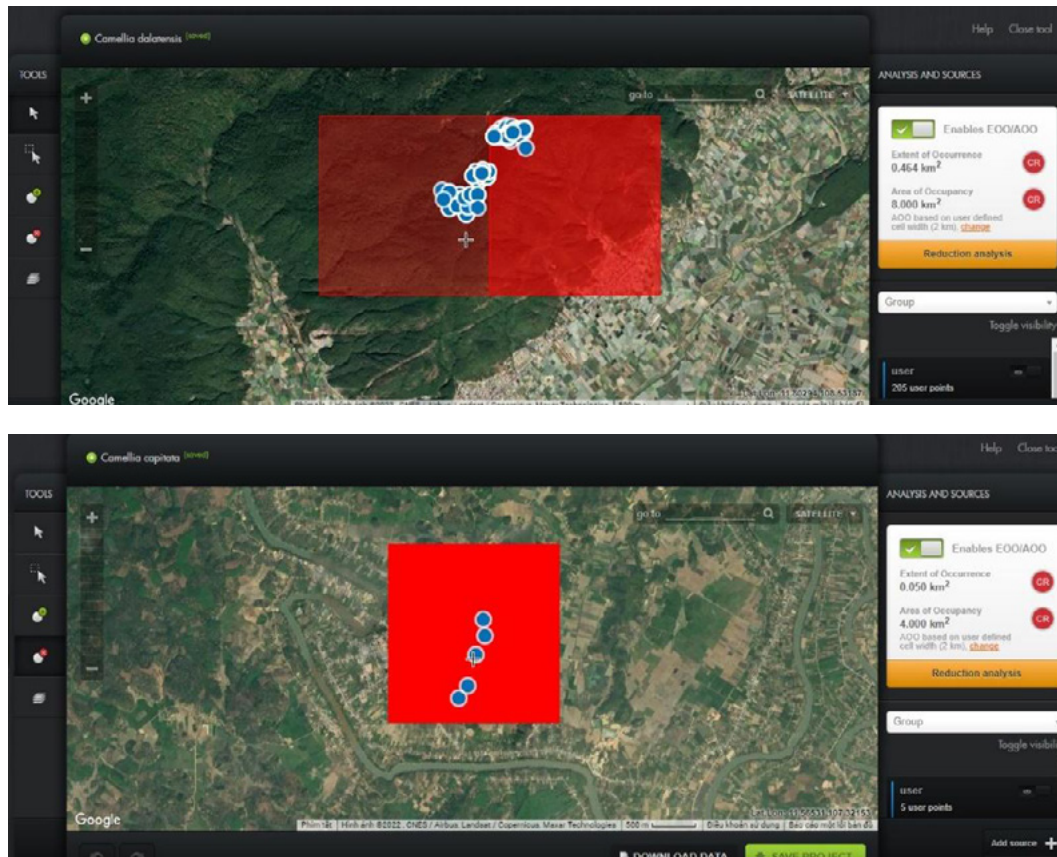


Figure 1 Extent of Occurrence (EOO) and Area of Occupancy (AOO) of *Camellia dalatensis* (upper panel) and *C. capitata* (lower panel)

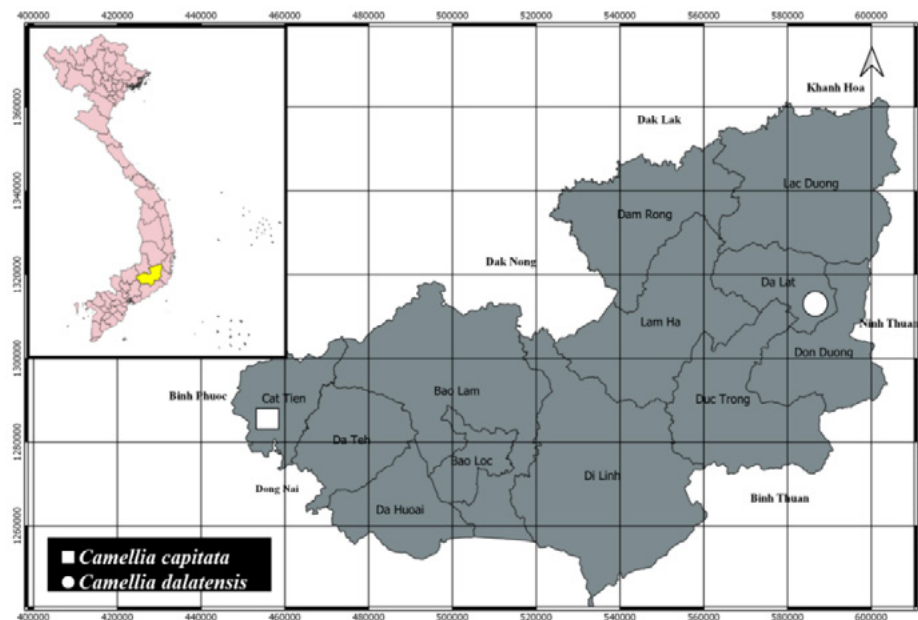


Figure 2 Distribution map of *Camellia capitata* and *C. dalatensis* in Lam Dong Province, Vietnam



Figure 3 Appearance of *Camellia capitata* and *C. dalatensis*
 Notes: a. Seedlings of *C. dalatensis* in their natural habitat; b. Flower of *C. dalatensis*; c. Flower of *C. capitata*; d. Seedlings of *C. capitata* in their natural habitat.

Differences in PGR requirements for the rooting of cuttings not only vary between plant species, but also depend on the position in the plant where the cuttings are sourced. Cuttings taken from mature trees often have reduced endogenous auxin content compared to cuttings taken from juvenile plants. Hence, it is necessary to undertake experiments on cuttings responses toward PGR dosages to ensure that optimal PGR dosages are being used for the correct cutting type. Also, at very high concentrations, the rooting efficiency can be significantly reduced, such as in *Prunus laurocerasus* L. at 2 g/L IBA (Sulusoglu & Cavusoglu 2010). Thus, depending on the type of tree and the age of the tree, the rooting auxin treatment needs to be adjusted to the appropriate concentration, and it is necessary to have specific studies for each tree species.

The effectiveness of using IBA in propagation of threatened *Camellia* species are shown in Tables 1 and 2. There were significant ($P < 0.05$) effects of IBA rates on survival percentage, rooting percentage, number of roots, root length, and rooting index.

For *C. dalatensis*, the survival percentage ranged from 65.56% to 80%; the rooting percentage ranged from 14.45% to 45.56%; the number of roots ranged from 0.57 to 3.80; the root length ranged from 1.67 cm to 4.23 cm; and the rooting index ranged from 1.12 to 16.32. The most effective treatment was 1,000 ppm IBA. For *C. capitata*, the survival percentage ranged from 28.89% to 40.00%; the rooting percentage ranged from 7.78% to 31.11%; the number of roots ranged from 0.30 to 1.90; the root length ranged from 0.40 cm to 2.13 cm; and the rooting index ranged from 0.15 to 4.09. The most effective treatment was 1,500 ppm IBA.

Table 1 Effect of IBA concentration on *Camellia dalatensis* root formation

| Treatment | Survival percentage (%) | Rooting percentage (%) | Number of roots per cutting | Root length (cm) | Rooting index |
|------------------|--------------------------------|-------------------------------|------------------------------|------------------------------|-------------------------------|
| 500 ppm | 74.45±2.22 ^{ab} | 32.22±2.94 ^{bc} | 1.73±0.35 ^{bcd} | 2.43±0.15 ^b | 4.29±1.11 ^c |
| 1,000 ppm | 77.78±1.11^{ab} | 45.56±2.94^a | 3.80±0.32^a | 4.23±0.38^a | 16.32±2.76^a |
| 1,500 ppm | 76.67±1.93 ^{ab} | 37.78±4.01 ^{ab} | 3.73±0.90 ^a | 2.90±0.75 ^b | 11.72±4.83 ^{ab} |
| 2,000 ppm | 76.67±1.93 ^{ab} | 31.11±2.94 ^{bc} | 2.50±0.61 ^{ab} | 2.80±0.45 ^b | 6.85±2.06 ^{bc} |
| 0.5% | 72.22±2.94 ^b | 22.22±2.94 ^{de} | 1.33±0.12 ^{bcd} | 2.87±0.15 ^b | 3.85±0.50 ^c |
| 1.0% | 80.00±1.92 ^a | 35.56±1.11 ^b | 2.10±0.35 ^{bc} | 2.83±0.38 ^b | 5.73±0.65 ^{bc} |
| 1.5% | 77.78±1.11 ^{ab} | 25.55±2.22 ^{cd} | 2.07±0.19 ^{bc} | 2.57±0.46 ^b | 5.29±1.10 ^{bc} |
| 2.0% | 65.56±1.11 ^c | 20.00±1.92 ^{de} | 0.63±0.29 ^{cd} | 1.70±0.32 ^b | 1.12±0.67 ^c |
| Not using PGRs | 74.44±1.11 ^{ab} | 14.45±2.22 ^e | 0.57±0.42 ^d | 1.67±0.22 ^b | 1.12±0.91 ^c |
| Mean | 75.06±0.93 | 29.38±1.95 | 2.05±0.25 | 2.67±0.18 | 6.25±1.10 ^c |
| P-value | 0.001 | 0.000 | 0.000 | 0.012 | 0.001 |

Notes: *Mean values in columns followed by different letters are statistically significantly different with $P \leq 0.05$ using Duncan's multiple range test.

Table 2 Effect of IBA concentration on *Camellia capitata* root formation

| Treatment | Survival percentage (%) | Rooting percentage (%) | Number of roots per cutting | Root length (cm) | Rooting index |
|------------------|-------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|
| 500 ppm | 38.89±1.11 ^{ab} | 16.67±1.93 ^c | 0.83±0.90 ^{bcd} | 1.67±0.12 ^c | 0.99±0.21 ^{bc} |
| 1,000 ppm | 40.00±1.92 ^a | 25.55±2.22 ^b | 1.37±0.12 ^{ab} | 1.53±0.12 ^{bc} | 2.10±0.25 ^b |
| 1,500 ppm | 40.00±1.92^a | 31.11±1.11^a | 1.90±0.15^a | 2.13±0.24^a | 4.09±0.70^a |
| 2,000 ppm | 31.11±1.11 ^{bcd} | 25.57±1.11 ^b | 0.87±0.09 ^{bcd} | 1.23±0.27 ^c | 1.05±0.23 ^{bc} |
| 0.5% | 36.67±3.85 ^{abc} | 13.33±1.93 ^c | 0.57±0.09 ^{cd} | 1.23±0.12 ^c | 0.68±0.06 ^{bc} |
| 1.0% | 32.22±2.94 ^{abcd} | 14.44±1.11 ^c | 1.00±0.47 ^{bc} | 1.80±0.15 ^{ab} | 1.94±1.05 ^b |
| 1.5% | 32.22±4.01 ^{abcd} | 14.44±2.94 ^c | 1.40±0.21 ^{ab} | 1.23±0.90 ^c | 1.71±0.23 ^b |
| 2.0% | 26.67±1.93 ^d | 11.11±1.11 ^{cd} | 0.97±0.15 ^d | 1.00±0.21 ^c | 1.02±0.35 ^{bc} |
| Not using PGRs | 28.89±2.22 ^{cd} | 7.78±1.11 ^d | 0.30±0.26 | 0.40±0.12 ^d | 0.15±0.11 ^c |
| Mean | 34.07±1.16 | 17.78±1.52 | 1.02±0.54 | 1.30±0.10 | 1.53±0.25 |
| P-value | 0.009 | 0.000 | 0.001 | 0.000 | 0.001 |

Notes: *Mean values in columns followed by different letters are statistically significantly different with $P \leq 0.05$ using Duncan's multiple range test.

For *C. dalatensis* and *C. capitata*, the liquid growth regulator was more effective compared to the charcoal powder in promoting rooting. Results on survival percentage and rooting percentage are compared in Figure 4.

Liquid IBA solutions are easy to prepare, while the phloem and xylem of cuttings have better access to exogenous hormones than hormones combined with powders. However, powder containing activated charcoal is an effective adsorbent for many pollutants (organic, inorganic, microbial, and biological) (Mohammad-Khah & Ansari 2009). Therefore, when the activated charcoal-contained powder is dry-mixed with a plant growth regulator, the dry mix can reduce infection. Therefore, in this research, we sought to identify

the most effective treatment, whether the activated charcoal-contained powder or liquid plant growth regulator.

Effect of Cutting Types on Root Formation

Cutting types are divided into softwood (non-lignified) cuttings, semi-hardwood (partly lignified) cuttings, and hardwood (lignified portion) cuttings (Griffin *et al.* 1998; Islam *et al.* 2010). Most propagators of woody plants use semi-hardwood cuttings for higher rooting efficiency compared to softwood and hardwood cuttings (Alkaç *et al.* 2022).

In each cutting type, the cutting position, whether terminal shoot or stem cuttings, also has a great impact on rooting efficiency. In most species,

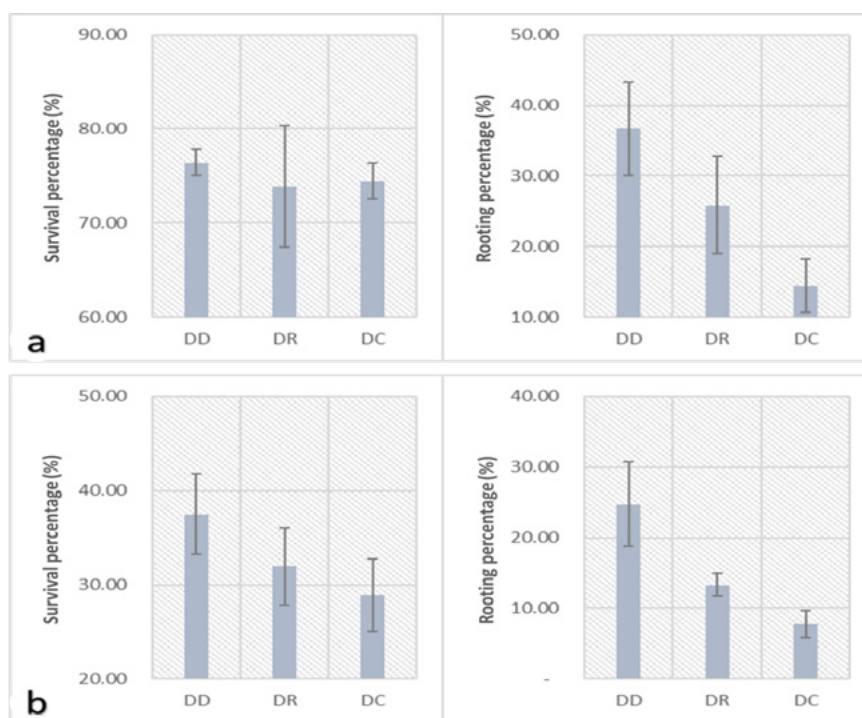


Figure 4 Comparisons on survival and rooting percentages for cuttings dipped in liquid IBA or IBA in charcoal for *Camellia dalatensis* (a) and *C. capitata* (b)

Notes: DD = IBA solution, DR = IBA powder, DC = no IBA.

Table 3 Effect of cutting types on *Camellia dalatensis* root formation

| Treatment | Survival percentage (%) | Rooting percentage (%) | Number of roots per cutting | Root length (cm) | Rooting index |
|-----------|-------------------------|------------------------|-----------------------------|------------------|---------------|
| TC | 90.00±1.92 | 58.89±1.11 | 5.10±0.59 | 4.50±0.12 | 23.08±3.20 |
| SC | 67.78±2.94 | 31.11±2.94 | 1.97±0.15 | 2.33±0.20 | 4.62±0.62 |
| Mean | 78.88±5.21 | 45.00±6.39 | 3.53±0.75 | 3.42±0.50 | 13.85±4.38 |
| P-value | 0.003 | 0.001 | 0.007 | 0.001 | 0.005 |

Notes: *Mean values in columns followed by different letters are statistically significantly different with $P \leq 0.05$ using Duncan's multiple range test; TC = Terminal shoot cuttings; SC = Stem cuttings.

Table 4 Effect of cutting type on *Camellia capitata* root formation

| Treatment | Survival percentage (%) | Rooting percentage (%) | Number of roots per cutting | Root length (cm) | Rooting index |
|-----------|-------------------------|------------------------|-----------------------------|------------------|---------------|
| TC | 45.56±1.11 | 25.56±1.11 | 1.80±0.06 | 1.87±0.19 | 3.36±0.37 |
| SC | 37.78±2.22 | 34.45±2.22 | 1.50±0.06 | 2.00±0.10 | 3.00±0.19 |
| Mean | 41.67±2.06 | 30.00±2.28 | 1.65±0.08 | 1.93±0.10 | 3.18±0.20 |
| P-value | 0.035 | 0.023 | 0.021 | 0.056 | 0.436 |

Notes: *Mean values in columns followed by different letters are statistically significantly different with $P \leq 0.05$ using Duncan's multiple range test; TC = Terminal shoot cuttings; SC = Stem cuttings.

cuttings from terminal shoot have higher rooting efficiency. However, the use of appropriate plant growth regulators can affect the rooting efficiency of stem cuttings and ensure propagation efficiency with the number of rooted cuttings higher than that of the terminal shoot cuttings (Solikin 2019). Choosing the right cuttings for propagation

and increasing the efficiency of propagation are necessary solutions.

For some *Camellia* spp., it is common to choose semi-hardwood cuttings because this cutting type provides the best results. In this study, semi-hardwood cutting was used and divided into terminal shoot cuttings and stem cuttings (Tables 3 and 4).

Based on Experiment 1, the IBA concentrations used were 1,000 ppm for *C. dalatensis* and 1,500 ppm for *C. capitata*. The parameters of survival percentage, rooting percentage, number of roots, root length, and rooting index were significantly different for *C. dalatensis* ($P < 0.05$). Terminal shoot cuttings provided better results compared to stem cuttings with the survival percentage of 90.00% compared to 67.78%, the rooting percentage reaching 58.89% compared to 31.11%, 5.10 roots per cutting compared to 1.97 roots per cutting, the root length reached 4.50 cm compared to 2.33 cm, the rooting index reached 23.08 compared to 4.62. Similar results have been reported by Wazir (2014) in propagating *C. japonica* by using terminal cuttings with 1,000 ppm IBA where the highest percentage of rooting was 84.96%, and the longest root length was 18.3 cm. For *C. capitata*, the parameters of survival percentage, rooting percentage, and number of roots per cutting were significantly different ($P < 0.05$). However, root length and rooting index were not statistically significantly different ($P > 0.05$). The survival percentage of terminal shoot cuttings compared to stem cuttings was 45.56% compared to 37.78%. However, the rooting index for terminal shoot cuttings was about 9% lower than that of stem cuttings (25.56% versus 34.45%), and the number of roots per cutting was 1.80 compared to 1.50. The root length ranged from 1.87 cm to 2.00 cm (average 1.93 cm) and the rooting index range was 3.00 to 3.36 (average 3.18) for both cutting types.

Comparing the rooting parameters, the effective cutting type for *C. capitata* propagation was semi-hardwood stem cuttings. At the time of gathering cuttings in the field, the *C. capitata* had begun to sprout. Therefore, the terminal shoot cuttings were very immature and weak, so they were easy to be infected by fungi. Besides, we collected the stem cuttings at the same time that the cuttings were partially lignifying, to have better auxin synthesis; thereby, the rooting percentage of stem cuttings was higher than that of terminal shoot cuttings in *C. capitata*.

Effect of Substrates on Root Formation

The growing substrate environment should have good porosity to support smooth water draining and provide a suitable temperature and humidity regimes for root development. The substrate media can be combined with other factors, such as plant growth regulators and cutting types, to bring the highest efficiency to the propagation process (Johnson *et al.* 2005; Silva *et al.* 2012).

The selected growing substrate for propagation in this study was a mixture of sand and coir dust. Sand ensures good drainage, and coir dust improves water retention in the rooting environment. In this experiment, 1,000 ppm IBA was used for semi-hardwood cuttings of *C. dalatensis*, while 1,500 ppm IBA was used for semi-hardwood cuttings of *C. capitata*. Rooting results are shown in Table 5 (*C. dalatensis*), Table 6 (*C. capitata*), and Figure 5.

Table 5 Effect of substrate type on *Camellia dalatensis* root formation

| Treatment | Survival percentage (%) | Rooting percentage (%) | Number of roots per cutting | Root length (cm) | Rooting index |
|-----------|-------------------------|-------------------------------|------------------------------|------------------------------|-------------------------------|
| A1 | 94.44±1.11 | 75.56±1.11 ^c | 4.93±0.30 ^c | 4.60±0.06 ^c | 22.73±1.65 ^c |
| A2 | 95.56±2.94 | 83.33±1.93 ^b | 5.13±0.18 ^{bc} | 5.33±0.15 ^b | 27.41±1.47 ^{bc} |
| A3 | 95.56±1.11 | 88.89±2.22^a | 6.87±0.27^a | 6.23±0.15^a | 42.88±2.71^a |
| A4 | 95.56±2.94 | 81.11±1.11 ^b | 6.10±0.50 ^{ab} | 5.60±0.20 ^b | 34.26±3.59 ^b |
| Mean | 95.28±0.10 | 82.22±1.61 | 5.76±0.27 | 5.44±0.19 | 31.82±2.53 |
| P-value | 0.978 | 0.003 | 0.011 | 0.000 | 0.002 |

Notes: *Mean values in columns followed by different letters are statistically significantly different with $P \leq 0.05$ using Duncan's multiple range test; A1 = 75% sand and 25% coir dust; A2 = 50% sand and 50% coir dust; A3 = 25% sand and 75% coir dust; A4 = 100% coir dust.

Table 6 Effect of substrate type on *Camellia capitata* root formation

| Treatment | Survival percentage (%) | Rooting percentage (%) | Number of roots per cutting | Root length (cm) | Rooting index |
|-----------|-------------------------|------------------------|------------------------------|------------------------------|------------------------------|
| A1 | 64.45±2.22 | 47.78±2.22 | 2.03±0.09 ^b | 2.33±0.12 ^b | 4.72±0.05 ^b |
| A2 | 65.55±2.22 | 52.22±2.94 | 2.83±0.20^a | 2.87±0.17^a | 8.12±0.69^a |
| A3 | 65.56±2.94 | 53.33±1.93 | 2.57±1.19 ^a | 1.83±0.07 ^c | 4.73±0.50 ^b |
| A4 | 66.66±3.33 | 52.22±2.22 | 2.57±0.33 ^a | 1.77±0.09 ^c | 4.54±0.28 ^b |
| Mean | 65.56±1.84 | 51.39±1.19 | 2.50±0.11 | 2.20±0.14 | 5.53±0.49 |
| P-value | 0.952 | 0.406 | 0.026 | 0.001 | 0.001 |

Notes: *Mean values in columns followed by different letters are statistically significantly different with $P \leq 0.05$ using Duncan's multiple range test; A1 = 75% sand and 25% coir dust; A2 = 50% sand and 50% coir dust; A3 = 25% sand and 75% coir dust; A4 = 100% coir dust.

For *C. dalatensis*, there was no statistically significant differences in survival percentage ($P > 0.05$) between treatments, with a mean value of 95.28%. However, rooting percentage, number of roots, root length, and rooting index were significantly different ($P < 0.05$). Rooting percentage ranged from 75.56% to 88.89% (average 82.22), number of roots per cutting ranged from 4.93 to 6.78 (average 5.76), root length ranged from 4.60 cm to 6.23 cm (average 5.44 cm), and rooting index ranged from 22.73 to 42.88 (average 31.82).

The treatment with the best results for *C. dalatensis* was 25% sand:75% coir dust, which may be related to the wet environment where this species occurs. *C. dalatensis* was distributed in Dalat City which has high humidity and high annual rainfall. For *C. capitata*, there was no significant difference between the survival and rooting percentages ($P > 0.05$), the average survival percentage was 65.56% and the average rooting percentage was 51.39%. The number of roots per cutting ranged from 2.03 to 2.83 (average 2.50), the root length ranged from 1.77 cm to 2.87 cm (average 5.44 cm) and the rooting index ranged from 4.54 to 8.12 (mean 5.53). Treatment with the best results for *C. capitata* was the treatment with equal parts of sand and coir dust. *C. capitata* occurs in Cat Tien District which has lower humidity and rainfall compared to that in Dalat City. Therefore, this species was able to grow better in the medium which had similar conditions to their natural habitat.

The effects of media types and composition on rooting efficiency have been reported in many plants (Eed & Burgoyne 2014). Air content and oxygen diffusion rate in media are important for rooting (Ercisli *et al.* 2002). The characteristics of the species also influence rooting success. In particular, *Camellia impressinervis* grows well in high moisture and well drained soil (Tran 2018), in which their cuttings had the best rooting results in a growth medium of 100% sand with high air content and oxygen diffusion capacity (Tran *et al.* 2020).



Figure 5 Rootings of *Camellia* cuttings

Notes: a. *C. dalatensis* terminal shoot cuttings with 1,000 ppm IBA in a substrate of 25% sand mixed with 75% coir dust; b. *C. capitata* semi-hardwood stem cuttings with 1,500 ppm IBA in a substrate of 50% sand mixed with 50% coir dust.

CONCLUSION

Camellia capitata and *C. dalatensis* have very limited distributions area. The declining natural populations face the risk of extinction due to fragmentation, deforestation, illegal logging, and clearing for agriculture. Vegetative propagation protocols are essential to help domesticate these species and conserve genetic diversity. For propagation by using cuttings, root formation was effective using liquid IBA at 1,000 ppm for *C. dalatensis* and at 1,500 ppm for *C. capitata*. Rooting formation was the highest for terminal shoot cuttings of *C. dalatensis* and semi-hardwood stem cuttings of *C. capitata*. Sand and coir dust substrates with a ratio of 25:75% for *C. dalatensis* and 50:50% for *C. capitata* were the best substrates for root formation of cuttings.

ACKNOWLEDGMENTS

The authors would like to thank BCGI (Botanic Gardens Conservation International) for their financial support and the Southern Institute of Ecology, Forest Science Institute of Central Highlands and South of Central Vietnam, Cat Tien National Park, and Lam Vien Special-use Forest Management Board created the most favorable conditions for us to complete this study.

REFERENCES

- Alkaç OS, Öcalan ON, Güneş M. 2022. Effect of silver nanoparticles treatments on some characteristics of "Santander" lily cultivar. *Turkish Journal of Agriculture-Food Science and Technology (TURJAF)* [Internet]; [cited 2024 May 10]; 10(2):125-8. DOI: 10.24925/turjaf.v10i2.125-128.4386
- Beech E, Barstow M, Rivers M. 2017. *The red list of Theaceae*. Richmond (UK): Botanic Gardens Conservation International.
- Bhupathireddy B, Saxena D, Sharma M, Rao VN, Kaur R. 2022. The impact of IBA and various growing media on pomegranate (*Punica granatum* L.) hardwood cuttings: A review. *Int J Botany Stud* [Internet]; [cited 2024 May 10]; 7(4):97-102. Available from: <https://www.botanyjournals.com/assets/archives/2022/vol7issue4/7-4-18-825.pdf>.
- Çelik H, Çetin F. 2021. Effect of cutting time and IBA application on the rooting of Goji berry cuttings. *Yuzuncu Yil University Journal of Agricultural Sciences* [Internet]; [cited 2024 May 10]; 31(2):294-304. DOI: 10.29133/yyutbd.885250.
- Chang HT, Bartholomew B. 1984. *Camellias*. Portland (US): Timber Press.
- Dewi S, Sabhara S. 2022. The effect of natural PGR combination on the growth of fig (*Ficus carica* L.) plant cuttings. *IOP Conference Series: Earth and Environmental Science* [Internet]. [cited 2024 May 15]; 985:012019. Available from: <https://iopscience.iop.org/article/10.1088/1755-1315/985/1/012019/pdf>.
- Eed A, Burgoyne A. 2014. Effect of different rooting media and plant growth regulators on rooting of joboba (*Simmondsia chinensis* (Link) Schneider) semi-hard wood cuttings under plastic tunnel conditions. In *Proceedings: 2014 Feb; Bali (Indonesia): The International Conference on Agricultural, Ecological, and Medical Sciences*. p. 6-7.
- Ercisli S, Anapali O, Esisken A, Sahin U. 2002. The effects of IBA, rooting media and cutting collection time on rooting of kiwifruit. *Gartenbauwissenschaft* 67(1):34-8.
- Gautam P, Tripathi SK, Kumar A, Prakash S, Sengar RS, Awasthi M, ..., Kumar A. 2022. Effect of different concentrations of PGRs on shooting and survival of stem cuttings in Lemon (*Citrus limon* Burm.) cv. Pant lemon-1, under Western UP conditions. *Biological Forum - An International Journal Sciences* [Internet]; [cited 2024 May 10]; 14(3):1084-8. Available from: https://www.researchtrend.net/bfij/pdf/184%20Effect%20of%20different%20Concentrations%20of%20PGRs%20on%20Shooting%20and%20Survival%20of%20Stem%20Cuttings%20in%20Lemon%20_Citrus%20limon%20Burm._%20cv.%20Pant%20lemon-1,%20under%20Western%20U.P.%20conditions%20Prashant%20Gautam.pdf.
- Griffin JJ, Blazich FA, Ranney TG. 1998. Propagation of Thuja x 'Green Giant' by stem cuttings: Effects of growth stage, type of cutting, and IBA treatment. *J Environ Hortic* [Internet]; [cited 2024 May 10]; 16(4):212-4. DOI: 10.24266/0738-2898-16.4.212.
- Islam AA, Yaakob Z, Anuar N, Osman M. 2010. Propagation potentials of genotypes and different physiological ages of stem cuttings in *Jatropha curcas* L. *J Agric Sci* [Internet]; [cited 2024 May 10]; 2(4):75-82. DOI: 10.5539/jas.v2n4p75.
- IUCN Standards and Petitions Subcommittee [Internet]. 2022. *Guidelines for using the IUCN Red List Categories and Criteria (Version 15.1)*. Prepared by the Standards and Petitions Subcommittee [cited 2024 May 20]. Available from: <https://www.iucnredlist.org/resources/redlistguidelines>.
- Johnson CN, Eakes DJ, Bruner LL, Wright AN, Sibley JL. 2005. Effect of substrates on rooting of stem cuttings of the endangered species *Clematis socialis*. *HortScience* [Internet]; [cited 2024 May 10]; 40(3):875d-875. DOI: 10.21273/HORTSCI.40.3.875d.
- Marković M, Grbić M, Djukić M. 2014. Effects of cutting type and a method of IBA application on rooting of softwood cuttings from elite tree of Cornelian cherry (*Cornus mas* L.) from Belgrade area. *Silva Balcanica* [Internet]; [cited 2024 May 10], 15(1):30-7. Available from: https://silvabalcanica.wordpress.com/wp-content/uploads/2014/08/sb_1512014_4.pdf.

- Ming TL, Bartholomew B. 2007. Theaceae. In Wu ZY, Raven PH, Hong DY (Editors), Flora of China Vol. 12. Hippocastanaceae through Theaceae. Beijing (CN): Science Press; St. Louis (US): Missouri Botanical Garden Press. p.366-478.
- Mohammad-Khah A, Ansari R. 2009. Activated charcoal: Preparation, characterization and applications: A review article. International Journal of ChemTech Research 1(4):859-64.
- Nguyen TT, Luong VD, Do ND. 2023. *Camellia maianbii* (Theaceae): A new species of red-flowered *Camellia* from the North Central coast region of Vietnam. J Bot Res [Internet]; [cited 2024 May 10]; 6(1):176-83. DOI: 10.36959/771/576.
- Nguyen VV, Nguyen TH, Tran VH. 2017. Effects of plant growth regulators and external factors on the propagation of yellow flower *Camellia* from cuttings. Vietnam Journal of Agricultural Science 15(11):1539-46.
- Nguyen XT, Vu TD, Pham VT, Nguyen TS, Nguyen HT. 2021. Rapid propagation of Tam Dao yellow tea (*Camellia tamdaoensis* Ninh et Hakoda) by using stem cutting on aeroponic system. Vietnam Journal of Agriculture and Rural Development 18:186-94.
- Orel G, Wilson PG, Curry AS, Luu HT. 2014. Four new species and two new sections of *Camellia* (Theaceae) from Vietnam. Novon: A Journal for Botanical Nomenclature [Internet]; [cited 2024 May 10]; 23(3):307-18. DOI: 10.3417/2012076.
- Quach VH, Tran N, Hoang TT, Tagane S. 2024. *Camellia hoabinhensis* (Theaceae: sect. Chrysantha), a new yellow-flowered species from Northern Vietnam. Dalat University Journal of Science [Internet]; [cited 2024 May 20]; 14(1):21-9. DOI: 10.37569/DalatUniversity.14.1.1143(2024).
- Ray S, Ali MN. 2017. Factors affecting macropropagation of bamboo with special reference to culm cuttings: A review update. New Zealand Journal of Forestry Science [Internet]. [cited 2024 May 15]; 47. DOI: 10.1186/s40490-017-0097-z.
- Rivers MC, Luu HT. [Internet] 2018. *Camellia dalatensis*. The IUCN Red List of Threatened Species 2018: e.T70561528A70561545; [cited 2024 May 10]. DOI: 10.2305/IUCN.UK.2018-1.RLTS.T70561528A70561545.en.
- Rivers MC. [Internet] 2018. *Camellia capitata*. The IUCN Red List of Threatened Species 2018: e.T70424105A176937766; [cited 2024 May 12]. DOI: 10.2305/IUCN.UK.2018-1.RLTS.T70424105A176937766.en.
- Royal Botanic Gardens [Internet]. 2024. GeoCAT software application [cited 2024 May 20]. Available from: <http://geocat.kew.org/editor>.
- Silva RCP, Maia S, Paiva EP, Silva A, Coelho M, Silva F. 2012. Effect of substrate composition on rooting of cuttings of *Hyptis suaveolens*. Revista Brasileira de Ciências Agrárias [Internet]. [cited 2024 May 10]; 7(2):219-25. DOI: 10.5039/agraria.v5i3a1245.
- Solikin S. 2019. Effect of node position and number of stem cutting on the growth and yield of 'katuk' (*Sauropus androgynus* (L.) Merr.). El-Hayah [Internet]; [cited 2024 May 10]; 6(4):126-35. DOI: 10.18860/elha.v6i4.6336.
- Sulusoglu M, Cavusoglu A. 2010. Vegetative propagation of Cherry laurel (*Prunus laurocerasus* L.) using semi-hardwood cuttings. African Journal of Agricultural Research [Internet]; [cited 2024 May 10]; 5(23):3196-202. Available from: <https://academicjournals.org/journal/AJAR/article-full-text-pdf/784CF8229017>.
- Tran N, Luong VD. 2012. *Camellia dalatensis*: a new species and precious gene should be conserved. VNU Journal of Science (Natural Sciences and Technology) 28(2S):34-5.
- Tran VD. 2018. Overview of Golden *Camellias* in Cao Bang [Scientific Report]. Retrieved from Silviculture Research Institute, Hanoi, Vietnam.
- Tran VD, Tran DM, Dao TD, Mai TL, Nguyen TT, Dang VT, ..., Pham DS. 2020. Effect of exogenous hormone and rooting medium on cutting propagation of golden *Camellia* (*Camellia impressinervis*). J Appl Hortic [Internet]. [cited 2024 May 30]; 22(2):159-63. DOI: 10.37855/jah.2020.v22i02.29.
- Trinh NB, Hoang TS. 2024. *Camellia pyriformis* (Theaceae, section Calpandria), a new species from Northern Vietnam. Dalat University Journal of Science [Internet]; [cited 2024 May 20]; 14(1):13-20. DOI: 10.37569/DalatUniversity.14.1.1160(2024).
- Trinh NB, Luong VD, Le NHN, PhamVT. 2023. *Camellia vanlangensis* (Theaceae, Sect. ArcheCamellia), a new yellow-flowered species from Vietnam. Taiwania [Internet]; [cited 2024 May 10]; 68(4):465-71. DOI: 10.6165/tai.2023.68.465.
- Trinh TD. 2022. Yellow Camellias: a review of chemical constituents and biological activities. Dalat University Journal of Science [Internet]; [cited 2024 May 10]; 12(3):117-44. DOI: 10.37569/DalatUniversity.12.3.977(2022).
- Wazir JS. 2014. Effect of NAA and IBA on rooting of *Camellia* cuttings. International Journal of Agricultural Sciences and Veterinary Medicine [Internet]. [cited 2024 May 30]; 2(1):234-9. Available from: <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=801819a2b8dec329c6a59be2aa3aaca1f7025cd5>.
- Yang SX, Kieu DT, Le TH, Khuong HT. 2024. *Camellia hoana* (Theaceae, section *Corallina*), a new species from Bu Gia Map national park in Southern Vietnam. Dalat University Journal of Science [Internet]; [cited 2024 May 20]; 14(1):37-44. DOI: 10.37569/DalatUniversity.14.1.1207(2024).